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CLINICAL RESEARCH

Voclosporin and the Antiviral Effect Against SARS-CoV-2 in Immunocompromised Kidney Patients

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Introduction: Immunocompromised kidney patients are at increased risk of prolonged SARS-CoV-2 infection and related complications. Preclinical evidence demonstrates a more potent inhibitory effect of voclosporin on SARS-CoV-2 replication than tacrolimus *in vitro*. We investigated the potential antiviral effects of voclosporin on SARS-CoV-2 in immunocompromised patients.

Methods: First, we conducted a prospective, randomized, open-label, proof-of-concept study in 20 kidney transplant recipients (KTRs) on tacrolimus-based immunosuppression who contracted mild to moderate SARS-CoV-2 infection. Patients were randomized to continue tacrolimus or switch to voclosporin. Second, we performed a *post hoc* analysis on SARS-CoV-2 infections in 216 patients with lupus nephritis (LN) on standard immunosuppression who were randomly exposed to voclosporin or placebo as part of a clinical trial that was conducted during the worldwide COVID-19 pandemic.

Results: The primary end point was clearance of SARS-CoV-2 viral load and that did not differ between voclosporin-treated KTRs (median 12 days, interquartile range [IQR] 8–28) and tacrolimus-treated KTRs (median 12 days, IQR 4–16) nor was there a difference in clinical recovery. Pharmacokinetic analyses demonstrated that, when voclosporin trough levels were on-target, SARS-CoV-2 viral load dropped significantly more (ΔCt 7.7 [3.4–10.7]) compared to tacrolimus-treated KTRs (ΔCt 2.7 [2.0-4.3]; $P = 0.035$). In voclosporin-exposed patients with LN, SARS-CoV-2 infection was detected in 6% (7/116) compared to 12% (12/100) in placebo-exposed patients (relative risk [RR] 1.4 [0.97–2.06]). Notably, no voclosporin-exposed patients with LN died from severe SARS-CoV-2 infection compared to 3% (3/100) in placebo-exposed patients (RR 2.2 [1.90–2.54]).

Conclusion: This proof-of-concept study shows a potential positive risk-benefit profile for voclosporin in immunocompromised patients with SARS-CoV-2 infection. These results warrant further investigations on voclosporin to establish an equipoise between infection and maintenance immunosuppression.

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Immunocompromised patients with systemic autoimmune diseases or KTRs had an increased risk to die from COVID-19 at the start of the pandemic,[1–3] and at a higher risk for not responding to vaccination. Notably, KTRs had an estimated 3 to 4 times increased risk of SARS-CoV-2 (COVID-19) related mortality[1] most likely due to preexisting comorbidity as well as vulnerability from immunosuppression. Establishing an individualized balance between adequate maintenance immunosuppression and risk of infection has been the main goal of physicians during the COVID-19 pandemic.

In this perspective, the calcineurin inhibitors (CNIs) cyclosporin-A (CsA) and voclosporin were particularly

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of interest during the COVID pandemic for 2 reasons. First, CNIs have well-described immunosuppressive efficacy in KTRs[4] and glomerular diseases, such as LN, membranous nephropathy and podocytopathies.[5] Second, from a mechanistic point of view, CsA and voclosporin exhibit their immunosuppressive effects by inhibiting calcineurin through the binding of cyclophilin A, whereas tacrolimus binds to FK-binding proteins for the inhibition of calcineurin. Cyclophilins have previously been identified as interaction partners of the CoV nonstructural protein 1 and inhibition of cyclophilins by CsA directly blocked the replication of coronaviruses.[6] In more detail, CsA was demonstrated to inhibit *in vitro* replication of human coronaviruses HCoV-NL63,[7] HCoV-229E,[8,9] MERS-CoV, and SARS-CoV.[10–12] Consequently, during the COVID-19 pandemic, several reports advocated to switch CNI-based immunosuppressive regimen to include CsA to benefit from previously described antiviral effects.[13,14] Moreover, there are several nonimmunosuppressive cyclosporin derivates that have potent suppressive effects on the replication of multiple coronaviruses.[15,16] In addition, 2 clinical cohort studies reported better outcomes in KTRs with a SARS-CoV-2 infection when CsA was part of their maintenance immunosuppression.[17,18]

Currently, the majority of KTRs have a CNI-based regimen as standard maintenance immunosuppression with a preference for tacrolimus over CsA because of its superiority on long-term renal graft function, allograft survival, and acute rejection rates.[4,19] Unlike CsA, voclosporin has demonstrated noninferiority to tacrolimus in KTRs to prevent graft rejection in a phase 2 randomized trial.[20] Mechanistically, we recently reported that voclosporin, a CNI that is structurally closely related to CsA and inhibits cyclophilin with significantly higher affinity, inhibited SARS-CoV-2 viral replication at approximately 10-fold lower concentrations than CsA.[15] Consequently, we postulated that voclosporin could be beneficial to immunocompromised patients by contributing to the viral clearance of SARS-CoV-2. Given that voclosporin is not registered for KTRs, the use of voclosporin in this trial is investigational of nature.

To address the null hypothesis, the current analysis used 2 approaches. First, we conducted a proof-of-concept study in SARS-CoV-2-positive KTRs, who were randomized to continue tacrolimus treatment or switch to voclosporin, to assess viral clearance of SARS-CoV-2 (VOCOVID study – clinicaltrials.gov NCT04701528, registered January 8, 2021). Second, we performed a *post hoc* analysis of SARS-CoV-2 infections and outcomes in a cohort of patients with LN on standard immunosuppression who were exposed to voclosporin or placebo within a randomized study conducted during the COVID-19 pandemic (AURORA-2 study - clinicaltrials.gov NCT03597464, registered July 24, 2018).

METHODS

VOCOVID Study Population

We conducted a randomized, open-label, proof-of-concept trial involving KTRs with mild to moderate symptoms from a COVID-19 infection. Trial enrollment was planned for 20 patients who were on stable maintenance immunosuppression with tacrolimus. Prior to study entry, KTRs with suspected COVID-19 infection needed confirmation by a positive polymerase chain reaction (PCR) test for SARS-CoV-2. If positive, according to national guidelines at the emergence of the pandemic, standard maintenance immunosuppressive therapy was reduced to dual therapy with corticosteroids and tacrolimus. Patients were then randomly assigned to either continue tacrolimus for the duration of the study or switch from tacrolimus to voclosporin.

Patients with mild to moderate symptoms were eligible, where mild symptoms were defined as nonhospitalized patients without the need for oxygen supplementation. and moderate symptoms were defined as the need for hospitalization with the need for oxygen therapy. Female patients of childbearing potential required a negative pregnancy test at screening. Patients were ineligible if they had severe COVID-19 symptoms defined as the requirement of admittance to a medium or high care unit with the need for positive pressure ventilation or mechanical intubation; if they were less than 3 months post-transplantation or had a documented organ rejection within the past 3 months; if they had a documented Chronic Kidney Disease Epidemiology Collaboration estimated glomerular filtration rate <15 ml/min within the 3 months before screening.

VOCOVID Study End Points

The primary objective of the study was to investigate the kinetics of the antiviral effects of voclosporin on SARS-CoV-2 in stable KTRs over the 56-days study period, compared to standard of care with tacrolimus. Therefore, predefined endpoints for "viral clearance" were assessed within this exploratory, proof-of-concept study and incrementally defined as follows: (i) time in days to the first negative PCR test, (ii) time to 2 consecutive negative PCR tests, and (iii) the quantitative log-reduction in viral RNA load based on PCR values.

Predefined clinical endpoints were assessed by the following: (i) time to clinical recovery defined as free of symptoms for 5 days or more, (ii) time to clinical

symptom relief, defined as free of symptoms for 1 day or more, (iii) time to hospital discharge for hospitalized subjects, and (iv) occurrence of treatment failures within the first 56 days defined as worsening of COVID infection requiring hospitalization for nonhospitalized subjects or requiring admittance to the ICU or death for hospitalized subjects.

The secondary objective was to assess the safety and tolerability of voclosporin in stable KTRs infected with SARS-CoV-2 over the total study period of 1 year, including adverse events (AEs), kidney graft function decline, serum creatinine concentrations, incidence of acute or late rejection, and the formation of *de novo* donor-specific antibodies.

In addition, considering that antiviral effects of voclosporin required adequate concentrations and tissue distribution, frequent whole blood trough concentrations and dried bloodspot abbreviated area-under-the-curve ($AUC_{0–12h}$) measurements on voclosporin and tacrolimus were performed.

VOCOVID Study Interventions, Sampling, and Follow-Up

Study Treatment Intervention

Patients who were randomized to switch to voclosporin received an initiating dose of voclosporin of 6 capsules (of 7.9 mg each) 2 times a day for a treatment period of at least 56 days with a possible extension up to 1 year. The duration of voclosporin treatment beyond 56 days was left to the discretion of the treating physician considering patient's physical recovery from COVID-19, comorbidities, complications, as well as and benefit-risk profile and side effects. Because the antiviral effects of voclosporin could only be expected at adequate levels, the study aimed at voclosporin trough concentrations between 30 to 60 ng/ml[20] and tacrolimus trough concentrations between 3 to 7 ng/ml.

Study Visits and Sampling

After randomization, patients were followed-up with for protocolized safety and efficacy visits on day 4, 8, 16, 28, and 56. In addition, from day 2 to day 14, daily home monitoring of vital parameters using the COVID-box with concomitant teleconsultations were performed.[21–23] From day 16 to day 28, teleconsultations were performed every other day. After day 56, patients were followed-up with in an extended safety period with visits on day 90, 180, 270, and 360 (end of study visit). Within the first 56 days of the study, specimen collection for SARS-CoV-2 nucleic acid testing by quantitative reverse transcription PCR included the self-collection of a saliva sample and a throat swab every 4 days. In addition, on day 0, 4, 8, 16, 28, and 56, a nasopharyngeal swab and blood sampling for routine lab measurements were collected by a research nurse.

VOCOVID Bio-Analytics

Voclosporin and tacrolimus trough concentrations and dried bloodspot AUC measurements were performed by the ISO 15,189 accredited Clinical Pharmaceutical Laboratory of the Department of Clinical Pharmacy and Toxicology in the Leiden University Medical Center. Quantification of tacrolimus was performed with a validated LC-MS/MS method as previously described.[24] Briefly, voclosporin quantification in whole blood with the LC-MS/MS method was performed using a Thermo Quantiva UPLC-MS/MS system, consisting of an Ultimate 3000 series UHPLC system, coupled to a TSQ Quantiva triple stage quadrupole mass spectrometer (all from Thermo Fisher Scientific BV/BVBA, Breda, The Netherlands). More details have been described earlier.[15] Both methods were validated according to the European Medicines Agency bioanalytical method validation guideline.[25] Whole blood concentrations were determined within 2 days after collection. Dried blood spot samples were stored at −20 °C until batch analysis every 2 weeks. Tacrolimus dried blood spot $AUC_{0–12h}$ estimation was performed as described earlier with finger prick samples obtained at time = 0, 1, 2, and 3 hours after dose intake.[26] Voclosporin dried blood spot $AUC_{0–12h}$ estimation was performed by capillary sampling obtained at 0, 1, 2, and 3 hours after dose intake and a developed 2 compartment population pharmacokinetic model first order absorption and with linear elimination comparable to the model, as previously published[27] with Monolix 2021R1 as Population PharmacoKinetic software (Lixoft, Antony, France).[28]

VOCOVID Safety Assessments

Safety assessments included the reporting and follow-up of AEs, severe AEs (SAEs), and suspected unexpected serious adverse reactions. AEs were defined as any undesirable experience occurring to a subject during the study, whether or not it is considered related to the investigational product. All AEs reported spontaneously by the subject or observed by the investigator or their staff were recorded. Only AEs which started on or after the date of first dose of study drug were summarized for this study. An SAE was defined as any untoward medical occurrence or effect that met 1 or more of the following criteria: resulted in death, was life threatening (at the time of the event), required in-patient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability or incapacity, was a congenital anomaly or birth defect, or any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgment by the investigator. An elective hospital admission will

not be considered as a SAE. Suspected unexpected serious adverse reactions were defined as adverse reactions that are all untoward and unintended responses to an investigational product related to any dose administered. In the current trial, all unexpected SAEs related to voclosporin usage were considered suspected unexpected serious adverse reactions.

SAEs and suspected unexpected serious adverse reactions, together with preparation and assessment of annual safety reports were summarized by system organ class. AEs of interest were defined as long-term effect on graft function, incidence of rejection and formation of donor-specific antibodies. A Data Safety Monitoring Board was not established because the conversion of tacrolimus to voclosporin was considered only a mild-risk intervention that would not exceed regular clinical interventions for transplant recipients such as the switch of tacrolimus to cyclosporin for side effects as neurotoxicity or posttransplant diabetes. However, due to the limited experience with and off-label use of voclosporin in stable KTRs beyond 1 year of transplantation, special attention was given by periodic evaluation of AEs and SAEs in a safety committee, with additional assessment of all SAEs by an independent physician to determine severity and causality (i.e., relation to study treatment). The independent physician was blinded to the treatment allocation and had adequate knowledge of epidemiology, immunology, etiology, and infectious diseases in KTRs.

VOCOVID Trial Oversight

The trial was investigator-initiated and designed, conducted, and analyzed by the authors. The trial was approved by the institutional review board of the Leiden University Medical Center and conducted in adherence with the International Council for Harmonization Good Clinical Practice guidelines, the Declaration of Helsinki, and local regulations on the conduct of clinical research. All the patients provided written informed consent before participation in the trial. The authors vouch for the completeness and accuracy of the data and for the adherence of the trial to the protocol.

VOCOVID Statistical Analysis

A per-protocol statistical analyses was performed using predominantly descriptive statistics and nonparametric Mann-Whitney U test for nonnormally distributed data in relation to the small patient numbers in this study. Chi-square tests were used to compare dichotomized outcomes between the groups. Survival functions and time-to-event analyses were plotted as Kaplan-Meier curves and groups compared by the log-rank test. Data on demographic and baseline characteristics are summarized for continuous variables with medians and interquartile ranges. Discrete variables e.g., ethnicity and gender (male/female) were summarized by proportions (percentages). Viral loads were calculated from standard PCR cycle time (Ct) values for SARS-CoV-2 detected in specimens from individual subjects and summarized over time. All clinical endpoints are summarized descriptively. Continuous and log-normalized data are summarized using median with interquartile ranges.

AURORA-2 Study Description

Study details on AURORA-2 are published elsewhere[29] (clinicaltrials.gov NCT03597464). Briefly, in AURORA-2, 216 patients with LN treated with a standard-of-care immunosuppressive regimen (i.e., prednisolone and mycophenolate mofetil) were treated for 3 years in a double-blinded fashion either add-on voclosporin or placebo. AURORA-2 patients completed a year of treatment in the AURORA-1 and continued the same double blind treatment for 2 additional years in AURORA-2. The primary objective of AURORA-2 was to assess the safety of long-term treatment of patients with LN with voclosporin. AURORA-2 investigated 2 years of continuation treatment and was conducted between June 12, 2018 and October 7, 2020 during the first and second wave of the COVID pandemic.

AURORA-2 *Post Hoc* Analysis

Within the AURORA-2 patient cohort, the incidence and outcome of SARS-CoV-2 infections in patients with LN was collected through reporting of AEs. RRs were calculated using Fisher exact test comparing voclosporin-exposed patients with LN to placebo-exposed patients.

RESULTS

Between November 2020 and June 2021, all KTRs that presented with COVID symptoms were screened at the Transplant Center of the Leiden University Medical Center. Of the 61 patients screened, 20 KTR were included in the study. Of the remaining patients, 31 did not meet inclusion criteria, 8 patients did not consent to participate, and 2 patients were found unsuitable based on communication problems or symptom duration of more than 14 days. Baseline characteristics are summarized in Table 1 and are generally well-balanced with respect to duration since transplantation, immunosuppressive regimens, SARS-CoV-2 infection related symptom severity, symptom duration, and vaccination status. Briefly, KTRs had a median duration of 8.3 (IQR: 4.2–10.6) years after transplantation with a median estimated glomerular filtration rate of 56 (IQR: 39–79) ml/min, a median urinary protein-to-creatinine ratio of 21 (IQR: 15–31) mg/g. In addition, at the time of study entry, mild COVID-related clinical characteristics were not

Table 1. Patient characteristics at baseline

Clinical characteristics	Voclosporin	Tacrolimus	*P*-value[a]	Total population
No. of patients	10	10		20
Age at transplantation in yrs (median [IQR])	62 (45–69)	50 (48–57)	0.7	53 (47–64)
Male gender (*n* [%])	5 (50%)	3 (30%)	0.6	8 (40%)
BMI in kg/m^2 (median [IQR])	25.5 (23.1–33.4)	25.6 (21.8–26.2)	0.4	25.6 (22.5–28.1)
Years after Tx (median [IQR])	8.6 (2.7–10.0)	8.1 (4.5–11.0)	0.6	8.3 (4.2–10.6)
Immunosuppresive regime at inclusion (*n* [%])				
TAC/MPA/PRED	3 (30%)	5 (50%)	NA	8 (40%)
TAC/EVL/PRED	3 (30%)	1 (10%)	NA	4 (20%)
TAC/MPA	1 (30%)	0 (10%)	NA	1 (5%)
TAC/PRED	3 (30%)	4 (40%)	NA	7 (35%)
Comorbidities (*n* [%])				
Hypertension	6 (60%)	6 (60%)	NA	12 (60%)
Cardiovascular disease[b]	4 (40%)	3 (30%)	NA	7 (35%)
Diabetes mellitus	5 (50%)	4 (40%)	NA	9 (45%)
Neurological disease[c]	1 (10%)	2 (20%)	NA	3 (15%)
Pulmonary disease[d]	2 (20%)	1 (10%)	NA	3 (15%)
Obesity Δ	1 (10%)	2 (20%)	NA	3 (15%)
Malignancy	2 (20%)	2 (20%)	NA	4 (20%)
SARS-CoV-2 infection details				
Vaccinated prior to study commencement (*n* [%])	3 (30%)	2 (20%)	1	5 (25%)
Days of symptoms at randomization (median [IQR])	5 (4–6)	5.5 (3.3–10)	0.5	5 (4–7)
Baseline viral load in Ct-value (median [IQR])	23.7 (20.4–24.7)	28.9 (22.3–33.2)	0.2	24.5 (20.6–31.3)
Viral typing (*n* [%])				
Wuhan wild type (lineages B.1)	3 (30%)	4 (40%)	NA	7 (35%)
Alpha variant (lineages B.1.1.7)	6 (60%)	4 (40%)	NA	10 (50%)
Vital parameters (median [IQR])				
Systolic blood pressure (mm Hg)	126 (123–138)	141 (115–153)	0.4	127 (118–143)
Pulse (beats per minute)	78 (74–81)	79 (70–81)	0.9	79 (73–81)
Temperature (°C)	36.8 (36.6–37.1)	37.0 (36.7–37.3)	0.6	36.9 (36.6–37.3)
Respiratory rate (breaths/min)	19 (17–20)	18 (16–24)	0.8	18 (16–23)
Oxygen saturation (%)	98 (97–100)	98 (96–99)	0.9	98 (97–99)
Laboratory parameters (median [IQR])				
Serum creatinine (μmol/l)	120 (77–152)	118 (96–145)	1	119 (87–149)
eGFR (ml/min)	53 (37–74)	59 (42–77)	0.8	56 (39–79)
UPCR (mg/g)	22 (16–33)	19 (10–28)	0.5	20 (15–31)
aCRP (mg/l)	13 (6–29)	12 (3–61)	1	13 (3–39)
CD4 count (absolute)	428 (173–529)	289 (196–438)	0.5	337 (189–509)
CD8 count (absolute)	331 (84–505)	215 (176–325)	1	259 (129–376)

BMI, body mass index; CT, cycle times; CRP, c-reactive protein; eGFR, estimated glomerular filtration rate; EVL, everolimus; IQR, interquartile range; MPA, mycophenolate mofetil; PRED, prednisolone; TAC, tacrolimus; Tx, treatment; UPCR, urinary protein-to-creatinine ratio.
[a]Analyzed by nonparameteric Mann-Whitney-U test.
[b]Myocardial infarction, stroke, peripheral arterial disease, chronic thrombo-embolic pulmonary hypertension.
[c]Neuropathy, parkinson, multiple sclerosis.
[d]Asthma, chronic obstructive pulmonary disorders.
Δ BMI $>$ 30.

significantly different for both arms at baseline with a median of 5 (IQR: 4–7) days of COVID-related symptoms, median of peripheral oxygen saturation of 98% (IQR: 97–99), median C-reactive protein level of 13 mg/l (IQR: 3–39) and a baseline SARS-CoV-2 viral load (in Ct-value) of 25 [IQR: 21–31]. These 20 KTRs were randomized to switch to voclosporin ($N = 10$) or continue tacrolimus ($N = 10$).

In Figure 1, we summarize the individual courses of SARS-CoV-2 viral load regression while incorporating relevant heterogeneity of COVID19-specific factors at baseline such as vaccination status, symptom duration, SARS-CoV-2 antibody positivity for nucleocapsid protein and outcomes. Viral clearance, defined as time (in days) to first negative PCR test, was not different between both arms (median 12 days [IQR: 8–28] in the voclosporin arm vs. 12 days [IQR: 4–16] in tacrolimus arm). In addition, no significant difference was observed between voclosporin-treated or tacrolimus-treated KTRs for the other incremental definitions of viral clearance (Figure 2a–d). Moreover, the end point of clinical recovery, predefined as being either >1 or >5 days free of symptoms, was not different between both arms (Figure 2e–f). Nor was there a difference in the progression to more severe COVID-19, which occurred in 3 patients (2 patients within the voclosporin arm and 1

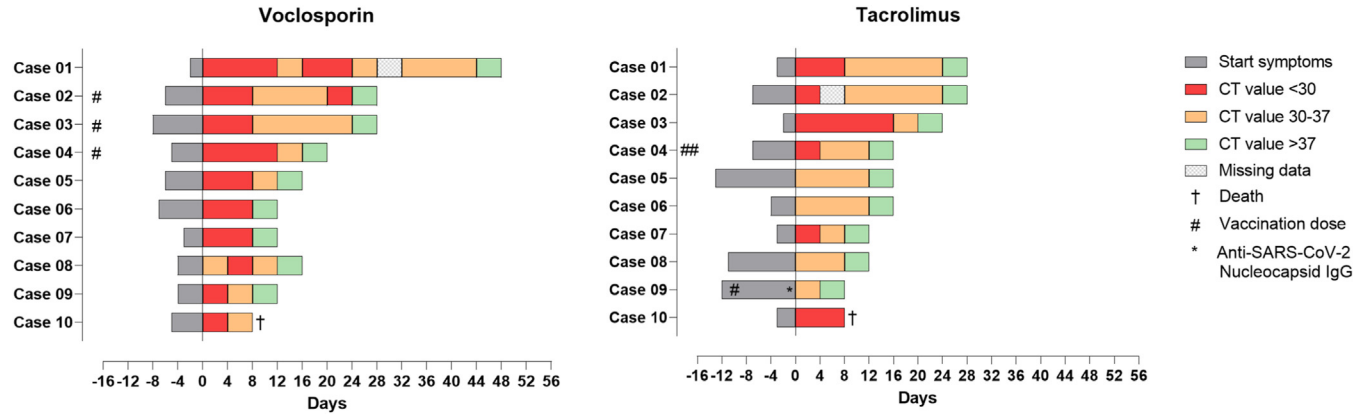

Figure 1. Individual courses of SARS-CoV-2 viral clearance, according to the prespecified definition of time in days to first negative PCR test, in 10 kidney transplant recipients (KTRs) randomized to voclosporin treatment versus continuation of tacrolimus treatment. Prerandomization COVID19-related symptom duration is depicted in gray; categories of PCR Ct-values are depicted in red (high viral load with Ct-value <30), orange (low viral load with Ct-value from 30–37) and green (no viral load with Ct-value >37). Symbol # corresponds to 1 vaccination dose.

patient in tacrolimus arm) with 2 subsequent COVID-19 related deaths (1 patient in each arm).

The number of AEs were similar between the voclosporin and tacrolimus group and are summarized in Supplementary Table S1. In 6 patients, a mild temporary mild decrease in estimated glomerular filtration rate occurred; 5 voclosporin-treated patients compared to 1 tacrolimus-treated patient. In 2 patients, 1 in each treatment arm, the estimated glomerular filtration rate-drop was considered an acute kidney injury related to high trough levels, which was reversible over time. There was no significant difference in the 1-year follow-up regarding kidney graft function, proteinuria, and potassium levels as canonical CNI-related AEs. SAEs were equally distributed over both groups. Lastly, throughout the study there were no events of rejection or formation of donor-specific antibodies observed.

We next performed a pharmacokinetic analysis to optimally assess a potential antiviral effect of voclosporin because trough concentrations of 30 to 60 ng/ml would be required in line with the preclinical data demonstrating increasing antiviral efficacy. After conversion from tacrolimus to voclosporin, we observed suboptimal, "below-target" trough concentrations in 50% of voclosporin-treated patients whereas, not unexpectedly, all KTRs that continued tacrolimus were on-target or above-target in the first 4 days after study entry (Figure 3a). Importantly, between days 4 to 8, all voclosporin-treated patients had reached on-target or above-target trough concentrations when simultaneously the SARS-CoV-2 clearance was significantly higher in voclosporin-treated KTRs (median increase in Ct value of 7.7 [3.4–10.7]) compared to tacrolimus-treated KTRs (median increase in Ct value of 2.7 [2.0–4.3]; $P = 0.035$) (Figure 3b). This difference weaned between days 8 and 12 (Figure 3c).

To further corroborate the antiviral properties of voclosporin, we assessed SARS-CoV-2 infections in a cohort of immunocompromised patients with LN who were randomly exposed to add-on voclosporin or placebo. We observed 7 of 116 patients (6%) testing positive for SARS-CoV-2 infection in the voclosporin-exposed group compared to 12 of 100 patients (12%) in the placebo-exposed group (RR 1.4 [95% confidence interval 0.97–2.06]). Notably, severe COVID-19-related deaths did not occur in the voclosporin-exposed patients compared to 3 of 100 (3%) in the placebo-exposed patients (RR 2.2 [95% confidence interval 1.90–2.54]).

DISCUSSION

In immunocompromised patients whose health state is critically dependent on chronic immunosuppression, establishing equipoise between risk of infection and immunosuppression is important, particularly during a pandemic. Voclosporin has previously shown its clinical efficacy as an immunosuppressant in KTRs and patients with LN, and we recently reported on increased inhibitory effects of voclosporin against SARS-CoV-2 compared to other CNIs *in vitro*. Therefore, we now demonstrated in a clinical proof-of-concept study the feasibility of KTRs with a SARS-CoV-2 infection to switch from tacrolimus to voclosporin. In this small cohort, the switch to voclosporin did not lead to a shorter time to viral clearance or quicker clinical recovery. However, our pharmacokinetic data provided evidence that voclosporin at time of adequately target concentrations was associated with a reduction of SARS-CoV-2 viral load compared to tacrolimus. Moreover, as part of a phase 3, randomized trial conducted during the COVID-19 pandemic, immunocompromised patients with LN exposed to

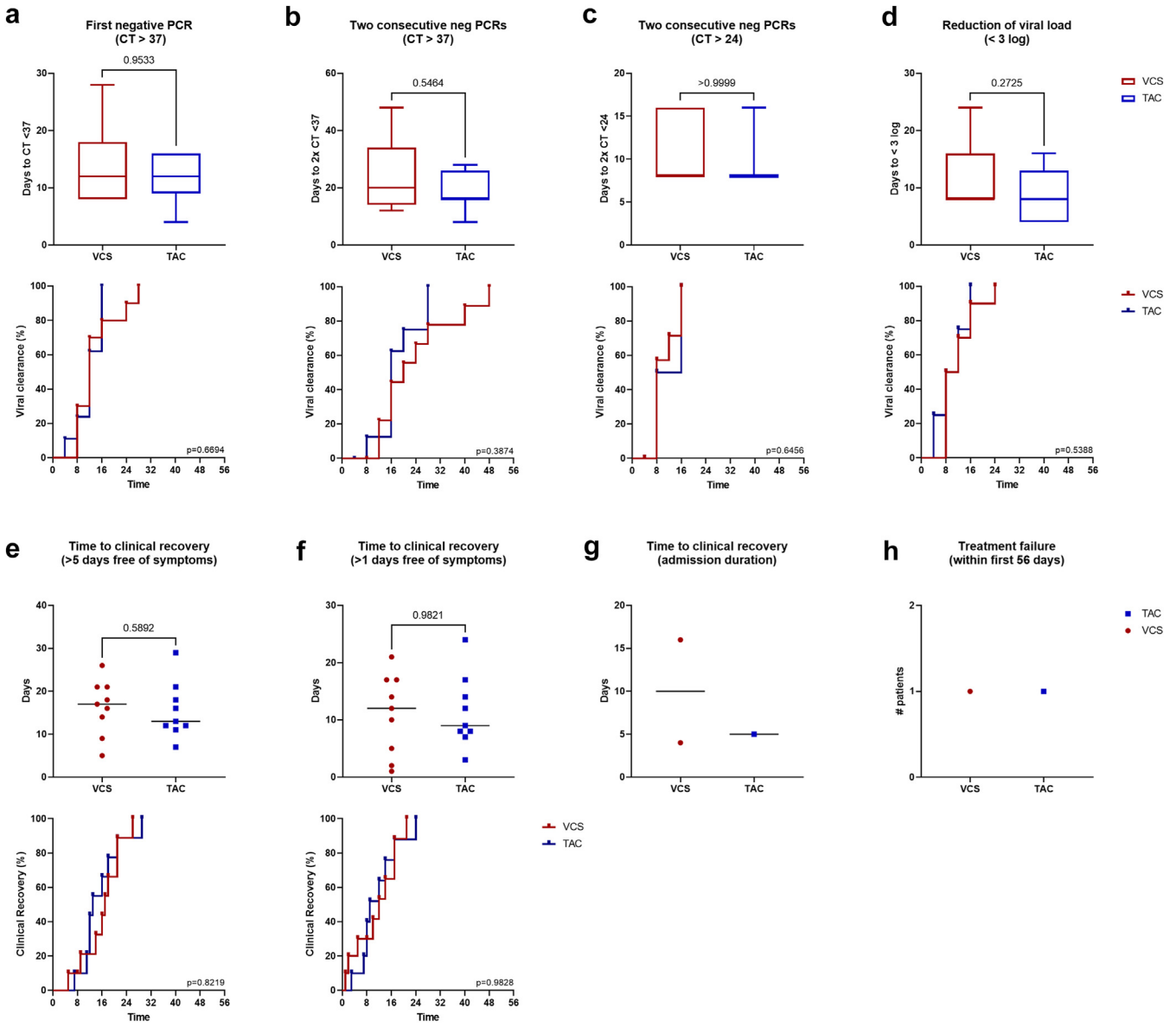

Figure 2. Summary by Whisker boxplots and Kaplan-Meier plots on SARS-CoV-2 viral clearance (a–d) and COVID19-related clinical courses (e–h). (a) Time to first negative PCR test with a cut-off Ct-value > 37. (b) Time to 2 consecutive negative PCR tests with a cut-off Ct-value >37. (c) Time to 2 consecutive negative PCR tests with a cut-off Ct-value > 24. (d) Time to a quantitative log-reduction in viral RNA load. (e) Time to clinical recovery defined as free of symptoms for at least 5 days. (f) Time to clinical symptom relief defined as free of symptoms for at least 1 day. (g) Time to hospital discharge for KTRs requiring hospital admission ($N = 3$). (h) Time to treatment failure within 56 days after randomization ($N = 2$).

voclosporin potentially had a lower risk of mortality as compared to placebo-exposed patients shown retrospectively in small patient numbers. Importantly, we found no short-term or long-term safety issues during conversion from tacrolimus to voclosporin. Taken together, these studies corroborate a potential positive risk-benefit profile for voclosporin in immunocompromised patients with a SARS-CoV-2 infection, giving reassurance to further studies that are needed to firmly establish the beneficial role of voclosporin in maintenance immunosuppressive regimens of SARS-CoV-2 infected KTRs.

The VOCOVID study is the first study to investigate voclosporin in the setting of SARS-CoV-2 infections in immunocompromised patients. Voclosporin was recently approved by the US Food and Drug Administration and European Medicines Agency for the treatment of active LN.[30] Therefore, the VOCOVID study provides a unique data set, because there has not been another clinical study conducted which could be important to practicing clinicians treating immunocompromised patients during a pandemic. Voclosporin is a novel CNI that was developed through modification of cyclosporine, in an attempt to

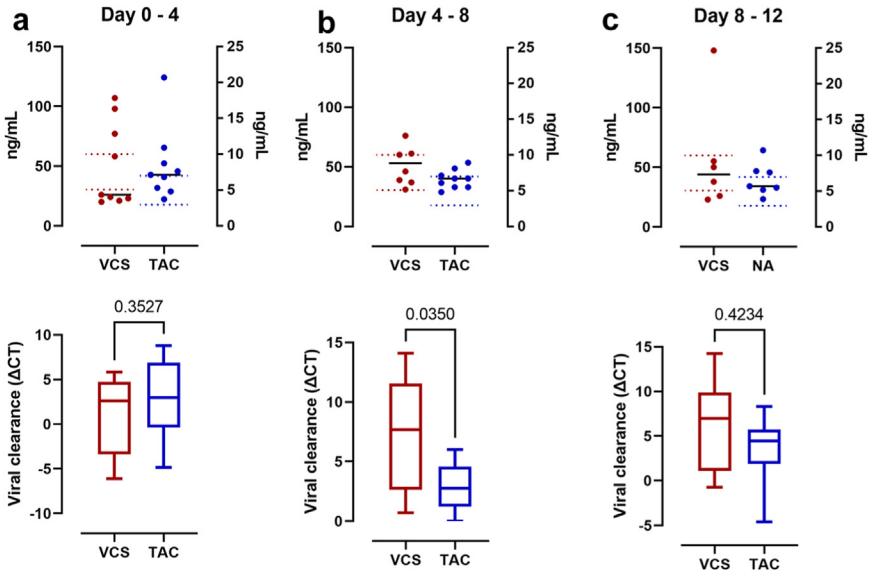

Figure 3. Pharmacokinetics (upper panels) of voclosporin (VCS-red) and tacrolimus (TAC-blue) in relation to SARS-CoV-2 viral clearance (lower panels) in KTRs. Each red/blue dot represents 1 patient; dotted lines illustrate the target trough concentrations for voclosporin 30–60 ng/ml (red) and tacrolimus 3–7 ng/ml. Upper panels shows trough levels of KTRs on voclosporin (red) and tacrolimus (blue) and in the lower panels the corresponding viral clearance (ΔCt) between (a) day 0 and day 4; (b) day 4 and day 8 and (c) day 8 and day 12 after study inclusion.

identify a compound with improved efficacy, metabolic stability, and safety. The structural modification (addition of a single carbon extension to the amino acid-1 position) produces a molecule with high potency and a favorable metabolic profile, without the need for therapeutic drug monitoring.[31] The antiviral effects of voclosporin was investigated in this study because the antiviral effects of CsA on coronaviruses by inhibition of cyclophilin binding has been reported.[6] This was further corroborated by preclinical evidence supporting a 10-fold potent antiviral effect of voclosporin on SARS-CoV-2[15] and a 3 to 4-fold more potent activity against norovirus than CsA.[32] From a safety perspective, the use of voclosporin as compared to tacrolimus in KTRs was well-studied, demonstrating reduced risks for common CNI-related side effects, such as hypertension, new-onset diabetes, renal insufficiency, and neurotoxicity.[20] Therefore, as a proof-of-concept study, investigating antiviral effects was most evident to be conducted in COVID-positive KTRs because CNIs form the cornerstone of their maintenance immunosuppressive treatment.

To determine an antiviral effect of voclosporin on SARS-oV-2 infections, the present study used the following 2 complimentary approaches: (i) a mechanistic proof-of-concept study to assess in humans whether SARS-CoV-2 viral clearance benefited from switching tacrolimus to voclosporin and (ii) the evaluation of an experiment-by-nature in which immunocompromised patients with LN were exposed to voclosporin during the initial phases of the worldwide COVID-pandemic as part of a clinical trial. Both studies provide complementary evidence on the translation of an *in vitro* antiviral effect by voclosporin toward clinical benefit. To achieve this, the design of the VOCOVID study was an explorative and translational study that required overcoming several practical challenges among which was to adequately monitor and sample subjects while bound by obligatory quarantine measures. The present study overcame these challenges by implementing home-monitoring with the COVID-box which was demonstrated to be safe and reduced COVID-related hospitalization,[21,22] by supporting subjects to self-perform viral PCR-testing, and by focusing primarily on KTRs with mild to moderate symptoms to allow for frequent out-patient clinic follow-up visits in an attempt to anticipate the limited hospital capacity during the waves of the COVID-19 pandemic. Despite facing these practical challenges, the study was able to corroborate *in vitro* observations that significant inhibition of SARS-CoV-2 viral replication with voclosporin, could be observed *in vivo*.[15] Briefly, pharmacokinetic studies established in healthy human adults that voclosporin has a large distribution volume and distribution clearance, indicating higher tissue concentrations compared to whole blood concentrations.[33] Indeed, in nonhuman primates, voclosporin concentration in lung were estimated to be 2.5-fold higher than the measured whole blood concentration.[34] Therefore, in the VOCOVID study, a target trough concentration of voclosporin ≥40 ng/ml was chosen, which was estimated to achieve a C_{max} of 250 ng/ml or higher in whole blood. Of note, by extrapolation, we estimated that a transient but even 2.5 higher lung

tissue concentration of voclosporin could be achieved, increasing the potential antiviral effects. Accordingly, it was remarkable to identify a significant larger drop in SARS-Cov-2 viral load at day 4 to 8 when on-target voclosporin levels were actually achieved in study patients. Indeed, we can conclude from the VOCOVID study that a follow-up study design to adequately compare the viral clearance between tacrolimus and voclosporin would ideally benefit when both treatment arms would be exposed to a COVID-19 pandemic at times that KTRs would be on adequate and stable trough levels. Although it is virtually impossible to conduct such a study, we further corroborated our VOCOVID study with data from an "experiment-of-nature" occurring in the AURORA-2 trial. This study progressed throughout the initial waves of the COVID pandemic, providing the opportunity to bypass the pharmacokinetic challenges of initiating, that is, switching to voclosporin requiring time to achieve stable concentrations.[34] Patients with LN participating in the AURORA-2 trial were exposed to either voclosporin or placebo added onto a background immunosuppression with low-dose steroids and mycophenolate[29] (clinicaltrials.gov NCT03597464). In this setting, immunocompromised patients were now exposed to voclosporin or placebo prior to the moment of an acute SARS-Cov-2 infection. The observed number of SARS-CoV-2 infections was low in both groups; however, considering that voclosporin-exposed patients with LN had triple immunosuppression versus dual immunosuppression in the placebo-exposed patients, we observed a trend toward reduced cases and fatalities from SARS-Cov-2 infections in the voclosporin arm. Importantly, these results were in line with 2 other reported cohort studies demonstrating reduced risk of severe COVID-related complications in CsA-exposed KTRs compared to other immunosuppressants.[17,18] Collectively, both VOCOVID and AURORA-2 provided supporting evidence that voclosporin has antiviral effects on SARS-CoV-2 infection in immunocompromised kidney patients.

Our study had some limitations which need addressing. First, results on viral clearance in the VOCOVID study were impacted by the initiation of SARS-CoV-2 vaccination program during the conduct of the study, the emergence of novel SARS-CoV-2 variants with variable symptom severity, a small disbalance of COVID-related symptom duration prerandomization (with numerically longer preentry symptoms in the tacrolimus-treated group) and a nonsignificant disbalance in baseline viral loads (with numerically lower load in the tacrolimus-treated group). Self-evidently, these factors provide room for a more complicated interpretation of the VOCOVID study results. Second, it needs recalling that at the time of initiating the VOCOVID study, voclosporin was not yet formally approved and therefore a large-scale study design was not realistic. However, the limited availability and supply of voclosporin influenced the compact, proof-of-concept study design during the COVID-19 pandemic and aimed at guiding future larger studies. Third, with respect to the observed viral clearance and pharmacokinetics of CNIs, it is impossible to rule out a delayed effect of underexposure to voclosporin after the first days of switching from tacrolimus. Reduced immunosuppression could accelerate viral clearance due to increased T-cell activity from initial underexposure after conversion. In defense against this hypothetical notion, reduced immunosuppression in KTRs is associated with higher risk of organ rejection and the formation of donor-specific antibodies which we did not observe over a follow-up of 1 year. Vice versa, in the tacrolimus arm, more frequent above-target exposure was noted in accordance with other reports,[35] which might have contributed to enhanced T-cell inhibition and reduced viral clearance.

In conclusion, the present study provides the first clinical results corroborating experimental data that voclosporin has a beneficial antiviral effect against SARS-CoV-2 infection in immunocompromised KTRs. As the COVID-19 pandemic persists, immunocompromised patients have a constrained response to vaccination and remain at high risk for severe COVID-19-related complications and comorbidities. Considering that voclosporin has become more widely available since achieving US Food and Drug Administration and European Medicines Agency approval, our study's results are reassuring and provide valuable information on effect sizes for future trials aimed at investigating the beneficial role of voclosporin in immunocompromised patients to establish an equipoise between reducing risks of infection while maintaining adequate and effective immunosuppression.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Summary of adverse events in VOCOVID.

CONSORT Checklist.

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