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Efficacy and safety of the combination of reduced duration prophylaxis followed by immuno-guided prophylaxis to prevent cytomegalovirus disease in lung transplant recipients (CYTOCOR STUDY): an open-label, randomised, non-inferiority clinical trial

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ABSTRACT

Introduction Prolonged use of antivirals to prevent the development of cytomegalovirus (CMV) disease in lung transplant patients has been shown to have significant side effects, for which alternatives are being sought to reduce their use. The monitoring of cell immunity against CMV could be an alternative as it has shown to be useful in identifying transplant patients at low risk of infection, who could benefit from shorter prophylaxis. The **aim** of the CYTOCOR study is to demonstrate that the combination of a reduced prophylaxis strategy with subsequent CMV-specific immunological monitoring would allow CMV infection to be controlled in lung transplant patients as effectively as the usual strategy (prophylaxis followed by pre-emptive therapy), while reducing the side effects of antivirals due to the shorter duration of prophylaxis.

Methods and analysis Phase III randomised, open, multicentre, parallel, non-inferiority clinical trial to study the efficacy and safety of the combination of a prophylaxis strategy up to month +3 post-transplant followed by immuno-guided prophylaxis using the QuantiFERON-CMV technique up to month +12 post-transplant to prevent CMV disease in CMV-seropositive lung transplant recipients. This strategy will be compared with a combination of a usual prophylaxis strategy up to month +6 post-transplant followed by pre-emptive therapy up to month +12. To study the incidence of CMV disease, patients will be followed up to 18 months post-transplantation. A total of 150 patients are expected to be recruited for the study.

Ethics and public dissemination The clinical trial has been approved by the Research Ethics Committees and authorised by the Spanish Agency of Medicines and Medical Devices (AEMPS).

If the hypothesis of this clinical trial is verified, the dissemination of the results could change clinical practice by increasing knowledge about the safety and efficacy of discontinuing valganciclovir prophylaxis in lung transplant recipients.

Trial registration number NCT03699254.

Strengths and limitations of this study

- If the hypothesis of this clinical trial is confirmed, the usual clinical practice for the management of cytomegalovirus (CMV) in lung transplant recipients could be modified by incorporating the monitoring of specific cell immunity against this virus, which would allow for the better identification of patients at risk of CMV disease.
- It would also reduce the time of antiviral prophylaxis in many of these patients with CMV-specific immunity and therefore prevent or limit the adverse effects of this antiviral and lead to economic savings.
- The main limitation of this study is the complexity of the design of the clinical trials, which can hinder the inclusion and follow-up of candidates. For this reason, a sample size has been calculated assuming a 5% loss to follow-up and a conservative inclusion rate in the estimated time.
- Another limitation is the QuantiFERON-CMV technique, as this technique only measures CD8 +specificT-cell response and does not cover patients with rare human leucocyte antigen class I alleles.

INTRODUCTION

Cytomegalovirus (CMV) infection is a significant cause of morbidity and mortality in solid organ transplant (SOT) patients. The risk of

CMV disease in SOT patients depends on several factors, among others the transplanted organ, the donor/recipient CMV serology and the immunosuppression therapy used.[1 2]

Depending on each patient's risk, the prevention strategy to be used is defined. There are two types of strategies: universal prophylaxis and pre-emptive treatment. Universal prophylaxis consists of the administration of antivirals such as ganciclovir or valganciclovir during the first months post-transplant. Pre-emptive therapy is based on the administration of antivirals once the CMV replication has been detected in blood or serum, for which it is necessary to monitor the patient at regular intervals.[2 3]

In the particular case of lung transplant recipients, both the international and Spanish consensus guidelines on the management of CMV infection in SOT patients recommend universal prophylaxis in all lung transplant recipients of a CMV-positive donor, with the serology of the recipient determining the duration of prophylaxis: 12 months in CMV-negative recipients (D+/R-) and 6 months in CMV-positive recipients (D+/R+).[1 2]

However, the application of universal prophylaxis has associated risks due to the side effects of prolonged antiviral use. The most frequent side effects are leucopenia, digestive discomfort (diarrhoea, vomiting, abdominal pain) and renal dysfunction. To prevent or reduce the adverse effects of these antivirals, recent research has focused on the search for immunological biomarkers to help identify transplant patients at low risk of CMV replication/reactivation in which prophylaxis could be reduced or even discontinued. In particular, the monitoring of cell-mediated CMV immunity has been shown to be useful in guiding clinical decision-making in transplanted patients.[3–8]

Several techniques are currently available to monitor cell-mediated immunity to CMV, including the use of multimers, intracellular staining, Enzyme-Linked Immunospot assay (ELISPOT) and QuantiFERON-CMV assay (QF-CMV). Regardless of the technique used, several authors have shown that the presence of cellular immunity to CMV in pre-transplantation or post-transplantation is associated with a lower risk of CMV replication and/or disease.[9–18] Specifically, our group has been working for years in the pre-transplant monitoring of cell immunity against CMV in SOT patients using the QF-CMV technique (Qiagen). This is a functional technique that quantifies interferon-γ (IFNG) released by CMV-specific CD8 +T cells when stimulated with 22 CMV peptides.[16 19] Our published results indicate that patients with CMV-specific cell response (QF-CMV Reactive; ≥0.2 IFNG IU/mL) prior to transplantation have a lower risk of CMV replication after transplantation.[16]

Recently published results of a clinical trial in lung transplant recipients have shown that the monitoring of CMV-specific cell immunity, also measured by QF-CMV, permits individualising the preventive management of CMV disease in these transplant recipients.[20] The patients in the study were randomised to receive standard prophylaxis for 5 months or experimental prophylaxis guided by QF-CMV. The authors observed that the QF-CMV-guided experimental prophylaxis arm had a lower incidence of CMV infection than the standard prophylaxis arm. Therefore, the standardisation and validation of these studies have the potential to significantly change the monitoring and treatment of CMV infection in transplanted patients and further individualise strategies to prevent CMV infection.[20 21]

Bearing in mind these results, we have formulated a new hypothesis based on the fact that lung transplant patients who are QF-CMV Reactive at month +3 after transplantation could benefit from reduced duration prophylaxis, as they have specific immunity to maintain the virus under control. Thus, by combining a strategy of reduced duration prophylaxis (henceforth reduced prophylaxis) with immunological monitoring of CMV-specific response at a later stage would control CMV replication in these patients in the same way as the current strategy (prophylaxis followed by pre-emptive therapy), while reducing the side effects of antivirals since the duration of prophylaxis is shorter. The aims of CYTOCOR study are: (1) To evaluate the efficacy of reduced prophylaxis (3 months) followed by immuno-guided prophylaxis (QF-CMV Reactive, cut-off 0.2 IU/mL) to prevent CMV disease in R+lung transplant recipients in comparison with the usual strategy of universal prophylaxis (6 months) followed by pre-emptive therapy for 6 months and (2) To assess whether, in the patients of experimental group who develop CMV disease, an IFNG cut-off point other than 0.2 IU/mL could predict protection against the disease more reliably.

METHODS AND ANALYSIS

Design

This is a phase III randomised, open, multicenter, parallel, non-inferiority clinical trial. The patients will be assigned to two groups (figure 1):

1. Control Group (universal prophylaxis+pre-emptive therapy; 6+6): In this patients the recommendation of the Spanish Consensus Document[1] will be followed according to this strategy: (i) u*niversal prophylaxis* with valganciclovir (900 mg/24 hours, corrected for renal function) up to month +6. The use of associated immunotherapy (eg, anti-CMV hyperimmune immunoglobulin) will depend on each centre's clinical practice; (ii) *pre-emptive therapy guided by viral load from month +6 to month +12*. For a viral load above >38 copies/mL (>35 IU/mL) and depending on each centre's clinical practice, treatment with valganciclovir may be initiated (900 mg/12 hours, corrected for renal function). Blips must be excluded before starting treatment.
2. Experimental Group (reduced prophylaxis+immuno-guided prophylaxis; 3+9): (i) u*niversal prophylaxis* with valganciclovir (900 mg/24 hours, corrected for renal function) up to month +3. The use of associated immunotherapy (eg, anti-CMV hyperimmune immu-

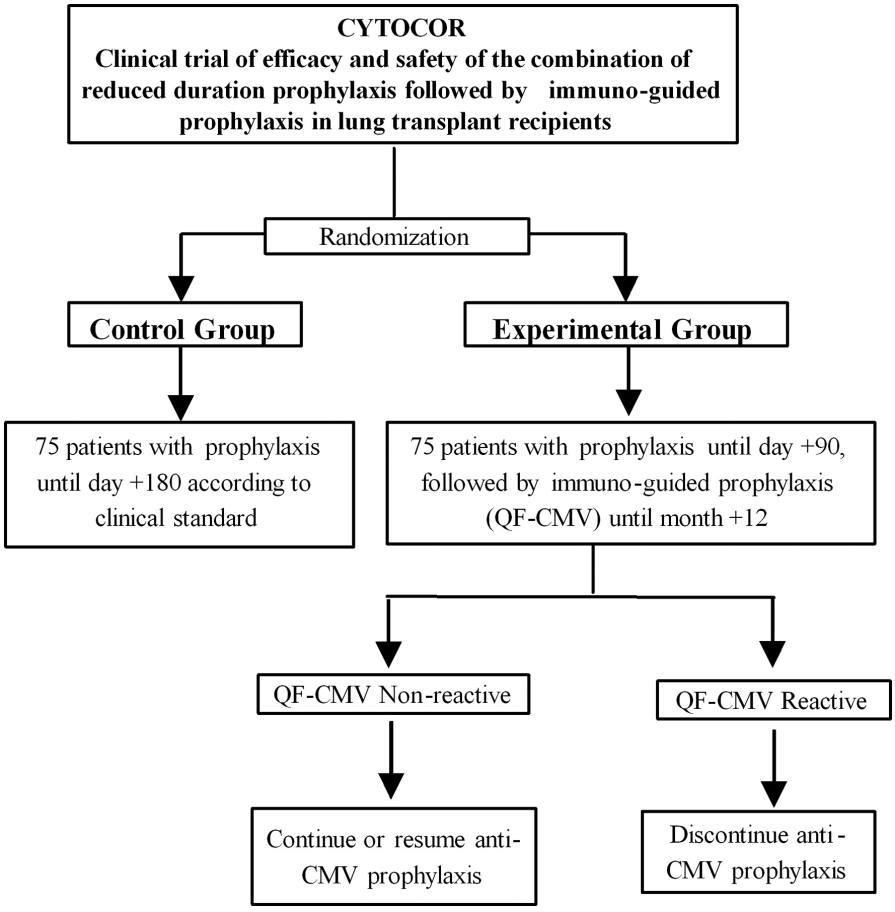

Figure 1 Flow diagram. CMV,cytomegalovirus; QF-CMV, QuantiFERON-CMV.

noglobulin) will depend on each centre's clinical practice; (ii) *immuno-guided prophylaxis*. This will consist of a monthly determination of cellular immunity by QF-CMV from month +3 to month +12. In this group, the strategy will be as follows (figure 2):

1. Following the first determination of positive specific immunity (QF-CMV Reactive), prophylaxis will be discontinued. Monthly monitoring of the specific immunity will continue until month +12. Cases in which the specific immunity is negative (QF-CMV Non-Reactive or Indeterminate) after initiating immuno-guided prophylaxis, valganciclovir prophylaxis will be resumed.
2. In patients in whom all cell immunity determinations are negative (QF-CMV Non-Reactive or Indeterminate), valganciclovir prophylaxis will be maintained until month +12.
3. At least one viral replication control will be performed with each cell immunity determination (monthly) and if positive (>38 copies/mL or >35 IU/mL), will be treated according to each centre's clinical practice.
4. If the patient in the experimental group continues with prophylaxis at month +12, the prophylaxis should be discontinued at this time.

In either of the two groups (control and experimental) and when indicated according to each centre's usual clinical practice, ganciclovir may be used (5 mg/kg/12 hours, corrected for renal function). All patients will be followed up to month +18 post-transplant to study CMV disease.

Study population and setting

The clinical trial is a multicentre project in which seven national lung transplant centres will participate. The trial will include lung transplant patients with positive CMV serology belonging to the participating centres. Patients who meet all the inclusion criteria and no exclusion criteria will be prospectively included in the study.

The inclusion and exclusion criteria for the trial are described in box 1.

Withdrawal criteria

Patients may withdraw from the study at any time, for any reason and without prejudice to future medical treatment. Patients who do not comply with the study procedure, have not been followed up or for whom no further information has become available since the date of withdrawal or the date of last contact shall be considered a study "withdrawal". The reasons for withdrawal will be examined in full accordance with bioethical principles regarding the guarantee of patients' rights and autonomous and informed consent. The criteria for withdrawal from the study are described below:

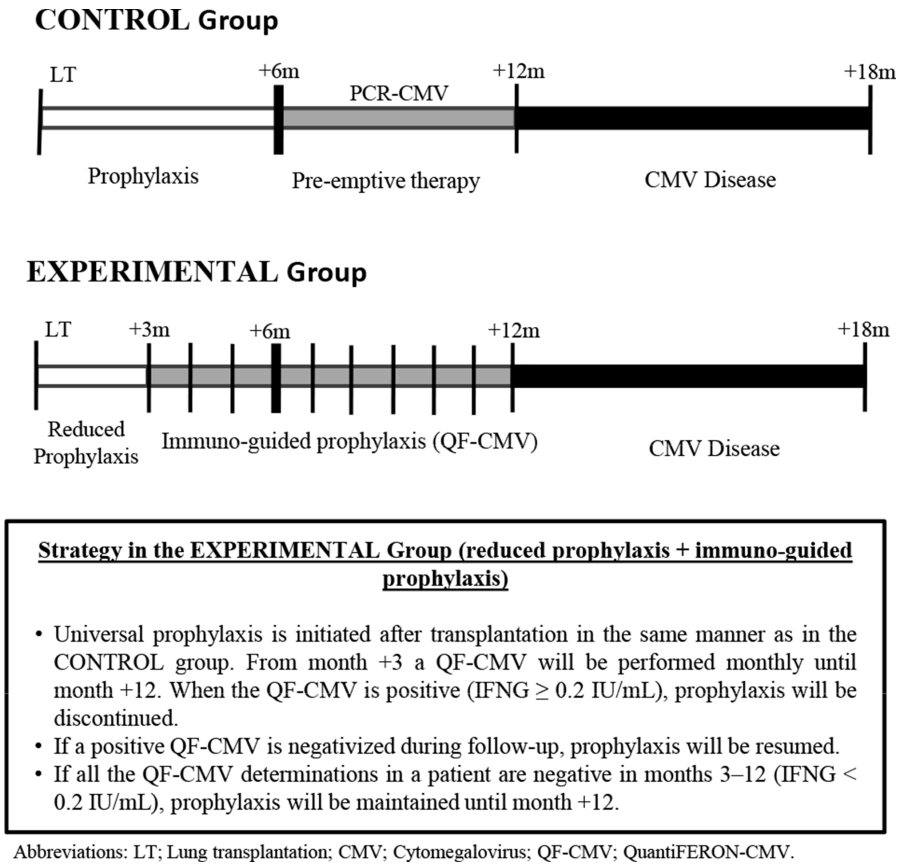

Figure 2 Study design. m, month; PCR, polymerase chain reaction.

1. On patient's request and withdrawal of patient's informed consent.
2. Protocol violation or deviation.
3. If considered clinically appropriate by the investigators when the patient's symptoms worsen.
4. Administrative decision by investigators, sponsor or a regulatory authority.
5. Loss to follow-up.
6. Serious adverse event or clinically relevant event at the discretion of the researcher.
7. Unexpected serious adverse reaction at the discretion of the researcher.
8. The endpoint of the study is reached:
   - In the experimental group (3+9): Loss to follow-up/Death/QF-CMV Non-reactive at month +12.
   - In the control group (6+6): Loss to follow-up/Death/CMV replication at month +12.

The investigator shall indicate whether the patient or the investigator made the decision to withdraw from the study and which of the following possible reasons led to the withdrawal:

1. Any patient in either group who develops CMV disease (symptomatic replication or organ disease without viraemia) will be withdrawn from the study (patients who develop asymptomatic replication will not be withdrawn from the study; they will be treated and the scheduled follow-up will continue).
2. On patient's request and withdrawal of informed consent.
3. Protocol violation or deviation (eg, non-compliance with treatment, need for prohibited treatment, etc).
4. If considered clinically appropriate by the investigators when the patient's medical condition worsens.
5. Administrative decision by investigators, sponsor or a regulatory authority.

Box 1 Study selection criteria

Inclusion criteria

1. Lung transplant recipients with positive pre-transplant CMV serology
2. Over 18 years of age
3. That the expected time of prophylaxis with valganciclovir is 6 months post-transplant
4. Patients who have given written informed consent

Exclusion criteria

1. Pre-transplant CMV-seronegative recipients
2. HIV-infected patients
3. Pregnant and/or lactating women
4. Intolerance to valganciclovir/ganciclovir
5. Multivisceral transplant patients
6. Patients who cannot comply with the follow-up protocol

CMV, cytomegalovirus.

Table 1 Definitions included in the study

Type	Definition
CMV replication	Can be diagnosed by growing the virus in vitro, finding evidence of viral infection by intra-cytoplasmic or intra-nuclear inclusions or by antibody-based staining techniques for CMV in histopathological sections or finding evidence of replication using nucleic acid based assays or antigenaemia studies.
CMV disease	Evidence of CMV infection with attributable symptoms. CMV disease can be sub-classified into CMV viral syndrome or tissue invasive disease. Definitive diagnosis of CMV pneumonia must be based on the histological demonstration of CMV invasive disease. Nevertheless, for this study we also accept the evidence of CMV infection (including detection of CMV-DNA in bronchoalveolar lavage (BAL)) with attributable symptoms once other potential causes has been ruled out.

CMV, cytomegalovirus.

Study variables

Efficacy variables

1. Primary outcome variable: Incidence of CMV disease at 18 months post-transplant. "CMV disease" is defined as evidence of CMV infection with attributable symptoms (table 1). CMV disease can be sub-classified as CMV viral syndrome or invasive tissue disease.[2]
2. Secondary outcome variables: Incidence of CMV replication (excluding replication blips in periods of prophylaxis). "CMV replication" is defined as a viral load greater than 38 copies/mL (equivalent to approximately 35 IU/mL) (table 1).[2]

Explanatory variables

The following demographic and clinical information will be collected from all the patients: age, sex and baseline disease, type of transplant (single lung or double lung), pre-transplant CMV serology, donor and recipient HLA typing, induction immunosuppressive therapy (dose and duration), maintenance immunosuppressive therapy (dose and duration), CMV antiviral treatment (dose and duration, including immunotherapy), other opportunistic infections not associated with CMV (bacterial, viral and fungal), acute or chronic graft rejection (time since transplantation, number of episodes and treatment) and adverse effects attributable to CMV antiviral treatment (total number of granulocyte-colony stimulating factor doses required or reduction in immune suppression on the basis of low white blood cells/neutrophil count).

Randomisation and masking

The number of patients will be 150 (75 in each group). Patients who meet the selection criteria will be randomised, and may be included in the control group or the experimental group. Randomisation will be carried out by means of electronic case report forms (eCRFs). The ratio will be 1:1 for each group and stratified by centres. The study design is open, but the investigator will not know the treatment assignment until the patient signs the informed consent form and randomisation is performed, thus minimising selection bias.

Study procedures

The duration of follow-up for each patient will be 18 months and will start from the moment the patient is transplanted. A total of 15 visits will be scheduled during the trial: one visit during the first 30 days post-transplant, 12 monthly visits up to the first 12 months post-transplant, one visit at 15 months post-transplant and one visit at 18 months post-transplant. The follow-up visits in the Control and Experimental groups will be scheduled as they are shown in tables 2 and 3, respectively. All visits may be made 7 days before or after the day indicated by the protocol without being considered deviation, as they will coincide with the visits made following usual clinical practice.

Both groups: As for determination of CMV replication, in the first 3 months it will not be compulsory to monitor viral replication (unless indicated according to the centre's clinical practice) nor will CMV-specific cell immunity be determined because both groups are receiving universal prophylaxis. It will only be compulsory to draw a sample for CMV viral load in patients in which prophylaxis has been discontinued. The first determination of CMV viral load will coincide with the day prophylaxis is discontinued and taken as a baseline determination. In centres where CMV viraemia is monitored by antigenaemia assay or the viral load is determined in whole blood, an aliquot should be sent to the laboratory of the coordinating centre to determine the CMV viral load.

Experimental group: To determine the CMV-specific cell immunity, all the samples will be sent to the laboratory of the coordinating centre for analysis. Depending on the results, the following procedures will be performed: (a) If the QF-CMV is Reactive (IFNG ≥0.2 UI/mL) at any of these visits, valganciclovir prophylaxis will be discontinued; (b) If the QF-CMV is Non-Reactive (IFNG <0.2 IU/mL), prophylaxis will be continued (or reinitiated if previously discontinued) and (c) In those cases in which the patient of the experimental group is on prophylaxis until month +12, prophylaxis will be discontinued.

Statistical analysis

The required sample size for a non-inferiority trial has been calculated assuming an 85% success rate of the

Table 2 Summary chart of visits (control group)

Procedures control group	Visit 1 (first 30 days)	Visit 2 to Visit 7 (month +1 to+6)	Visit 8 to Visit 13 (month +7 to +12)	Visit 14 and Visit 15 (month +15 and +18)
Informed consent	X			
Inclusion/exclusion criteria	X			
Randomisation	X			
Medical history/anamnesis	X	X	X	X
Physical examination*	X	X	X	X
Pregnancy test	X			
Antiviral prophylaxis	X	X		
CMV PCR sample†		X	X	
Haemogram/biochemistry‡	X	X	X	X
Adverse events/concomitant medication	X	X	X	X

*Physical examination: weight, heart rate, blood pressure, temperature, abdominal palpation and presence of oedemas
†CMV PCR will be compulsory when antiviral prophylaxis is discontinued. The first CMV PCR will be performed at Visit 7 (month +6), coinciding with the discontinuation of antiviral prophylaxis. At Visits 14 and 15 it will not be compulsory to draw samples for viral load (unless indicated according to the center's clinical practice).
‡Haemogram: red blood cells, haemoglobin, leukocytes, neutrophils and platelets. Biochemistry: alkaline phosphatase, gamma-glutamyltransferase, alanine aminotransferase, aspartate aminotransferase, C-reactive protein, bilirubin, albumin and creatinine.
CMV, cytomegalovirus; PCR, polymerase chain reaction.

control group, a non-inferiority margin of 7%, an alpha risk of 0.05, a power of 80% (beta risk 0.20) and an estimated follow-up loss of 5%. The total number of patients required per group is 75 (total sample of 150 patients).[22] The sample size was determined in order to address the primary objective of the study, that is, to evaluate if the experimental regimen is not less effective than the control regimen (non-inferiority study) in terms of the incidence of CMV disease in the 18 months post-transplant (primary endpoint).

Clinical data will be collected by means of eCRFs. All analyses will be performed using PASW Statistics software

Table 3 Summary chart of visits (experimental group)

Procedures experimental group	Visit 1 (first 30 days)	Visit 2 to Visit 3 (month +1 to +2)	Visit 4 to Visit 13 (month +3 to +12)	Visit 14 and Visit 15 (month +15 and +18)
Informed consent	X			
Inclusion/exclusion criteria	X			
Randomisation	X			
Medical history/anamnesis	X	X	X	X
Physical examination*	X	X	X	X
Pregnancy test	X			
Antiviral prophylaxis†	X	X	X	
CMV PCR ‡,§		X	X	
QF-CMV sample§			X	
Haemogram/biochemistry¶	X	X	X	X
Adverse events/concomitant medication	X	X	X	X

*Physical examination: weight, heart rate, blood pressure, temperature, abdominal palpation and presence of edemas
†In month +3 post-transplant, the patient will continue with antiviral prophylaxis depending on the QF-CMV results.
‡CMV PCR will be compulsory when antiviral prophylaxis is discontinued. The first CMV PCR will coincide with the day prophylaxis is discontinued and will be taken as a baseline determination.
§At Visits 14 and 15 it will not be compulsory to draw samples for viral load or for QF-CMV (unless indicated according to the center's clinical practice)
¶Haemogram: red blood cells, haemoglobin, leukocytes, neutrophils and platelets. Biochemistry: alkaline phosphatase, gamma-glutamyltransferase, alanine aminotransferase, aspartate aminotransferase, C-reactive protein, bilirubin, albumin and creatinin
CMV, cytomegalovirus; PCR, polymerase chain reaction; QF-CMV, QuantiFERON-CMV.

V.15.0 (IBM Corporation) and R software (V.3.5.0). Frequencies and percentages will be calculated for the qualitative variables and compared using the X^2 test or Fisher's test. For quantitative variables, the mean and SD will be calculated. Normality will be analysed using the Kolmogorov-Smirnov test and comparisons will be made using the Student's t-test or the Mann-Whitney test depending on whether or not they follow a normal distribution, respectively. For the comparison of three or more groups, the analysis of variance (ANOVA) or Kruskal-Wallis tests will be performed. The incidence of CMV disease according to the strategy used will be calculated by Kaplan-Meier curves which will be compared using the log-rank test. If patients in the Experimental Group (3+9) develop CMV disease, a multivariate Cox proportional hazards regression model will be used. The Receiver Operating Characteristic (ROC) curve will be used to calculate if there is a cut-off in IFNG levels other than 0.2 UI/mL that could better predict protection against CMV disease.

ETHICAL ISSUES AND DISSEMINATION PLAN

This clinical trial will be conducted in accordance with the protocol, the principles set out in the current revised version of the Declaration of Helsinki (Fortaleza, 2013) and in accordance with the applicable regulatory requirements, in particular the ICH Tripartite Guideline "Standards of Good Clinical Practice", Royal Decree 1090/2015 regulating clinical trials with medicinal products in Spain, and Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use.

The protocol, the informed consent form, the patient information form and any documents applicable to the study have required approval by the appropriate regulatory agencies. The study has been approved by the Coordinating Committee for Biomedical Research Ethics. Authorisation has also been obtained from the Spanish Agency of Medicines and Medical Devices (AEMPS).

The trial is registered in publicly accessible databases such as the Spanish Clinical Studies Registry (REec) and ClinicalTrials.gov (NCT03699254).

DISCUSSION

Immunological monitoring of CMV infection in transplanted patients has been shown to be useful in identifying patients who are protected against infection by the virus after transplantation and in whom antiviral treatment may be reduced or discontinued. However, the vast majority of studies that have addressed this issue are observational,[4–19] and do not provide solid evidence that is strong enough for rapid implementation in routine clinical practice. It is therefore a major challenge to perform intervention studies to demonstrate that the monitoring of cell immunity against CMV in transplanted patients is a very useful in routine clinical practice.

In the particular case of lung transplant patients, the results of the first clinical trial in these patients in which immunological monitoring is used to individualise the duration of universal prophylaxis have recently been published.[20] In this study, patients in the arm with experimental prophylaxis immuno-guided by QF-CMV showed a lower incidence of CMV infection than in the arm with standard prophylaxis for a duration of 5 months.

However, if the hypothesis of our study is confirmed, the duration of CMV antiviral prophylaxis in lung transplant patients could be further shortened, as it could be reduced to only 3 months in patients who already present specific CMV-specific immunity at 3 months. This would reduce the toxicity associated with the prolonged use of antivirals and lead to greater economic savings of antiviral drugs.

On the other hand, there is scientific evidence of late-onset CMV disease at 12 months after lung transplantation.[23] Therefore, our study could prove whether in the subgroup of patients who reach month +12 without cell immunity to the virus (QF-CMV Non-Reactive) have a higher incidence of late CMV disease. These results would serve to evaluate the possibility of prolonging prophylaxis with antivirals in this small subgroup of patients.

As for the technique we intend to use to monitor cell immunity against CMV in our study population, we have chosen QF-CMV because it is a standardised technique with a well-defined cut-off, requires minimal sample manipulation, is easy to use, provides negative and positive controls for each patient and is automatable. Additionally, our group has used this technique for years and we therefore have broad experience and a highly qualified staff.

In conclusion, the CYTOCOR study aims to individualise the management of CMV infection in lung transplant patients by monitoring CMV-specific immunity. If our hypothesis is confirmed, the management of CMV infection in lung transplant patients could be individualised in such a way that: (1) The toxicity associated with the prolonged use of antivirals would be reduced, (2) Economic costs would be reduced by decreasing the antiviral treatment, (3) Costs of virological monitoring would be saved and (4) Morbidity and costs associated with late disease would be saved after inadequate discontinuation of prophylaxis in these patients.

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Contributors JT-C, AP-V, SC and JMV were responsible for formulating the research question, the methodological design of the study and writing the first draft of the manuscript. JT-C, AP-V and SC were responsible for obtaining financial support for the study. EV and ABP revised the statistical analysis plan and the methodological aspects of the study. JT-C, AP-V and JMV collaborated in the selection of the hospitals participating in the study. AL-P and MAL-A drafted the drug safety surveillance portion of the study. JT-C is the coordinating investigator. PU, RA-M, AP, IO-G, VM and DI have collaborated in the critical revision of the manuscript. All authors have read and approved the final manuscript.

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Patient consent for publication Not required.

Ethics approval In accordance with Royal Decree 1090/2015 of 4 December concerning the regulation of clinical trials with medicinal products in Spain, multicenter clinical trials only require the approval of a single Medicinal Research Ethics Committee (reference MREC) and of the Spanish Agency of Medicines and Medical Devices (AEMPS). This clinical trial has been approved by the reference MREC of the province of Cordoba and the AEMPS (Code: FCO-CYT-2018-01; EudraCT Number: 2018-003300-39).

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