Immunoguided Discontinuation of Prophylaxis for Cytomegalovirus Disease in Kidney Transplant Recipients Treated with Antithymocyte Globulin: A Randomized Clinical Trial

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Summary: In CMV-seropositive kidney transplants receiving ATG induction, immunoguided prevention is not inferior to standard prophylaxis to prevent CMV complications. Prophylaxis can be prematurely discontinued when CMV-CMI is recovered with no significant increase in the incidence of CMV replication or disease.

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ABSTRACT

Background. Antiviral prophylaxis is recommended in cytomegalovirus (CMV)-seropositive kidney transplant (KT) recipients receiving antithymocyte globulin (ATG) as induction. An alternative strategy of premature discontinuation of prophylaxis after CMV-specific cell-mediated immunity (CMV-CMI) recovery (immunoguided prevention) has not been studied. The aim of this study was to evaluate whether it is effective and safe to discontinue prophylaxis when CMV-CMI is detected and to continue with preemptive therapy.

Methods. In this open-label, non-inferiority clinical trial, patients were randomized 1:1 to follow immunoguided strategy, receiving prophylaxis (valganciclovir 900 mg daily) until CMV-CMI recovery or to receive fixed-duration prophylaxis until day +90. After prophylaxis, preemptive therapy (valganciclovir 900 mg twice daily) was indicated in both arms until month 6. The primary and secondary outcomes were incidence of CMV disease and replication, respectively, within the first 12 months. Desirability of outcome ranking (DOOR) assessed two deleterious events (CMV disease/replication and neutropenia).

Results. A total of 150 CMV-seropositive KT recipients were randomly assigned. There was no difference in the incidence of CMV disease (0% vs. 2.7%; $P = 0.149$) and replication (17.1% vs. 13.5%; log-rank test, $P = 0.422$) between both arms. Incidence of neutropenia was lower in the immunoguided arm (9.2% vs. 37.8%; OR, 6.0; $P < 0.001$). A total of 66.1% of patients in the immunoguided arm showed a better DOOR, indicating a greater likelihood of a better outcome.

Conclusions. Prophylaxis can be prematurely discontinued in CMV-seropositive KT patients receiving ATG when CMV-CMI is recovered since no significant increase in the incidence of CMV replication or disease is observed.

Clinical Trials Registration: NCT03123627.

Abbreviations: ATG, antithymocyte globulin; CMV, cytomegalovirus; CMV-CMI, CMV-specific cell-mediated immunity; IFNG, interferon-gamma; KT, kidney transplant; QF, QuantiFERON-CMV**.**

INTRODUCTION

Immunosuppression modulates the risk of cytomegalovirus (CMV) infection after solid organ transplantation [1,2]. In CMV-seropositive kidney transplant (KT) recipients preemptive therapy is indicated, which consists of monitoring patients with a sensitive diagnostic technique to detect asymptomatic replication and treat it with an antiviral drug before disease develops [1-3]. However, when CMV-seropositive KT recipients receive induction therapy with antithymocyte globulin (ATG), antiviral prophylaxis is recommended for a minimum of 3 months [3]. This recommendation is based on the high risk of CMV disease [3-5], although the published evidence is contradictory [6,7].

ATG is a potent immunosuppressive drug that acts by reducing T-cell immunity and the incidence of acute rejection [8]. Currently, the indications for ATG have been notably extended in KT recipients. This means that prophylaxis, instead of preemptive therapy, is indicated in >40% of CMV-seropositive KT for the sole reason of receiving ATG induction.

Today it is possible to individualize the preventive management of KT by assessing the risk of each patient ("individual pathogenic balance") using techniques that quantify cell-mediated immunity [9–12]. Specifically, we know that pre-transplant CMV-specific cell-mediated immunity (CMV-CMI) defines the risk of post-transplant CMV infection [13]. There is also evidence that >80% of KT recipients with pre-transplant CMV-CMI treated with ATG recover (or maintain) this immunity by the first trimester (≈30% in the first month) [14]. The aim of this study was to evaluate whether it would be effective and safe to prematurely discontinue antiviral prophylaxis when CMV-CMI is detected after induction treatment and to continue with preemptive therapy (immunoguided prevention).

MATERIAL AND METHODS

Study design and participants

This is a multicentre, randomized, open-label, non-inferiority clinical trial of immunoguided discontinuation of antiviral prophylaxis followed by preemptive therapy (immunoguided prevention) vs. fixed-duration prophylaxis using valganciclovir in CMV-seropositive KT recipients who received ATG induction (Supplementary Table 1). Patients from eight centres of the Spanish Network for Research in Infectious Diseases (REIPI) and five centres of the Spanish Kidney Disease Network (RedInRen) were enrolled between August 2016 and October 2018. The Ethics Committee (Institutional Review Board) of the coordinating hospital (Reina Sofia University Hospital, Code FCO-TIM-2015-01) approved the protocol. Other centres approved the protocol when necessary. All patients or their legal representatives signed the informed consent. The study was conducted following the guidelines of Good Clinical Practice (Helsinky Declaration) and applicable Spanish law. The trial was registered in EudraCT (number, 2015-004406-42).

Patients

Eligible patients were KT recipients; aged ≥18 years; CMV-seropositive; pre-transplant positive CMV-CMI; receiving ATG (accumulate dose ≥ 1mg/kg for a maximum of 10 days); and had a negative pregnancy test (female of childbearing potential). All patients were advised about the potential teratogen effect of valganciclovir to avoid pregnancy. ATG was indicated in high-risk immunological patients and recipients of organs donated after circulatory death following the clinical protocols of each participating centre. High-risk immunological patients were defined as: (i) candidates with a panel reactivity antibody (PRA) >30%; (ii) candidates with donor-specific antibodies and (iii) retransplantation with loss of allograft due to rejection. PRA was defined as the proportion of HLA antigens singly or in combination out of a panel reacting with a patient's serum. Exclusion criteria included multiple organ transplant (including kidney-pancreas), HIV infection and patients unable to follow the protocol.

Maintenance immunosuppression consisted of the association of a calcineurin inhibitor (tacrolimus or cyclosporine), mycophenolate mofetil/mycophenolic acid and steroids. When indicated, mTOR inhibitors were used.

Randomization and masking

Patients were included in the trial when CMV-CMI was tested before transplantation. Patients were randomized within 15 days after transplantation in a 1:1 ratio to immunoguided prevention or fixed-duration prophylaxis by a computer-generated web-based allocation using permuted blocks of ten.

Intervention

Valganciclovir prophylaxis (900 mg orally once daily adjusted by creatinine clearance) was indicated after transplantation when oral medication was tolerated. The protocol allowed the use of intravenous ganciclovir (5 mg/kg/day adjusted by creatinine clearance) until oral medication was tolerated. Patients in the immunoguided arm underwent CMV-CMI assessment at days +30, +45, +60 and +90 after transplantation, with discontinuation of prophylaxis when positive CMV-CMI was achieved. All patients received a minimum of 30 days of prophylaxis. Patients with a Negative or Indeterminate CMV-CMI at day +90 discontinued the prophylaxis and continued with preemptive therapy until day +180, in accordance with clinical practice. Preemptive therapy was indicated when prophylaxis was discontinued before day +90 and until day +180, following the protocols of each centre. A CMV viral load was performed at least every two weeks. Valganciclovir 900 mg (adjusted by creatinine clearance) orally twice daily was indicated when clinically significant (see below). In the control arm, patients received valganciclovir 900 mg (adjusted by creatinine clearance) orally once daily for 90 days followed by preemptive therapy until day +180.

Ganciclovir and valganciclovir doses were adjusted based on calculated creatinine clearance (CrCl, Cockcroft-Gault formula) in accordance with standard recommendations. Patients in which valganciclovir was interrupted for any reason could resume medication in the study once the cause was determined, provided they had not missed >14 consecutive days. Otherwise, the patient was excluded from the study.

Patients were followed up for 12 months or until lost to follow-up, exclusion or death (whichever occurred first).

Outcomes

The primary outcome was the proportion of patients with CMV disease in the 12 months after transplantation. CMV disease was defined in accordance with current recommendations [1–3] and the CMV Drug Development Forum recommendations for use in clinical trials [14]: 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 organ disease.

Secondary outcomes included the proportion of patients with CMV replication (incidence).

Desirability of Outcome Ranking (DOOR)

Desirability of Outcome Ranking (DOOR) analysis for assessing CMV disease or replication (disease/replication) and neutropenia (<1.500 mm^3) was performed (Table 1). It was defined *post-hoc* knowing that CMV disease was not observed in the immunoguided arm. The best outcome was defined as no CMV disease/replication without neutropenia and the worst as CMV disease/replication with neutropenia. The categories between these two extremes were no CMV

disease/replication with neutropenia and CMV disease/replication without neutropenia. DOOR is a method for comparing arms using a single, ordinal patient-centred outcome that represents a global assessment of patient outcome, 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 [15,16], whereas a probability greater than 50%, combined with a 95% CI that excludes 50%, indicates a significantly greater likelihood of a better outcome in one group compared to the other (and vice-versa).

Determination of CMV viral load

CMV load was analysed in plasma or whole blood by real-time PCR using the technique implemented at each centre. Samples available in each laboratory were analysed, both those carried out according to the protocol and by indication of the responsible physician. Clinically significant CMV replication was defined as >1500 IU/mL in plasma or >5000 IU/mL in whole blood. CMV replication was considered asymptomatic when it was not accompanied by CMV disease (CMV syndrome or CMV disease) [1–3].

Determination of CMV-CMI

CMV-CMI was assessed using the QuantiFERON-CMV (QF) assay, performed according to the manufacturer's instructions (Cellestis, a QIAGEN Company, Melbourne, Australia). In brief, 1 mL of heparinized whole blood was collected in 3 QF 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 (phytohemagglutinin). After incubation for 16–24 h at 37 ºC, supernatants were harvested and analysed for interferon-gamma (IFNG) (IU/mL) by standard ELISA. A result for the CMV antigens was "Reactive" (positive CMV-CMI) when the CMV antigen response minus the negative control response was ≥ 0.2 IU/mL

of IFNG. A result was "Indeterminate" when the IFNG level in the CMV antigen tube was less than 0.2 IU/mL and in the mitogen tube was less than 0.5 IU/mL.

Clinical assessment and other variables

Efficacy and safety were evaluated by clinical assessment including vital signs, laboratory analysis, CMV viral load and adverse events.

Data were collected on basal characteristics, age and gender, retransplantation, type of dialysis, donor type, basal renal disease, PRA, HLA (typed at each centre), immunosuppression, pretransplant donor/recipient CMV-serostatus, ATG dose and duration, post-transplant CMV-CMI in the immunoguided arm, valganciclovir side effects, concomitant medication, CMV replication and disease. A senior clinical research monitor revised all data.

Statistical analysis

The sample size was calculated based on the non-inferiority of the primary endpoint (incidence of CMV disease in the 12 months post-transplant). We have assumed that the incidence of CMV disease in the fixed-duration prophylaxis arm would not be more than 3% (data on file). With this estimate (alpha error = 0.05, power = 0.80, lost 5%, double tail, and a non-inferiority limit of 7%), the calculated sample size was 150 patients, 75 patients in each arm**.**

The analysed population included all randomized patients, who received at least one dose of valganciclovir and who had at least one postrandomization safety assessment (intent-to-treat [ITT] population). The results were expressed as medians (interquartile range [IQR]) for the quantitative variables and as percentages for the qualitative variables. Continuous variables were analysed using the Mann–Whitney *U* test. Categorical variables were compared using the Chi-square test or

Fisher's exact test. The hypothesis of no difference in the proportion of patients with CMV disease in each arm was analysed using the phi coefficient. Kaplan-Meier curves were used to calculate the cumulative hazard function, which considers the instantaneous risk of CMV replication/disease among patients still at risk of these events. The cumulative hazard by strategy was compared using the log-rank test. For the DOOR analysis, desirability of outcome ranking probabilities were calculated. Since the sample size was calculated for the primary endpoint, the findings for the analysis of secondary endpoints should be interpreted as exploratory. *P* values ≤ 0.05 were considered statistically significant, and all tests were two-sided. SPSS 25.0 software (SPSS Inc.) was used for the statistical analysis.

The study protocol is available online.

This study is registered, ClinicalTrials.gov Identifier: NCT03123627.

RESULTS

Participants

Figure 1 shows a diagram of the patient flow in the trial. Of the 336 patients included before transplantation, 150 patients were randomized at a median of 9.5 days (IQR, 6.0–13.0 days): 74 patients to the fixed-duration prophylaxis arm and 76 patients to the immunoguided arm. Sixty-seven (90.5%) and 69 patients (90.8%) completed the planned follow-up in each arm, respectively. Nevertheless, the 150 patients were analysed since all of them met the criteria for inclusion in the ITT population.

Table 2 shows the baseline characteristics of the ITT population analysed. There were no differences between the two groups regarding the dose of ATG received (median, 4.4 vs. 4.0 mg/kg; *P* = 0.736). Seven patients in the immunoguided group (9.2%; median, 4 days) and 11 patients in the prophylaxis group (14.9%; median, 3 days) received intravenous ganciclovir until oral medication was tolerated.

Primary outcome. Desirability of Outcome Ranking (DOOR) analysis

Only two patients (2.7%) developed CMV disease in the fixed-duration prophylaxis group. One patient suffered from viral syndrome on day +39 and another from disseminated disease on day +181. Both patients responded to intravenous ganciclovir and were cured. No patient developed CMV disease in the immunoguided group (n=76) (Phi Coefficient; *P* = 0.149) (Table 3).

Table 3 also shows the classification of patients in the four DOOR mutually exclusive hierarchical levels in descending order of desirability. A total of 66.1% (95% CI, 64.4–67.7%) of patients in the immunoguided arm showed a better DOOR than those in the control arm.

Secondary outcomes

A detailed report of the QF assay results during the follow-up period is shown in Table 4. The QF test results were provided to the clinicians at a median of 4.5 days (IQR 3.0-7.0 days) from the time they were available. The clinicians took a median of 4 days (IQR 2.0-6.0 days) to discontinue the medication, although prophylaxis was discontinued later in three patients (+16, +21 and +36).

Prophylaxis was prematurely discontinued in 45 patients (59.2 %) of the immunoguided group: 32 at day +30, 7 at day +45 and 6 at day +60. Therefore, duration of prophylaxis was significantly reduced in this group, with the antiviral being administered for a median of 57 days

(IQR, 35.5–86.5 days) and for a median of 90 days (IQR, 83.7–97.0 days) in the control group (P < 0.001). After prophylaxis discontinuation, valganciclovir was preemptively administered for a median of 38 days (IQR, 27.5–54.5 days) in the immunoguided group and for 58 days (IQR, 22.5–90.0 days) in the control group (P = 0.294).

CMV replication was observed in 13 patients in the immunoguided group and in 10 patients in the control group (incidence, 17.1% vs. 13.5%; odds ratio 1.32, 95% CI 0.54-3.23). All CMV replication episodes in both groups occurred after prophylaxis was discontinued and the patients received antiviral treatment.

Figure 2 shows the cumulative hazard curves for CMV disease/replication at 12 months according to the strategy. All episodes of CMV replication in the control arm occurred later than in the immunoguided arm. The curves begin to separate after the first month, when prophylaxis began to be suspended in patients with positive CMV-CMI in the immunoguided arm but continued in the fixed-duration prophylaxis arm. Afterwards, both curves joined when prophylaxis was suspended in the control group. The median time until the appearance of CMV replication was 95 days (IQR, 79.0–118.0) in the immunoguided group compared to 149.5 days (IQR, 123.7–169.7) in the fixed-duration prophylaxis group (P = 0.003).

Adverse events

Although no significant differences were observed in the global incidence of adverse events, a lower incidence of neutropenia (<1500 mm^3) was observed in the immunoguided arm (9.2% [7/76] vs. 37.8% [28/74]; OR 6; 95% CI 2.4–14.8; P = <0.001) (Table 5).

DISCUSSION

In this randomized clinical trial in CMV-seropositive KT patients receiving ATG induction, immunoguided prevention was not inferior to fixed-duration prophylaxis for the prevention of CMV disease during the first 12 months after transplantation. Nor were differences observed in other secondary outcomes considered exploratory. Additionally, no differences were found in the incidence of clinically significant viral replication. The incidence of viral replication increased several weeks after the antiviral was discontinued in both groups. Due to the trial design, the prophylaxis time was longer in the control group, which explains why CMV replication appeared later in this group but in a similar proportion to that of the immunoguided arm.

It is well known that the efficacy of valganciclovir prophylaxis is limited by the incidence of neutropenia [17], which sometimes requires the antiviral to be discontinued [18]. Preemptive therapy is not exempt from this problem either, but with a much lower incidence [19]. This study shows that although both regimens do not differ in efficacy, the immunoguided regimen had the advantage of safety since the prophylaxis time with valganciclovir was significantly reduced. Therefore, the incidence of neutropenia, the main adverse effect of this drug, was much lower in the immunoguided arm. When we applied a DOOR analysis, which takes into account efficacy and safety (neutropenia), the immunoguided strategy was superior to fixed-duration prophylaxis. In other words, by changing prophylaxis to preemptive therapy when CMV-CMI is reactive, preventive efficacy is not lost and neutropenia is reduced.

There are two high-risk scenarios for CMV disease in KT recipients in which prophylaxis is recommended [1,3]: transplants from CMV-seropositive donors to CMV-seronegative recipients (D+/R-) and patients receiving ATG induction. It has recently been reported that early therapy is associated with a lower incidence of CMV disease than prophylaxis in D+/R- liver transplant recipients [20]. Although there is no evidence of this in kidney transplantation, preemptive therapy could be an option in those groups with the logistical capacity to do so. Our study provides evidence

that prophylaxis can also be avoided in the other risk scenario, treatment with ATG. Both strategies can prevent the adverse effects of prolonged valganciclovir therapy.

Numerous publications have demonstrated the usefulness of different CMV-CMI techniques to identify transplanted patients at risk of CMV disease/replication [11–14,21]. A recently published study has validated the usefulness of CMV-CMI monitoring to guide preemptive therapy in CMV-seropositive KT not receiving ATG treatment [22]. Our trial is complementary to this, as it demonstrates the usefulness of CMV-CMI monitoring in CMV-seropositive KT recipients receiving induction with ATG. Both interventional studies demonstrate that the time has come to apply CMV-CMI monitoring in clinical practice [23].

Our study has several strengths: (i) the preventive strategy was allocated randomly; (ii) the primary efficacy outcome (CMV disease) is clinically relevant; (iii) the proportion of patients who developed CMV disease in the control group was predetermined in the calculation of the sample size; (iv) the combination of efficacy and safety has been taken into account in a DOOR analysis; (v) QF determinations, which were crucial in the experimental arm, were done centrally at the coordinating centre and (vi) the preemptive therapy has taken into account the clinical practice of each centre, thus reflecting what can happen in real practice.

The trial also has limitations: (i) for logistical reasons the trial could not be blinded; (ii) CMV-CMI monitoring was not included in the fixed-duration prophylaxis group; (iii) to detect CMV replication, the biological sample (plasma or whole blood) and the PCR technique used varied across centres; (iv) adherence to virological monitoring protocols in real-life practice may be different; (v) our results refer to KT and cannot be extrapolated to other types of transplants with different risk and immunosuppression protocols; (vi) we enrolled patients at a potentially low risk for CMV replication since only CMV-seropositive patients with positive CMV-CMI were recruited, which may be a limitation for external validation.

In conclusion, in CMV-seropositive KT receiving ATG induction, immunoguided prevention is not inferior to standard prophylaxis to prevent CMV complications. Therefore, antiviral prophylaxis can be prematurely discontinued in CMV-seropositive KT patients receiving ATG when CMV-CMI is recovered since no significant increase in the incidence of CMV replication or disease is observed.

Notes

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Potential conflicts of interest.

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FIGURE LEGENDS

Figure 1. Patient flow through the study.

Figure 2. Cumulative hazard curves of CMV disease/replication according to the strategy followed by the patients (immunoguided prevention versus fixed-duration prophylaxis).

APPENDIX

TIMOVAL Study group

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University of Barcelona (UAB), Barcelona, Spain). All participated in data collection; however, they do not follow ICMJE conditions for authorship.

Table 1. Ordinal outcomes for efficacy, safety and benefit-risk analyses with categories in ascending order of desirability[a]

	Outcome
Efficacy	1. CMV replication/disease
	2. No CMV disease/replication
Safety	1. Neutropenia (<1500/mm^3)
Benefit-Risk	1. No CMV disease/replication without neutropenia
	2. No CMV disease/replication with neutropenia
	3. CMV disease/replication without neutropenia
	4. CMV disease/replication with neutropenia

[a] Desirability of outcome ranking (DOOR) analysis was defined *post-hoc*.

Table 2. Baseline characteristics of the study population and Ganciclovir/Valganciclovir use during the trial (N=150).

	Immunoguided prevention (n=76)	**Fixed-duration prophylaxis (n=74)**	***P-value*[a]**
Age (years), median (IQR)	60 (50.2-67.0)	59.5 (50.5-68.0)	0.855
Gender, no. (%)			0.003
Female	29 (38.2)	46 (62.2)	
Male	47 (61.8)	28 (37.8)	
Hemodialysis, no. (%)	54 (71.1)	55 (74.3)	0.197
Retransplantation (yes), no. (%)	32 (42.1)	25 (33.8)	0.317
Donor status, no. (%)			0.952
CMV seropositive	60 (78.9)	58 (78.4)	
CMV seronegative	13 (17.1)	12 (16.2)	
Unknown	3 (3.9)	4 (5.4)	
Source of donor organ, no. (%)			0.057
Donor after brain death	47 (61.8)	39 (52.7)	
Donor after circulatory death	29 (38.2)	30 (40.5)	
Living	0 (0.0)	5 (6.8)	
Hyperimmunized, no. (%)	30 (39.5)	39 (52.7)	0.140
Chronic kidney disease, no. (%)			0.705
Glomerulonephritis	12 (15.8)	17 (23.0)	
Unknown	23 (30.3)	15 (20.3)	

Polycystic kidney disease	9 (11.8)	12 (16.2)	
Diabetes mellitus	6 (7.9)	4 (5.4)	
Autoimmune	6 (7.9)	8 (10.8)	
Hypertension	6 (7.9)	6 (8.1)	
Others	14 (18.4)	12 (16.2)	
Immunosuppression			
ATG total dose (mg/Kg), median (IQR)	4.4 (2.9-5.4)	4 (3.0-5.8)	0.736
mTOR, no. (%)	13 (17.1)	9 (12.2)	0.392
mTOR (days), median (IQR)	180 (35.0-231.5)	184 (19.0-195.0)	0.764

Abbreviations: no, number; IQR, interquartile range; ATG, antithymocyte globulin; mTOR, mechanistic Target of Rapamycin.

[a] Chi-squared or Fisher Test were used.

Table 3. Outcome of selected secondary endpoints (intent-to-treat population).

	Immunoguided prevention (n=76)	Fixed-duration prophylaxis (n=74)	*P*
Primary outcome, no. (%)			
Incidence of CMV disease	0 (0.0)	2 (2.7)	0.243
Secondary outcomes, no. (%)			
Incidence of CMV replication	13 (17.1)	10 (13.5)	0.542
DOOR at 12 months, no. (%)			
No CMV disease/replication without neutropenia	57 (75.0)	39 (52.7)	<0.001
No CMV disease/replication with neutropenia	6 (7.9)	25 (33.8)	<0.001
CMV disease/replication without neutropenia	12 (15.8)	7 (9.5)	0.248
CMV disease/replication with neutropenia	1 (1.3)	3 (4.1)	0.323
DOOR components at 12 months, no. (%)			
CMV disease/replication	13 (17.1)	10 (13.5)	0.542
Neutropenia (<1500/mm^3)	7 (9.2)	28 (37.8)	<0.001

Desirability of Outcome Ranking (DOOR) analysis was performed. For this analysis, the composite variable of incidence of CMV disease or replication was considered.

Given that the two patients with CMV disease also had CMV replication, only one event was considered in these patients.

Table 4. Results of the QuantiFERON-CMV assay performed in the immunoguided group (76 patients).

	Time points			
Parameters	**Day +30**	**Day +45**	**Day +60**	**Day +90**
Patients with QF assay results, no. (%)[a]	74 (97.3)	73 (96.1)	72 (94.7)	72 (94.7)
Negative, no. (%)	16 (21.1)	22 (28.9)	23 (30.3)	18 (23.7)
Indeterminate, no. (%)	24 (31.6)	11 (14.5)	4 (5.3)	2 (2.6)
Positive, no. (%)	34 (44.7)	40 (52.6)	45 (59.2)	52 (68.4)
IFNG (IU/mL), median (IQR)	2.4 (0.9-9.4)	2.9 (1.0-11.7)	3.6 (1.2-14.0)	8.1 (1.0-16.3)
Discontinuation of prophylaxis, no. (%)[b]	32 (42.1)	7 (9.2)	6 (7.9)	28 (36.8)

Abbreviations: IFNG, interferon-gamma; IQR, interquartile range; no, number.

[a] QF results were not available for some patients due to either investigator/patient decisions or technical reasons.

[b] A total of 31 patients reached day +90 with prophylaxis, although three of them withdrew from the study (1 by investigator's decision, 1 patient decision and 1 death).

Note: Once prophylaxis was discontinued, the clinical decisions were based on viral load monitoring. No further clinical decision was taken based on the negativization of CMV-CMI since the serum from the QF tests performed at the timepoints after discontinuation were frozen at -80º C and the results were analysed *a posteriori*.

Table 5. Overview of safety and common adverse events (incidence ≥10%) in either arm.

	Immnunoguided prevention (n=76)	Fixed-duration prophylaxis (n=74)	p^d
Overview of safety, number (%)			
Patients with any adverse event	44 (57.9)	51 (68.9)	0.161
Patients with serious adverse events	14 (18.4)	18 (24.3)	0.378
All-cause mortality at 12 months	1 (1.3)	3 (4.1)	0.363
Common adverse events[a], number (%)			
Neutropenia (<1500/mm^3)[b]	7 (9.2)	28 (37.8)	<0.001
Increased blood creatinine (>2.5 mg/dL)	23 (30.3)	19 (25.7)	0.532
Urinary tract infection[c]	12 (15.8)	11 (14.9)	0.875
Biopsy-proven acute rejection	12 (15.8)	8 (10.8)	0.370
Diarrhoea	8 (10.5)	4 (5.4)	0.248

[a] Occurring in ≥10% of patients between time of first drug intake and 28 days after last drug intake. Multiple occurrences of same adverse event in one patient counted only once.

[b] All patients who had neutropenia also had leukopenia.

[c] Urinary tract infection included BK virus infection.

[d] Chi-squared or Fisher Test were used.

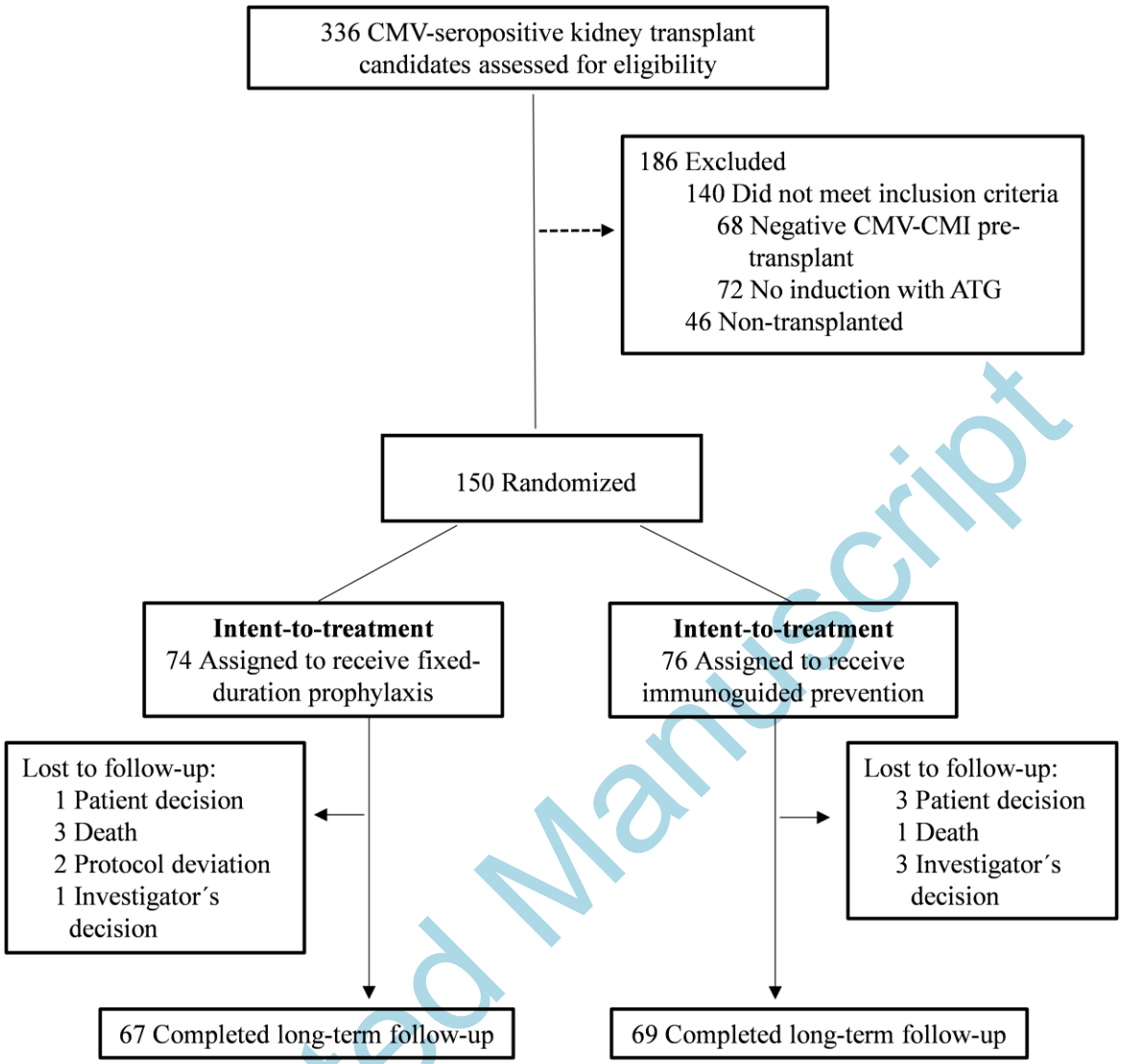
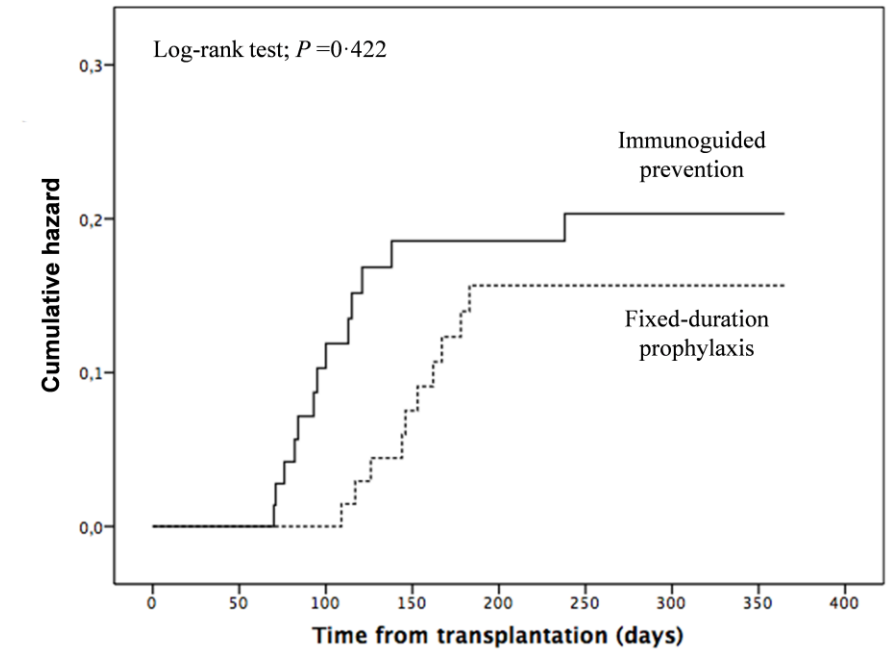


Figure 1



Number at risk								
Immunoguided prevention	76	74	62	58	58	56	56	56
Fixed-duration prophyalxis	74	72	69	64	59	58	58	58

Figure 2