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MAJOR ARTICLE

Immune monitoring-guided *vs* fixed duration of antiviral prophylaxis against cytomegalovirus in solid-organ transplant recipients. A Multicenter, Randomized Clinical Trial

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Background: The use of assays detecting cytomegalovirus (CMV)-specific T-cell-mediated immunity may individualize the duration of antiviral prophylaxis in transplant recipients.

Methods: In this open-label randomized trial, adult kidney and liver transplant recipients from six centers in Switzerland were enrolled if they were CMV-seronegative with seropositive donors or CMV-seropositive receiving anti-thymocyte globulins. Patients were randomized to a duration of antiviral prophylaxis based on immune-monitoring (intervention) or a fixed duration (control). Patients in the control group were planned to receive 180 days (CMV-seronegative) or 90 days (CMV-seropositive) of valganciclovir. Patients were assessed monthly with a CMV-specific interferon gamma release assay (T-Track® CMV); prophylaxis in the intervention group was stopped if the assay was positive. The primary outcomes were the proportion of patients

with clinically significant CMV infection and reduction in days of prophylaxis. Between-group differences were adjusted for CMV serostatus.

Results: Overall, 193 patients were randomized (92 in the immune-monitoring and 101 in the control group) of which 185 had evaluation of the primary endpoint (87 and 98 patients, respectively). Clinically significant CMV infection occurred in 26/87 (adjusted percentage, 30.9%) in the immune-monitoring group and in 32/98 (adjusted percentage, 31.1%) in the control group (adjusted risk difference -0.1, 95%CI -13.0%, 12.7%; p=0.064). The duration of antiviral prophylaxis was shorter in the immune-monitoring group (adjusted difference -26.0 days, 95%-CI -41.1 to -10.8 days, p<0.001).

Conclusions: Immune monitoring resulted in a significant reduction of antiviral prophylaxis, but we were unable to establish noninferiority of this approach on the co-primary endpoint of CMV infection.

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Keywords: cell-mediated immunity, transplant, personalized medicine, prevention, viral infection

INTRODUCTION

Cytomegalovirus (CMV) causes a viral illness and may decrease allograft survival in solid-organ transplant recipients.(1) Patients at the highest risk for CMV complications are CMV-seronegative and receive an organ from a seropositive donor. CMV-seropositive patients receiving anti-thymocyte globulins are considered to be at intermediate risk.(2, 3) These patients usually receive prophylaxis with an antiviral drug during the early post-transplant period.(4) While efficacious, antiviral prophylaxis is associated with toxicity and increased costs. Tailoring the duration of prophylaxis in a personalized health-precision approach may therefore improve the management of transplant recipients.(5) Assays measuring CMV-specific T-cell-mediated immunity can be used to stratify CMV risk after transplantation,(6-8) but their clinical application has only been studied in two small randomized trials. (9, 10)

In this randomized trial, we determined whether an immune monitoring guided approach to tailor the duration of antiviral prophylaxis based on the result of a CMV cell-mediated immune assay (11) is associated with a non-inferior incidence of clinically significant CMV infection, while reducing the duration of prophylaxis in comparison with the current standard.

METHODS

Study design and participants

This was an open-label non-inferiority randomized clinical trial of an individualized duration of antiviral prophylaxis according to a commercial interferon-gamma release assay to measure CMV-specific immunity vs. a fixed duration of prophylaxis in transplant recipients. Patients were recruited at six transplant centers in Switzerland (Basel, Bern, Geneva, Lausanne, St. Gallen and Zurich) participating in the Swiss Transplant Cohort Study (STCS).(12)

CMV-seronegative kidney and liver transplant recipients aged ≥18 years who received an organ from a seropositive donor (CMV-seronegative) and CMV-seropositive recipients who received anti-thymocyte globulins (CMV-seropositive) were enrolled during the first month post transplantation if they were scheduled to receive CMV antiviral prophylaxis. Exclusion criteria were inability to provide consent and/or unwillingness to comply with the study protocol.

All participants provided written informed consent. The study protocol was approved by local Ethics committees (PB_2016-00862) and is registered at ClinicalTrials.gov (NCT02538172). The authors vouch for the completeness and accuracy of the data and for the fidelity of the trial to the protocol.

Randomization

Eligible patients were centrally randomized within 30 days post-transplantation through an interactive web-based response system (secuTrial®, interActive Systems GmbH, Berlin) in a 1:1 ratio to either an immune monitoring guided duration of prophylaxis or a fixed duration (control). The protocol prespecified that randomization was stratified by transplanted organ (kidney and liver) and CMV serostatus of recipients, and blocked with fixed block sizes, but was only stratified by transplanted organ and remained unblocked due to human error when programming of the interactive web-based response system.

Procedures

Antiviral prophylaxis (valganciclovir 900 mg once daily adapted to kidney function) was started within the first 15 days after transplantation in all patients. Patients underwent assessments of CMV-specific cell-mediated immunity every four weeks from day 30 after transplantation using a commercially available CMV-specific interferon gamma ELISpot (T-Track®CMV, Mikrogen, Neuried, Germany; formerly Lophius Biosciences, Regensburg, Germany), which enumerates the CD4+ and CD8+ T-cells after stimulation of peripheral blood mononuclear cells with CMV antigens (pp65 and IE1). We hereafter refer the T-Track® CMV assay as the CMV-immune assay. (13) CMV-seronegative patients were monitored for six months while CMV-seropositive patients were monitored for three months.

In the control group, results of the CMV-immune assay were neither communicated to treating physicians nor to patients and durations of prophylaxis were planned to be 180 days in CMV-seronegative patients and 90 days in CMV-seropositive patients, in line with international guidelines.(4) In the immune-monitoring group, patients were started on the same antiviral prophylaxis as in the control group, but their treating physicians received results of the CMV-immune assay within 48-72h of blood sampling. In case of a positive result, valganciclovir was discontinued. If the CMV-immune assay was negative or invalid, valganciclovir was continued until the first positive assay or the maximal duration of prophylaxis was reached (180 or 90 days, according to CMV serostatus), whichever occurred first.

Rules for interpretation of the CMV-immune assay results are shown in the appendix. Briefly, test results were considered positive if the geometric mean of four-replicate SFC values resulting from IE-1 and/or pp65 stimulation was ≥ 10 SFC/200,000 PBMC and if the ratio of the SFC geometric mean of the stimulated vs. unstimulated condition was ≥ 2.5. A test was considered negative when test results for both IE-1 and pp65 antigens were negative and the positive control was positive (≥ 10 SFC/200,000 PBMC). A negative test together with a negative positive control was considered invalid. A positive test together with a negative positive control was valid and evaluated as positive.

After discontinuation of antiviral prophylaxis, CMV-DNAemia was monitored by PCR at two weeks and then monthly until the end of follow-up by local laboratories. Clinicians used similar cutoffs for starting antiviral therapy in case of asymptomatic CMV-DNAemia: >500-1000 CMV DNA IU/ml in plasma or >5000-10,000 CMV DNA IU/ml in whole blood, as per routine clinical practice at each center. Patients were followed for a maximum of 12 months after transplantation.

Outcomes

The first co-primary outcome was the proportion of patients with a clinically significant CMV infection up to 12 months after transplantation. The second co-primary outcome was the reduction in days of antiviral prophylaxis. Clinically significant CMV infection included both CMV disease and treated asymptomatic CMV infection.(14) CMV infection was defined as evidence of CMV replication regardless of symptoms (14) and CMV disease as CMV infection with attributable symptoms. The diagnosis and classification of CMV events were done by the clinician in charge of the patient and then validated by study site investigators. The duration of prophylaxis was calculated from the day of starting the antiviral drug after transplantation to the day of discontinuation due to end of prophylaxis period, a positive assay, valganciclovir toxicity, or a clinician's decision. Secondary outcomes were the incidence of all CMV events including untreated CMV replication, high-level CMV-DNAemia (>1000 IU/ml in plasma or 10,000 IU/ml in whole blood), and the incidence of acute rejection, allograft/patient survival at 1-year follow-up. Safety outcomes included the proportion of patients discontinuing antiviral prophylaxis due to toxicity and the occurrence of leukopenia, grade 4 leukopenia, and anemia.

Statistical analysis

The sample size was driven by the primary study hypothesis of non-inferiority of immune-monitoring versus a fixed duration of prophylaxis in the risk of first co-primary outcome of at least one clinically significant CMV infection up to one-year post-transplantation. The incidence was assumed to be 8% in both groups.(3) A sample size of 192 patients would provide 80% power to detect non-inferiority on a risk difference scale at a margin of 12% and a one-sided alpha of 2.5%. Non-inferiority would be declared if the upper limit of the two-sided 95%-confidence interval (CI) for the risk difference was below 12%. For the second co-primary outcome of duration of antiviral prophylaxis, this sample size resulted in more than 88% power to detect superiority using a Wilcoxon rank-sum test at a two-sided alpha of 2.5% based on simulations using 10 chains with 10,000 iterations each and assuming a difference in means of 15 days and a common standard deviation of 30 days.

The modified intention-to-treat (mITT) population consisted of participants who were randomized and had at least one CMV-DNAemia assessment. In addition, at least one valid CMV-immune assay result was required for patients in the immune-monitoring group. The per-protocol population excluded patients with major protocol violations, defined as continuation of antiviral prophylaxis for more than four weeks despite a positive CMV-immune assay or termination of prophylaxis despite a negative CMV-immune assay in the immune-monitoring group, and deviation from the specified prophylaxis period by more than four weeks in the control group.

The primary analysis used the Mantel-Haenszel method stratified by CMV serostatus to estimate an adjusted risk difference for the first co-primary outcome of clinically significant CMV infection. In pre-specified secondary analyses, we modelled the first co-primary outcome on an odds ratio scale using mixed logistic regression with random intercept for center.(15) These models included age and sex as covariates, and used covariate adjustment or stratified analyses to adjusted for CMV serostatus. In addition to Kaplan-Meier estimates for the time to participants' first clinically significant CMV infection, we used cumulative incidence functions that considered patients' death as competing event.(16) For the second co-primary outcome of antiviral prophylaxis duration, we used a Wilcoxon rank-sum test stratified by CMV serostatus to test for superiority and performed stratified analysis with inverse-variance weights to adjust between-group differences in mean duration of antiviral prophylaxis by CMV serostatus. Safety outcomes were compared between groups by a two-sided binomial test with significance level of 5%. Analyses were performed using R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria) and Stata software version 17.0 (StataCorp, College Station, Texas, USA).

Role of the funding source

Lophius Biosciences provided the CMV interferon-gamma release assays and organized quarterly quality controls of the implementation of the assay throughout the study. The funders

of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

Patients

From January 2016 to October 2019, 193 patients were randomized, 92 to immune-monitoring and 101 patients to control. Two patients did not have CMV-DNAemia assessments at follow-up and three patients did not have a valid CMV-immune assay in the immune-monitoring group, whereas three patients lacked CMV-DNAemia at follow-up in the control group. Therefore, 87 and 98 patients were included in the mITT analysis, respectively (Figure 1). Patients' demographic characteristics were similar in both groups, except for CMV serostatus, with 43/87 (49.4%) and 58/98 (59.2%) CMV-seronegative recipients assigned to immune-monitoring and control, respectively (Table 1). Twenty-two and 18 patients were excluded from the per-protocol analysis (Figure 1 and Supplement Table 1).

Primary outcomes

In the modified intention-to-treat analysis, 26 out of 87 patients allocated to immune-monitoring (adjusted percentage, 30.9%) and 32/98 control patients (31.1%) had a clinically significant CMV infection (Mantel-Haenszel risk difference of -0.1, 95%-CI -13.0 to 12.7, p for non-inferiority=0.064; Table 2 and Figure 2A). The first clinically significant CMV infection tended to occur earlier with immune-monitoring than control, but at 12 months, the cumulative incidence was comparable between groups (Figure 3A). The duration of antiviral prophylaxis was shorter with immune-monitoring (adjusted difference, -26.0 days, 95%-CI -41.1 to -10.8 days, p<0.001) (Table 2 and Figure 2B). In the per-protocol analysis, 22/65 patients with immune-monitoring (34.7%) and 26/80 control patients (30.7%) had a clinically significant CMV infection (Mantel-Haenszel risk difference of 4.2, 95%-CI -10.6 to 19.1, p for non-inferiority=0.31; Supplement Table 2 and Supplement Figure 1). The duration of antiviral prophylaxis was shorter with immune-monitoring (adjusted difference, -38.4 days, 95%-CI -47.5 to -29.2 days, p<0.001; Supplement Table 2 and Supplement Figure 1).

Subgroup analysis by CMV serostatus

In CMV-seronegative recipients, clinically significant CMV infection was seen in 17/43 patients with immune-monitoring (39.5%) and in 27/58 control patients (46.6%) (risk difference -7.0%, 95% CI -26.5% to 12.4%). In CMV-seropositive recipients, clinically significant CMV infection was seen in 9/44 (20.5%) and 5/40 (12.5%), respectively (risk difference 8.0%, 95% CI -7.8% to 23.7%, p for interaction with CMV-serostatus 0.23; Figure 2A). The first clinically significant CMV infection tended to occur earlier with immune-monitoring group than control irrespective of CMV-serostatus (Figures 3B and 3C). Symptomatic CMV disease was diagnosed in 8 patients

(9.2%) with immune-monitoring (all among CMV-seronegative patients) and 10 control patients (10.2%; in 9 CMV-seronegative and 1 CMV-seropositive patient).

The mean duration of antiviral prophylaxis was shorter with immune-monitoring as compared to the control group among CMV-seronegative patients (-16.7 days, 95% CI -40.5 to 7.0) and among CMV-seropositive patients (-32.4 days, 95% CI -52.1 to -12.6; p for interaction with CMV-serostatus 0.36; Table 3 and Figure 2B).

In the per-protocol analysis, the incidence of clinically significant CMV infection was higher with immune-monitoring among CMV-seropositive patients (risk difference 17.6%, 95% CI 0% to 35.3%), but numerically lower in CMV-seronegative patients (risk difference -8.1%, 95% CI -30.7% to 14.5%, p for interaction 0.047; Supplement Table 3 and Supplement Figure 1). The duration of antiviral prophylaxis was shorter in both CMV-seronegative (-23.3 days, 95% CI -42.2 to -4.4) and CMV-seropositive patients (-42.9 days, 95% CI -53.4 to -32.5, p for interaction 0.070; Supplement Table 3 and Supplement Figure 1).

Secondary and safety outcomes

The total number of CMV events was 62 (adjusted incidence rate 71.1 per 100 patient-years) in patients with immune-monitoring and 66 in control patients (66.1 per 100 patient-years), including 30 episodes of high-level CMV-DNAemia in the immune-monitoring group (adjusted incidence rate 36.0 per 100 patient-years) and 38 episodes in the control group (37.0 per 100 patient-years; Table 2). The management of CMV events is summarized in the Supplement Table 4 and 5. The rates of allograft rejection are shown in Table 2. Six (7.6%) and 7 patients (6.8%) discontinued antiviral prophylaxis due to drug toxicity in immune-monitoring and control groups, respectively. Leukopenia was seen in 47/87 (55.6%) patients with immune-monitoring and 59/98 (60.2%) control patients, with only 1% of patients (1/87 and 1/98, respectively) having severe leukopenia (Table 2).

In a post-hoc analysis, 38/87 patients with immune-monitoring (43.7%) stopped prophylaxis because of a positive CMV assay, 37/87 (42.5%) because the patient reached the end of prophylaxis period, and 12/87 (13.7%) for other reasons. Prophylaxis was discontinued due to a positive CMV-immune assay in the immune-monitoring group in 9/43 (20.9%) in CMV-seronegative patients and 29/44 (65.9%) in CMV-seropositive patients. A description of the results of the CMV-immune assay according to CMV serostatus is shown in the supplementary Table 6. While most CMV-seropositive patients showed a positive CMV-immune assay result within the first month post transplant, only one-fourth of CMV-seronegative patients had a positive test up to six months post transplant. Results were similar for both immune-monitoring and control groups. Figure 4 shows the kinetics of cell-mediated immunity for both pp65 and IE1 antigens. Overall, a detectable immune response appeared earlier in CMV-seropositive patients, and it was mostly driven by a response to the CMV pp65 antigen. Additional analyses of

efficacy and safety outcomes are presented in the Supplementary Tables 7 to 14 and Supplementary Figures 2 to 5.

DISCUSSION

In this randomized trial of 193 solid-organ transplant recipients, immune monitoring resulted in a significant reduction in the duration of antiviral prophylaxis, but we were unable to establish non-inferiority of this approach on the co-primary outcome of clinically significant CMV infection.

Observational studies have suggested that a detectable CMV-specific cellular immune response is associated with a lower incidence of subsequent CMV infection.(8, 17, 18) A recent trial by Páez-Vega et al. used a CMV-specific interferon gamma assay for determining the duration of prophylaxis in 150 CMV-seropositive kidney transplant recipients.(9) As observed in our trial, immune monitoring resulted in a significant reduction of antiviral prophylaxis in transplant recipients. The incidence of the primary outcome of CMV disease was low (0 versus 2 events), but the 95% confidence interval for the risk difference in the more frequent secondary outcome of any CMV infection was wide and did not rule out a clinically relevant disadvantage of immune monitoring. Taken together, the two trials suggest that immune monitoring results in a clinically relevant reduction in duration of antiviral prophylaxis without increasing the risk of CMV disease, but results are less robust for outcomes that include CMV replication.

We encountered important differences in the results of cell-mediated immunity assays according to CMV serostatus. This was expected according to previous literature,(19) thus the trial was designed in a manner to take these differences into account. Although CMV-seropositive patients receiving anti-thymocyte globulins might have low CD3+ T-cell counts for weeks after transplant,(20) these patients were able to mount a detectable CMV-specific interferon-gamma response rapidly in our trial. Notably, no case of CMV disease was observed in CMV-seropositive patients in the immune-monitoring group. On the contrary, CMV-negative patients showed impaired cell-mediated immunity, in particular during effective antiviral prophylaxis. Accordingly, an alternative approach needs to be explored in this subgroup of patients.(10)

Although our findings were obtained using an Elispot assay, alternative methods for assessing cell-mediated responses against CMV exist, such as employing an Elisa test (Quantiferon-CMV) or intracellular cytokine staining. While some studies suggest that the Elispot assay may exhibit superior diagnostic performance (21), our results closely align with those from the clinical trial conducted by Paez-Vega et al., which used the Quantiferon-CMV assay (9). Consequently, while caution is needed when extending our findings, the notion that a positive cell-mediated immune assay could safely support the discontinuation of antiviral prophylaxis in transplant recipients seems applicable to scenarios using different assays.

This study has several limitations. Importantly, there was a clinically relevant baseline imbalance in CMV-serostatus between immune-monitoring and control group due to lack of stratification by CMV-serostatus due to human error. In order to account to this limitation, all group-specific estimates and between-group differences were therefore adjusted for CMV serostatus using stratified analyses with appropriate statistical weights. Second, some deviations from the protocol were seen during the clinical trial, including the extension of prophylaxis in patients with a detectable immunity in the intervention arm. This may have been due to clinicians' discomfort with stopping prophylaxis in patients with perceived higher risk for CMV complications.

In conclusion, immune monitoring resulted in a significant reduction of antiviral prophylaxis duration in transplant recipients. Even though we were unable to establish non-inferiority on the co-primary outcome of CMV infection, the risk of CMV-related complications, in particular CMV disease, seems low in this population.

Contributors

OM, MP, TF, CvD, HHH, and NJM conceived and designed the study. HHH, MJK, KK, SD, CH, NS, DS, UHD, LNW, NE, KH, DN, DG, MM, IB, AS, PEC, KH, TFM, AVB, HH, MH, FSR, JV, and AZ were responsible for the acquisition of data. ML, SS, and PJ performed the analyses and interpreted the results in collaboration with all other authors. OM, ML, CH and PJ wrote the first draft of the report. All authors critically revised the report for important intellectual content and approved the final version.

Declaration of interests

Oriol Manuel received research grants from Lophius Biosciences (meanwhile acquired by Mikrogen), the Novartis Foundation and the Swiss Transplant Cohort Study, and has participated in DSMB of Syneos and in advisory boards of MSD, Biotest, and Takeda; and reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Takeda, paid to institution. Hans H. Hirsch has received grants from Moderna paid to his institution, consulting fees from AiCuris, Allovir, Moderna, VeraTX and Roche, and has received honoraria from VeraTX, Takeda, Biotest and Gilead. Peter Jüni serves as unpaid member of the steering group of a trial funded by Terumo; he has received research grants to the institution from Appili Therapeutics, but has not received personal payments by any pharmaceutical company or device manufacturer. All other authors: no conflicts of interest.

Data sharing statement

Deidentified, individual participant data that underlie this Article, along with a data dictionary describing variables in the dataset, are available to researchers whose proposed purpose of use is approved by the Scientific Committee of the Swiss Transplant Cohort Study. Related documents

such as the study protocol and informed consent form will be made available on request. To request the dataset, please send a signed data request form to oriol.manuel@chuv.ch.

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Table 1. Baseline characteristics of the patients included in the modified intention-to-treat analysis, N (%)

Baseline Characteristics	Immune-monitoring (N=87)	Control (N=98)
Age, median (IQR), y	53.0 (43.5-60.0)	57.5 (45.25-65.0)
Female – no. (%)	29 (33.3)	31 (31.6)
Deceased donor – no. (%)	58 (66.7)	71 (71.6)
Organ		
Kidney	77 (88.5)	87 (88.8)
Liver	10 (11.5)	11 (11.2)
CMV serostatus		
CMV-seropositive	44 (50.6)	40 (40.8)
CMV-seronegative	43 (49.4)	58 (59.2)
Underlying kidney disease (n=164)		
Autosomal dominant polycystic kidney disease	6 (7.8)	16 (18.4)
Allograft nephropathy	4 (5.2)	2 (2.2)
Diabetic nephropathy	4 (5.2)	12 (13.8)
Glomerulonephritis	16 (20.8)	23 (26.4)
Hypertensive nephropathy	4 (5.2)	12 (13.8)
Other	43 (55.8)	22 (25.3)
Underlying liver disease (n=21)		
Alcoholic liver disease	4 (40.0)	1 (9.1)
Chronic viral hepatitis	3 (30.0)	0 (0)
Primary sclerosing cholangitis	1 (20.0)	1 (9.1)
Other	2 (10.0)	9 (81.8)
MELD score at transplantation, median (IQR)	17.0 (11.0-21.0)	17.0 (8.75-23.5)
Induction therapy		
Anti-thymocyte globulins	52 (59.8)	51 (52.0)
Basiliximab	37 (42.5)	50 (51.0)
Rituximab	1 (1.1)	2 (2.0)
Intravenous immunoglobulins	5 (5.7)	9 (9.2)
None	6 (6.9)	6 (6.1)
Maintenance therapy		
Prednisone	79 (90.8)	91 (92.9)
Mycophenolate	66 (75.9)	82 (83.7)
Tacrolimus	75 (86.2)	85 (86.7)
Cyclosporine	9 (10.3)	9 (9.2)
Azathioprine	9 (10.3)	10 (10.2)

Abbreviations: IQR, interquartile range; MELD, Model for End-Stage Liver Disease

Table 2. Clinical outcomes in patients randomized to immune-monitoring or control in the modified intention-to-treat analysis

	Immune-monitoring (n = 87)	Control (n = 98)	Effect estimate (95% CI)	P-value
			Risk difference	
Clinically significant CMV infection, No. of patients (%)*	26 (30.9)	32 (31.1)	-0.1 (-13.0, 12.7)	0.064**
Tissue-invasive disease§	2 (2.5)	2 (1.9)	0.7 (-3.6, 4.9)	-
Viral syndrome§	6 (7.6)	8 (7.7)	-0.1 (-7.6, 7.4)	-
Treated asymptomatic replication§	18 (20.7)	22 (21.5)	-0.6 (-12.5, 11.1)	-
			Difference in means	
Days of antiviral prophylaxis, mean (SD)*	113.7 (47.6)	145.5 (37.9)	-26.0 (-41.1, -10.8)	< 0.001
			Incidence rate difference	
Episodes of CMV infection, No. of episodes (IR)°	62 (71.1)	66 (66.1)	-1.9 (-25.7, 21.9)	-
Tissue-invasive disease	2 (2.5)	2 (1.9)	0.0 (NA)	-
Viral syndrome	6 (7.6)	10 (9.8)	-2.0 (-8.3, 4.3)	-
Treated asymptomatic replication	25 (29.4)	30 (29.3)	-0.3 (-14.8, 14.2)	-
Untreated asymptomatic replication	29 (31.5)	24 (25.1)	- 1.6 (-15.6, 12.3)	-
High-level CMV DNAemia, No. of episodes (IR)°	30 (36.0)	38 (37.0)	-1.1 (-16.3, 14.2)	-
Safety endpoints, No. of patients (%)			Risk difference	
Discontinuation of prophylaxis due to toxicity	6 (7.6)	7 (6.8)	0.8 (-6.5, 8.1)	-
Leucopenia	47 (55.6)	59 (60.2)	-4.7 (-19.0, 9.6)	-
Grade 4 leucopenia	1 (1.3)	1 (0.9)	0.3 (-2.7, 3.3)	-
Anemia	20 (23.5)	16 (16.2)	7.2 (-4.3, 18.8)	-
Allograft rejection	9 (10.2)	6 (5.6)	4.6 (-3.2, 12.5)	-
Graft loss	2 (2.3)	1 (0.9)	1.4 (-2.3, 5.1)	-
Death	1 (1.0)	2 (2.1)	-1.0 (-4.6, 2.5)	-

Effect estimates are adjusted for CMV-serostatus using stratified analyses with Mantel-Haenszel weights for risk differences and inverse variance weights for differences in means and incidence rates; percentages, means and incidence rates in immune-monitoring and control groups are adjusted to have the same distribution of CMV-serostatus as seen in both groups combined (101 CMV-seronegative and 84 CMV-seropositive patients). *Co-primary endpoint. **One-sided p-value for non-inferiority; all

remaining p-values are two-sided for superiority. §In case of several episodes of clinically significant CMV infection, the most severe episode was included for each patient. °Estimates in brackets are incidence rates per 100 patient-years.

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Table 3. Clinical outcomes in patients included in the immune-monitoring group and the control group by CMV risk status in the modified intention-to-treat analysis

	CMV-seronegative			CMV-seropositive		
	Immune-monitoring (n = 43)	Control (n = 58)	Effect estimate (95% CI)	Immune-monitoring (n = 44)	Control (n = 40)	Effect estimate (95% CI)
			Risk difference			Risk difference
Clinically significant CMV infection, No. of patients (%)*	17 (39.5)	27 (46.6)	-7.0 (-26.5, 12.4)	9 (20.5)	5 (12.5)	8.0 (-7.8, 23.7)
Tissue-invasive disease§	2 (4.6)	2 (3.4)	1.2 (-6.7, 9.1)	0 (0)	0 (0)	0
Viral syndrome§	6 (13.9)	7 (12.1)	1.9 (-11.4, 15.2)	0 (0)	1 (2.5)	-2.5 (-7.3, 2.3)
Treated asymptomatic replication§	9 (20.9)	18 (31.0)	-10.1 (-27.1, 6.9)	9 (20.5)	4 (10.0)	10.5 (-4.7, 25.6)
			Difference in means			Difference in means
Days of antiviral prophylaxis, mean (SD)*	158.6 (67.3)	175.3 (48.9)	-16.7 (-40.5, 7.0)	69.8 (54.8)	102.1 (36.6)	-32.4 (-52.1, -12.6)
			Incidence rate difference			Incidence rate difference
Episodes of CMV infection, No. of episodes (IR)°	29 (67.4)	47 (81.1)	-13.7 (-47.4, 20.1)	33 (75.5)	19 (48.0)	27.5 (-6.1, 61.1)
Tissue-invasive disease	2 (4.7)	2 (3.5)	1.2 (-6.8, 9.2)	0 (0)	0 (0)	0
Viral syndrome	6 (14.0)	8 (13.8)	0.1 (-14.6, 14.9)	0 (0)	2 (5.1)	-5.1 (-12.1, 1.9)
Treated asymptomatic replication	15 (34.9)	25 (43.1)	-8.3 (-32.7, 16.2)	10 (22.9)	5 (12.6)	10.2 (-7.7, 28.2)
Untreated asymptomatic replication	6 (14.0)	12 (20.7)	-6.8 (-22.9, 9.4)	23 (52.6)	12 (30.3)	22.3, (-5.2, 49.8)
High-level CMV DNAemia, No. of episodes (IR)°	21 (48.8)	32 (55.2)	-6.4 (-34.7, 21.9)	9 (20.6)	6 (15.2)	5.4 (-12.7, 23.5)
Safety end points, No. of patients (%)			Risk difference			Risk difference
Discontinuation of prophylaxis due to toxicity	6 (14.0)	6 (10.3)	3.6 (-9.4, 16.6)	0 (0)	1 (2.5)	-2.5 (-7.3, 2.3)
Leucopenia	30 (69.8)	35 (60.3)	9.4 (-9.2, 28.0)	17 (38.6)	24 (60.0)	-21.4 (-42.3, -0.4)
Grade 4 leucopenia	1 (2.3)	1 (1.7)	0.6 (-5, 6.2)	0 (0)	0 (0)	0
Anemia	12 (27.9)	10 (17.2)	10.7 (-5.9, 27.2)	8 (18.2)	6 (15.0)	3.2 (-12.7, 19.1)
Allograft rejection	4 (9.3)	6 (10.3)	-1 (-12.7, 10.7)	5 (11.4)	0 (0)	11.4 (2, 20.7)
Graft loss	0 (0)	1 (1.7)	0.6 (-5, 6.2)	1 (2.3)	0 (0)	2.3 (-2.1, 6.7)
Death	0 (0)	1 (1.7)	-1.7 (-5.1, 1.6)	1 (2.3)	1 (2.5)	-0.2 (-6.8, 6.3)

*Co-primary endpoint. §In case of several episodes of clinically significant CMV infection, the most severe episode was included for each patient. °Estimates in brackets are incidence rates per 100 patient-years.

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FIGURES LEGEND

Figure 1. Trial profile.

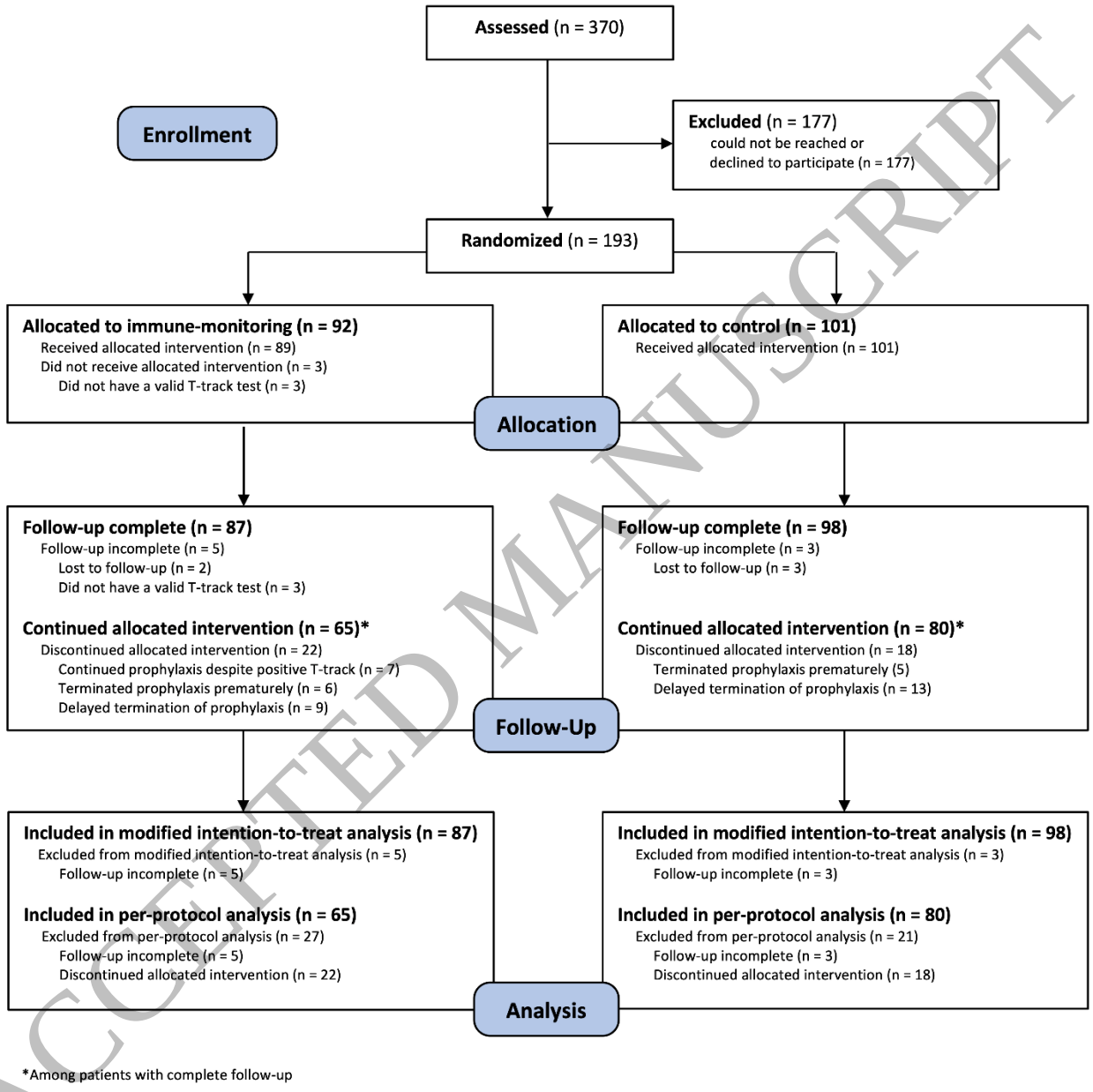

Figure 2. Primary outcomes overall and by CMV serostatus.

(A) Difference in proportion of patients with at least one clinically significant CMV infection. Non-inferiority would be established if the upper limit of the two-sided 95% CI were less than 12% (non-inferiority margin, dashed line). The p-value for non-inferiority is one-sided with set at 0.025. (B) Difference in mean prophylaxis duration. The p-value for superiority is two-sided from a stratified Wilcoxon test with set at 0.025. Analyses were done in the modified intention-to-treat population. Overall estimates were adjusted for CMV-serostatus using stratified analyses with Mantel-Haenszel

weights for the risk difference of clinically significant CMV infection (A) and inverse variance weights for the difference in mean prophylaxis duration (B). The p-values for interaction between intervention and CMV serostatus were 0.226 for CMV infection (A) and 0.361 for mean prophylaxis duration (B).

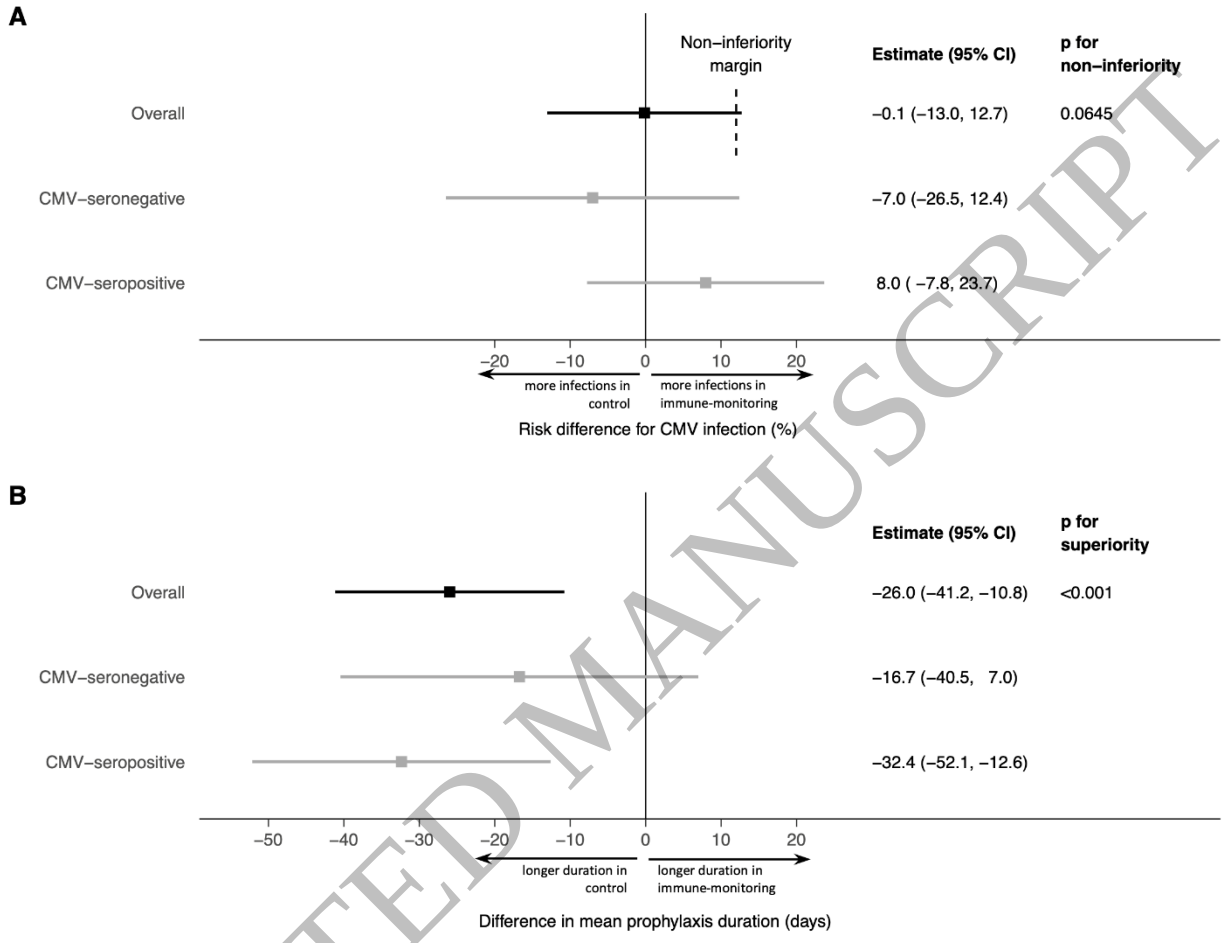

Figure 3. Kaplan-Meier estimates of the probability of clinically significant CMV infection.

(A) Overall, (B) CMV-seronegative patients, (C) CMV-seropositive patients. IRR, Incidence rate ratio. Overall Kaplan-Meier estimates and corresponding incidence rate ratio are unadjusted for CMV serostatus.

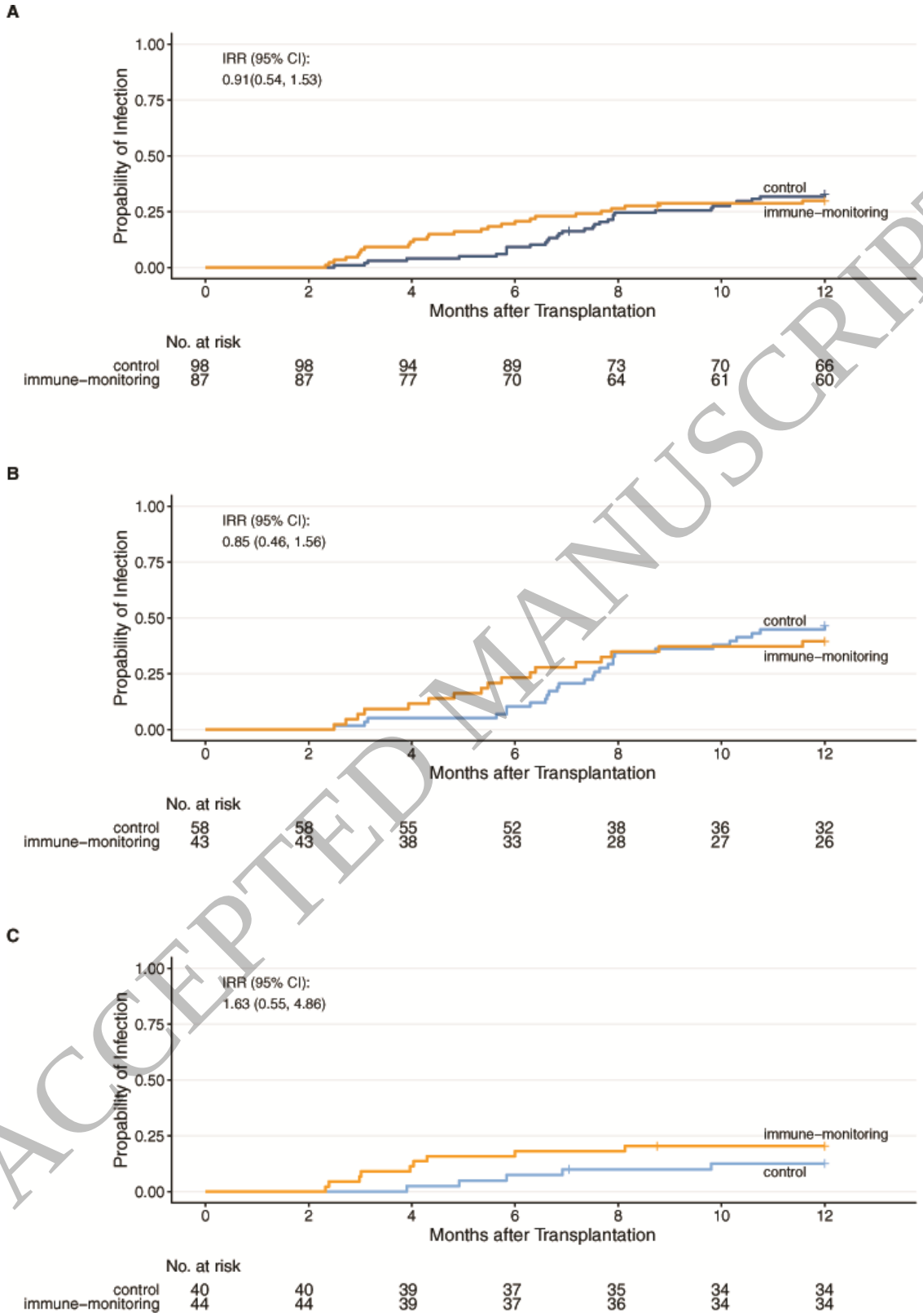

Figure 4. Results of the CMV immune assay. Boxplot of the results of the T-Track assays in the immune-monitoring group and the control group, according to CMV risk status (CMV-seronegative and

CMV-seropositive). Results of the T-track assays in the control group were not communicated to the treating physicians. A: pp65 CMV antigen. B: IE1 CMV antigen. The boxes indicate the lower and upper quartiles and the lines inside the boxes indicate the median. The whiskers extend to the furthest observations from the lower and upper quartiles that are still within 1.5 x the interquartile range. Observations beyond that range are shown as circles. The dashed line indicates the primary cutoff of the -Track® CMV assay.