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## OBSTETRICS

# In utero treatment of congenital cytomegalovirus infection with valacyclovir in a multicenter, open-label, phase II study



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**BACKGROUND:** Congenital infection with human cytomegalovirus is a major cause of morbidity and mortality. A randomized controlled trial showed that high-dosage valacyclovir prevents cytomegalovirus disease in transplant recipients. Fetuses showing ultrasound features of infection are at high risk of being symptomatic at or before birth. In a pilot study, oral administration of high-dosage valacyclovir to mothers significantly decreased viral load and produced therapeutic concentrations in the blood of infected fetuses. A randomized controlled trial comparing prenatal treatment with valacyclovir against placebo in infected fetuses failed to recruit because women declined randomization. Randomized controlled trials in fetal medicine have often proven unacceptable by women who decline termination of pregnancy and are not prepared to resign themselves to the odds of the natural history of the disease.

**OBJECTIVE:** We evaluated the efficacy of oral valacyclovir, 8 g daily, for pregnant women carrying a symptomatic cytomegalovirus-infected fetus, targeting a high-risk group for developing both neurosensory and neurological impairment.

**STUDY DESIGN:** We designed a multicenter, open-label, phase II study with 1 arm, using one of Simon's optimal 2-stage designs. Symptomatic fetuses were defined by the presence of measurable extracerebral or mild cerebral ultrasound symptoms. They were treated in utero from prenatal diagnosis at a median of 25.9 weeks' gestation until delivery or termination of pregnancy. Fetuses with severe brain anomalies on ultrasound were not included as were cases completely asymptomatic at presentation, because treatment was unlikely to modify either outcome. The primary endpoint was the proportion of asymptomatic neonates born to treated mothers.

**RESULTS:** At the interim analysis, 8 of 11 women delivered an asymptomatic neonate (required:  $\geq 7$ ). In step 2, 32 additional cases were included for a total of 43; the final number of asymptomatic neonates was 34, more than the 31 required to indicate efficacy according to the Simon 2-stage design. They remained asymptomatic at 12 months. High-dosage valacyclovir given for a median of 89 days to pregnant women carrying a moderately infected fetus was efficient at giving birth to asymptomatic neonates. Fetal blood viral loads decreased and platelet counts increased, both significantly ( $P = .01$  and  $P < .001$ , respectively), between treatment initiation and birth after treatment completion, regardless of duration of fetal infection. Compared with a historical cohort obtained by a meta-analysis of the literature, the use of valacyclovir (8 g daily) significantly increased the proportion of asymptomatic neonates from 43% without treatment to 82% with treatment. Although the pill burden was high (16 pills a day) adherence to treatment was  $>90\%$ . Finally, valacyclovir at this high dosage was extremely well tolerated.



**CONCLUSION:** Our results indicate that high-dosage valacyclovir given in pregnancy is effective for improving the outcome of moderately symptomatic infected fetuses. Although this study is not a randomized controlled trial, this is the first study reporting the efficacy of an antiviral drug to treat cytomegalovirus-infected fetuses. Moreover, this first study will allow new trials to be conducted, using valacyclovir as a baseline safe and effective treatment in pregnancy, to be compared to the new emerging and more potent anticytomegalovirus drugs that have not currently been tested in pregnancy.

**Key words:** congenital infection, cytomegalovirus, fetal therapy, fetus, symptomatic, valacyclovir

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## Introduction

Congenital cytomegalovirus (CMV) infection affects 0.7% of live births and is the leading cause of congenital neurological disease of infectious origin.<sup>1</sup> Among all neonates positive for infection on screening, 20% eventually have neurodevelopmental impairment with permanent sequelae.<sup>2</sup> Around 10% of infected neonates are symptomatic at birth; their risk of sequelae reaches 58%, including sensorineural hearing loss or

cognitive or motor defects. The risk of sequelae in newborns who were asymptomatic at birth is around 13%, mainly due to progressive hearing loss.<sup>2</sup>

An infected fetus's risk of symptoms at birth is assessed by interpreting the results of both prenatal imaging and laboratory tests.<sup>3-8</sup> Fetal CMV disease is progressive: early symptoms of systemic infection can be expressed as extracerebral findings at prenatal ultrasound; fetal brain involvement usually does not show

**TABLE 1**  
**Main inclusion criteria**

At least 1 extracerebral abnormality compatible with fetal CMV infection

Fetal growth restriction <sup>42</sup>
Abnormal amniotic fluid volume
Ascites and/or pleural effusion
Skin edema
Hydrops
Placentomegaly >40 mm <sup>43</sup>
Hyperechogenic bowel <sup>a</sup>
Hepatomegaly >40 mm <sup>44</sup>
Splenomegaly >30 mm <sup>45</sup>
Liver calcifications

And/or 1 isolated cerebral abnormality

Moderate isolated ventriculomegaly (<15 mm)
Isolated cerebral calcification
Isolated intraventricular adhesion
Vasculopathy of lenticulostriate vessels

And/or laboratory findings of generalized CMV infection in fetal blood

Fetal viremia >3000 copies/mL
Fetal platelet count <100,000/mm <sup>3</sup>

All ultrasound examinations leading to inclusion in study were reviewed by principal investigator at each center. Measurements of spleen, liver, and placenta were standardized according to literature.<sup>43-45</sup>

CMV, cytomegalovirus.

<sup>a</sup> Since diagnosis of hyperechogenic bowel can be subjective and associated with high interobserver and intraobserver variability, diagnosis of hyperechogenic bowel was only considered for grade-2 or grade-3 hyperechogenic bowel<sup>46</sup>; this semiquantitative analysis was chosen to limit subjectivity sometimes associated with ultrasound findings.

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until several weeks later.<sup>9</sup> Severe brain lesions seen on prenatal ultrasound predict a dismal outcome.<sup>4</sup> This leaves a window of opportunity for treatment of symptomatic fetuses without brain involvement.<sup>10</sup>

Vaccination is not available<sup>11</sup> and no prenatal treatment of congenital CMV has yet been validated. The use of CMV-specific hyperimmune globulin to prevent transmission from mother to fetus has produced conflicting results.<sup>12,13</sup> Neonatal antiviral treatment with either ganciclovir or valganciclovir improves auditory and neurological outcomes in symptomatic newborns,<sup>14</sup> but these drugs, highly genotoxic in vitro, are not approved in pregnancy. Although valacyclovir is less effective

than ganciclovir against CMV in vitro,<sup>15</sup> high-dosage valacyclovir has proven clinically efficient to prevent CMV disease in transplant recipients.<sup>16</sup> The mechanism of acyclovir's anti-CMV activity in clinical settings remains unexplained. Valacyclovir also has the best safety profile of the anti-CMV drugs. Neither cell transformation nor increased risk of neoplasia has been reported in vitro, and no increased risk of birth defects has been detected in the offspring of thousands of women exposed during pregnancy.<sup>17,18</sup> Finally, valacyclovir is well tolerated with rare side effects. In a pilot study, we found that oral administration of high-dosage valacyclovir to mothers significantly decreased viral load and produced

therapeutic concentrations in fetal blood with a mean fetal blood plasma concentration of >17  $\mu\text{mol/L}$ . These results suggested the value of a clinical trial to investigate this therapeutic option further.<sup>10</sup> We failed to complete a randomized controlled trial comparing prenatal treatment with valacyclovir against placebo in moderately symptomatic infected fetuses due to failure to recruit (Cymeval NCT01037712). In this open-label phase II trial with 1 arm we show that high-dosage valacyclovir given in pregnancy is safe and appears effective for improving the outcome of moderately symptomatic fetuses.

## Materials and Methods

### Patients

Eligible women were pregnant with an infected fetus identified by a positive CMV polymerase chain reaction assay in amniotic fluid, sampled by amniocentesis >21 weeks,<sup>19-22</sup> together with the presence of  $\geq 1$  extracerebral ultrasound features compatible with CMV infection and/or 1 isolated cerebral abnormality and/or 1 of the following laboratory findings in fetal blood: fetal platelet count <100,000/mm<sup>3</sup> or CMV DNA viral load >3000 copies/mL (Table 1). The presence of severe ultrasound brain abnormalities (Table 2) and the absence of any ultrasound feature of infection or laboratory abnormality in fetal blood were exclusion criteria. The detailed eligibility criteria are listed in the [supplementary Appendix](#). Fetal blood sampling by cordocentesis under ultrasound guidance was offered to all participants to evaluate fetal platelets and viral DNA load to help refine the fetal prognosis.<sup>4</sup> Cordocentesis was not, however, required for study eligibility, as this invasive procedure was not the standard of care in all participating centers.

### Procedures

#### Study design

The trial was a multicenter, open-label, phase II study with 1 arm, based on 1 of Simon's<sup>23</sup> optimal 2-stage designs. All participants received oral valacyclovir (2 g, 4 times a day, therefore 8 g daily). The medication was continued until delivery or 24 treatment weeks, whichever was sooner. The study drug was

purchased from GlaxoSmithKline (Marly Le Roi, France), which had no other role in the study. Visits for clinical and ultrasound examinations, questions about clinical side effects (headache, nausea, neurologic effects listed in the product monograph), and adherence assessment by pill count were scheduled every 2 weeks until delivery. Maternal plasma levels of aspartate aminotransferase, alanine aminotransferase, and creatinine were assessed once a month during treatment. All newborns were examined between days 4 and 7 of life by a trained pediatrician according to a standardized clinical evaluation. Auditory brainstem responses, cranial ultrasound, fundoscopy, and laboratory tests (see criteria for primary endpoint in [supplementary data](#)) were also performed.

The ethics committee of Poissy-Saint Germain Hospital approved the study (2011-001610-34). Participants gave written informed consent before inclusion. Study oversight was provided by an independent data and safety monitoring board (Clinical Research Unit, Cochin-Necker, Paris).

### Study endpoints

The primary study endpoint was the proportion of asymptomatic neonates born to women treated with valacyclovir. An asymptomatic neonate was a neonate without growth restriction (that is, with birthweight  $\geq 10$ th percentile), normal clinical examination, normal laboratory findings, no severe features of infection on cerebral imaging, normal fundoscopic examination, and normal audiology findings (see the [supplementary Appendix](#) for details).

The secondary endpoints included adverse events related to the study medication and adherence to treatment. CMV DNA levels and platelet counts were compared in pretreatment fetal blood when available and cord blood at birth and in symptomatic and asymptomatic neonates. The effect of the duration of maternal treatment was also assessed.

### Historical comparator group

Our systematic review on PubMed using 3 key words (“cytomegalovirus,” “congenital,” “ultrasound”) yielded 216

**TABLE 2**  
**Main exclusion criteria**

Presence of at least 1 severe cerebral ultrasound abnormality among following:

Ventriculomegaly $\geq 15$ mm
Periventricular hyperechogenicity
Hydrocephaly
Microcephaly $< 3$ SD
Mega-cisterna magna $> 10$ mm
Vermian hypoplasia
Porencephaly
Lissencephaly
Periventricular cysts
Abnormal corpus callosum

Or absence of any ultrasound feature of infection or laboratory abnormality in fetal blood

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articles, but only 3 were written in English and included detailed tables that described both prenatal ultrasound findings and neonatal outcome and also included postmortem findings in termination of pregnancy cases.<sup>10,24,25</sup> These 3 suitable studies included 724 pregnancies with a maternal CMV primary infection, 217 with an infected fetus. The review of the ultrasound symptoms of these 217 fetuses showed no ultrasound abnormalities in 142 of them and severe cerebral ultrasound abnormalities in 28. Ultrasound abnormalities matching our inclusion criteria were therefore seen in 47 cases, which formed the historical comparator group. Among them 20 (42.55%) neonates were born asymptomatic. Twenty fetuses were terminated and all underwent postmortem examination showing both macroscopic and microscopic evidence of brain damage in all cases. We therefore assumed that these fetuses would have been born symptomatic and classified then into the symptomatic group. See [Table S1](#) for details in the [supplementary Appendix](#).

### Laboratory assays

CMV serology and viral load quantification were centralized and analyzed at the Necker Hospital virology laboratory. Maternal CMV primary infection was

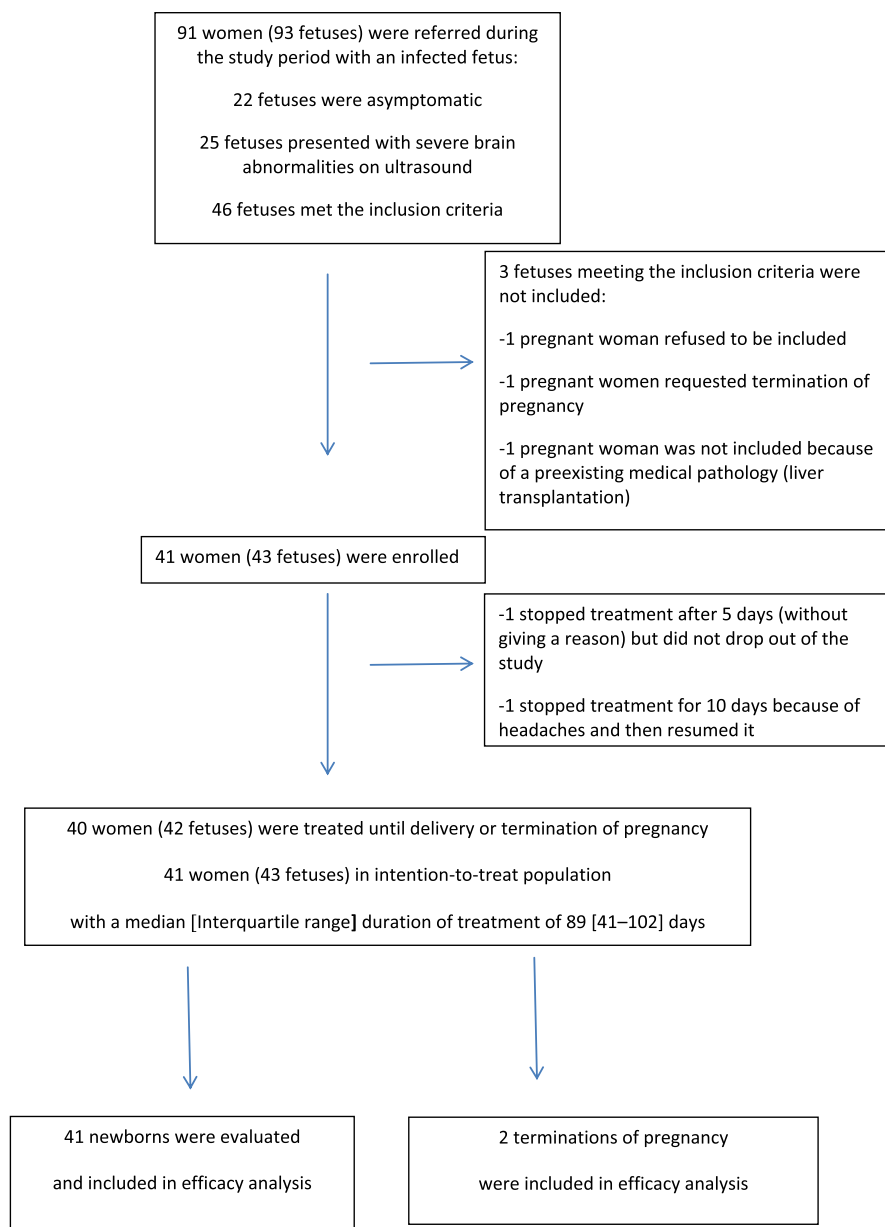
diagnosed by seroconversion or the concomitant presence of CMV-specific IgM antibodies and low IgG avidity.<sup>22</sup> After extracting DNA from fetal whole blood, cord whole blood, and neonatal urine with the MagnaPure LC platform (Roche Diagnostic, Meylan, France), we performed quantitative polymerase chain reaction for CMV with the CMV-R gene kit (Argene, BioMerieux, Marcy-L'Etoile, France). The limit of detection was 446 copies (or 178 IU)/mL.

### Statistical analysis

To estimate the sample size according to Simon<sup>23</sup> optimal 2-stage design, we assumed that a proportion of asymptomatic neonates of  $< 60\%$  was not clinically relevant in relation to valacyclovir efficacy, while a proportion of  $> 80\%$  was deemed acceptable. With a type I error fixed at 0.05 and a power of 80%, we needed 11 infected fetuses for the first stage: if at least 8 cases in stage 1 had a good outcome (were asymptomatic), then 32 additional infected fetuses would be included in stage 2 for a total of 43. If at least 31 of the 43 cases were asymptomatic at birth, valacyclovir would be judged to have a positive effect.

Analyses of efficacy data were performed for all included fetuses (intention to treat). The proportion of

**FIGURE 1**  
Flowchart of enrollment and outcomes



Enrollment and outcomes.

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asymptomatic neonates and its 95% confidence interval were calculated with an unbiased estimator.<sup>26</sup> In the historical comparator group, we used a random effects model to calculate the overall proportion of asymptomatic neonates and a 95% confidence interval for all 47 cases from the 3 studies.

We used nonparametric tests for all secondary analyses.

Platelet counts at birth and in fetal blood were compared to the gestational age-specific reference range published by Meher-Homji et al.<sup>27</sup> The average individual deviations of values at birth and in fetal blood from the published mean in noninfected fetuses were compared to 0. We also compared individual differences in platelet counts between fetal blood and birth to the slope of the reference

mean in noninfected fetuses. Two-sided *t* tests were used for these analyses.

R software (Version 2.15; R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical analyses and its “meta” package for the metaanalysis.

## Results

### Enrollment and baseline characteristics

From January 2012 through December 2014, 41 pregnant women and their 43 fetuses (39 singletons and 4 twins from 2 pairs of dichorionic twins) were enrolled at 6 centers, as described in Figure 1. Table 3 summarizes their baseline characteristics. The median gestational age at inclusion was 26 (range 22–35) weeks. All but 1 woman had a primary infection at a median gestational age of 10 weeks. In all, 54% of all fetuses presented with at least 2 eligible ultrasound symptoms, 39% with only 1, and 3 showed only viremia >3000 copies/mL (4500; 90,000; and 101,000 copies/mL, respectively).

### Primary endpoint

At the planned interim analysis, 8 of the first 11 women included had given birth to an asymptomatic neonate. This number exceeded the number of asymptomatic neonates ( $\geq 7$ ) required to continue to step 2, which included 32 additional fetuses, for a total of 43. The final analysis showed 34 asymptomatic neonates, more than the 31 required to suggest efficacy according to the Simon<sup>23</sup> optimal 2-stage design in the population studied (Table 4).

Comparison of results of our trial with those of the historical cohort indicates that valacyclovir significantly increased the proportion of asymptomatic neonates, from 43% (range, 29–57) without treatment to 82% (range, 67–88) with treatment (and without any overlapping of confidence intervals) (Figure 2).

The 9 symptomatic cases include 2 terminations of pregnancy performed because a severe brain abnormality subsequently appeared on ultrasound (Table 5). There was only 1 significant difference in maternal and fetal characteristics at baseline (Table 6a, 6b)

between the cases with good (asymptomatic neonates) and poor (symptomatic neonates or termination of pregnancy) outcomes: fetal platelet count at inclusion, which was significantly lower in the poor outcome group ( $P < .007$ ) (Table 6b). The duration of maternal treatment did not differ significantly between the 2 groups ( $P = .236$ ) (Table 6a).

Table S2 reports all details on pregnancy outcome (gestational age at delivery, birthweight, clinical examination, imaging, and laboratory data).

## Secondary endpoints

### Adverse events

Valacyclovir was well tolerated. Only 2 women reported headaches, and treatment was suspended for 10 days in only 1. Although maternal alanine aminotransferase and aspartate aminotransferase levels increased after 3 months of treatment, this increase was not clinically relevant; all values were  $<40$  IU/L, and creatinine levels did not change throughout treatment (Figure S1). Adherence in the subgroup of 27 women evaluated for it was  $>90\%$  (Table S3).

### Viral loads and platelet counts

Although the duration of maternal treatment was not correlated with CMV DNA levels in either cord blood ( $P = .65$ ) or neonatal urine ( $P = .24$ ), longer duration was associated with a higher platelet count at birth ( $P = .018$ ) (Figure S2 and Table S4). Neither viral loads nor platelet counts at birth were correlated with the time of maternal infection in pregnancy and therefore with the duration of fetal infection (Table S4). Symptomatic and asymptomatic neonates did not differ significantly for viral DNA load levels in either neonatal cord blood ( $P = .391$ ) or neonatal urine ( $P = .081$ ), but the neonatal platelet count was significantly lower in symptomatic neonates ( $P < .001$ ) (Table S5). Blood viral load decreased and platelet count increased, both significantly, between the fetal blood obtained in utero before maternal treatment began and the cord blood sampled at birth ( $P = .01$  and  $P < .001$ , respectively) (Table 7). The mean

**TABLE 3**  
Characteristics of population at baseline

Characteristics	Median [interquartile range] or n (%)
<b>Women (N = 41)</b>	
Age at inclusion, y	31.2 [28.6–33.9]
Body mass index before pregnancy	21.6 [19.8–23]
<b>Parity</b>	
0	11 (26.8)
$\geq 1$	30 (73.2)
No. of pregnancies	2 [2–3]
Primary infection <sup>a</sup>	40 (97.6)
Gestational age at primary infection, wk	10 [7.8–16.2]
Gestational age at inclusion, wk	25.9 [24.1–31.7]
Interval between primary infection and inclusion, wk	16 [12.3–18.6]
<b>Fetuses (N = 43)</b>	
Only 1 symptom at ultrasound	17 (39.5)
$>1$ Symptom at ultrasound	23 (53.5)
Fetal blood CMV DNA load $>3000$ copies/mL	3 (7)
Fetal growth restriction	3 (7)
Abnormal amount of amniotic fluid	3 (7)
Ascites and/or pleural effusion	1 (2.3)
Placentomegaly	13 (30.2)
Hyperechogenic bowel	25 (58.1)
Hepatomegaly	6 (14)
Splenomegaly	9 (20.9)
Liver calcification	1 (2.3)
Moderate cerebral abnormality	5 (11.6)

CMV, cytomegalovirus.

<sup>a</sup> Forty women had primary infection and 1 woman was diagnosed with secondary infection.

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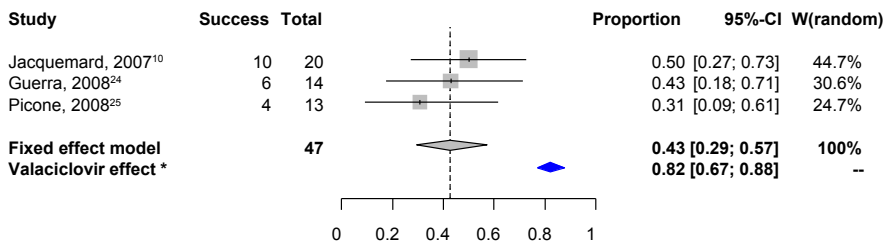
increase in platelet counts from the beginning to the end of valacyclovir treatment was significantly higher than the expected corresponding increase in noninfected fetuses ( $P = .008$ ) (Figure S3).

**TABLE 4**  
Primary endpoint and efficacy

	First step	Second step
<b>Outcome no.</b>		
Asymptomatic neonates	8	34
Symptomatic neonates or termination of pregnancy	3	9
<b>Total</b>	<b>11</b>	<b>43</b>

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**FIGURE 2**  
Valacyclovir effect compared to historical control group



Proportion of asymptomatic neonates and 95% confidence interval (CI) were calculated with unbiased estimator.<sup>26</sup> In historical control group, we used random effects model to calculate overall proportion of asymptomatic neonates and 95% CI for 43 cases reported in 3 suitable published studies. \*Unbiased estimation of binomial probability in multistage design.

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The 5-year follow-up of the children is obviously not completed. They have currently been followed up for a median of 12 (range 4-36) months. Among the 33 children who were asymptomatic at birth, all were still asymptomatic at 12 months of age. All 7 neonates with symptoms were treated with valganciclovir for 6 weeks and none showed subsequent worsening of neurosensory hearing loss.

### Historical cohort

Analysis of the historical group showed that any proportion of asymptomatic

neonates <60% would be unacceptable for Simon<sup>23</sup> optimal 2-stage design. Moreover, comparison of our trial results with those of the historical cohort indicates that valacyclovir significantly increased the proportion of asymptomatic neonates, from 43% without treatment to 82% with treatment (and without any overlapping of confidence intervals) (Figure 2).

### Comment

This is the first trial of antiviral treatment of symptomatic fetuses with CMV disease. This approach followed a pilot

study conducted from 2003 through 2005 that suggested treatment with high-dosage valacyclovir given orally to pregnant women carrying a symptomatic fetus infected with CMV could decrease viral DNA loads and achieve therapeutic concentrations in fetal blood.<sup>10</sup> A phase II randomized placebo-controlled trial was planned to test the efficacy of valacyclovir given to women with a symptomatic infected fetus (Cymeval NCT01037712) in 2008. It failed, however, to recruit enough participants, including only 6 women over 2 years. To avoid what appeared to be an unacceptable placebo arm, we shifted to a trial based on a Simon<sup>23</sup> optimal 2-stage design with only 1 arm—treated patients (Cymeval II NCT01651585). Severe fetal cerebral lesions are known to have a dismal prognosis and are unlikely to be reversible under treatment.<sup>28</sup> Their odds ratio for a poor outcome was 40.6<sup>4</sup>; we therefore excluded them. Women carrying an infected fetus that appeared completely asymptomatic on ultrasound and had normal laboratory results were similarly excluded, because their outcome is known to be generally good.<sup>4,9,29,30</sup> In a previous study, we showed that odds ratio for a poor outcome was 4.4 when there was any

**TABLE 5**  
Characteristics of 9 cases with poor outcome

Case	Gestational age at primary infection, wk	Gestational age at inclusion, wk	Inclusion criteria	Days of treatment	Symptoms at birth or termination of pregnancy
1	10	23	Oligohydramnios, hyperechogenic bowel	92	Bilateral hearing loss
2		23	Hyperechogenic bowel, intraventricular adhesion	18	Termination of pregnancy <sup>a</sup>
3	13	35	Intraventricular adhesion	5	Termination of pregnancy <sup>a</sup>
4	9	32	Fetal growth restriction placentomegaly, intraventricular adhesion	6	Bilateral hearing loss
5	9	25	Hyperechogenic bowel, fetal thrombocytopenia (55,000/mm <sup>3</sup> )	97	Thrombocytopenia (59,000/mm <sup>3</sup> )
6	6	23	Hyperechogenic bowel	99	Unilateral hearing loss
7	7	24	Hyperechogenic bowel, fetal thrombocytopenia (66,000/mm <sup>3</sup> )	103	Unilateral hearing loss
8	8	22	Hyperechogenic bowel	15	Unilateral hearing loss
9	Secondary maternal infection	26	Fetal growth restriction	68	Growth restriction

<sup>a</sup> Two terminated fetuses underwent postmortem examination and in both cases severe brain lesions were identified.

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TABLE 6

**Comparison of maternal and fetal characteristics at baseline between cases with good (asymptomatic neonate) and poor (symptomatic neonate or termination of pregnancy) outcomes**

Table 6a.

## Women's characteristics

	Total	Good outcome	Poor outcome	P
	Median [interquartile range] (N = 41)	Median [interquartile range] (N = 33)	Median [interquartile range] (N = 8)	
Maternal age at inclusion, y	31.2 [28.6–33.9]	31.5 [28.8–33.9]	30.2 [28.1–33.5]	.717
Body mass index before pregnancy	21.6 [19.8–23]	20.4 [19.4–22.7]	22.9 [21.8–23.9]	.058
Parity, n (%)				
0	11 (26.8)	8 (24.2)	3 (37.5)	.744
1	20 (48.8)	17 (51.5)	3 (37.5)	
2	9 (22)	7 (21.2)	2 (25)	
3	1 (2.4)	1 (3)	0 (0)	
No. of pregnancies	2 [2–3]	2 [2–3]	2 [1.8–2.5]	.73
Primary infection, n (%)	40 (97.6)	33 (100)	7 (87.5)	.195
Gestational age at primary infection, wk	10 [7.8–16.2]	11 [8–17]	9 [7.5–9.5]	.199
Gestational age at inclusion, wk	25.9 [24.1–31.7]	27 [24.6–31.7]	24.6 [23.8–27.6]	.411
Interval between primary infection and inclusion, wk	16 [12.3–18.6]	15.9 [12.1–18.6]	17.1 [15.9–20]	.182
Treatment interruption, n (%)	2/39 (5.1)	1/33 (3)	1/6 (16.7)	.287
Duration of treatment, d	89 [41–102]	89 [43–102]	80 [15–97.5]	.236

Table 6b.

## Fetal characteristics

	Total	Good outcome	Poor outcome	P
	Median [interquartile range] (N = 43)	Median [interquartile range] (N = 34)	Median [interquartile range] (N = 9)	
Inclusion criteria, n (%)				
Only 1 symptom at ultrasound	17 (39.5)	15 (44.1)	2 (22.2)	.332
Fetal blood DNA load >3000 copies/mL	3 (7)	3 (8.8)	0 (0)	
>1 Symptom at ultrasound	23 (53.5)	16 (47.1)	7 (77.8)	
Fetal growth restriction, n (%)	3 (7)	1 (2.9)	2 (22.2)	.106
Abnormal amount of amniotic fluid, n (%)	3 (7)	2 (5.9)	1 (11.1)	.515
Ascites and/or pleural effusion, n (%)	1 (2.3)	1 (2.9)	0 (0)	1
Placentomegaly, n (%)	13 (30.2)	11 (32.4)	2 (22.2)	.699
Hyperechogenic bowel, n (%)	25 (58.1)	19 (55.9)	6 (66.7)	.712
Hepatomegaly, n (%)	6 (14)	5 (14.7)	1 (11.1)	1
Splenomegaly, n (%)	9 (20.9)	7 (20.6)	2 (22.2)	1
Liver calcifications, n (%)	1 (2.3)	0 (0)	1 (11.1)	.209
Moderate cerebral anomalies, <sup>a</sup> n (%)	5 (11.6)	2 (5.9)	3 (33.3)	.054
Fetal viremia at inclusion in log <sub>10</sub> IU/mL	4.4 [4–5]	4.3 [3.8–4.8]	5.1 [4.4–5.9]	.1
Fetal platelet count at inclusion/mm <sup>3</sup>	174,000 [145,000–208,000]	177,500 [155,250–208,000]	67,000 [61,000–88,000]	.006

<sup>a</sup> Moderate isolated ventriculomegaly (<15 mm) or isolated cerebral calcification or isolated intraventricular adhesion or vasculopathy of lenticulostriate vessels.Leruez-Ville et al. In utero treatment of congenital cytomegalovirus infection. *Am J Obstet Gynecol* 2016.



TABLE 7

**Correlation of fetal and neonatal laboratory indicators (viral DNA load and platelet count, from cord blood, compared with viral DNA load and fetal platelet count obtained in utero before inclusion)**

	Fetal blood before beginning maternal treatment	Neonatal cord blood	Differences <sup>a</sup>	P
Viral DNA in blood, log <sub>10</sub> IU/mL				
Median (interquartile range)	4.0 (3.55–4.6)	3.05 (2.57–3.92)	−0.5 (−2.075 to −0.075)	.01
N	28	32	24	
Platelet count/mm <sup>3</sup>				
Median (interquartile range)	173,000 (141,500–201,500)	245,000 (193,000–274,000)	101,000 (47,500–122,000)	<.001
N	27	41	27	

<sup>a</sup> Differences between values obtained for same case in utero in fetal blood before maternal treatment and in neonatal cord blood after treatment. Participants with viral DNA load less than threshold value of 178 IU were considered to have viral DNA load of 89 IU (1.94 IU).

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noncerebral ultrasound abnormality and 1.13 for each 10,000/mm<sup>3</sup> decrease in fetal platelet count <100,000/mm<sup>3</sup>.<sup>4</sup> Another observational study showed that a fetal platelet count <50,000/mm<sup>3</sup> had 80% predictive value for poor outcome.<sup>6</sup> We therefore enrolled women with either extracerebral or nonsevere cerebral ultrasound features, a low platelet count, or a high viral load in blood sampled in utero.<sup>4,6,31</sup> These criteria were selected specifically to target a group at high risk for progressive damage, on the hypothesis that prenatal treatment could lower this risk that could lead to neonatal neurodevelopmental impairment.

According to Simon<sup>23</sup> optimal 2-stage design, high-dosage valacyclovir given orally to pregnant women was effective in improving the outcome of pregnancies with a fetus infected with symptomatic CMV, shown by either extracerebral or nonsevere cerebral ultrasound features or abnormal fetal laboratory results. Moreover, when compared with a historical cohort of similar cases collected from the literature,<sup>10,24,25,32,33</sup> an unbiased estimation confirmed the efficacy of valacyclovir compared to no treatment. Among the 43 symptomatic fetuses treated in utero, there were only 4 cases with an indisputably poor outcome (2 terminations of pregnancy for severe brain abnormalities and 2 cases of bilateral hearing loss), while the other 5 infants symptomatic at birth showed only mild

disease (3 with unilateral hearing loss, 1 with isolated thrombocytopenia, and another with growth restriction). The clinical impact of valacyclovir may be explained by its direct inhibition of CMV replication. In renal transplantation and in HIV infection, high-dose regimens of valacyclovir are effective in preventing CMV disease and suppressing CMV viremia.<sup>16,34,35</sup> A sort of placebo effect may have also participated in the effect of valacyclovir. That is, although the participants were informed that the efficacy of valacyclovir in utero was unknown and that they could request a termination of pregnancy in accordance with French law, only 2 terminations were requested, both after fetuses developed severe cerebral symptoms visible on ultrasound. The availability of treatment may have alleviated parental anxiety and dissuaded women from requesting termination of pregnancy by offering them something more than helpless anxiety and expectant management.

Although the valacyclovir dosage used in this study was much higher than that used for treatment of herpes simplex infection in pregnancy (8 g per day vs 1 g per day), the maternal clinical and laboratory tolerances were excellent and no adverse effect was observed in the neonates.

Moreover, despite the burden of taking 16 tablets throughout the day, cumulative adherence to treatment was >90%.

Overall, 40% of the women met the inclusion criterion of 1 ultrasound

abnormality suggestive of CMV, 53% had >1, and 7% had only a high fetal blood viral load (>3000 copies/mL). The latter criterion was selected on the basis of previous work by Boppana et al,<sup>31</sup> who reported that no neonates with a viral load <3000 copies developed hearing loss. However, a more recent study reports that fetal viral load >30,000 copies/mL is a predictive marker for poor outcome.<sup>6</sup> Therefore, the cut-off of 3000 copies/mL might have been too low. Nonetheless, only 3 cases were included based on this criterion alone, and 2 of them had much higher viral loads (110,000 and 90,000 copies/mL). None of these 3 patients had a poor outcome.

At birth, viral loads in cord blood were significantly lower and platelet counts significantly higher than in fetal blood obtained in utero before treatment began. The antiviral effects of valacyclovir can easily explain these changes. Nonetheless, both differences might also be due either to chance or the natural course of these markers since little is known about the spontaneous kinetics of blood viral loads or platelet counts over time after acute fetal infection. The similarity of blood viral DNA loads reported in neonates born to untreated mothers, regardless of the trimester of pregnancy of her primary infection,<sup>36</sup> suggests that no spontaneous change in viral load is likely to be significant over the few weeks of intrauterine life. Postnatal decreases in viral load have been evaluated over a much longer period

of time.<sup>37</sup> Another reason for the differences in the prenatal and postnatal courses evolutions of viremia is the closed intrauterine circuit across the fetal-placental circulation. Moreover, the 1.0 log reduction we observed in blood viral load between fetal blood sampled before treatment and cord blood at birth after antiviral treatment is similar to the 1.3 log reduction reported in a controlled trial of HIV-infected patients also treated with high-dose valacyclovir.<sup>34</sup> We observed no correlation between the duration of fetal infection and neonatal platelet counts or viral loads. Although platelet counts are reported to increase during pregnancy, their increase in our study was correlated with the duration of valacyclovir treatment and was significantly higher than expected in a population of noninfected fetuses (17). A spontaneous decrease in fetal viral loads and an increase in platelet counts over time, independently of treatment, is very unlikely.

Blood viral load levels have been reported to be significantly higher in symptomatic neonates.<sup>31,38,39</sup> In our study viral loads in neonatal blood and in neonatal urine showed only a trend to being higher in the symptomatic group. This could be due to the small number of symptomatic cases in the study (N = 7), which may not allow reaching statistical significance. One could also speculate that this gap in viral load levels between symptomatic and asymptomatic neonates during the natural history of the infection might have been reduced in a population of treated fetuses.

The main limitation of our study is that it is not randomized. It therefore remains difficult to assess definitively the respective roles of true antiviral effect and a placebo effect to explain the positive effect of treatment demonstrated in this setting. However, conducting a randomized placebo-controlled trial in pregnant women with a symptomatic CMV-infected fetus proved utterly impracticable. This difficulty has previously been encountered for another rare fetal condition carrying a risk of death or severe handicap.<sup>40</sup> This is especially relevant for women who choose not to terminate the pregnancy, at least as long

as a potential good outcome may exist. Because a classic randomized controlled trial proved to be too difficult to achieve, a possibility to strengthen the results of the present study could be to follow the design described by Relton et al<sup>41</sup> of a cohort multiple randomized controlled trial. In such trial, half of the eligible patients would be randomly selected to be treated while the other half would receive usual care; this would allow keeping comparable arms while avoiding the unacceptability of classic randomization. ■

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### References

1. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev Med Virol* 2007;17:253-76.
2. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev Med Virol* 2007;17:355-63.
3. Guerra B, Lazzarotto T, Quarta S, et al. Prenatal diagnosis of symptomatic congenital cytomegalovirus infection. *Am J Obstet Gynecol* 2000;183:476-82.
4. Benoist G, Salomon LJ, Jacquemard F, Daffos F, Ville Y. The prognostic value of ultrasound abnormalities and biological parameters in blood of fetuses infected with cytomegalovirus. *BJOG* 2008;115:823-9.
5. Simonazzi G, Guerra B, Bonasoni P, et al. Fetal cerebral periventricular halo at mid-gestation: an ultrasound finding suggestive of fetal cytomegalovirus infection. *Am J Obstet Gynecol* 2010;202:599.e1-5.
6. Fabbri E, Revello MG, Furione M, et al. Prognostic markers of symptomatic congenital human cytomegalovirus infection in fetal blood. *BJOG* 2011;118:448-56.
7. Feldman B, Yinon Y, Tepperberg Oikawa M, Yoeli R, Schiff E, Lipitz S. Pregestational, periconceptual, and gestational primary maternal cytomegalovirus infection: prenatal diagnosis in 508 pregnancies. *Am J Obstet Gynecol* 2011;205:342.e1-6.
8. Society for Maternal-Fetal Medicine (SMFM), Hughes BL, Gyamfi-Bannerman C. Society for Maternal-Fetal Medicine (SMFM) consult no. 39: diagnosis and antenatal management of congenital cytomegalovirus (CMV) infection. *Am J Obstet Gynecol* 2016;214:B5-11.
9. Benoist G, Salomon LJ, Mohlo M, Suarez B, Jacquemard F, Ville Y. Cytomegalovirus-related

fetal brain lesions: comparison between targeted ultrasound examination and magnetic resonance imaging. *Ultrasound Obstet Gynecol* 2008;32:900-5.

10. Jacquemard F, Yamamoto M, Costa J-M, et al. Maternal administration of valacyclovir in symptomatic intrauterine cytomegalovirus infection. *BJOG* 2007;114:1113-21.
11. Griffiths PD. Burden of disease associated with human cytomegalovirus and prospects for elimination by universal immunization. *Lancet Infect Dis* 2012;12:790-8.
12. Nigro G, Adler SP, La Torre R, Best AM; Congenital Cytomegalovirus Collaborating Group. Passive immunization during pregnancy for congenital cytomegalovirus infection. *N Engl J Med* 2005;353:1350-62.
13. Revello MG, Lazzarotto T, Guerra B, et al. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. *N Engl J Med* 2014;370:1316-26.
14. Kimberlin DW, Jester PM, Sánchez PJ, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. *N Engl J Med* 2015;372:933-43.
15. Tyms AS, Scamans EM, Naim HM. The in vitro activity of acyclovir and related compounds against cytomegalovirus infections. *J Antimicrob Chemother* 1981;8:65-72.
16. Lowance D, Neumayer HH, Legendre CM, et al. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group. *N Engl J Med* 1999;340:1462-70.
17. Stone KM, Reiff-Eldridge R, White AD, et al. Pregnancy outcomes following systemic prenatal acyclovir exposure: conclusions from the international acyclovir pregnancy registry, 1984-1999. *Birth Defects Res A Clin Mol Teratol* 2004;70:201-7.
18. Pasternak B, Hviid A. Use of acyclovir, valacyclovir, and famciclovir in the first trimester of pregnancy and the risk of birth defects. *JAMA* 2010;304:859-66.
19. Duff P. A thoughtful algorithm for the accurate diagnosis of primary CMV infection in pregnancy. *Am J Obstet Gynecol* 2007;196:196-7.
20. Cahill AG, Odibo AO, Stamilio DM, Macones GA. Screening and treating for primary cytomegalovirus infection in pregnancy: where do we stand? A decision-analytic and economic analysis. *Am J Obstet Gynecol* 2009;201:466.e1-7.
21. Guerra B, Simonazzi G, Banfi A, et al. Impact of diagnostic and confirmatory tests and prenatal counseling on the rate of pregnancy termination among women with positive cytomegalovirus immunoglobulin M antibody titers. *Am J Obstet Gynecol* 2007;196:221.e1-6.
22. Lereuz-Ville M, Sellier Y, Salomon LJ, Stirnemann JJ, Jacquemard F, Ville Y. Prediction of fetal infection in cases with cytomegalovirus immunoglobulin M in the first trimester of pregnancy: a retrospective cohort. *Clin Infect Dis* 2013;56:1428-35.

23. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials* 1989;10:1-10.
24. Picone O, Simon I, Benachi A, Brunelle F, Sonigo P. Comparison between ultrasound and magnetic resonance imaging in assessment of fetal cytomegalovirus infection. *Prenat Diagn* 2008;28:753-8.
25. Guerra B, Simonazzi G, Puccetti C, et al. Ultrasound prediction of symptomatic congenital cytomegalovirus infection. *Am J Obstet Gynecol* 2008;198:380.e1-7.
26. Jung S-H, Kim KM. On the estimation of the binomial probability in multistage clinical trials. *Stat Med* 2004;23:881-96.
27. Meher-Homji NJ, Montemagno R, Thilaganathan B, Nicolaides KH. Platelet size and glycoprotein Ib and IIIa expression in normal fetal and maternal blood. *Am J Obstet Gynecol* 1994;171:791-6.
28. Gaytant MA, Steegers EAP, Semmekrot BA, Merkus HMMW, Galama JMD. Congenital cytomegalovirus infection: review of the epidemiology and outcome. *Obstet Gynecol Surv* 2002;57:245-56.
29. Picone O, Vauloup-Fellous C, Cordier AG, et al. A series of 238 cytomegalovirus primary infections during pregnancy: description and outcome. *Prenat Diagn* 2013;33:751-8.
30. Farkas N, Hoffmann C, Ben-Sira L, et al. Does normal fetal brain ultrasound predict normal neurodevelopmental outcome in congenital cytomegalovirus infection? *Prenat Diagn* 2011;31:360-6.
31. Boppana SB, Fowler KB, Pass RF, et al. Congenital cytomegalovirus infection: association between virus burden in infancy and hearing loss. *J Pediatr* 2005;146:817-23.
32. Liesnard C, Donner C, Brancart F, Gosselin F, Delforge ML, Rodesch F. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. *Obstet Gynecol* 2000;95:881-8.
33. Lipitz S, Hoffmann C, Feldman B, Tepperberg-Dikawa M, Schiff E, Weisz B. Value of prenatal ultrasound and magnetic resonance imaging in assessment of congenital primary cytomegalovirus infection. *Ultrasound Obstet Gynecol* 2010;36:709-17.
34. Griffiths PD, Feinberg JE, Fry J, et al. The effect of valacyclovir on cytomegalovirus viremia and viruria detected by polymerase chain reaction in patients with advanced human immunodeficiency virus disease. *AIDS Clinical Trials Group Protocol 204/Glaxo Wellcome 123-014 International CMV Prophylaxis Study Group. J Infect Dis* 1998;177:57-64.
35. Emery VC, Sabin C, Feinberg JE, Grywacz M, Knight S, Griffiths PD. Quantitative effects of valacyclovir on the replication of cytomegalovirus (CMV) in persons with advanced human immunodeficiency virus disease: baseline CMV load dictates time to disease and survival. *The AIDS Clinical Trials Group 204/Glaxo Wellcome 123-014 International CMV Prophylaxis Study Group. J Infect Dis* 1999;180:695-701.
36. Zavattoni M, Lombardi G, Rognoni V, et al. Maternal, fetal, and neonatal parameters for prognosis and counseling of HCMV congenital infection. *J Med Virol* 2014;86:2163-70.
37. Forner G, Abate D, Mengoli C, Palù G, Gussetti N. High cytomegalovirus (CMV) DNAemia predicts CMV sequelae in asymptomatic congenitally infected newborns born to women with primary infection during pregnancy. *J Infect Dis* 2015;212:67-71.
38. Vauloup-Fellous C, Ducroux A, Couloigner V, et al. Evaluation of cytomegalovirus (CMV) DNA quantification in dried blood spots: retrospective study of CMV congenital infection. *J Clin Microbiol* 2007;45:3804-6.
39. Walter S, Atkinson C, Sharland M, et al. Congenital cytomegalovirus: association between dried blood spot viral load and hearing loss. *Arch Dis Child Fetal Neonatal Ed* 2008;93:F280-5.
40. Morris RK, Malin GL, Quinlan-Jones E, et al. Percutaneous vesicoamniotic shunting versus conservative management for fetal lower urinary tract obstruction (PLUTO): a randomized trial. *Lancet* 2013;382:1496-506.
41. Relton C, Torgerson D, O' Cathain A, Nicholl J. Rethinking pragmatic randomized controlled trials: introducing the "cohort multiple randomized controlled trial" design. *BMJ* 2010;340:c1066.
42. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. *Am J Obstet Gynecol* 1985;151:333-7.
43. Grannum PA. Ultrasound examination of the placenta. *Clin Obstet Gynaecol* 1983;10:459-73.
44. Vintzileos AM, Neckles S, Campbell WA, Andreoli JW, Kaplan BM, Nochimson DJ. Fetal liver ultrasound measurements during normal pregnancy. *Obstet Gynecol* 1985;66:477-80.
45. Hata T, Deter RL. A review of fetal organ measurements obtained with ultrasound: normal growth. *J Clin Ultrasound* 1992;20:155-74.
46. Slotnick RN, Abuhamad AZ. Prognostic implications of fetal echogenic bowel. *Lancet* 1996;347:85-7.

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