Safety and efficacy of immunoguided prophylaxis for cytomegalovirus disease in low-risk lung transplant recipients in Spain: a multicentre, open-label, randomised, phase 3, noninferiority trial

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Summary

Background The standard prophylaxis treatment for cytomegalovirus (CMV) disease in CMV-seropositive lung transplant recipients is six months of prophylaxis with valganciclovir followed by six months of pre-emptive therapy. This protocol is associated with adverse events and risk of resistance. We have previously shown that prophylaxis can be suspended in CMV-seropositive kidney transplant recipients receiving thymoglobulin without increasing the risk of CMV disease and reducing the incidence of neutropenia. The objective of the current study is to demonstrate that immunoguided prophylaxis is effective and safe in seropositive lung transplant recipients.

Methods A phase III, multicentre, randomised, open-label, noninferiority clinical trial was conducted in adult lung transplant recipients. Patients were randomised (1:1) to two groups: (1) immunoguided prophylaxis (IP), consisting of 3 months of universal prophylaxis followed by CMV-specific cell-mediated immunity-guided discontinuation, or (2) standard prophylaxis (SP), consisting of 6 months of prophylaxis followed by pre-emptive therapy, both for a total of 12 months. The noninferiority margin was 7%. The primary and secondary efficacy endpoints were CMV disease and asymptomatic CMV replication at month 18. The primary and secondary safety endpoints were incidence of neutropenia (defined as neutrophil count <1500 cells/μL), incidence of rejection and number of days of valganciclovir prophylaxis. This trial was registered in EudraCT (2018-003300-39) and ClinicalTrials.gov (NCT03699254). This trial has been completed.

Findings Patients were recruited between April 2019 and December 2021 in seven Spanish centres. A total of 150 patients were randomised (75 patients per group). Incidence of CMV disease at month 18 did not differ among groups (18·7% [14 patients] vs. 16·0% [12 patients]; risk difference [RD] −0·03 [95% CI −0·15% to 0·06%]; $P = 0·620$) but occurred earlier in the IP group compared to the SP group. The proportion of patients who developed CMV disease at ≤180 days after transplant was higher in the IP group compared with the SP group (8% [6 patients] vs. 0% [0 patients]; RD −0·08 [95% CI −0·14 to −0·02; $P = 0·009$]). Asymptomatic CMV replication was reduced in the IP

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group vs. the SP group (4·0% [3 patients] vs. 16·0% [12 patients]; adjusted RD 0·12 [95% CI 0·03–0·21; $P = 0·009$]). A total of 30 patients (40%) in the IP group did not require prophylaxis from month 4 to 12. No significant difference was observed in the proportion of patients with neutropenia during months 4 to 7 (14·7% [11 patients] vs. 25·3% [19 patients]; RD 0·11 [95% CI –0·02 to 0·23]; $P = 0·090$) or rejection (33·3% [25 patients] vs. 30·7% [23 patients]; RD –0·03 [95% CI –0·18 to 0·12; $P = 0·690$]). The median days of valganciclovir was lower in the IP group than in the SP group (137 [92–266] vs. 198 [173–281]; $P < 0.001$).

Interpretation Immunoguided prophylaxis was noninferior to the standard of care in preventing CMV disease in lung transplant recipients. It could be considered for implementing in clinical practice in CMV-seropositive lung transplant recipients upon considering the study limitations.

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Keywords: Cytomegalovirus infection; Clinical trial; Immunoguided prophylaxis; IGRA-CMV; Interferon-gamma; Lung transplantation

Research in context

Evidence before this study

We searched PubMed for studies on cytomegalovirus prevention on lung transplant patients (SOT) published prior to May 30, 2024. The search terms included ["cytomegalovirus"] AND ["prophylaxis" OR "prevention"] and ["lung transplant" OR "solid organ transplant"]. We also reviewed guidelines published by major international scientific societies and retrieved additional studies from the authors' personal reference lists and the reference lists of the included publications. No restriction on publication type or language was applied. We identified one international consensus and one Spanish consensus with specific recommendations for lung transplant patients. Antiviral prophylaxis is recommended for 6 months followed by 6 months of pre-emptive therapy.

Regardless of the technique used, the presence of CMV-CMI is associated with the risk of CMV disease. A randomized clinical trial in lung transplant patients has shown that it is safe to withdraw prophylaxis when CMV-CMI is protective. Another randomized clinical trial has shown that prophylaxis can be suspended in CMV-seropositive kidney transplant recipients receiving thymoglobulin without increasing the risk of CMV disease and reducing the incidence of neutropenia.

Added value of this study

In this randomized, open-label trial, the median number of days with antiviral prophylaxis was significantly lower in the immunoguided prophylaxis group compared to the standard prophylaxis group. Nonetheless, the immunoguided prophylaxis was noninferior to the standard of care for preventing CMV disease and no significant reduction in neutropenia was observed. It could be considered for implementing in clinical practice upon considering the study limitations.

Implications of all the available evidence

Immunoguided prophylaxis could be considered for implementing in clinical practice in CMV-seropositive lung transplant recipients upon considering the study limitations.

Introduction

Cytomegalovirus (CMV) infection causes extensive morbidity and mortality in lung transplantation.[1,2] The standard of care for transplant recipients who are CMV-seropositive is 6 months of prophylaxis with valganciclovir.[3,4] After prophylaxis, pre-emptive therapy is frequently administered until month 12 and guided by CMV viral load. Prolonged use of valganciclovir is associated with myelosuppression which requires the discontinuation of prophylaxis or the use of granulocyte colony-stimulating factor.[5] Furthermore, prolonged use of valganciclovir, particularly when the adjusted dose is subtherapeutic, may be associated with resistance, due to mutations in the UL97 gene.[6–8]

Strategies to avoid these adverse events (AEs) include the use of non-myelotoxic drugs such as letermovir[9] or the application of immunological biomarkers that help identify transplant patients at low risk of CMV disease in whom prophylaxis could be avoided. Specifically, the monitoring of CMV-specific cell-mediated immunity (CMV-CMI) has shown to be useful in individualising the preventive management of solid organ transplantation.[10–12] Several techniques are currently available to monitor CMV-CMI, including the use of

multimers, intracellular staining, and the commercially available assays ELISPOT and QuantiFERON-CMV (QF-CMV).[13–17] QF-CMV measures the release of interferon-gamma (IFNG) by CMV-specific CD8+ T lymphocytes *in vitro* when stimulated with CMV peptides.[18] Regardless of the technique used, the presence of CMV-CMI is associated with the risk of CMV disease.[16,19,20] A randomised clinical trial has shown that prophylaxis can be safely withdrawn when CMV-CMI is positive.[21] The TIMOVAL clinical trial showed that prophylaxis can be suspended in CMV-seropositive kidney transplant recipients receiving thymoglobulin without increasing the risk of CMV disease, while also reducing the incidence of neutropenia.[22] The objective of the current study is to demonstrate that immunoguided prophylaxis (IP) is effective and safe in CMV-seropositive lung transplant recipients.

Methods

Study design and participants

This phase III, multicentre, randomised, open-label, noninferiority clinical trial evaluated the efficacy and safety of immunoguided discontinuation of valganciclovir prophylaxis vs. standard prophylaxis (SP) for CMV disease in CMV-seropositive lung transplant recipients. Eligible patients were CMV-seropositive adult lung transplant recipients with an expected duration of 6 months of prophylaxis. Patients who were CMV-seronegative, HIV-positive, pregnant or lactating women, multivisceral transplant recipients, or unable to follow the protocol were excluded. Patients were recruited between April 2019 and December 2021 in seven Spanish centres.

The Ethics Committee (Institutional Review Board) of the coordinating hospital (Reina Sofia University Hospital, Code FCO-CYT-2018-01-4027, Date of approval 29/October/2018) approved the protocol. Other centres approved the protocol when necessary. The original protocol was not amended. All patients or their legal representatives signed informed consent. All personal data were self-reported by participants to describe the population. De-identification has been achieved by pseudonymization replacing subjects' direct identifiers and assigning every subject with a unique code that can only be traced back to the subject through a unique key stored separately. The study was conducted in accordance with the Good Clinical Practice guideline (International Conference on Harmonisation) and complies with Spanish law. Patients could withdraw from the study at any time. Patients who did not comply with the study procedure were considered a study 'withdrawal'. Patients were followed up for 18 months or until lost to follow-up, exclusion or death (whichever occurred first). The trial was registered in EUDRACT (2018-003300-39) and ClinicalTrials.gov (NCT03699254).

Randomisation and masking

Screening and randomisation were performed on the first visit, within 30 days of transplantation. Patients were randomised in a 1:1 ratio to IP or SP by a computer-generated web-based allocation using permuted blocks of ten. Patients and investigators were not masked to intervention. All patients were managed following the protocol of each centre until randomisation to reduce selection bias.

Intervention

Supplementary Figure S1 shows the CMV prevention strategy for both groups. Patients in the SP group received the recommended prophylaxis with valganciclovir for 6 months and continued with pre-emptive therapy until month 12.[3,4] Pre-emptive therapy was guided by plasma CMV viral load performed with the PCR commercial kit used in standard clinical practice at each centre. CMV viral load testing was performed at least every 2 weeks. The cut-off point to initiate treatment was according to the centre's standard clinical practice.

Patients in the IP group received 3 months of universal prophylaxis followed by IP until month 12. All patients were monitored monthly for CMV-CMI with QF-CMV. Prophylaxis was continued when CMV-CMI was negative (i.e. non-reactive or indeterminate QF-CMV) and was discontinued when CMV-CMI was positive (i.e. reactive QF-CMV). Both universal prophylaxis and pre-emptive therapy were discontinued after month 12. CMV viral load testing was performed monthly in parallel with CMV-CMI. Treatment was permitted when CMV disease was diagnosed at any time and with any viral load.

Endpoints

The primary efficacy endpoint was CMV disease at month 18. CMV disease was defined in accordance with current recommendations,[3,4] that is, evidence of CMV replication in any body fluid or tissue specimen with attributable symptoms. CMV disease can be further categorized as a viral syndrome (i.e, fever, malaise, leukopenia, and/or thrombocytopenia) or as an organ disease.

Subgroups for sensitivity analysis included age, gender, pre-transplant immunosuppression, basiliximab use, rejection, use of CMV-specific immunoglobulins (CMV-Ig) and presence of mechanistic target of rapamycin (mTOR) inhibitors. The secondary efficacy endpoint was asymptomatic CMV replication. The primary safety endpoint was neutropenia (neutrophil count <1500 cells/μL). Since both groups received prophylaxis in the first 3 months, we only analysed the period between months 4 and 7 post-transplantation. Secondary safety endpoints were rejection and the number of days of valganciclovir prophylaxis.

AEs were recorded at all study visits, and laboratory tests were performed at each centre. An AE was considered serious when it required the suspension of prophylaxis or hospitalisation, was life-threatening, or resulted in death or disability.

Clinical assessment and other variables

Valganciclovir was used for universal prophylaxis (900 mg once daily). Intravenous ganciclovir was also permitted when necessary. The dose was adjusted with creatinine clearance (calculated with the Cockcroft–Gault formula) following standard recommendations. Prophylaxis could be temporarily interrupted for any reason, but the patient was excluded if prophylaxis was not reinitiated within 14 days. Adherence was assessed at each visit. CMV-Ig were allowed in both groups following the protocol of each centre.

Patients were treated according to local clinical practice. Induction immunosuppression with basiliximab was permitted. Maintenance immunosuppression consisted of the association of tacrolimus, mycophenolate mofetil/mycophenolic acid and steroids. When indicated, mTOR inhibitors were used. CMV disease was treated with full dose ganciclovir (5 mg/kg every 12 h) or valganciclovir (900 mg twice daily) according to severity and local protocols.

Follow-up in all patients, including those with CMV disease, continued until month 18. A summary of visits in both groups is shown in Supplementary Tables S1 and S2. During the period of home confinement due to the COVID-19 pandemic, the inclusion of patients and some follow-up visits were cancelled or delayed. Telephone visits were acceptable according to the indications of the Spanish Agency for Medicines and Health Products (AEMPS, informative note reference MUH 04/2020 of 16 March 2020 on exceptional measures applicable to clinical trials to manage problems derived from the COVID-19 emergency; www.aemps.gob.es). The delay or rescheduling of visits was not considered a major deviation. When a face-to-face visit was delayed in the IP group, patients continued their current treatment (i.e. with or without prophylaxis) until the next visit at which CMV-CMI determination was available.

Efficacy and safety endpoints were clinically assessed at all study visits. Other variables recorded were age, gender, lung disease, type of transplant, retransplantation, pre-transplant immunosuppression, level of immunoglobulin G (IgG) at month 3, rejection and basal immunosuppression. A senior clinical research monitor reviewed all data.

Assessment of CMV-CMI

CMV-CMI was assessed in the IP group using the QF-CMV assay and performed according to the manufacturer's instructions (QIAGEN, Germany). In brief, 1 mL of heparinised whole blood was collected in 3 QF-CMV blood collection tubes. The tubes contained either (i) a mix of 22 CMV peptides; (ii) a negative control (no antigens) or (iii) a positive mitogen control (containing phytohaemagglutinin). After collection, the tubes were shaken vigorously and incubated for 16–24 h at 37 °C. Supernatants were subsequently harvested and analysed for IFNG (IU/mL) by standard ELISA. According to the manufacturer's instructions, a result was considered 'reactive' (positive CMV-CMI) when the CMV antigen tube response minus the negative control response was ≥0·2 IU/mL of IFNG and 'nonreactive' (negative CMV-CMI) when the level was <0·2 IU/mL. A result was 'indeterminate' when the IFNG level was <0·2 IU/mL in the CMV antigen tube and <0·5 IU/mL in the mitogen tube (once the negative control was subtracted).

Similarly, for the classification and regression tree (CART) analysis (see the Statistics section below for further details), the quantitative IFNG level (UI/mL) in the CMV antigen tube response minus the negative control response was used.

DOOR analysis

A DOOR analysis was performed to assess the composite variable CMV disease plus neutropenia. The best endpoint was defined as no CMV disease without neutropenia and the worst endpoint as CMV disease with neutropenia (Supplementary Table S3). The categories between these two extremes were no CMV disease with neutropenia and CMV disease without neutropenia.

DOOR is a method for comparing groups using a single, ordinal patient-centred endpoint that represents a global assessment of patient endpoint, including efficacy and safety variables. The analysis consists of estimating the probability of a more desirable result in one group relative to another. A probability of 50% implies equality of groups. In contrast, a probability greater than 50%, combined with a 95% confidence interval (CI) that excludes 50%, indicates a significantly greater likelihood of a better endpoint in one group compared with the other (and vice-versa).

Sample size calculation and statistical analysis

The sample size was calculated based on the noninferiority of the primary efficacy endpoint (proportion of CMV disease in the 18 months post-transplant <7%). We assumed a CMV disease incidence of 15% in the SP group. With this estimation (alpha error = 0·05, power = 0·80, lost to follow-up 5%, double tail, and a noninferiority limit of 7%), the calculated sample size was 150 patients (75 patients in each group). Since the sample size was calculated for the primary efficacy endpoint, the analysis of other endpoints must be interpreted as exploratory.

The results were expressed as medians and interquartile range (IQR) for the quantitative variables and as frequencies (percentages) for the qualitative variables. Normality was analysed using the Kolmogorov–Smirnov

test. Continuous variables were analysed using the Student's *t*-test or the Mann–Whitney U test. Categorical variables were compared using the Chi-square test or Fisher exact test.

The analysis population for efficacy and safety endpoints included all randomised patients who initiated valganciclovir treatment. The proportion of missing data per variable is shown in Supplementary Table S4. The little MCAR test was used to verify that missing data were at random and imputation was done using the Markov chain Monte Carlo method. To ensure consistency with the analytical model, the imputation model included all relevant covariates as well as the outcome variable (CMV disease) to improve the quality of imputations. Collinearity among variables was assessed and considered during the imputation process to maintain the integrity of the data relationships. Discontinuations from the study were considered 'failures'.

The risk difference (RD) between both groups and the two-sided 95% CI were calculated using the Mantel–Haenszel method. The efficacy endpoints were analysed by comparing the proportion of CMV disease and asymptomatic CMV replication between both groups at month 18. To compare the event probability of CMV disease and neutropenia in both groups (one minus the overall survival probability) the two-sided log-rank test was used. An additional stratified analysis of RD was performed using the Mantel–Haenszel method. *P*-values were obtained by the Wald test. Number of days on valganciclovir, including prophylaxis and preemptive treatment, were compared and the RD for the two strati (180 days) was also calculated.

We conducted a mixed-effects multivariable logistic regression analysis to evaluate CMV disease at 18 months as the primary outcome. Fixed effects were included for the independent variables of interest (including age, gender, strategy of prevention [IP/SP], use of basiliximab, type of transplant [uni/bipulmonary] and rejection [yes/no]). The centre was modelled as a random effect (intercept) to account for variability between centres. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated for the fixed effects, while the variability associated with the random effects was captured through the variance component. In addition to the logistic regression model, we conducted a sensitivity analysis using a parametric survival model (Weibull model) to assess time to event. This analysis was performed after confirming the non-compliance with the proportional hazards assumption using Schoenfeld residual plots.

A classification and regression tree (CART) analysis was also performed to evaluate the variables that best grouped patients according to those who required prophylaxis from months 4 to 12 and those who did not. To control for the site effect, centres were classified into those with low or high risk of receiving prophylaxis using TreeNet and considering all other variables (Supplementary Figure S2). Centres classified as low risk had a low prophylaxis rate after consideration of patients' features. This analysis was confirmed in an adjusted model using multivariable logistic regression. The Akaike information criterion was used to select the final logistic models.

Data were censored at the last assessment. *P*-values of $\leq$0·05 were considered statistically significant, and all tests were two-sided. SPSS 25·0 software (SPSS Inc.), R software, CART, and TreeNet (both version SPM 8·3) were used for the statistical analysis.

Deviations from the protocol

This study was carried out during the COVID-19 pandemic, which has led to some deviations from the original protocol as follows: (i) some patients were unable to attend follow up visits; (ii) CMV-CMI monitoring could not always be performed exactly when indicated per protocol in IP group, potentially leading to some positive CMV-CMI tests going undetected and prophylaxis not discontinued. This has led to the prolongation of prophylaxis in some patients thus reducing the risk of CMV disease and increasing the risk of neutropenia. Failure to attend follow-up visits also could have impacted the SP group resulting in irregular virological monitoring, thus potentially increasing the risk of CMV disease.

Role of the funding source

The funder of the study was the Carlos III Health Institute (grant numbers PI18/00099) and the Andalusian Society of Organ and Tissue Transplantation (SATOT). QIAGEN supported the professional editing service and the open-access publication fee. No funder had a role in the study design, data acquisition, analysis or writing up.

Results

Participants

Fig. 1 shows the patient flow chart during the trial. One hundred and fifty patients were randomised at a median of 9·5 days (IQR 6·0–13·0 days): 75 patients to the SP group and 75 to the IP group. Sixty-seven (89·3%) and 65 patients (86·7%) completed the planned follow-up in each group, respectively. All 150 patients received at least one prophylaxis dose and were included in the analysis. Table 1 shows the baseline characteristics of the analysed population. The proportion of missing data for each variable is presented in Supplementary Table S4.

Efficacy endpoints

Fourteen patients (18·7%) in the IP group developed CMV disease (1 organ disease and 13 viral syndromes), vs. 12 patients (16·0%) in the SP group (4 organ disease and 8 viral syndromes) (RD: –0·03 [95% CI –0·15 to

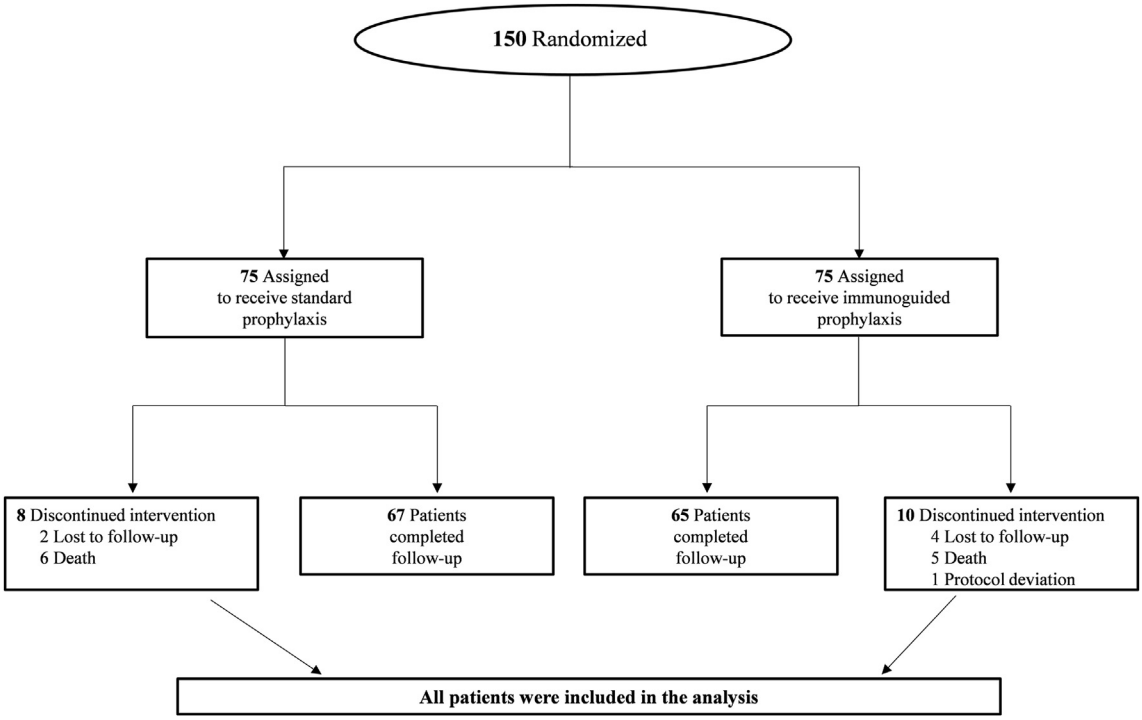


Fig. 1: Patient's flow through the study.

0·06]; P = 0·620 Table 2). Time to CMV disease was shorter in the IP group than in the SP group (190 days [156–204] vs. 260 days [244–343]; P < 0·001). The proportion of patients who developed CMV disease within 180 days post-transplant was significantly higher in the IP group than in the SP group (8% [6 patients] vs. 0% [0 patients]; RD –0·08: [95% CI –0·14 to –0·02]; P = 0·009). Therefore, the incidence of CMV disease did not differ among groups but occurred earlier in the IP group. The mixed-effects logistic regression analysis revealed that the immunoguided prophylaxis in experimental group was not significantly associated with the risk of developing CMV disease at 18 months (OR = 0·97; 95% CI: 0·41–2·29; P = 0·95) (Supplementary Table S5). The sensitivity analysis using a parametric survival model with a Weibull distribution showed no significant difference in time to event for the experimental group, with a hazard ratio (HR) of 1·03 (95% CI: 0·57–1·87; P = 0·920).

Fig. 2 presents the event probability for CMV disease (P = 0·554, log rank test) and neutropenia (P = 0·081) in the SP and IP groups. Sensitivity analysis of the primary endpoint did not reveal significant differences in any subgroup except for patients with prior rejection (RD 0·25 [95% CI 0·05–0·45]; P = 0·01) (Fig. 3). In patients with rejection, the incidence of CMV disease was lower in the IP group (4·2%; 1/24) than in the SP group (29·2%; 7/24). Neither group experienced any cases of CMV disease during the prophylaxis period.

Asymptomatic CMV replication was significantly lower in the IP group vs. the SP group (4·0% [3 patients]

Parameters	Standard prophylaxis (n = 75)	Immunoguided prophylaxis (n = 75)	P-value[a]
Age (years), median (IQR)	62 (57–64)	62 (58–66)	0·380
Female, n (%)	19 (25·3)	29 (38·7)	0·080
Retransplantation, n (%)	2 (2·7)	1 (1·3)	0·847
Pre-transplant immunosuppression, n (%)	17 (22·7)	18 (24·0)	1·000
Bipulmonar transplant, n (%)	46 (61·3)	53 (70·7)	0·228
Lung disease			0·417
Restrictive, n (%)	29 (38·7)	22 (29·3)	
Obstructive, n (%)	36 (48·0)	39 (52·0)	
Others (septic and vascular), n (%)	10 (13·3)	13 (18·7)	
IgG level at month 3 (mg/dL), median (IQR)	648 (525–775)	655 (541–776)	0·304
Prophylaxis with CMV-Ig, n (%)	3 (4·0)	6 (8·0)	0·494
Induction therapy (basiliximab)[b], n (%)	61 (81·3)	56 (74·7)	0·324
Basal immunosuppression[c]			
Micophenolate/Micophenolic acid, n (%)	73 (97·3)	74 (98·7)	0·500
mTOR (yes), n (%)	11 (14·7)	5 (6·7)	0·113
Rejection (yes), n (%)	23 (30·7)	25 (33·3)	0·726

Abbreviations: IQR, interquartile range; mTOR, mechanistic Target of rapamycin; CMV-Ig, cytomegalovirus-specific immunoglobulins. [a]Continuous variables were analysed using the Student's *t*-test or the Mann–Whitney U test. When indicated, categorical variables were compared using the Chi-square test or Fisher exact test. [b]All patients with induction therapy received basiliximab (one patient received 20 mg and the rest 40 mg). [c]All patients received tacrolimus and steroids.

Table 1: **Baseline characteristics of the study population (N = 150).**

vs. 16·0% [12 patients]; RD 0·12 [95% CI 0·03–0·21]; $P = 0·009$) (Table 2). The monthly episodes of viral replication in the SP group (months 7–12) are shown in Supplementary Table S6.

Safety endpoints

Supplementary Figure S4 shows median neutrophil count in the period between months 4 and 7 post-transplantation. As shown in Table 2, there were no differences in the proportion of patients with neutropenia during month 4–7 for the IP group vs. the SP group (14·7% [11 patients] vs. 25·3% [19 patients]; RD 0·11 [95% CI –0·02 to 0·23; $P = 0·090$]), although a trend toward a difference was observed. Fig. 2b displays the event probability of neutropenia up to 18 months, showing a tendency towards a higher incidence in the SP group.

No difference was observed in the proportion of rejection between the IP and SP groups (33·3% [25 patients] vs. 30·7% [23 patients]; RD –0·03 [95% CI –0·18 to 0·12; $P = 0·69$]).

The median number of days that patients received antiviral prophylaxis was lower in the IP group than in the SP group (137 [92–266] vs. 198 [173–281]; $P < 0·001$), as was the proportion of patients with >180 days of antiviral prevention using valganciclovir (40% [30 patients] vs. 62·7% [47 patients]; RD 0·23 [0·07–0·38]; $P = 0·004$).

DOOR analysis

Table 2 shows the classification of patients in the four DOOR mutually exclusive hierarchical levels in descending order of desirability. A total of 52·5% (95% CI 44·5–60·4%) of patients in the IP group showed a better DOOR than those in the SP group.

CMV-CMI monitoring in the IP group

Supplementary Table S7 shows CMV-CMI monitoring at each time point and the proportion of patients receiving valganciclovir prophylaxis each month, including those for whom prophylactic valganciclovir was discontinued or reinitiated. CMV-CMI testing was not performed in a number of patients due to COVID-19 mobility restrictions. After 3 months of prophylaxis, CMV-CMI was positive in 82·8% of patients (48/58) and prophylaxis was discontinued in all cases (48/75 patients, 64%). In 40% of patients (30/75), prophylaxis was unnecessary after the third month. Eight percent of patients (6/75) continued with prophylaxis until month 12. Prophylaxis was required for variable periods of time in 52% of patients (39/75).

All 14 patients with CMV disease in the IP group had a positive CMV-CMI. Nevertheless, 2 patients had a low level of IgG, and the rest were treated with additional immunosuppression due to COVID-19 infection or rejection in the previous month.

Endpoints	Standard prophylaxis (n = 75)	Immunoguided prophylaxis (n = 75)	Risk difference[a] (95% CI)	P-value[b]
Primary efficacy endpoint[c], n (%)				
CMV disease	12 (16)	14 (18·7)	−0·03 [−0·15; 0·06]	0·620
CMV viral syndrome	8 (10·7)	13 (17·3)	−0·07 [−0·18; 0·04]	0·230
CMV organic disease	4 (5·3)	1 (1·3)	0·04 [−0·02; 0·1]	0·190
Time to CMV disease (days)				
≤180	0 (0)	6 (8)	−0·08 [−0·14; −0·02]	0·009
>180	12 (16)	8 (10·7)	0·05 [−0·06; 0·10]	0·190
Secondary efficacy endpoint[c], n (%)				
Asymptomatic CMV replication	12 (16)	3 (4)	0·12 [0·03; 0·21]	0·009
Safety endpoints, n (%)				
Neutropenia (<1500 cells/μL)[d]	19 (25·3)	11 (14·7)	0·11 [−0·02; 0·23]	0·090
Rejection	23 (30·7)	25 (33·3)	−0·03 [−0·18; 0·12]	0·690
Days with valganciclovir[e]				
≤180	28 (37·3)	45 (60)	−0·23 [−0·38; −0·07]	0·004
>180	47 (62·7)	30 (40)	0·23 [0·07; 0·38]	0·004
DOOR analysis, n (%)				
No CMV disease without neutropenia	47 (62·7)	52 (69·3)	−0·07 [−0·22; 0·08]	0·380
No CMV disease with neutropenia	16 (21·3)	9 (12)	0·09 [−0·03; 0·21]	0·130
CMV disease without neutropenia	9 (12)	12 (16)	−0·04 [−0·15; 0·07]	0·480
CMV disease with neutropenia	3 (4)	2 (2·7)	0·01 [−0·04; 0·07]	0·630

Abbreviations: CMV, cytomegalovirus; DOOR, desirability of outcome ranking. [a]Difference between groups using Mantel–Haenszel method. [b]P-values were calculated using the Wald test, based on the observed risk differences and standard errors derived from the confidence intervals. [c]Patients followed up for 18 months. [d]Month 4–7. [e]Included prophylaxis and/or pre-emptive therapy with ganciclovir/valganciclovir.

Table 2: **Efficacy and safety endpoints.**

In the IP group, 30 patients (40%) had positive CMV-CMI between months 4 and 12 and thus did not require prophylaxis during this time period. A CART analysis showed that this subpopulation could be defined by two variables: (1) the quantification of IFNG in the first QF-CMV test performed after prophylaxis (cut-off, 2·16 IU/mL) and (2) the centre (high vs. low risk of needing prophylaxis). All 30 of these patients had an IFNG level of >2·16 UI/mL in response to CMV antigens. Additionally, among these patients, the CART analysis identified the type of centre (low or high risk of needing prophylaxis) as the most important variable for distinguishing patients who did not require prophylaxis (82·6% in low-risk centres) from those who did (37·5% in high-risk centres; Supplementary Figure S3). The multivariable analysis confirmed that having a level of IFNG >2·16 IU/mL in the first QF-CMV test after prophylaxis (adjusted odds ratio [OR] 33·7 [95% CI 6·01–189·1]; $P < 0·0001$) and being treated at a low-risk centre (adjusted OR 10·8 [95% CI 2·77–42·4]; $P < 0·001$) were both associated with positive CMV-CMI (and thus

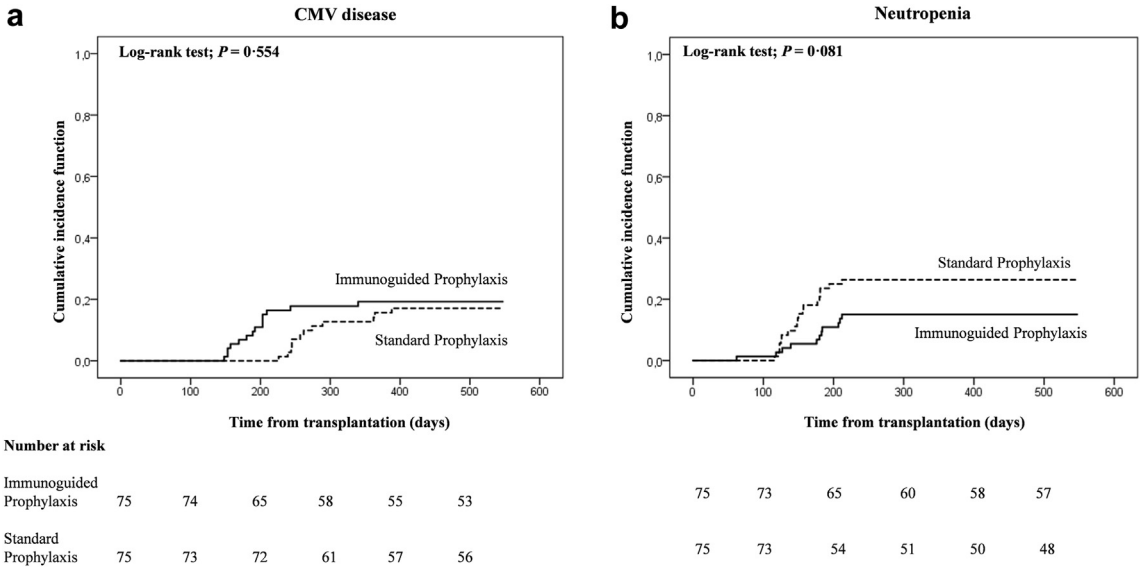
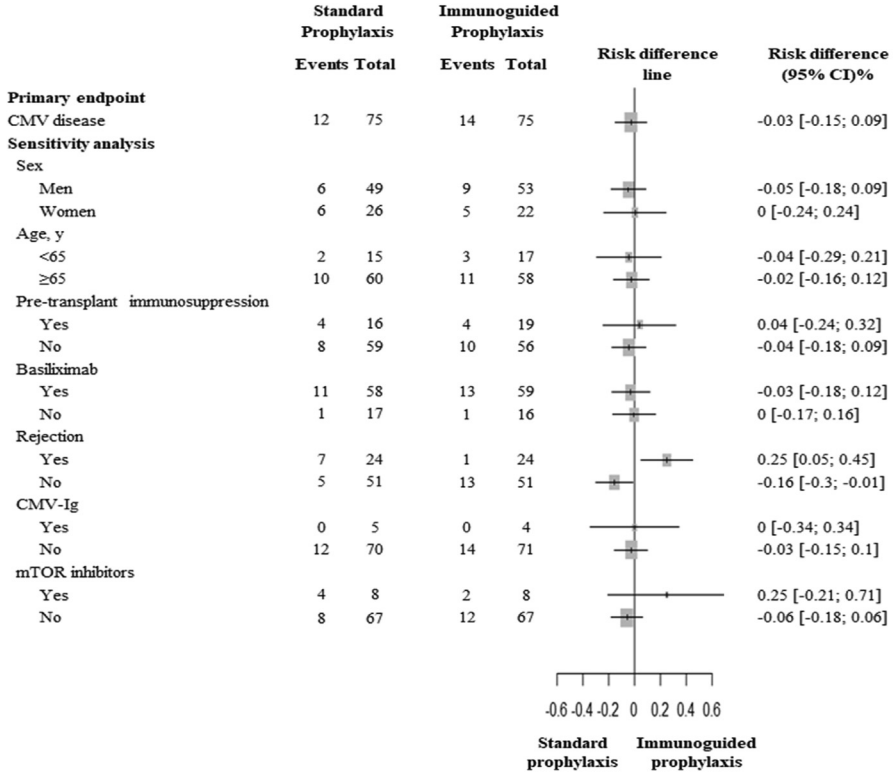


Fig. 2: Event probability of CMV disease (a) and neutropenia (<1500 cells/µL) (b) according to the strategy followed by the patients.



a) A Forest Plot is a graphical representation used to display the results of multiple studies in a single image. The vertical line in the center of the graph represents the neutral or reference value. Studies to the left of this line favor the standard prophylaxis group, while studies to the right favor the immunoguided prophylaxis group.

b) The risk difference (RD) with 95% CI were calculated using the Mantel-Haenszel method. P-values were calculated with the Wald test.

Fig. 3: Primary endpoint of CMV disease with standard prophylaxis vs. immunoguided prophylaxis in the full analysis set.

not requiring antiviral prophylaxis) at all time points after month 3 (Supplementary Table S8).

Adverse events

The three most frequent AEs across both groups were neutropenia, rejection and diarrhoea. No differences were observed in the proportion of patients with any AEs, serious adverse events, or all-cause mortality across both groups (Supplementary Table S9). No deaths were considered related to CMV disease.

Discussion

In this randomised, open-label trial, IP was found to be noninferior to SP for preventing CMV disease in CMV-seropositive lung transplant recipients. CMV disease occurred in 18.7% of patients in the IP group (all had mild CMV syndrome) up to month 18 post-transplantation compared with 16% of patients in the SP group. No cases of CMV disease occurred during universal prophylaxis periods. Four cases (5·3%) of probable CMV disease without biopsy confirmation were observed in the SP group during pre-emptive therapy, showing a higher severity of CMV disease in this group compared with the IP group. Nevertheless, no deaths were considered related to CMV disease. The IP appeared to be superior to SP for the reduction of CMV disease in those patients with prior rejection. Nevertheless, this cannot be considered a definitive conclusion since this was an exploratory endpoint.

A positive CMV-CMI does not always prevent the development of CMV disease, although timely intervention as a result of a positive test may be able to reduce disease severity. All patients with CMV disease in the IP group were CMV-CMI positive; nevertheless, all were over-immunosuppressed, the majority due to the treatment of rejection, severe COVID-19 infection, or hypogammaglobulinemia.

It is important to consider that 40% of the patients in the IP group did not need to restart prophylaxis once the initial period of 3 months had ended, and 77·3% of patients were able to discontinue prophylaxis at month 4. The remaining patients could not be monitored due to mobility restriction measures related to the COVID-19 pandemic.

Prolonged prophylaxis is a risk factor for resistance to ganciclovir, especially when the dose has to be adjusted to renal function or reduced due to the onset of side effects.[6,8] However, all cases of CMV replication and disease in this study responded to treatment with ganciclovir. Therefore, it can be assumed that no resistance occurred, although this was not confirmed with genotypic studies as this was not clinically indicated.

In a clinical trial of IP with antithymocyte globulin to prevent CMV in kidney transplant recipients, patients treated with IP had a reduced incidence of neutropenia when compared with patients receiving SP.[22] In the present trial, IP did not significantly reduce the proportion of neutropenia. However, a trend towards reduced incidence of neutropenia was observed and the number of days of valganciclovir (prophylaxis or anticipated therapy) was significantly lower in this group vs. the SP group. Furthermore, DOOR analysis indicated the superiority of IP vs. SP when efficacy and safety were analysed together. The incidence of other AEs, including severe AEs, was similar in both groups and to those reported in the literature,[21,22] as they were not related to CMV prophylaxis.

Our study confirms that the level of IFNG in the first QF-CMV determination performed after fixed prophylaxis of 3 months is essential to define a subgroup of patients who maintain CMV-CMI throughout follow-up and who will not need prophylaxis. Thus, a IFNG cut-off >2·16 IU/mL along with the type of centre (low risk) were significantly associated with a lower risk of requiring prophylaxis. These variables could potentially be used to define a population in whom monitoring may not be necessary.

This trial has important limitations. Firstly, it was underpowered since the sample size was limited due to a very optimistic anticipated effect size. The lack of significant differences between both groups, such as in incidence of neutropenia, could be due to the sample size, meaning clinically relevant differences cannot be ruled out. This study was carried out during the COVID-19 pandemic, which has led to some deviations from the original protocol as follows: (i) some patients were unable to attend follow up visits and (ii) CMV-CMI monitoring could not always be performed exactly when indicated per protocol. IP was not performed in 17 patients (22·7%) after prophylaxis. Assuming 83% of those 17 patients could be CMV-CMI positive, we estimated that prophylaxis was not stopped in 14 cases. This circumstance was repeated on subsequent visits, increasing the duration of prophylaxis, reducing the risk of CMV disease, and increasing the risk of neutropenia. Failure to attend follow-up visits could have impacted on the SP group, resulting in irregular virological monitoring, thus potentially increasing the risk of CMV disease. Consequently, we cannot completely rule out effects of COVID-19 pandemic on the incidence of CMV disease and neutropenia. Another limitation was related to the QF-CMV assay used to analyse the CMV-CMI, which, like other laboratory assays, is not 100% sensitive. Its ability to evaluate a broader spectrum of immune cells functionalities could be potentially improved with a higher cut-off value. Further clinical trials using alternative cut-off values of QF-CMV and other assays may be needed to better understand a clinical value of the IP strategy. A further limitation is that long-term endpoints associated with CMV disease were not evaluated after month 18. The study was not blinded or placebo-controlled, and does not include a cost-effectiveness analysis. Lastly, although effort was made to avoid biases, some baseline imbalances were observed.

In conclusion, IP was noninferior to the standard of care for preventing CMV disease in adult CMV-seropositive lung transplant recipients. It could be considered for implementing in clinical practice upon considering the study limitations.

Contributors

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Critical revision of the manuscript for important intellectual content: All authors.

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Obtained funding: J T-C.

Final approval of the version to be published: All authors.

Administrative, technical, or material support: MAL-A, A P-V and J T-C.

Data sharing statement

Within 12 months of this publication, anonymised participant data will be available for researchers upon request, that must be approved by the steering committee (J Torre-Cisneros, A Páez-Vega, S Cantisan). Requests should be submitted to Dr Julian Torre-Cisneros: julian.torre.sspa@juntadeandalucia.es or Dr Sara Cantisan: sara.cantisan.sspa@juntadeandalucia.es. Data will be provided after a signed data access agreement with IMIBIC.

Declaration of interests

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A P-V, R F-M, A C, JJ C, I M, B G-G and S C are staff collaborators at the Centre for Biomedical Research Network on Infectious Diseases (CIBERINFEC).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanepe.2025.101268.

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