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ORIGINAL ARTICLE

Effects of valganciclovir on spermatogenesis in renal transplant patients – results of a multicenter prospective nonrandomized study

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SUMMARY

Ganciclovir (GCV) inhibits spermatogenesis in preclinical studies but long-term effects on fertility in renal transplant patients are unknown. In a prospective, multicenter, open-label, nonrandomized study, male patients were assigned to Cohort A [valganciclovir (VGCV), a prodrug of GCV] ($n = 38$) or B (no VGCV) ($n = 21$) by cytomegalovirus prophylaxis requirement. Changes in semen parameters and DNA fragmentation were assessed via a mixed-effects linear regression model accounting for baseline differences. Sperm concentration increased post-transplant, but between baseline and treatment end (mean 164 days Cohort A, 211 days Cohort B), the model-based change was lower in Cohort A (difference: 43.82×10^6/ml; $P = 0.0038$). Post-treatment, sperm concentration increased in Cohort A so that by end of follow-up (6 months post-treatment) changes were comparable between cohorts (difference: 2.09×10^6/ml; $P = 0.92$). Most patients' sperm concentration improved by end of follow-up; none with normal baseline concentrations ($\geq 20 \times 10^6$/ml) were abnormal at end of follow-up. Changes in seminal volume, sperm motility/morphology, DNA fragmentation, and hormone levels were comparable between cohorts at end of follow-up. Improvement in semen parameters after renal transplant was delayed in men receiving VCGV, but 6 months post-treatment parameters were comparable between cohorts.

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Introduction

Cytomegalovirus (CMV) infection is a potentially serious solid-organ transplantation complication, which may result in hematologic, gastrointestinal, and respiratory complications, and contribute to acute and/or chronic graft rejection [1–3]. Valganciclovir (VGCV), the L-valyl ester prodrug of ganciclovir (GCV), is approved for prophylaxis and/or treatment of CMV infection in adult renal, heart, or kidney–pancreas transplant recipients and pediatric kidney or heart transplant recipients [4]. Following oral administration, VGCV is rapidly converted to GCV, with almost all absorbed drug appearing as GCV in the circulation [5]; thus, the effects of VGCV are attributed to GCV, which inhibits rapidly dividing cells, reduces sperm count, and impairs

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fertility in male rats at exposures below therapeutic levels [6]. In animals, GCV effects on male fertility appear to be reversible 24 weeks after stopping treatment [6], with partial reversibility seen from 12 weeks [Roche data on file]; however, little is known about long-term effects on fertility in patients receiving VGCV.

In men with end-stage renal disease, spermatogenesis and fertility are frequently impaired, but improve after renal transplant [7,8]. Given the effects of GCV on spermatogenesis in animals [6], it is important to determine whether patients receiving VGCV benefit from the long-term fertility improvements observed after renal transplantation. We conducted a trial in renal transplant recipients, comparing parameters of spermatogenesis and fertility in patients receiving up to 200 days' VGCV post-transplant, compared with a cohort not treated with VGCV.

Materials and methods

This was a multicenter, open-label, nonrandomized, prospective study conducted in North America (WV25651; NCT01663740) in male patients aged 20–50 years receiving their first renal transplant, with no known history of infertility and willing and able to provide semen samples.

The study was conducted at renal transplant centers with high volumes of patients and local facilities for immediate assessment of semen parameters to reduce time-related variability and limited to 13 sites within one geographical region (USA/Mexico) to minimize variability in population and diversity in immunosuppression protocols, many of which impact fertility [9].

Patients were excluded if they had received GCV or VGCV within 3 months of enrollment, any investigational drug within 3 months of transplant, an organ transplant other than a kidney, alkylating agents or other medications known to affect fertility or male hormone levels, or had a history of any condition likely to interfere with their ability to participate.

All patients agreed to use a barrier contraceptive throughout or for at least 90 days after VGCV treatment, and provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board/Ethical Committee at each center.

Patients were assigned to one of two cohorts: Patients requiring VGCV prophylaxis (e.g., CMV-negative recipients of CMV-positive organs) were assigned to Cohort A and received VGCV as indicated by local prescribing information and practice, while patients not needing CMV prophylaxis (e.g., where donors were CMV-negative) were assigned to Cohort B and received no VGCV initially, although they could subsequently receive VGCV at any time if clinically indicated. This was a safety study with no efficacy component, and patients were analyzed according to treatment received: any patient initially assigned to Cohort B who subsequently received VGCV for >90 days was to be analyzed as part of Cohort A.

Patients were assessed at screening (within 6 weeks before surgery), at baseline (≤4 weeks post-transplant), at the end of treatment (within 28 days of completing VGCV treatment for Cohort A, and Week 28 ± 28 days for Cohort B), and at follow-up (6 months after the end of treatment ± 28 days, but no later than Week 52). At each visit, blood and semen samples were collected, if possible. Taking into account the high incidence of erectile dysfunction in patients with chronic kidney disease [10], it was anticipated that semen collection might impose a burden on patients and so the screening and baseline values for semen parameters were averaged as a single time point. A sample was requested at the end of treatment and at follow-up, with a second semen sample 1 week later, if possible. Samples were split into two for analysis of semen parameters locally [seminal volume, sperm concentration, total motility, and morphology (% normal)] and for DNA fragmentation analysis by terminal uridine nick-end labeling (TUNEL) [11] at a central laboratory; analysis was performed according to WHO guidelines [12], with a conservative lower limit of normal of 20×10^6/ml for sperm count. Semen collection was performed according to standard practice at each center, and procedures for collection, handling, and shipping of blood samples were specified in a laboratory manual supplied to all centers.

Blood was analyzed for hormone levels [total testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin], inhibin B (a spermatogenesis biomarker) [13], and creatinine, for calculation of creatinine clearance (CrCl). Concomitant medications, adverse events (AEs; reported descriptively, with no formal comparisons between cohorts) related to GCV, VGCV, CMV, and sexual dysfunction, and VGCV dose and frequency, were recorded at baseline and end-of-treatment visits.

Valganciclovir treatment

Patients received the currently approved VGCV dose (900 mg orally daily until 200 days post-transplant) [4]

or similar regimens of shorter duration (e.g., 100 days), according to local practice, adjusted as needed for renal function, based on calculated CrCl (no adjustment for CrCl ≥60 ml/min, 450 mg daily for CrCl 40–59 ml/min, 450 mg every 2 days for CrCl 25–39 ml/min, 450 mg twice weekly for CrCl 10–24 ml/min, dose interrupted for CrCl < 10 ml/min or for patients receiving dialysis) [4].

Endpoints

The primary endpoint was change in mean sperm concentration from baseline to end of treatment. Given the nonrandomized nature of the study, differences in baseline sperm concentration and in other factors likely to affect spermatogenesis (age and duration of pretransplant dialysis) were included in the statistical model as covariates (see "Statistical considerations"). Secondary endpoints were as follows: changes in sperm concentration from baseline to end of follow-up and from end of treatment to end of follow-up; changes in other semen parameters and hormone levels between baseline, end of treatment, and end of follow-up; and proportions of patients with abnormal sperm concentration, improved sperm concentration, and improved TUNEL score, at follow-up versus end of treatment and baseline. Changes in these parameters were similarly analyzed by models accounting for differences in baseline factors.

For each semen parameter, the baseline value for each patient was the mean of baseline and screening (if both samples were taken), or either baseline or screening if only one was taken. For end of treatment and follow-up, if two samples were available the mean was used.

Statistical considerations

In the absence of reliable historical data, no formal sample size calculations were performed when the study was designed. The protocol stated that a sample size of approximately 20 patients in the VGCV-treated cohort was deemed realistic and with this sample size, an effect size of approximately 0.7–0.8 for continuous variables can be detected with 80% power at the 5% significance level.

The originally planned primary analysis of change from baseline in sperm concentration was a mixed-effects repeated measures model that included the following as covariates: cohort, visit, and cohort by visit interaction as well as baseline sperm concentration, age (20–35, 36–50 years), and duration of pretransplant dialysis (<6, ≥6 months); an unstructured covariance matrix accounted for the two visits for each patient. Following submission of the study report to the Division of Antiviral Products of the US Food and Drug Administration (FDA), the agency requested that separate analysis of covariance models be fit to estimate change from baseline to end of treatment and change from baseline to end of follow-up. The analyses as requested by the FDA, and included in the product label, are presented here, with results according to the original model provided in the Supporting Information. Secondary continuous endpoints were analyzed in a similar fashion to the primary endpoint. Secondary categorical endpoints were analyzed by estimating the difference between independent Cohorts A and B with cohort effect, standard error, and 95% confidence interval (CI) reported. No adjustment for multiple testing was made for secondary endpoints. Numbers at each time point were variable due to missing samples: All available data at each time point were used in the analyses.

Results

Patients

Of 59 patients enrolled from 13 sites (11 in the USA and two in Mexico), 38 were assigned to Cohort A (VGCV) and 21 to Cohort B (no treatment). Compared with Cohort B, more patients in Cohort A had azoospermia at baseline (20% vs. 10%) and fewer had normal sperm concentration (30% vs. 40%). Half of patients in both cohorts had oligozoospermia (Table 1). Patients in Cohort A had been on dialysis for a mean of 39.3 months compared with 24.1 months in Cohort B.

In Cohort A, three patients did not receive a transplant and four received no VGCV; these patients were not included in the analyses so 31 patients were included from Cohort A. All 21 patients in Cohort B received a transplant and were included in the analyses.

Average time from transplant to end-of-treatment visit was 164 days in Cohort A and 211 days in Cohort B, and from transplant to end-of-follow-up visit was 337 days in Cohort A and 396 days in Cohort B. In Cohort A, mean duration of VGCV exposure (±standard deviation) was 118 ± 63 days (range 1–293 days). Eighteen patients were exposed to VGCV for <100 days and three for < 60 days. In Cohort B, one patient received VGCV dose by error, three received VGCV as concomitant medication, and six received GCV as concomitant medication; only three of these received VGCV or GCV for longer than 8 days, and these patients did not provide any samples after baseline and

Table 1. Baseline characteristics.

	Cohort A (VGCV) $n = 38$	Cohort B (no VGCV) $n = 21$
Median age, years (range)	34 (22–49)	33 (20–41)
Race, *n* (%)		
White	22 (59.5)	10 (47.6)
Black or African American	7 (18.9)	2 (9.5)
Asian	2 (5.4)	3 (14.3)
Hawaiian or Pacific Islander	1 (2.7)	0
Other	5 (13.5)	6 (28.6)
Pretransplant dialysis, *n* (%)	33 (89.2)	15 (71.4)
Median duration, months	21.0	13.0
Sperm concentration, *n* (%)	$n = 30$	$n = 20$
Normal ($\geq 20 \times 10^6$/ml)	9 (30.0)	8 (40.0)
Oligozoospermia	15 (50.0)	10 (50.0)
Mild (>10 to $\leq 15 \times 10^6$/ml)	3 (10)	0
Moderate (>5 to $\leq 10 \times 10^6$/ml)	6 (20.0)	2 (10.0)
Severe ($\leq 5 \times 10^6$/ml)	6 (20.0)	8 (40.0)
Azoospermia	6 (20.0)	2 (10.0)
History of infertility	0	0
Previously fathered children, *n* (%)	16 (42.1)	11 (52.4)

VGCV, valganciclovir.

Denominators for percentages are based on the number of patients in the safety population with nonmissing data in each cohort for the relevant variable.

were excluded from the analyses. The short duration of exposure in the other patients was not expected to affect outcomes, and they were included in the analyses.

Mean sperm concentration

In Cohort B, mean sperm concentration increased from $23.2 \pm 24.90 \times 10^6$/ml to $59.4 \pm 70.73 \times 10^6$/ml at the end-of-treatment visit and to $73.2 \pm 55.76 \times 10^6$/ml at the end of follow-up. In Cohort A, mean sperm concentration decreased during treatment, from $21.0 \pm 28.33 \times 10^6$/ml to $13.8 \pm 31.40 \times 10^6$/ml but increased to $60.5 \pm 67.03 \times 10^6$/ml by the end of follow-up (Fig. 1). No conclusions could be drawn from these raw means, with different numbers of patients assessed at each time point. Adjusting for baseline differences, the model-based difference in the change in mean sperm concentration between baseline and end of treatment (Cohort A–Cohort B) was -43.82×10^6/ml (95% CI: -72.48 to -15.16; $P = 0.0038$); however, sperm concentration recovered following the end of treatment in Cohort A, meaning that there was no significant difference between cohorts for the change from baseline to end of follow-up (Table 2). Mean sperm concentration in both cohorts was within the normal range ($\geq 20 \times 10^6$/ml) by the end of follow-up.

Improvements in sperm concentration between baseline and end of treatment were seen in 33.3% of patients in Cohort A and 64.3% in Cohort B. By the end of follow-up, improvements in sperm concentration from baseline were seen in 90% of patients in Cohort A and 80% in Cohort B.

No patients in either cohort who had a sperm concentration within the normal range at baseline had an abnormal sperm concentration at end of follow-up, while 40% of patients in Cohort A and 20% in Cohort B shifted from abnormal to normal between these time points. While 6/24 (25%) patients in Cohort A and 1/14 (7%) in Cohort B shifted from normal to abnormal sperm concentration between baseline and the end-of-treatment visit, all recovered by end of follow-up. Of the patients with azoospermia at baseline (six in Cohort A, two in Cohort B), none of those who provided post-baseline samples showed recovery to normal levels either at end of treatment or end of follow-up.

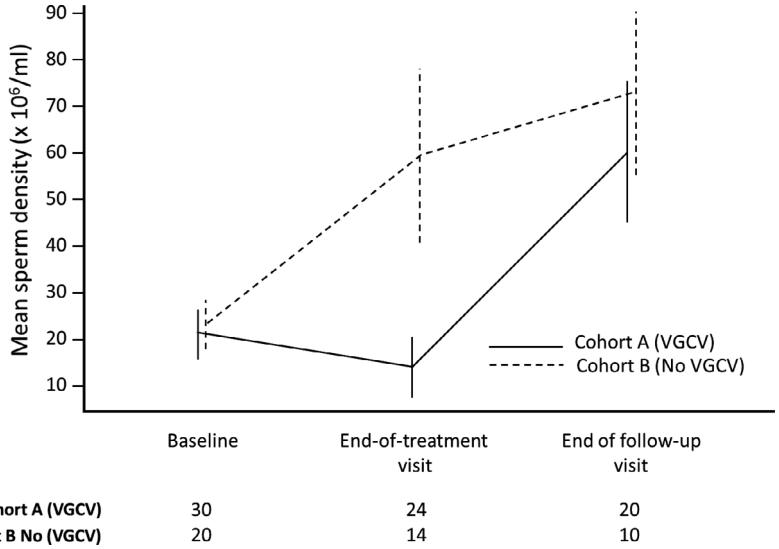
For secondary endpoints, model-based data are shown here. Data using raw means, from which it is not possible to draw conclusions due to variations in baseline and small patient numbers, are shown in Figs S1–S5.

Seminal volume

Model-based changes in seminal volume were similar over time in the two cohorts and differences between cohorts were considered not clinically meaningful. Estimated mean changes from baseline to end of treatment were -0.14 ml (95% CI: -0.64 to 0.37) for VGCV-treated patients and -0.32 ml (95% CI: -0.94 to 0.31) for untreated controls, and from baseline to end of follow-up were -0.39 ml (95% CI: -0.84 to 0.07) and -0.30 ml (95% CI: -0.90 to 0.29), respectively (Table 3).

Total motility of sperm

Model-based changes in sperm total motility showed a similar pattern to sperm concentration: Motility recovered more quickly and was numerically greater at all time points in controls than in VGCV-treated patients. Estimated mean changes from baseline to end of treatment were 2.76% (95% CI: -9.40 to 14.92) in VGCV-treated patients and 25.79% (95% CI: 10.41 to 41.17) in controls, and from baseline to end of follow-up were 27.08% (95% CI: 16.47 to 37.69) and 30.55% (95% CI: 16.52 to 44.59), respectively (Table 3). These data



n	Baseline	End-of-treatment visit	End of follow-up visit
Cohort A (VGCV)	30	24	20
Cohort B No (VGCV)	20	14	10

Figure 1 Unadjusted arithmetic mean sperm concentration over visits. Vertical bars represent 95% CIs. This figure displays the unadjusted arithmetic means and 95% CI of all observations available per visit in each cohort. As the number of patients contributing data differs from visit to visit, conclusions cannot be drawn from this display and should only be made from the model-based results. CI, confidence interval; VGCV, valganciclovir.

suggest that VGCV treatment appeared to inhibit improvement in sperm total motility following renal transplant, with marked improvement observed following VGCV treatment cessation.

Sperm morphology

There were no clinically relevant differences in the model-based changes from baseline between groups at any time point: estimated mean changes from baseline to end of treatment were 5.16% (95% CI: −4.41 to 14.73) in VGCV-treated patients and 8.14% (95% CI: −2.84 to 19.10) in controls, and from baseline to end of follow-up were 5.64% (95% CI: −0.42 to 11.70) and 7.90% (95% CI: −1.31 to 17.12), respectively (Table 3).

DNA fragmentation

Similar decreases in mean TUNEL score were seen in both cohorts from baseline to end of treatment and from baseline to end of follow-up. There were no relevant differences between cohorts, suggesting that VGCV did not appear to affect sperm DNA integrity. Model-based estimates showed no significant differences between cohorts at any time points (Table 4).

Hormone levels

Changes in levels of testosterone, LH, FSH, prolactin, and inhibin B were largely comparable between cohorts, and model-based estimates showed no clinically relevant differences between cohorts for the change from baseline to end of treatment (Table 4). A larger decrease in mean LH from baseline to end of follow-up was observed in VGCV-treated patients compared with controls: in both cohorts, the overall change appeared to be mostly due to a change from end of treatment to end of follow-up. Changes in testosterone, FSH, prolactin, and inhibin B showed no relevant differences between cohorts at any time point (Table 4).

Comparisons between the planned analyses of continuous endpoints (linear mixed-effects models with cohort, visit, and cohort by visit interaction in addition to baseline covariates) and separate models with and without covariates for end-of-treatment and end-of-follow-up visits were completed and are shown in the Supplementary Appendix. Overall, there was consistency between the results, indicating that conclusions were robust.

Adverse events

The safety profile of VGCV is well established and, given the nonrandomized nature of the trial, no formal comparison of AEs between cohorts was conducted. Most patients reported at least one AE (Cohort A 87%, Cohort B 95%) with a similar mean number of AEs per patient (8.3 and 8.4, respectively) (Table S2); the most common AEs in Cohort A being hyperkalemia (25.8%), anemia (22.6%), hypomagnesemia (22.6%), tremor (22.6%), and transplant rejection (22.6%). A similar incidence of anemia (23.8%) and tremor (23.8%) was seen in Cohort B, with a lower incidence of hyperkalemia (9.5%), hypomagnesemia (no events), and transplant rejection (9.5%). Most AEs were mild or moderate in severity and unrelated to treatment, with eight patients in Cohort A reporting treatment-related AEs, one of whom reported three severe, related AEs (anemia, arthralgia, and groin pain). Severe AEs were

Table 2. Model-based changes in sperm concentration between baseline, end of treatment, and end of follow-up.

Change from	Sperm concentration × 10^6/ml (95% CI)		Difference	*P* value
	Cohort A (VGCV)	Cohort B (no VGCV)		
Baseline to end of treatment	*n* = 24–11.13 (−29.79 to 7.53)	*n* = 1432.69 (9.67 to 55.71)	−43.82 (−72.48 to −15.16)	0.0038
End of treatment to end of follow-up	*n* = 1957.15 (20.38 to 93.92)	*n* = 95.88 (−50.97 to 62.74)	51.27 (−24.63 to 127.16)	0.1756
Baseline to end of follow-up	*n* = 2040.94 (15.29 to 66.58)	*n* = 10 43.03 (9.57 to 76.49)	−2.09 (−44.18 to 40.00)	0.9193

CI, confidence interval; VGCV, valganciclovir.
n numbers reflect the number of patients with observed values for the endpoint (no imputation for missing data at baseline or follow-up).

reported in five (16.1%) patients in Cohort A and five (23.8%) in Cohort B. One life-threatening AE (transplant rejection) was reported, in Cohort A.

Discussion

In this nonrandomized study in renal transplant recipients, the cohort of patients receiving VGCV prophylaxis for up to 200 days had comparable changes in sperm parameters between baseline and end of follow-up to those of the untreated cohort. The increase in sperm concentration in the post-transplant period was greater in untreated patients compared with VGCV-treated patients, but VGCV-treated patients experienced a greater increase after the end of treatment. A similar pattern was seen for other sperm parameters.

Patients with end-stage renal disease on dialysis frequently experience impaired fertility, which improves following transplant [7,8], and our data indicate that the use of VGCV prophylaxis to prevent CMV disease can delay the post-transplant improvement in spermatogenesis and parameters of fertility until after VGCV treatment. Average sperm concentration as well as sperm motility was inhibited in adult male renal transplant recipients who received VGCV treatment for up to 200 days, but recovered by the follow-up visit approximately 6 months after cessation of VGCV treatment and was comparable to that in untreated controls. Patients treated with VGCV did not show increased sperm DNA fragmentation compared with untreated patients.

Notably, similar proportions of patients in each cohort showed improvements in sperm counts from abnormal to normal by the end of follow-up, and no patient in either cohort experienced a shift from normal to abnormal between baseline and end of follow-up. There were no meaningful differences in outcome or clinically important conclusions whether the original model (including multiple time points and covariates) or the revised model (without covariates, and testing time points separately) was used. Overall, hormonal changes were comparable between cohorts: model-based estimates of LH and FSH decreased, and inhibin B increased, from baseline to end of follow-up, indicative of normalization of hormone levels post-transplantation. The fall in LH was more pronounced in the VGCV cohort both from baseline to end of treatment ($P = 0.014$) and from baseline to end of follow-up ($P = 0.021$) (Table S2), but this did not translate to a difference in levels of total testosterone between cohorts. For FSH and inhibin B, model-based estimates increased in the VGCV cohort from baseline to end of

Table 3. Model-based changes in seminal volume, sperm total motility, and sperm morphology.

Mean change from	Cohort A (VGCV)	Cohort B (no VGCV)	Difference	*P* value
Seminal volume (ml), mean change (95% CI)				
Baseline to end of treatment	*n* = 25 −0.14 (−0.64 to 0.37)	*n* = 14 −0.32 (−0.94 to 0.31)	−0.18 (−0.59 to 0.94)	0.6398
End of treatment to end of follow-up	*n* = 20 −0.10 (−0.55 to 0.35)	*n* = 9 0.02 (−0.61 to 0.66)	−0.13 (−0.89 to 0.63)	0.7323
Baseline to end of follow-up	*n* = 20 −0.39 (−0.84 to 0.07)	*n* = 10 −0.30 (−0.90 to 0.29)	−0.09 (−0.83 to 0.66)	0.8154
Sperm total motility (%), mean change (95% CI)				
Baseline to end of treatment	*n* = 24 2.76 (−9.40 to 14.93)	*n* = 14 25.79 (10.41 to 41.17)	−23.03 (−44.02 to −2.04)	0.0325
End of treatment to end of follow-up	*n* = 19 8.95 (−4.28 to 22.19)	*n* = 9 20.64 (1.88 to 39.39)	−11.68 (−37.04 to 13.68)	0.3505
Baseline to end of follow-up	*n* = 20 27.08 (16.466 to 37.686)	*n* = 10 30.55 (16.519 to 44.587)	−3.48 (−22.03 to 15.08)	0.7028
Sperm morphology (%), mean change (95% CI)				
Baseline to end of treatment	*n* = 21 5.16 (−4.41 to 14.73)	*n* = 12 8.14 (−2.84 to 19.10)	−2.97 (−17.91 to 11.96)	0.6866
End of treatment to end of follow-up	*n* = 17 −0.71 (−9.80 to 8.37)	*n* = 7 2.24 (−10.01 to 14.48)	−2.95 (−19.19 to 13.29)	0.7080
Baseline to end of follow-up	*n* = 19 5.64 (−0.42 to 11.70)	*n* = 8 7.90 (−1.31 to 17.12)	−2.260 (−13.56 to 9.04)	0.6823

CI, confidence interval; VGCV, valganciclovir.

n numbers reflect the number of patients with observed values for the endpoint (no imputation for missing data at baseline or follow-up).

Table 4. Model-based changes in TUNEL score and total testosterone, luteinizing hormone, follicle-stimulating hormone, prolactin, and inhibin B levels.

Change from	Cohort A (VGCV)	Cohort B (no VGCV)	Difference	*P* value
TUNEL score, mean change (95% CI)				
Baseline to end of treatment	*n* = 22 −4.70 (−8.47 to −0.93)	*n* = 14 −5.78 (−10.00 to −1.56)	1.08 (−4.32 to 6.47)	0.6864
End of treatment to end of follow-up	*n* = 16 4.45 (0.136 to 8.759)	*n* = 9 1.58 (−3.702 to 6.859)	2.87 (−4.001 to 9.739)	0.3940
Baseline to end of follow-up	*n* = 18 −2.85 (−6.95 to 1.26)	*n* = 10 −5.04 (−10.39 to 0.31)	2.19 (−4.63 to 9.02)	0.5131
Testosterone (nmol/l), mean change (95% CI)				
Baseline to end of treatment	*n* = 26 0.63 (−1.53 to 2.79)	*n* = 14 2.57 (−0.29 to 5.42)	−1.94 (−5.37 to 1.50)	0.2597
End of treatment to end of follow-up	*n* = 19 −0.94 (−2.99 to 1.11)	*n* = 11 −0.98 (−3.46 to 1.49)	0.05 (−3.06 to 3.16)	0.9753
Baseline to end of follow-up	*n* = 19 0.95 (−1.33 to 3.23)	*n* = 11 1.50 (−1.32 to 4.32)	−0.55 (−4.07 to 2.97)	0.7494
Luteinizing hormone (mU/ml), mean change (95% CI)				
Baseline to end of treatment	*n* = 26 −0.22 (−1.02 to 0.59)	*n* = 13 −0.15 (−1.26 to 0.95)	−0.06 (−1.40 to 1.27)	0.9237
End of treatment to end of follow-up	*n* = 20 −1.48 (−2.02 to −0.95)	*n* = 11 −0.42 (−1.08 to 0.24)	−1.07 (−1.90 to −0.23)	0.0143
Baseline to end of follow-up	*n* = 20 −1.89 (−2.50 to −1.29)	*n* = 11 −0.75 (−1.52 to 0.01)	−1.14 (−2.10 to −0.19)	0.0210
Follicle-stimulating hormone (U/l), mean change (95% CI)				
Baseline to end of treatment	*n* = 26 1.39 (−0.86 to 3.64)	*n* = 13 −0.18 (−3.21 to 2.85)	1.57 (−2.13 to 5.26)	0.3958
End of treatment to end of follow-up	*n* = 20 −3.46 (−4.50 to −2.43)	*n* = 11 −1.99 (−3.25 to −0.73)	−1.47 (−3.14 to 0.19)	0.0798
Baseline to end of follow-up	*n* = 20 −2.84 (−3.63 to −2.05)	*n* = 11 −1.81 (−2.80 to −0.83)	−1.03 (−2.29 to 0.23)	0.1054
Prolactin (mU/ml), mean change (95% CI)				
Baseline to end of treatment	*n* = 25 17.64 (−12.58 to 47.86)	*n* = 13 19.12 (−20.48 to 58.72)	−1.48 (−49.45 to 46.49)	0.9502
End of treatment to end of follow-up	*n* = 19 −7.43 (−30.08 to 15.22)	*n* = 11 −16.17 (−44.04 to 11.70)	8.74 (−26.44 to 43.92)	0.6134
Baseline to end of follow-up	*n* = 20 4.65 (−21.70 to 31.00)	*n* = 11 −21.74 (−53.89 to 10.40)	26.39 (−14.18 to 66.96)	0.1927
Inhibin B (pg/ml), mean change (95% CI)				

Table 4. Continued.

Change from	Cohort A (VGCV)	Cohort B (no VGCV)	Difference	*P* value
Baseline to end of treatment	*n* = 22 4.47 (−11.30 to 20.24)	*n* = 12 −4.88 (−25.45 to 15.69)	9.35 (−16.01 to 34.70)	0.4569
End of treatment to end of follow-up	*n* = 18 40.33 (11.66 to 68.99)	*n* = 9 14.45 (−22.66 to 51.56)	25.88 (−20.02 to 71.79)	0.2548
Baseline to end of follow-up	*n* = 17 52.83 (26.68 to 78.99)	*n* = 10 14.22 (−18.35 to 46.80)	38.61 (−2.83 to 80.05)	0.0663

CI, confidence interval; TUNEL, terminal uridine nick-end labeling; VGCV, valganciclovir.

n numbers reflect the number of patients with observed values for the endpoint (no imputation for missing data at baseline or follow-up).

treatment but decreased in the untreated cohort, although none of the differences were significant. It is possible that meaningful differences could have been detected using a larger patient group. The safety profile of VGCV in this study was consistent with that previously observed in other studies [4].

These findings are relevant to the renal transplantation setting but cannot be extrapolated to recipients of other organ transplants. Limitations of the study include the nonrandomized design, with potential for imbalances between cohorts. Patients in Cohort B were younger and had shorter duration of dialysis, which may result in higher sperm concentrations, as shown by higher semen parameters in this cohort; however, age, duration of pretransplant dialysis, and the baseline value of the parameter to be analyzed were included as covariates in the statistical models to adjust the change from baseline estimates for potential imbalances in these known confounders. Assessment of pregnancy or live birth rate was not considered realistic as a measure of fertility in a clinical trial of this size, in this setting, and also may not be specific to a drug effect on male fertility. Therefore, the proxy measures of semen analysis and DNA fragmentation were used.

The delay in recovery of spermatogenesis in the VGCV treatment group may have been due to a number of factors, including direct negative effects of CMV infection, VGCV treatment, or other treatments received. The rate of transplant rejection was higher in Cohort A, leading to a higher rate of corticosteroid use in this cohort.

Baseline sperm parameters were a mean of those obtained at the screening and baseline visits, as available. Because the baseline visit could occur up to 28 days after transplant and was a median of 15 days in Cohort A and 17 days in Cohort B, patients in Cohort A could have already been exposed to VGCV at baseline. Spermatogenesis in humans takes 68–72 days, and it is not likely that this exposure to VGCV would have a meaningful effect on spermatogenesis. Similarly, short-term, limited exposure to GCV/VGCV in Cohort B is not expected to influence the results. Of six patients exposed to GCV, five had short exposure before baseline and one had no postbaseline semen assessments. Of the four patients exposed to VGCV, only two, with 1 and 8 days' exposure, had postbaseline semen assessments. These data indicate that in renal transplant recipients CMV prophylaxis with VGCV does not impair parameters of spermatogenesis and fertility in the long term. The improvement conferred by renal transplantation is

delayed during VGCV treatment post-transplant. These data indicate that the post-transplant improvement in semen parameters seen in untreated patients occurs in VGCV-treated patients but may take longer. Nevertheless, within 6 months of stopping VGCV, semen parameters improve to the same extent as in patients who had no VGCV treatment after transplant.

Authorship

PM and DAP were responsible for the design of the study. MA, PM, and NM were responsible for analysis of data. PM was responsible for drafting the article. PM, DAP, MA, RH, SM, and NM were responsible for interpretation of data, providing intellectual content, revising the article, and final approval of the published version.

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Conflicts of interest

PM reports employment by Covance, Inc. (Princeton, NJ, USA), the clinical research organization retained by F. Hoffmann-La Roche Ltd (Basel, Switzerland) for execution of the trial. DAP reports grants and other payments to his institution by F. Hoffmann-La Roche Ltd and Covance, Inc. MA, RH, SM, and NM report employment by F. Hoffmann-La Roche Ltd. All authors received third-party medical writing support funded by F. Hoffmann-La Roche Ltd.

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Data-sharing statement

Qualified researchers may request access to individual patient-level data through the clinical study data request platform: www.clinicalstudydatarequest.com. Further details on Roche's criteria for eligible studies are available here: https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Roche.aspx. For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here: https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Shifts in sperm concentration over visits.
Table S2. Summary of post-transplant adverse events.
Figure S1. Mean seminal volume over visits.
Figure S2. Mean sperm motility over visits.
Figure S3. Mean sperm morphology over visits.
Figure S4. Mean TUNEL scores over visits.
Figure S5. Mean serum levels of testosterone, luteinizing hormone, follicle-stimulating hormone, prolactin, and inhibin B over visits.

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