# 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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Abstract: 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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*Clinical Infectious Diseases*

MAJOR ARTICLE

# Immune Monitoring-Guided Versus Fixed Duration of Antiviral Prophylaxis Against Cytomegalovirus in Solid-Organ Transplant Recipients: A Multicenter, Randomized Clinical Trial

**Oriol Manuel,[1,2] Mirjam Laager,[3] Cédric Hirzel,[4] Dionysios Neofytos,[5] Laura N. Walti,[4] Gideon Hoenger,[6] Isabelle Binet,[7] Aurelia Schnyder,[7] Susanne Stampf,[8] Michael Koller,[8] Matteo Mombelli,[1,2] Min Jeong Kim,[8,9] Matthias Hoffmann,[10,11] Katrin Koenig,[8,12] Christoph Hess,[6,13] Anne-Valérie Burgener,[6,14] Pietro E. Cippà,[15,16] Kerstin Hübel,[15] Thomas F. Mueller,[15] Daniel Sidler,[17] Suzan Dahdal,[17] Franziska Suter-Riniker,[18] Jean Villard,[19] Andrea Zbinden,[20] Giuseppe Pantaleo,[21] Nasser Semmo,[22] Karine Hadaya,[23,24] Natalia Enríquez,[5] Pascal R. Meylan,[1] Marc Froissart,[25] Dela Golshayan,[2] Thomas Fehr,[14,26] Uyen Huynh-Do,[17] Manuel Pascual,[2] Christian van Delden,[5] Hans H. Hirsch,[27,28] Peter Jüni,[29,a] and Nicolas J. Mueller,[30,a] the Swiss Transplant Cohort Study (STCS)**

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***Background.*** The use of assays detecting cytomegalovirus (CMV)–specific T cell–mediated immunity may individualize the duration of antiviral prophylaxis after transplantation.

***Methods.*** In this randomized trial, kidney and liver transplant recipients from 6 centers in Switzerland were enrolled if they were CMV-seronegative with seropositive donors or CMV-seropositive receiving antithymocyte 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 ELISpot assay (T-Track CMV); prophylaxis in the intervention group was stopped if the assay was positive. The co-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 group and 101 in the control group), of whom 185 had evaluation of the primary outcome (87 and 98 patients). CMV infection occurred in 26 of 87 (adjusted percentage, 30.9%) in the immune-monitoring group and in 32 of 98 (adjusted percentage, 31.1%) in the control group (adjusted risk difference, −0.1; 95% confidence interval [CI], −13.0% to 12.7%; $P = .064$). The duration of prophylaxis was shorter in the immune-monitoring group (adjusted difference, −26.0 days; 95%, CI, −41.1 to −10.8 days; $P < .001$).

***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 outcome of CMV infection.

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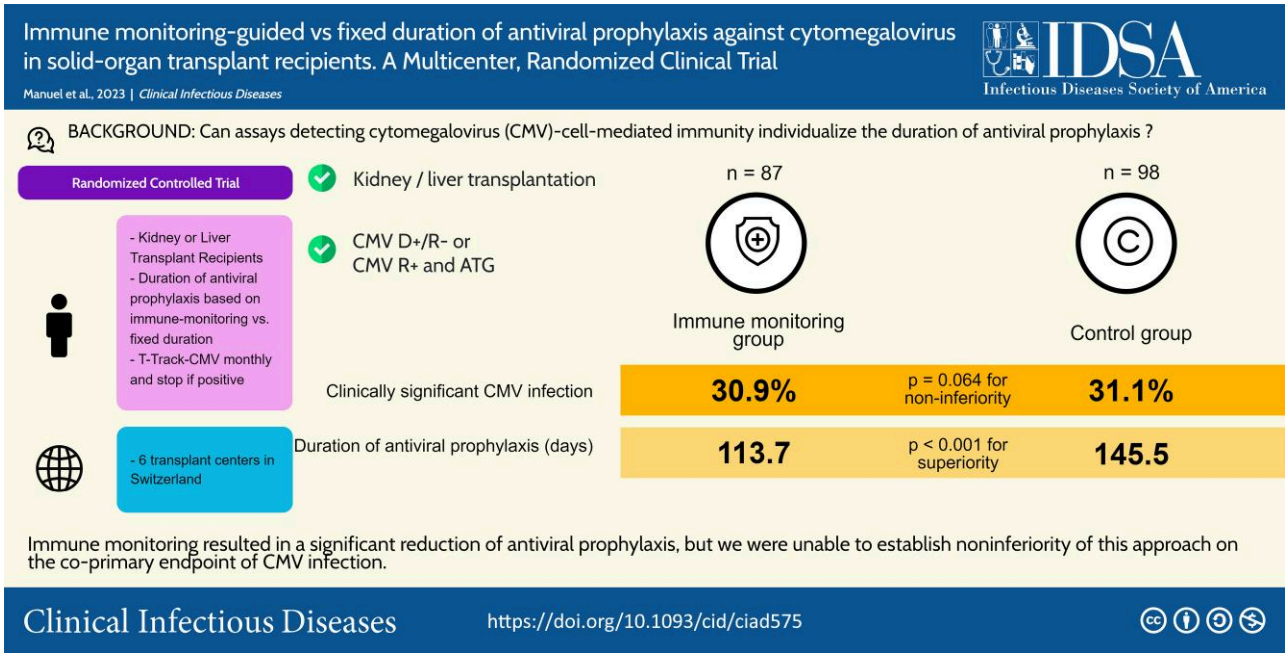
*Graphical Abstract*




This graphical abstract is also available at Tidbit: https://tidbitapp.io/tidbits/immune-monitoring-guided-vs-fixed-duration-of-antiviral-prophylaxis-against-cytomegalovirus-in-solid-organ-transplant-recipients-a-multicenter-randomized-clinical-trial-1cd729ef-d7a6-4dca-a470-d73a1f6a3b41

**Keywords.** cell-mediated immunity; transplant; personalized medicine; prevention; viral infection.

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 who receive antithymocyte 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 that measure 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 2 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 noninferior 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, noninferiority, randomized clinical trial of an individualized duration of antiviral prophylaxis according to a commercial interferon-gamma release assay to measure CMV-specific immunity versus a fixed duration of prophylaxis in transplant recipients. Patients were recruited at 6 transplant centers in Switzerland (Basel, Bern, Geneva, Lausanne, St. Gallen, and Zurich) that participated in the Swiss Transplant Cohort Study [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 antithymocyte 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. 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 be stratified by transplanted organ (kidney and liver) and CMV serostatus of recipients and blocked with fixed block sizes; however, it was only stratified by transplanted organ and remained unblocked due to human error when the interactive web-based response system was programmed.

#### 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 4 weeks from day 30 after transplantation using a commercially available CMV-specific interferon-gamma enzyme-linked immunosorbent spot (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). Hereafter, we refer to the T-Track CMV assay as the CMV-immune assay [13]. CMV-seronegative patients were monitored for 6 months, and CMV-seropositive patients were monitored for 3 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 48–72 hours 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 provided in the Supplementary Materials. Briefly, test results were considered positive if the geometric mean of 4 replicate spot-forming cells (SFC) values resulting from IE1 and/or pp65 stimulation was ≥10 SFC/200 000 peripheral blood mononuclear cells (PBMCs) and if the ratio of the SFC geometric mean of the stimulated versus unstimulated condition was ≥2.5. A test was considered negative when test results for both IE1 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 using polymerase chain reaction at 2 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, 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 was defined 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 ending of the 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), the incidence of acute rejection, and allograft/patient survival at 1-year follow-up. Safety outcomes included the proportion of patients who discontinued antiviral prophylaxis due to toxicity and the occurrence of leukopenia, grade 4 leukopenia, and anemia.

#### Statistical Analyses

The sample size was driven by the primary study hypothesis of noninferiority of immune monitoring versus a fixed duration of prophylaxis in the risk of first co-primary outcome of at least 1 clinically significant CMV infection up to 1 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 noninferiority on a risk difference scale at a margin of 12% and a 1-sided alpha of 2.5%. Noninferiority would be declared if the upper limit of the 2-sided 95% confidence interval (CI) for the risk difference was less than 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 2-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 1 CMV-DNAemia assessment. In addition, at least 1 valid CMV-immune assay result was required for patients in the immune-monitoring group. The per-protocol population

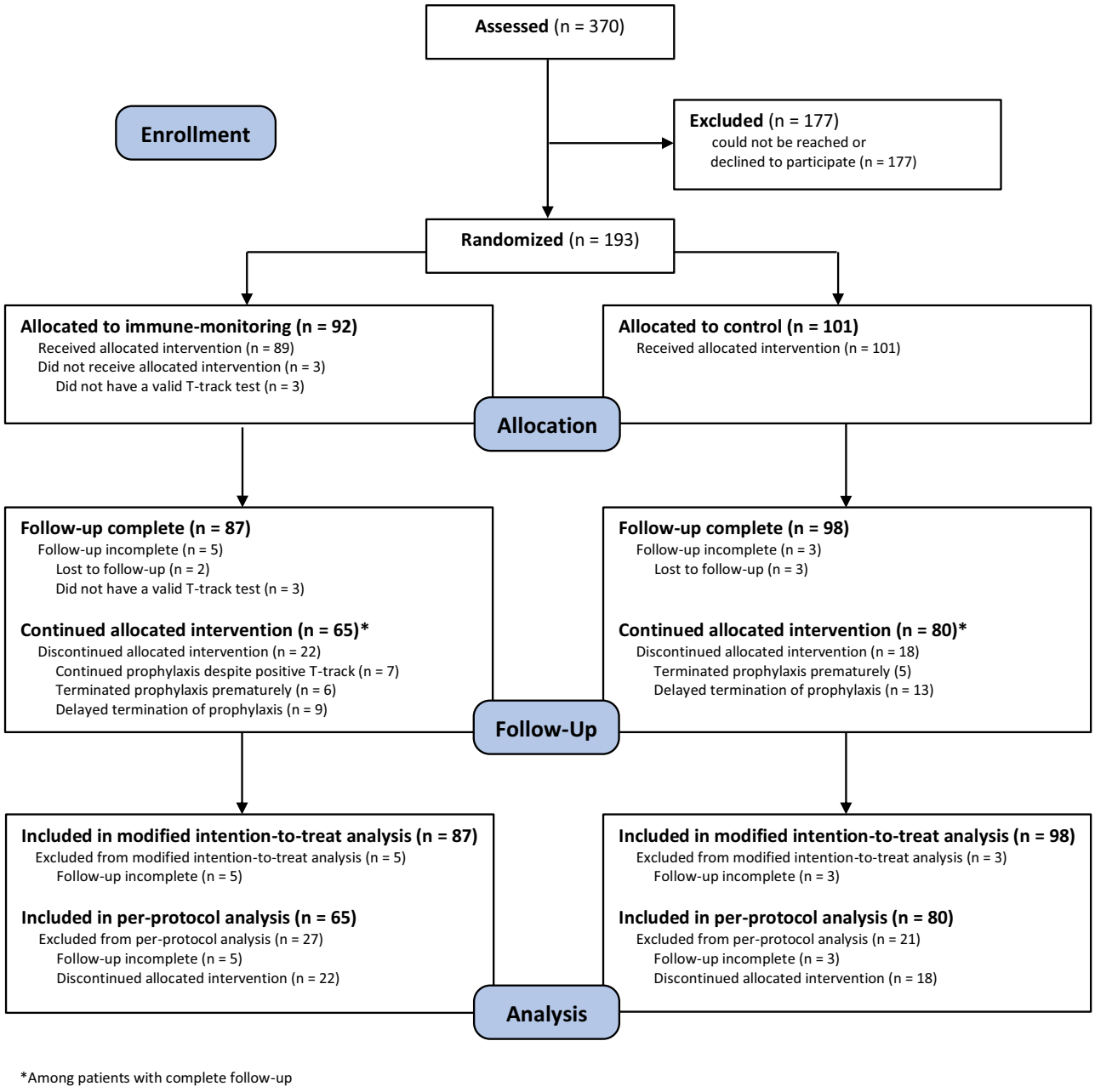



**Figure 1.** Trial profile.

excluded patients with major protocol violations, defined as continuation of antiviral prophylaxis for more than 4 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 4 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 prespecified secondary analyses, we modeled the first co-primary outcome on an odds ratio scale using mixed logistic regression with random intercept for the center [15]. These models included age and sex as covariates and used covariate adjustment or stratified analyses to adjust 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 a 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

**Table 1. Baseline Characteristics of the Patients Included in the Modified Intention-to-Treat Analysis, N (%)**

| Baseline Characteristic | 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) |
| Cytomegalovirus serostatus | | |
| Seropositive | 44 (50.6) | 40 (40.8) |
| 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) |
| Model for end-stage liver disease score at transplantation, median (IQR) | 17.0 (11.0–21.0) | 17.0 (8.75–23.5) |
| Induction therapy | | |
| Antithymocyte 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) |

Abbreviation: IQR, interquartile range.

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 using a 2-sided binomial test with a 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, TX).

## RESULTS

### Patients

From January 2016 to October 2019, 193 patients were randomized, 92 to the immune-monitoring group and 101 to the control group. Two patients did not have CMV-DNAemia assessments at follow-up, and 3 patients did not have a valid CMV immune assay in the immune-monitoring group, whereas 3 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 of 87 (49.4%) and 58 of 98 (59.2%) CMV-seronegative recipients assigned to the immune-monitoring and control groups, respectively (Table 1). Twenty-two and 18 patients were excluded from the per-protocol analysis (Figure 1, Supplementary Table 1).

### Primary Outcomes

In the mITT analysis, 26 of 87 patients allocated to the immune-monitoring group (adjusted percentage, 30.9%) and 32 of 98 patients allocated to the control group (31.1%) had a clinically significant CMV infection (Mantel–Haenszel risk difference, −0.1; 95% CI, −13.0 to 12.7; $P$ for noninferiority = .064; Table 2, Figure 2*A*). The first clinically significant CMV infection tended to occur earlier in the immune-monitoring group than in the control group; however, at 12 months, the cumulative incidence was comparable between groups (Figure 3*A*). The duration of antiviral prophylaxis was shorter with immune monitoring (adjusted difference, −26.0 days; 95% CI, −41.1 to −10.8 days; $P < .001$; Table 2, Figure 2*B*). In the per-protocol analysis, 22 of 65 patients in the immune-monitoring group (34.7%) and 26 of 80 patients in the control group (30.7%) had a clinically significant CMV infection (Mantel–Haenszel risk difference, 4.2; 95% CI, −10.6 to 19.1; $P$ for noninferiority = .31; Supplementary Table 2, Supplementary 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; $P < .001$; Supplementary Table 2, Supplementary Figure 1).

### Subgroup Analysis by CMV Serostatus

In CMV-seronegative recipients, clinically significant CMV infection was seen in 17 of 43 patients with immune monitoring (39.5%) and in 27 of 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 of 44 (20.5%) and 5 of 40 (12.5%), respectively (risk difference, 8.0%; 95% CI, −7.8% to 23.7%; $P$ for interaction with CMV serostatus, .23; Figure 2*A*). The first clinically significant CMV infection tended to occur earlier in the immune-monitoring group compared with the control group irrespective of CMV serostatus (Figure 3*B* and *C*). Symptomatic CMV disease was diagnosed in 8 patients (9.2%) with immune-monitoring (all among CMV-seronegative patients) and in 10 control patients (10.2%; in 9 CMV-seronegative patients and 1 CMV-seropositive patient).

The mean duration of antiviral prophylaxis was shorter in the immune-monitoring group compared with the control group

**Table 2. Clinical Outcomes in Patients Randomized to Immune-Monitoring or Control in the Modified Intention-to-Treat Analysis**

| Outcome | Immune Monitoring (n = 87) | Control (n = 98) | Effect Estimate (95% Confidence Interval) | *P* Value |
|---|---|---|---|---|
| | | | Risk difference | |
| Clinically significant CMV infection, no. of patients (%)[a] | 26 (30.9) | 32 (31.1) | −0.1 (−13.0 to 12.7) | .064[b] |
| Tissue-invasive disease[c] | 2 (2.5) | 2 (1.9) | 0.7 (−3.6 to 4.9) | … |
| Viral syndrome[c] | 6 (7.6) | 8 (7.7) | −0.1 (−7.6 to 7.4) | … |
| Treated asymptomatic replication[c] | 18 (20.7) | 22 (21.5) | −0.6 (−12.5 to 11.1) | … |
| | | | Difference in means | |
| Days of antiviral prophylaxis, mean (standard deviation)[a] | 113.7 (47.6) | 145.5 (37.9) | −26.0 (−41.1 to −10.8) | <.001 |
| | | | Incidence rate difference | |
| Episodes of CMV infection, no. of episodes (IR)[d] | 62 (71.1) | 66 (66.1) | −1.9 (−25.7 to 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 to 4.3) | … |
| Treated asymptomatic replication | 25 (29.4) | 30 (29.3) | −0.3 (−14.8 to 14.2) | … |
| Untreated asymptomatic replication | 29 (31.5) | 24 (25.1) | − 1.6 (−15.6 to 12.3) | … |
| High-level CMV-DNAemia, no. of episodes (IR)[d] | 30 (36.0) | 38 (37.0) | −1.1 (−16.3 to 14.2) | … |
| Safety end points, no. of patients (%) | | | Risk difference | |
| Discontinuation of prophylaxis due to toxicity | 6 (7.6) | 7 (6.8) | 0.8 (−6.5 to 8.1) | … |
| Leucopenia | 47 (55.6) | 59 (60.2) | −4.7 (−19.0 to 9.6) | … |
| Grade 4 leucopenia | 1 (1.3) | 1 (0.9) | 0.3 (−2.7 to 3.3) | … |
| Anemia | 20 (23.5) | 16 (16.2) | 7.2 (−4.3 to 18.8) | … |
| Allograft rejection | 9 (10.2) | 6 (5.6) | 4.6 (−3.2 to 12.5) | … |
| Graft loss | 2 (2.3) | 1 (0.9) | 1.4 (−2.3 to 5.1) | … |
| Death | 1 (1.0) | 2 (2.1) | −1.0 (−4.6 to 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 the 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).

Abbreviations: CMV, cytomegalovirus; IR, incidence rate; NA, not applicable.

[a]Co-primary end point.

[b]One-sided *P* value for noninferiority; all remaining *P* values are 2-sided for superiority.

[c]In case of several episodes of clinically significant CMV infection, the most severe episode was included for each patient.

[d]Estimates in brackets are incidence rates per 100 patient-years.

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, .36; Table 3, Figure 2*B*).

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, .047; Supplementary Table 3, Supplementary 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, .070; Supplementary Table 3, Supplementary Figure 1).

### Secondary and Safety Outcomes

There were 62 CMV events (adjusted incidence rate, 71.1 per 100 patient-years) in immune-monitoring patients 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 Supplementary Tables 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 the immune-monitoring and control groups, respectively. Leukopenia was seen in 47 of 87 (55.6%) immune-monitoring patients and 59 of 98 (60.2%) control patients, with only 1% of patients (1 of 87 and 1 of 98, respectively) having severe leukopenia (Table 2).

In a post hoc analysis, 38 of 87 patients with immune monitoring (43.7%) stopped prophylaxis because of a positive CMV assay, 37 of 87 (42.5%) because the patient reached the end of the prophylaxis period, and 12 of 87 (13.7%) for other reasons. Prophylaxis was discontinued due to a positive CMV-immune assay in the immune-monitoring group in 9 of 43 (20.9%) in CMV-seronegative patients and 29 of 44 (65.9%) in CMV-seropositive patients. A description of the results of the CMV-immune assay according to CMV serostatus is provided in Supplementary Table 6. While most CMV-seropositive patients showed a positive CMV-immune assay result within the first month post-transplant, only

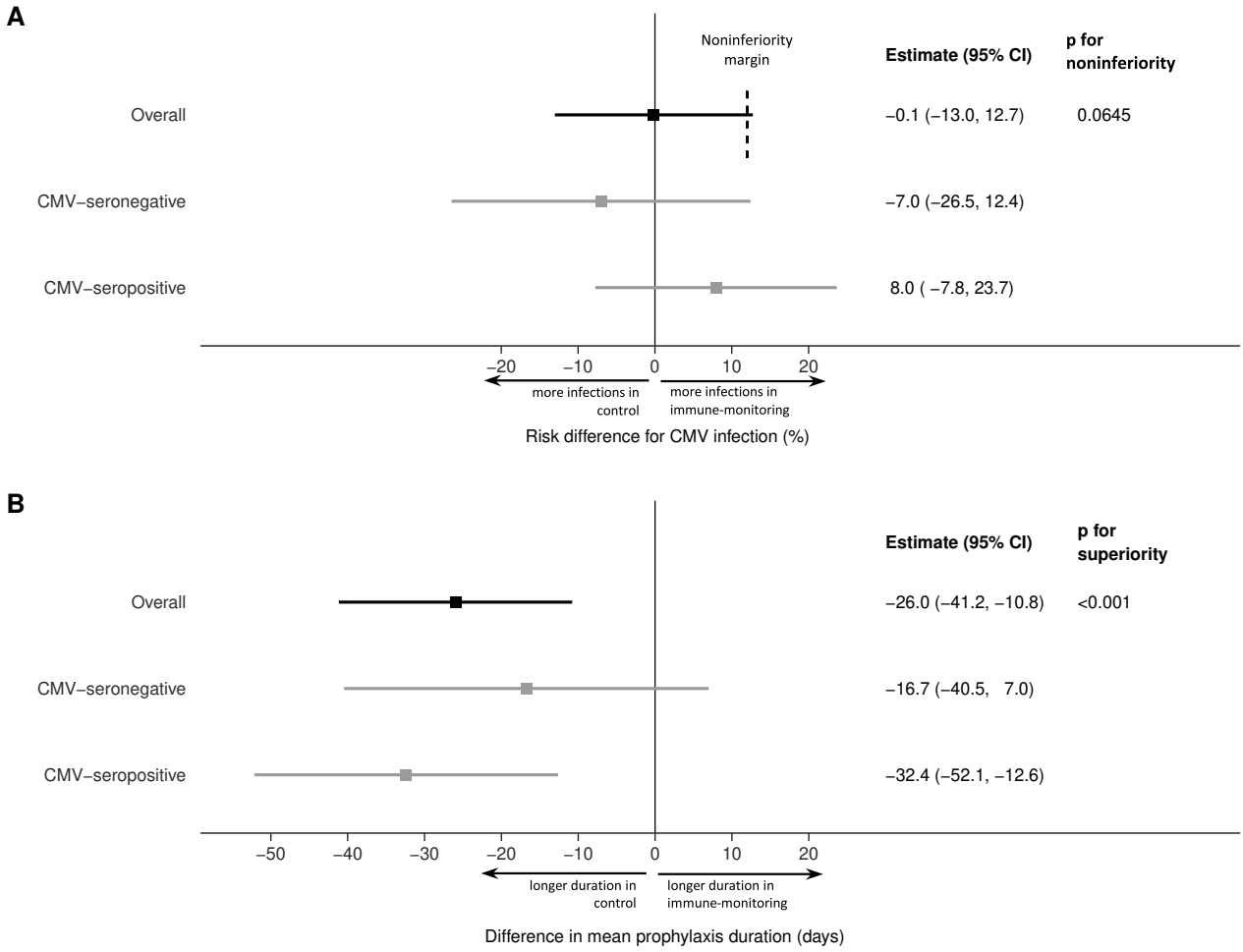



**Figure 2.** Primary outcomes overall and by CMV serostatus. *A*, Difference in proportion of patients with at least 1 clinically significant CMV infection. Noninferiority would be established if the upper limit of the 2-sided 95% CI was less than 12% (noninferiority margin, dashed line). The *P* value for noninferiority is 1-sided with α set at 0.025. *B*, Difference in mean prophylaxis duration. The *P* value for superiority is 2-sided from a stratified Wilcoxon test with α 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*). Abbreviations: CI, confidence interval; CMV, cytomegalovirus.

one-fourth of CMV-seronegative patients had a positive test up to 6 months post-transplant. Results were similar for both the 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 Supplementary Tables 7–14 and Supplementary Figures 2–5.

## DISCUSSION

In this randomized trial of 193 solid-organ transplant recipients, immune monitoring resulted in a significant reduction in the duration of antiviral prophylaxis. However, we were unable to establish noninferiority 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]. In a recent trial, Páez–Vega et al used a CMV-specific interferon-gamma assay to determine 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% CI for the risk difference in the more frequent secondary outcome of any CMV infection was wide and did not rule

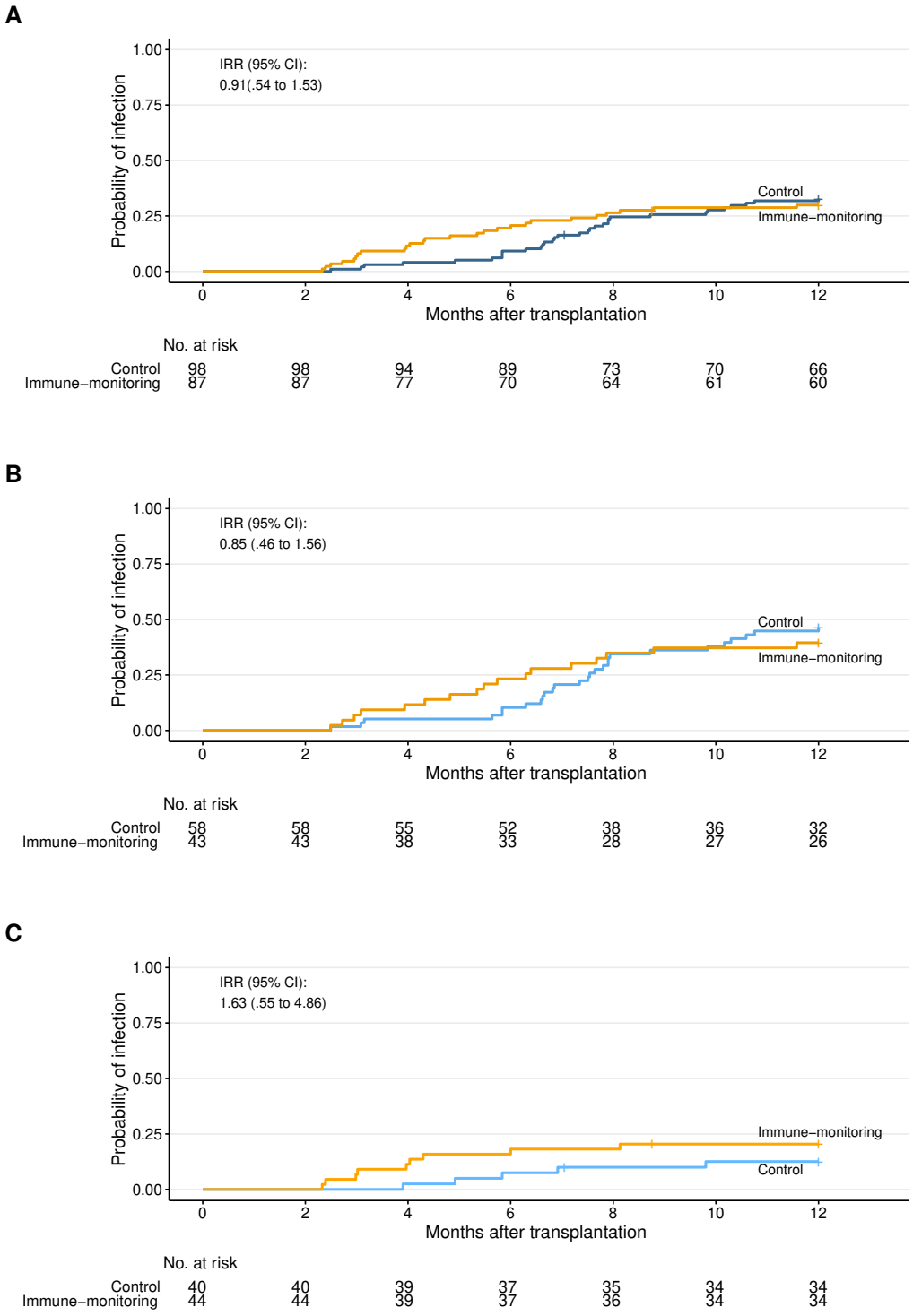



**Figure 3.** Kaplan–Meier estimates of the probability of clinically significant CMV infection: overall (*A*), CMV-seronegative patients (*B*), CMV-seropositive patients (*C*). Overall Kaplan–Meier estimates and corresponding IRRs are unadjusted for CMV serostatus. Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IRR, incidence rate ratio.

out a clinically relevant disadvantage of immune monitoring. Taken together, the 2 trials suggest that immune monitoring results in a clinically relevant reduction in the duration of antiviral prophylaxis without increasing the risk of CMV disease, but results are less robust for outcomes that include CMV replication.

**Table 3. Clinical Outcomes in Patients Included in the Immune-Monitoring and Control Groups by Cytomegalovirus Risk Status in the Modified Intention-to-treat Analysis**

| | CMV-Seronegative | | | CMV-Seropositive | | |
|---|---|---|---|---|---|---|
| Outcome | 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 (%)[a] | 17 (39.5) | 27 (46.6) | −7.0 (−26.5 to 12.4) | 9 (20.5) | 5 (12.5) | 8.0 (−7.8 to 23.7) |
| Tissue-invasive disease[b] | 2 (4.6) | 2 (3.4) | 1.2 (−6.7 to 9.1) | 0 (0) | 0 (0) | 0 |
| Viral syndrome[b] | 6 (13.9) | 7 (12.1) | 1.9 (−11.4 to 15.2) | 0 (0) | 1 (2.5) | −2.5 (−7.3 to 2.3) |
| Treated asymptomatic replication[b] | 9 (20.9) | 18 (31.0) | −10.1 (−27.1 to 6.9) | 9 (20.5) | 4 (10.0) | 10.5 (−4.7 to 25.6) |
| | | | Difference in means | | | Difference in means |
| Days of antiviral prophylaxis, mean (standard deviation)[a] | 158.6 (67.3) | 175.3 (48.9) | −16.7 (−40.5 to 7.0) | 69.8 (54.8) | 102.1 (36.6) | −32.4 (−52.1 to −12.6) |
| | | | Incidence rate difference | | | Incidence rate difference |
| Episodes of CMV infection, no. of episodes (IR)[c] | 29 (67.4) | 47 (81.1) | −13.7 (−47.4 to 20.1) | 33 (75.5) | 19 (48.0) | 27.5 (−6.1 to 61.1) |
| Tissue-invasive disease | 2 (4.7) | 2 (3.5) | 1.2 (−6.8 to 9.2) | 0 (0) | 0 (0) | 0 |
| Viral syndrome | 6 (14.0) | 8 (13.8) | 0.1 (−14.6 to 14.9) | 0 (0) | 2 (5.1) | −5.1 (−12.1 to 1.9) |
| Treated asymptomatic replication | 15 (34.9) | 25 (43.1) | −8.3 (−32.7 to 16.2) | 10 (22.9) | 5 (12.6) | 10.2 (−7.7 to 28.2) |
| Untreated asymptomatic replication | 6 (14.0) | 12 (20.7) | −6.8 (−22.9 to 9.4) | 23 (52.6) | 12 (30.3) | 22.3 (−5.2 to 49.8) |
| High-level CMV-DNAemia, no. of episodes (IR)[c] | 21 (48.8) | 32 (55.2) | −6.4 (−34.7 to 21.9) | 9 (20.6) | 6 (15.2) | 5.4 (−12.7 to 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 to 16.6) | 0 (0) | 1 (2.5) | −2.5 (−7.3 to 2.3) |
| Leucopenia | 30 (69.8) | 35 (60.3) | 9.4 (−9.2 to 28.0) | 17 (38.6) | 24 (60.0) | −21.4 (−42.3 to −.4) |
| Grade 4 leucopenia | 1 (2.3) | 1 (1.7) | 0.6 (−5 to 6.2) | 0 (0) | 0 (0) | 0 |
| Anemia | 12 (27.9) | 10 (17.2) | 10.7 (−5.9 to 27.2) | 8 (18.2) | 6 (15.0) | 3.2 (−12.7 to 19.1) |
| Allograft rejection | 4 (9.3) | 6 (10.3) | −1 (−12.7 to 10.7) | 5 (11.4) | 0 (0) | 11.4 (2 to 20.7) |
| Graft loss | 0 (0) | 1 (1.7) | 0.6 (−5 to 6.2) | 1 (2.3) | 0 (0) | 2.3 (−2.1 to 6.7) |
| Death | 0 (0) | 1 (1.7) | −1.7 (−5.1 to 1.6) | 1 (2.3) | 1 (2.5) | −0.2 (−6.8 to 6.3) |

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IR, incidence rate.

[a]Co-primary end point.

[b]In case of several episodes of clinically significant CMV infection, the most severe episode was included for each patient.

[c]Estimates in brackets are incidence rates per 100 patient-years.

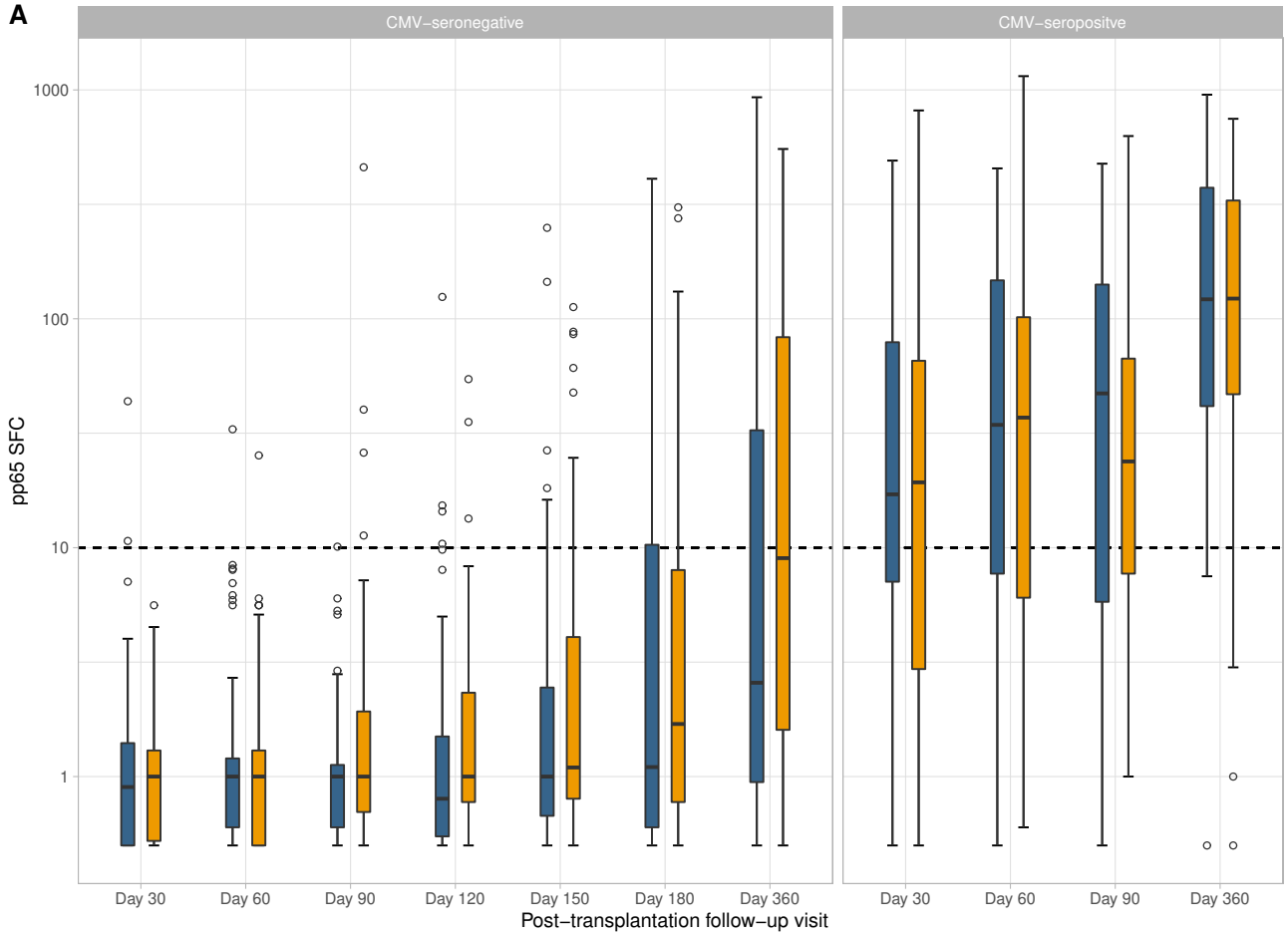



**Figure 4.** Results of the CMV immune assay: pp65 CMV antigen (*A*) and IE1 CMV antigen (*B*). Box plot of results from the T-Track assays in the immune-monitoring and control groups according to CMV risk status (CMV-seronegative and CMV-seropositive). Results of the T-track assays in the control group were not communicated to the treating physicians. 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× the interquartile range. Observations beyond that range are shown as circles. The dashed line indicates the primary cutoff of the -Track CMV assay. Abbreviations: CMV, cytomegalovirus; SFC, spot-forming cells.

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 antithymocyte 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 using an enzyme-linked immunosorbent assay 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, who 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 the immune-monitoring and control groups due to lack of stratification by CMV serostatus due to human error. To account for this limitation, all group-specific estimates and between-group differences were adjusted for CMV serostatus using stratified analyses with appropriate

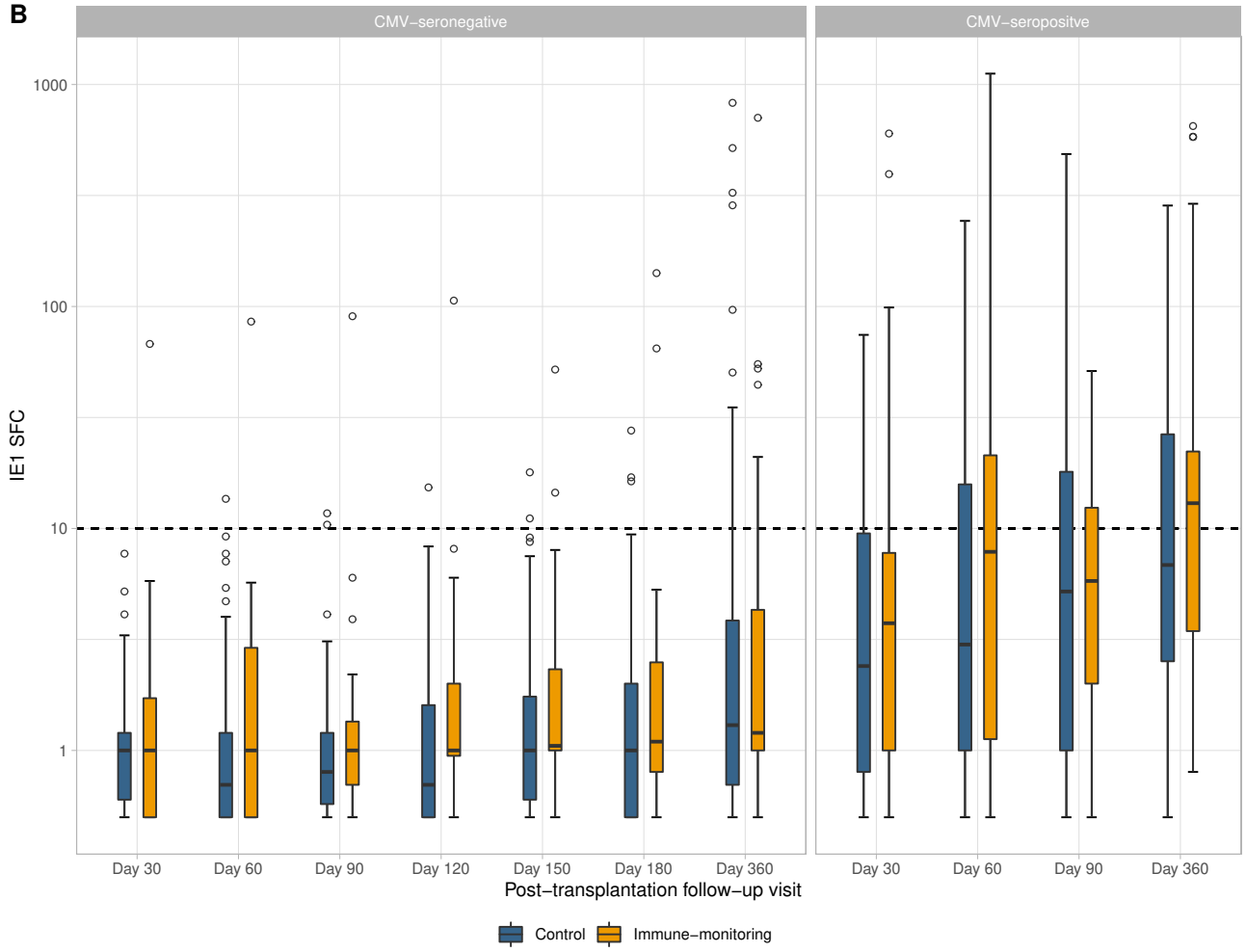



**Figure 4.** Continued

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 in antiviral prophylaxis duration in transplant recipients. Even though we were unable to establish noninferiority on the co-primary outcome of CMV infection, the risk of CMV-related complications, in particular CMV disease, seems low in this population.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

***Author Contributions.*** O. M., M. P., T. F., C. v. D., H. H. H., and N. J. M. conceived of and designed the study. H. H. H., M. J. K., K. K., S. D., C. H., N. S., D. S., U. H. D., L. N. W., N. E., K. H., D. N., D. G., M. M., I. B., A. S., P. E. C., K. H., T. F. M., A. V. B., H. H., M. H., F. S. R., J. V., and A. Z. acquired the data. M. L., S. S., and P. J. performed the analyses and interpreted the results in collaboration with all other authors. O. M., M. L., C. H., and P. J. wrote the first draft of the manuscript. All authors critically revised the first draft for important intellectual content and approved the final version.

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***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, are available on request. To request the dataset, please send a signed data request form to oriol.manuel@chuv.ch.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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