Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kimberlin DW, Whitley RJ, Wan W, et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. N Engl J Med 2011;365:1284-92.

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METHODS

Study Design and Population

Parallel, identical studies were performed to avoid confounding of outcome. The studies were designed by Drs. Kimberlin and Whitley, and were approved by the ethics boards at all participating centers.

The Bayley Scales of Infant Development is the standard neurodevelopmental tool available for and validated in infants beginning at 12 months of age.

All supplies of active and placebo study medication were labeled centrally, provided to study sites in blinded packaging, and dispensed by site research pharmacists.

Randomization was stratified by study site and blocked within each site.

This duration was selected based upon prior evidence that three or more cutaneous recurrences during the first six months following acute disease correlated with increased risk of neurologic sequelae in patients with SEM disease.[1, 2] Compliance with study medication administration was not systematically assessed throughout the lengthy treatment period due to the impracticality and cost of doing so; study results reflect the "real world" challenges and benefits of daily medication administration for prolonged periods of time. With skin recurrences while on blinded study medication, subjects had a lumbar puncture if the sick visit occurred within 12 hours of skin vesicle development. If the CSF indices were reassuring and CSF HSV PCR was negative, the subject was treated with episodic open-label oral acyclovir (80 mg/kg/day administered in

four divided doses) for 5 days, then blinded study drug suppression was resumed. If the subject had a second cutaneous recurrence and therefore met the secondary endpoint, the subject again was evaluated for CNS involvement and received episodic open-label treatment, but blinded study drug suppression was not resumed. Instead, the subject was offered open-label oral acyclovir suppression at the enrolling physician's discretion. If a subject had CNS disease while on blinded study drug, he/she was treated with another 21 day course of intravenous acyclovir, and the blinded medication was not resumed.

Virologic Assessments

Viral cultures were performed at the study sites. The CSF was tested for HSV DNA by PCR at the CASG Central Laboratory at the University of Alabama at Birmingham.[3]

Statistical Analyses

The site Principal Investigator and his/her study team gathered the study data, completed the Case Report Forms, and submitted the data to the CASG Central Unit where they were entered into the database.

The assumption of a normal distribution for mental and motor scores was confirmed by the normal probability plot and the Shapiro-Wilks normality test. If a score was not normally distributed ($P<0.05$), then the Kruskal-Wallis test was used to compare the two groups. For both secondary efficacy analyses,

For the tertiary safety analyses, frequencies of side effects were summarized by grade using frequencies and percentages.

Ms. Cloud led the statistical team, and Dr. Kimberlin led the overall data analysis assessments. Dr. Kimberlin and all coauthors attest to the validity of the data, decided to publish the study results, and wrote all drafts of the study manuscript.

RESULTS

Subject Demographics and Characteristics

Of the 24 subjects in the CNS study randomized to acyclovir suppression, 15 completed the six months on blinded active medication, 7 completed the six months on open-label active medication, and 2 stopped active drug suppression prior to completing the six months (Figure 1a). Of the 21 subjects in the CNS study randomized to placebo, 8 completed the six months on blinded placebo medication, 9 were switched per protocol to open-label active acyclovir suppression during the six month treatment course, and 4 stopped all study medication prior to completing the six months (Figure 1a).

Of the 15 subjects in the SEM study randomized to acyclovir suppression, 8 completed the six months on blinded active medication, 5 completed the six months on open-label active medication, and 2 stopped active drug suppression prior to completing the six months (Figure 1b). Of the 14 subjects in the SEM study randomized to placebo, 4 completed the six months on blinded placebo medication, 9 were switched per protocol to open-label active acyclovir

suppression during the six month treatment course, and 1 stopped all study medication prior to completing the six months (Figure 1b).

Cutaneous Recurrences

Two months after beginning blinded study medication, all babies randomized to acyclovir remained on blinded therapy, compared with three-quarters of babies randomized to placebo. By six months after randomization, four-fifths of babies randomized to acyclovir remained on blinded therapy, compared with one-half of babies randomized to placebo.

Cutaneous recurrences appeared as “typical” HSV mucocutaneous lesions, with small clusters of vesicles on erythematous bases.

Cutaneous recurrences occurred both during and following completion of antiviral suppression. By 6 months after study enrollment, 11 (28%) of the 39 subjects randomized to acyclovir and 16 (46%) of the 35 subjects randomized to placebo had experienced two or more skin recurrences. Between 6 and 12 months of life, 4 additional subjects randomized to acyclovir and 3 additional subjects randomized to placebo had experienced a second or subsequent cutaneous recurrence.

Safety Assessments

No differences between randomization groups were seen for other hematologic (white blood cell count, hemoglobin, hematocrit, and platelets) or chemistry (blood urea nitrogen, creatinine, bilirubin, aspartate aminotransferase, and alanine aminotransferase) laboratory tests, or for adverse events or serious adverse events.

DISCUSSION

The study endpoints were selected because of their high clinical relevance. Improved neurodevelopmental outcome has been a major goal of all therapeutic clinical trials of neonatal herpes for over 30 years.[4-7] Skin recurrences have both clinical implications (pain, psychosocial for child and parents, infectivity to others) and have been correlated with increased risk of poor neurologic outcomes.[1] An earlier small Phase I/II trial implicated neutropenia as possibly being associated with oral acyclovir suppressive therapy following neonatal herpes,[8] so systematic assessment of safety with a grading scale was imperative in these controlled studies.

Table 1. Demographics and Clinical Characteristics By Study Drug Assignment

Study	CASG 103				CASG 104	
Disease Classification	**CNS Disease**		**Disseminated Disease with CNS Involvement**		**SEM Disease**	
Study Drug Assignment	Acyclovir N=21	Placebo N=16	Acyclovir N=3	Placebo N=5	Acyclovir N=15	Placebo N=14
Gender (%)						
Male	11 (52)	7 (44)	3 (100)	4 (80)	10 (67)	8 (57)
Female	10 (48)	9 (56)	0 (0)	1 (20)	5 (33)	6 (43)
P-value	0.74		--		0.71	
Race (%)						
Caucasian, not Hispanic	18 (86)	9 (56)	2 (67)	3 (60)	9 (60)	9 (64)
African American	2 (10)	3 (19)	1 (33)	1 (20)	3 (20)	4 (29)
Hispanic	1 (5)	2 (13)	0 (0)	1 (20)	2 (13)	0 (0)
Asian or Pacific Islander	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)
Native American or Alaskan	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	0 (0)	2 (13)	0 (0)	0 (0)	1 (7)	0 (0)
P-value	0.19		--		0.58	
Birth Weight (lbs)						
Median	6.4	7.5	7.8	6.9	7.3	7.2
Range	2.0-8.5	6.0-9.9	6.9-8.6	6.4-8.5	2.4-9.9	2.3-9.0
P-value	0.01		--		0.70	
Head Circumference at Birth (in)						
Median	12.8	13.5	13.5	13.4	13.4	13.6
Range	9.3-14.2	12.2-15.2	13.2-13.8	13.4-13.4	9.6-15.0	10.0-14.8
P-value	0.03		--		0.64	
Gestational Age (wks)						
Median	37	38	40	39	38	39
Range	25-41	35-40	40-41	38-40	27-40	27-41
P-value	0.43		--		0.30	
Enrollment Weight (lbs)						
Median	7.4	8.7	8.6	8.3	7.2	8.4
Range	1.9-10.0	5.6-10.3	7.3-10.2	7.2-8.9	2.3-11.5	2.3-10.3
P-value	0.01		--		0.03	
HSV Type (%)						

Type I	2 (14)	3 (27)	1 (50)	1 (33)	5 (42)	5 (45)
Type II	12 (86)	8 (73)	1 (50)	2 (67)	7 (58)	6 (55)
Unknown	7	5	1	2	3	3
P-value	0.62		1.00		1.00	
CSF WBC at Presentation (cells/mm^3)						
Median	91.5	109.0	3.0	2.0	4.0	6.0
Range	10-1216	5-58,080	1-18	0-13	0-20	0-33
P-value	0.69		--		0.78	
CSF Protein at Presentation (mg/dL)						
Median	127.5	121.0	84.0	54.0	91.0	89.0
Range	69-307	51-310	49-272	47-91	43-116	39-169
P-value	0.58		--		0.58	
CSF PCR at Presentation						
Positive (%)	17 (81%)	12 (75%)	2 (67%)	3 (60%)	0 (0%)	0 (0%)
Negative (%)	4 (19%)	4 (25%)	1 (33%)	2 (40%)	14 (100%)	13 (100%)
P-value	0.70		--		--	
MRI Evidence of HSV Disease	10 (63%)	9 (64%)	1 (33%)	0 (0%)	0 (0%)	0 (0%)
P-value	1.00		--		--	
Abnormal EEG	9 (69%)	10 (71%)	1 (100%)	0 (0)	N/A	N/A
P-value	1.00		--			

N/A = not applicable

Table 2. Demographics and Clinical Characteristics of Subjects With and Without 12 Month Bayley Scales of Infant Development Assessments

	CNS Study		**SEM Study**	
	Bayley N=28	**No Bayley N=17**	**Bayley N=15**	**No Bayley N=14**
Gender (%)				
Male	14 (50)	11 (65)	12 (80)	6 (43)
Female	14 (50)	6 (35)	3 (20)	8 (57)
P-value	0.37		0.12	
Race (%)				
Caucasian, not Hispanic	20 (71)	12 (70)	8 (53)	10 (71)
African American	5 (18)	2 (12)	5 (33)	2 (14)
Hispanic	2 (7)	2 (12)	0 (0)	2 (14)
Asian or Pacific Islander	0 (0)	0 (0)	1 (7)	0 (0)
Native American or Alaskan	0 (0)	0 (0)	0 (0)	0 (0)
Other	1 (4)	1 (6)	1 (7)	0 (0)
P-value	0.89		0.21	
Birth Weight (lbs)				
Median	6.7	7.4	7.0	7.4
Range	2.4-9.2	2.0-9.9	2.3-9.9	2.4-9.0
P-value	0.33		0.43	
Head Circumference at Birth (in)				
Median	13.3	13.0	13.6	13.4
Range	9.7-15.2	9.3-14.4	10.0-15.0	9.6-14.8
P-value	0.67		0.42	
Gestational Age (wks)				
Median	39.5	38	38	39
Range	28-41	25-40	27-41	27-40
P-value	0.17		0.69	
Enrollment Weight (lbs)				
Median	7.8	8.2	7.8	7.8
Range	2.4-10.2	1.9-10.3	2.3-11.5	2.3-10.3
P-value	0.45		0.78	
HSV Type (%)				
Type I	7 (37)	0 (0)	2 (15)	8 (80)
Type II	12 (63)	11 (100)	11 (85)	2 (20)
Unknown	9	6	2	4
P-value	0.03		0.003	
CSF WBC at Presentation (cells/mm^3)				

Median	65	71	6	5
Range	1-58,080	0-1216	0-20	2-33
P-value	0.98		0.48	
CSF Protein at Presentation (mg/dL)				
Median	121	101	99	73
Range	49-310	47-307	40-169	39-162
P-value	0.74		0.18	
CSF PCR at Presentation				
Positive (%)	19 (68%)	15 (88%)	0 (0%)	0 (0%)
Negative (%)	9 (32%)	2 (12%)	15 (100%)	12 (100%)
P-value	0.16		0.22	
MRI Evidence of HSV Disease	13 (57%)	7 (54%)	0 (0%)	0 (0%)
P-value	1.00		--	
Abnormal EEG	14 (74%)	6 (50%)	N/A	N/A
P-value	0.26			

N/A = not applicable

Table 3. Summary of Serious Adverse Events and Possibly/Probably/Related Adverse Events

	CNS*		Disseminated*		SEM*	
	Acyclovir N (%)	Placebo N (%)	Acyclovir N (%)	Placebo N (%)	Acyclovir N (%)	Placebo N (%)
Discontinuation of Therapy Due to AE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Number of Subjects with AE Possibly Related to Study Drug	11 (68.7)	5 (31.3)	1 (50.0)	1 (50.0)	8 (72.7)	3 (27.3)
Number of Adverse Events Possibly Related to Study Drug, by Intensity						
Mild	7 (33.3)	4 (57.1)	0 (0)	1 (100.0)	6 (46.2)	2 (33.3)
Moderate	10 (47.6)	3 (42.9)	1 (100.0)	0 (0)	5 (38.5)	2 (33.3)
Severe	4 (19.0)	0 (0)	0 (0)	0 (0)	2 (15.4)	2 (33.3)
Number of Adverse Events Possibly Related to Study Drug, By Body System						
Body as a Whole	1	0	0	0	0	0
Digestive	2	1	0	0	4	0
Hematopoietic	16	4	1	0	7	5
Hepatic/Biliary	1	2	0	0	1	1
Neurologic	0	0	0	0	1	0
Skin	1	0	0	1	0	0
Discontinuation of Therapy Due to SAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Number of Subjects With SAE	15 (40.5)	12 (32.4)	2 (25.0)	3 (37.5)	8 (53.3)	5 (35.7)
Number of Subjects with Related SAE	9 (42.9)	4 (25.0)	1 (33.3)	0 (0.0)	3 (20.0)	4 (28.6)
Number of SAEs	25	23	3	4	17	10
Number of Serious Adverse Events, by Body System						
Body as a Whole	1	3	0	1	5	1
Digestive	5	0	0	0	3	1
Ear/Nose/Throat	2	1	0	0	2	0
Hematopoietic	9	4	1	0	5	3
Neurologic	2	5	0	0	0	0

Respiratory	2	1	1	0	1	0
Skin	4	9	1	3	1	5

* P-value for all comparisons > 0.05

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