A randomized, double-blind, placebo-controlled MRI study of anti–herpes virus therapy in MS

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Abstract—*Objective:* To evaluate the effect of treatment with the antiherpes drug valacyclovir on MRI-evident lesions in patients with relapsing-remitting MS in a phase 2, randomized, double-blind, placebo-controlled study. *Background:* It has been postulated from virologic studies that herpesvirus infection could play a role in the progression of MS. *Methods:* Patients were eligible for the study if they had had two or more MS relapses in the 2-year period before enrollment. Seventy patients with Expanded Disability Status Scale scores of 0 to 5.5 were randomly assigned to receive 1 gram of valacyclovir (n = 36) or placebo (n = 34) three times daily for 24 weeks. Patients underwent MRI every fourth week for 32 weeks: twice during pretreatment, six times during treatment, and once after treatment. Scoring of neurologic disability was performed at the start and end of the treatment period. The primary endpoint was the number of new active MRI-evident lesions over 24 weeks of treatment. Secondary endpoints included other MRI measures and clinical endpoints. *Results:* The mean number of new active lesions ± SD per patient during 24 weeks of treatment with valacyclovir was 11.9 ± 17.6 and that during placebo treatment was 14.5 ± 21.4. A protocol-planned exploratory analysis stratified patients according to baseline activity; this analysis showed that patients with high levels of disease activity in the valacyclovir treatment group (n = 17) developed fewer new active lesions per scan than did those in the placebo treatment group (n = 11). The median number (Q_1, Q_3 range) of active lesions was 2.0 (1.38, 3.96) in the valacyclovir treatment group and 6.5 (2.63, 9.0) in the placebo treatment group. *Conclusions:* Valacyclovir treatment did not reduce the formation of active lesions in patients with relapsing-remitting MS who had two or more relapses during the previous 2-year period. In a subgroup of patients with high levels of disease activity who had more than one active MRI-evident lesion during 4 weeks, valacyclovir treatment was associated with a reduced number of new active MRI-evident lesions and with an increase in the number of scans free of new active lesions. The results of the exploratory subgroup analysis provide support for further studies of antiherpes therapy for patients with MS and high levels of MRI-evident disease activity.

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Epidemiologic studies on the prevalence of MS indicate that infection might play a role in its pathogenesis.[1,2] Repeated attempts to identify a specific viral agent associated with MS have had some success. Several recent studies have focused on the role of Epstein–Barr virus (EBV),[3-5] herpes simplex virus (HSV),[6,7] and human herpesvirus 6 (HHV-6)[8,9] in MS.[10] Because the natural history of HHV is one of periodic reactivation resulting in latent and active phases, members of this family are interesting candidates for the pathogenesis of MS. They are ubiquitous, and some herpesviruses infect cells of the nervous system, cells involved with immune responsiveness, or both. After primary infection, herpesviruses enter a latent phase, from which they may reactivate and cause symptomatic or asymptomatic disease.[3,6,10] The mechanism of action of antiherpes drugs against herpesvirus reactivation and replication involves targeting virus-specific kinases and inhibition of viral DNA polymerases.[11,12] In a previous study of acyclovir treatment of patients with MS, a 34% reduction in the relapse rate among these patients was observed after 2 years of treatment (p = 0.08).[13] The dosage of 2.4 grams of acyclovir daily is

probably only sufficient to effectively suppress reactivation of HSV-1 and HSV-2. Some of the other herpesviruses thought to play a role in MS (e.g., EBV and HHV-6) would not be inhibited.[12]

To broaden the spectrum to include other herpesviruses, we studied valacyclovir: a pro-drug of acyclovir that increases acyclovir bioavailability three- to fivefold.[14] Here we evaluate the effect of specific antiherpes therapy on MRI-evident lesions in patients with relapsing–remitting MS (RRMS) in a phase 2, randomized, double-blind, placebo-controlled study.

Subjects and methods. *Study design.* This randomized, double-blind, placebo-controlled trial was conducted at two university centers in Gothenburg, Sweden, and Aarhus, Denmark.

Subjects. Seventy patients with a clinical diagnosis of active RRMS according to the criteria of Poser et al.[15] were enrolled in the study between July 1996 and November 1997. Active RRMS was defined as at least two clinically verified or anamnestic relapses of MS during the preceding 2-year period.

Men and women aged 18 to 55 years who were willing to give informed consent and to comply with the study protocol were eligible for enrollment if they had had RRMS for <20 years and had an Expanded Disability Status Scale (EDSS)[16] score of <6.

Patients were excluded from the study if they had frequent recurrences of labial or genital herpes (more than four per year) or had been treated with other antiviral medication 6 months before study entry. Patients treated with immunosuppressives (excluding steroids) or receiving medication along with interferon within 1 year before study entry and patients with claustrophobia, severe ataxia, ferromagnetic implants, or pregnancy also were excluded from the study. Other exclusion criteria included previous adverse reactions to acyclovir and clinically significant gastrointestinal, cardiovascular, hepatic, renal, hematologic, neoplastic, neurologic (other than MS), respiratory, or psychiatric disorders. Female patients were required to have a negative pregnancy test before study entry and to use adequate contraception.

Ethics and permissions. The study was conducted in accordance with Good Clinical Practice rules and was approved by the Institutional Review Boards and National Boards of Health at the participating institutions. All patients gave written informed consent before initiation of study procedures.

Study objectives. The primary objective of the study was to investigate the effect of valacyclovir treatment on the number of active MRI-evident lesions in patients with RRMS; in addition, valacyclovir was compared with placebo in terms of safety and tolerability for these patients. The number of active lesions at baseline was expected to be an important prognostic factor for response to treatment. Therefore, an exploratory statistical analysis examining the impact of disease activity during the pretreatment period (weeks 0 to 4) on treatment response was declared a priori.

Procedures. Each patient was scheduled for nine visits at which time MRI and evaluations (including blood sampling and clinical interviews with assessment of side effects and patient compliance) were performed. Clinical scoring of disability was determined immediately before the start and end of treatment and during relapses. Patient inclusion was at week 0 following physical examination and MRI to confirm diagnosis and eligibility. The patients received a diary card for recording disease progression (i.e., a relapse), adverse events, and other medication. The baseline MRI scan was obtained 4 weeks later (at week 4), and patients were then treated with valacyclovir or placebo according to randomization performed at week 0. If a patient developed a clinical relapse during the 4-week run-in period, the baseline scan was postponed until the patient's condition stabilized (4 weeks later at the earliest).

MRI and neurologic examination, including determination of scores for EDSS and the Regional Functional Scoring System (RFSS), were performed at baseline (week 4). Medication for a 4-week period was dispensed starting at week 4. At 4-week intervals, MRI was performed, and a study nurse interviewed patients regarding clinical relapses, adverse events, and treatment compliance; after the evaluation, medication was provided for the next 4-week period. At the week 28 visit (after 24 weeks of treatment), patients underwent clinical examination to determine EDSS and RFSS scores and to stop treatment. A final MRI scan was obtained at 32 weeks.

Randomization and blinding. Patients were randomly allocated to receive either two 500-mg tablets of valacyclovir three times daily or matching placebo in a one-to-one ratio, according to a computer-generated code. A separate randomization list was generated for each country with use of appropriate block sizes. Study centers were blinded to the study medication, and the identity and regimen of the study medication were contained in individual sealed envelopes in case there were problems.

Clinical assessments. Two physicians assessed each patient. A treating neurologist was responsible for overall medical management of the patient, including treatment of side effects. An assessing neurologist was responsible for the neurologic scoring and follow-up of relapses, which were defined as the appearance of new symptoms or worsening of old symptoms lasting for at least 24 hours.[17] Patients with relapses were asked to attend the study center within 48 hours for confirmation and evaluation of severity by the assessing neurologist. Relapses could be treated with a standard regimen of 1.0 gram of intravenous methylprednisolone for three consecutive days. All patients underwent neurologic assessment at the start of treatment at week 4 and at the end of the treatment period at week 28, including MRI within 24 hours.

MRI procedures. MRI was performed at each center in accordance with a standardized scanning protocol. The scanning protocol included strict positioning techniques that used internal and external landmarks. A double spin echo sequence (repetition time, 2,700 milliseconds; echo times, 20 and 80 milliseconds) was used. Furthermore, a T1-weighted image (repetition time, 547 milliseconds; echo time, 16 milliseconds) was obtained before and after the administration of 0.2 mL of gadolinium/kg of body weight. Slice thickness was 5 millimeters in all sequences. Examination was performed with use of 1.5-Tesla equipment (Philips Medical Systems, Eindhoven, the Netherlands) at both sites. Two neuroradiologists who were blinded to treatment and clinical course performed analyses of se-

quential MRI scans. Reproducibility was accomplished by reevaluation of seven randomly chosen patients.

MRI endpoints. The primary endpoint of the study was the number of new active lesions during the 24 weeks of treatment. Active lesions were defined as follows: new enhancing lesions (appearing on T1-weighted scans that were obtained after gadolinium administration; new non-enhancing lesions not observed previously (new lesions on T2-weighted scans that occurred at the location where previous MRI had shown enhancement were not counted); enlarged nonenhancing lesions (identified when enlargement of a previous stable appearing lesion occurred); and recurrent lesions (reappearing at sites where an earlier lesion had disappeared).

Secondary MRI endpoints were the above-mentioned four lesion types analyzed individually as well as the total number of scans revealing activity.

Exploratory analysis was performed with respect to disease activity during the pretreatment period as stated in the protocol. Patients were stratified according to the number of active MRI-evident lesions at baseline (week 4), and the number of new active MRI-evident lesions during the treatment period in the valacyclovir treatment group was compared with that in the placebo treatment group.

Clinical endpoints. Clinical endpoints were secondary and included relapse-free periods, proportion of relapse-free patients, progression of disability (increase in EDSS score of at least one point sustained over a 3-month period), need for steroid therapy, and MS-related hospital admission.

Statistical analysis. In a previous study,[18] the bootstrap method with use of the Mann–Whitney test showed that 30 patients in each treatment arm would provide at least an 80% power to detect a 70 to 80% reduction in active MRI-evident lesions after 6 months. To verify the sample sizes reported,[18] data were used to perform a similar simulation of 1,000 trials. Within each trial, the response rate for the placebo treatment arm was fixed at various levels (i.e., 80%), while the individual patient response was allowed to vary. The power of our study was computed as the proportion of trials that were statistically different by using the two-sided Mann–Whitney test and a 5% significance level. For 70 patients, a treatment effect of 80% would be detected by a power of 99%, a treatment effect of 70% would be detected by a power of 92%, and a treatment effect of 60% would be detected by a power of 74%.

Independent personnel entered all information into a database. The population for the intent-to-treat analysis was defined as all patients who were randomized, and data for this population were used for analyses of efficacy and safety. The Mann–Whitney test was used to evaluate the effect of treatment on the primary endpoint (median number of lesions during the treatment period) and on all secondary endpoints ($p = 0.05$). The level of significance for the exploratory analysis used for hypothesis generation was 0.05. Fisher's exact test, not specified in the protocol, was used to compare the difference in the proportion of patients with relapses during the 4-week run-in period.

Results. *Patient disposition.* There were 70 patients eligible for the study, and data for these patients were included in the analysis (figure 1). Sixty-eight patients completed treatment; one patient withdrew from the study
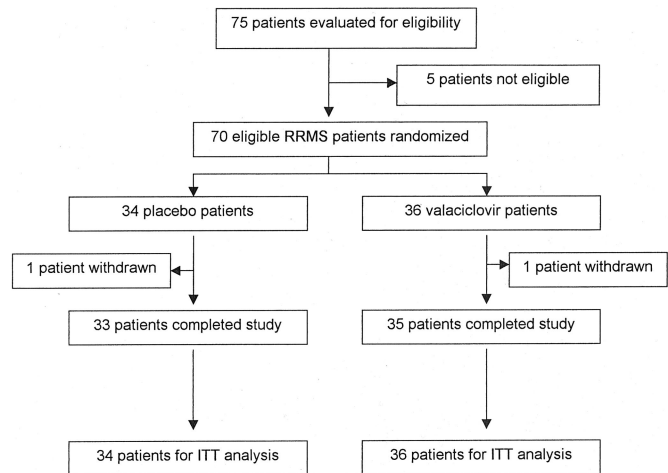
Figure 1. Flow diagram used in a study of the effect of valacyclovir treatment on MRI-evident lesions in patients with relapsing-remitting MS (RRMS). ITT = intent-to-treat.

at week 4, and one patient was not compliant to the study medication. MRI scans without enhancement were obtained for one patient who developed an adverse reaction to gadolinium.

Clinical characteristics at baseline. Demographics and disease characteristics were similar in the two treatment groups at study entry (table 1). During the pretreatment baseline activity period (weeks 0 to 4), clinical relapses occurred more frequently in the placebo treatment group than in the valacyclovir treatment group (7/35 vs 1/36, respectively; $p = 0.02$). However, exclusion of patients with relapses during the first 4 weeks of the study did not change the outcome of the planned or the exploratory MRI analysis.

Safety and tolerability. Valacyclovir was well tolerated. Adverse effects were described in 94% of patients in both groups. The most common adverse events were headache, rhinitis, and upper respiratory infection. There were no differences in the reported frequency of the various side effects among patients in the two treatment groups.

Clinical endpoints. No clinical differences were observed between valacyclovir and placebo for any of the clinical endpoints (table 2).

MRI analysis. The pretreatment baseline activity period (weeks 0 to 4) was prolonged because of relapses in one valacyclovir recipient and seven placebo recipients ($p = 0.025$; Fisher's exact test). Eleven of 35 valacyclovir recipients and 14 of 27 placebo recipients with active lesions were free of relapses during the run-in period (not significant). During the treatment period (weeks 4 to 28), 210 of 216 planned scanning procedures were performed in the valacyclovir treatment group, whereas 201 of 204 planned scanning procedures were performed in the placebo treatment group. In the valacyclovir treatment group, 55% of the MRI procedures (111 scans) revealed active lesions compared with 47% (95 scans) in the placebo treatment group. The median number (Q_1, Q_3 range) of active lesions at week 0 was 1 (0, 4) in the valacyclovir group and 0 (0, 3) in the placebo group (not significant). At week 4, the median number (Q_1, Q_3 range) of new active lesions was 0 (0, 3) in the valacyclovir treatment group and 1 (0, 4) in the placebo treatment group (not significant).

Table 1 *Baseline clinical characteristics of patients in a study of the effect of valacyclovir treatment on MS*

Characteristics	Valacyclovir recipients (n = 36)	Placebo recipients (n = 34)	Total (n = 70)
Mean age, y (SD)	36.0 (9.7)	33.1 (7.2)	34.6 (8.7)
Sex, F/M	23/13	21/13	44/26
Mean height, cm (SD)	171.4 (9.2)	172.3 (8.2)	171 (8.7)
Mean body weight, kg (SD)	67.6 (12.3)	67.3 (13.1)	67.5 (12.6)
Mean no. of MS relapses in the last 2 y (SD)	2.6 (0.9)	2.7 (0.9)	2.6 (0.9)
Mean duration of MS, y (SD)	9.6 (6.3)	9.5 (5.8)	9.5 (6.0)
Median EDSS score (range)	2.5 (0–5)	3.0 (0–6)	2.5 (0–6)
Patients with EDSS score of >4, n (%)	5 (14)	2 (6)	7 (10)
Patients with MS relapses during weeks 0–4, n (%)	1 (3)*	7 (21)	8 (11)

* Fisher's exact test; $p = 0.0256$.

EDSS = Expanded Disability Status Scale.

During treatment, the mean number of new active lesions per scan ± SD per patient was 1.9 ± 2.9 in the valacyclovir treatment group and 2.6 ± 4.1 in the placebo treatment group ($p = 0.44$). The number of new active lesions in each patient during the 24 weeks of treatment is shown in figure 2. The primary endpoint (the median number of new active lesions in each patient) was seven in the valacyclovir treatment group[1,12] and three in the placebo treatment group.[1,18] The mean number of new active lesions ± SD was 11.9 ± 17.6 in the valacyclovir treatment group and 14.5 ± 21.4 in the placebo treatment group (figure 2). The discrepancy in the mean and median values for treatment effect indicates that patients with MS with high and low levels of disease activity might react differently.

Exploratory MRI analysis. Twenty-four of 36 valacyclovir recipients and 18 of 34 placebo recipients did not have active lesions at the end of the 4-week pretreatment baseline activity period. At the start of the study (week 0), the median number of active lesions in patients with high levels of MRI-evident disease activity who had more than one new active MRI-evident lesion was 4.5 in the valacyclovir treatment group and 5 in the placebo group ($p = 0.554$). Four weeks later at the start of treatment, the median number was 3.5 in the valacyclovir treatment group and 8 in the placebo treatment group ($p = 0.13$).

The study was designed to analyze the effect of baseline activity on the treatment effect. Table 3 shows the effect of baseline activity during the pretreatment period (weeks 0 to 4) on the formation of new active lesions. It appears that valacyclovir-treated patients with one or more lesions at baseline had fewer lesions than did placebo-treated patients according to four cutoffs used for baseline activity (see table 3).

Results of exploratory subgroup MRI analysis at baseline are shown in figure 3. The median number (Q_1, Q_3 range) of new lesions per 4 weeks during the treatment period in patients with more than one active MRI-evident lesion at baseline was 6.5 (2.63, 9.0) in the placebo treatment group (n = 11) and 2.0 (1.38, 3.96) in the valacyclovir treatment group (n = 17) ($p = 0.025$). For patients with high-level disease activity who had more than one active lesion during the baseline period, the proportion of scans without evidence of active lesions was 28% (27/96 scans) during valacyclovir treatment compared with 5% (4/76 scans) during placebo treatment ($p < 0.001$; χ^2 test).

Of the patients in the high-level disease activity subgroup who had relapses during the run-in period, five were randomized to placebo treatment, and one was randomized to valacyclovir treatment. Exclusion of all six patients produced similar results for the exploratory subgroup analysis of patients with more than one active MRI-evident lesion during the baseline activity period (median number of new lesions: valacyclovir treatment group, 2.1; placebo treatment group, 7.4).

Discussion. In this randomized, double-blind, placebo-controlled study of the effect of antiherpes treatment on MRI-evident lesions in patients with

Table 2 *Clinical endpoints in a study of the effect of valacyclovir on MS*

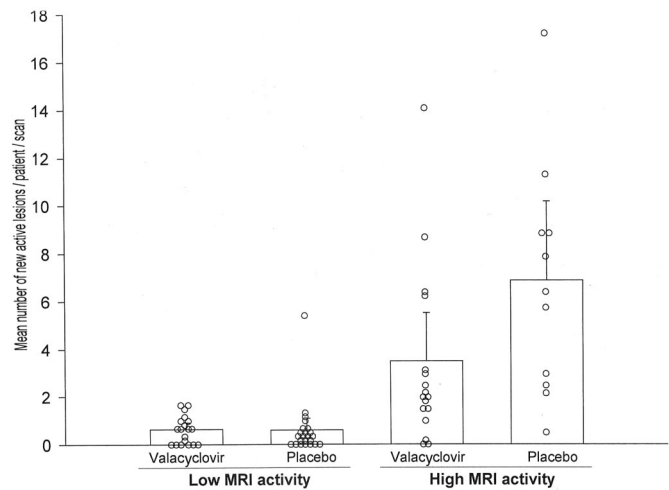| Assessment	Valacyclovir recipients (n = 36)	Placebo recipients (n = 34)	*p* Value
Disability (median difference)			
Change* in EDSS score	0.0 (−0.5 to 0.5)	0.0 (−0.5 to 0.0)	0.309
Change* in RFSS score	0.0 (−2.0 to 2.0)	0.0 (−3.0 to 2.0)	0.555
No. of relapses/no. of patients	27/20	22/17	0.821

* Median (Q_1, Q_3 range).

EDSS = Expanded Disability Status Scale; RFSS = Regional Functional Scoring System.
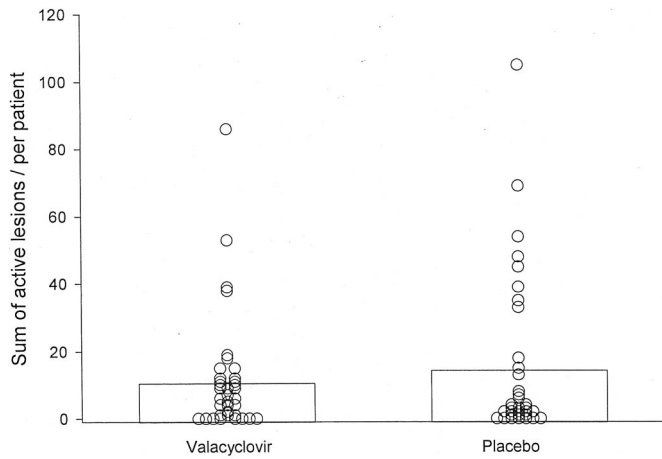
Figure 2. The number of new active MRI-evident lesions in patients with relapsing-remitting MS after 24 weeks of treatment with valacyclovir (n = 36) or placebo (n = 34). Column heights indicate mean values.

Figure 3. The number of active MRI-evident lesions in patients with relapsing-remitting MS after treatment with valacyclovir (n = 36) or placebo (n = 34). Patients are stratified into two groups: low MRI activity, one or fewer active MRI-evident lesions at baseline (valacyclovir: median, 0.67; mean ± SD, 0.63 ± 0.6; placebo: median, 0.33; mean ± SD, 0.59 ± 1.1); and high MRI activity, more than one active MRI-evident lesion at baseline (valacyclovir: median, 2.0; mean ± SD, 3.49 ± 3.8; placebo: median, 6.5; mean ± SD, 6.86 ± 4.9). Column heights and bars indicate mean values and SE, respectively.

MS, the effect of valacyclovir given during 24 weeks was evaluated primarily by monitoring the formation of active MRI-evident lesions in patients with RRMS. The power calculation, based on the effect of interferon-β on MRI-evident lesions in MS, anticipated a reduction by 80% of new active lesions.[19] Intravariability as well as intervariability of disease activity is pronounced in RRMS. Low-level disease activity at baseline correlated with low levels of MRI-evident disease activity during the study period, as expected, and high-level disease activity at baseline correlated with high levels of MRI-evident disease activity. Similar observations of variation in disease activity have been made in other studies.[18,20] Twenty percent of subjects in each treatment group did not develop new MRI-evident lesions over the entire study period. Consequently, stratification of patients with MS into groups with low and high levels of MRI-evident disease activity has recently been recommended for clinical trials of MS.[20]

Exploratory subgroup analysis of the effect of high-level MRI-evident disease activity on treatment response applied the pretreatment baseline activity for definition of activity level. The applied statistical

Table 3 *Number of new active MRI-evident lesions per scan during the treatment period (weeks 4–28) in valacyclovir and placebo recipients with more than 0, 1, 2, or 5 new active MRI-evident lesions during the pretreatment baseline period (weeks 0–4) in a study of the affect of valacyclovir on MS*

	No. of lesions		
Cutoff	Valacyclovir recipients (no. of patients)	Placebo recipients (no. of patients)	*p* Value
>0	1.67 (24)	2.75 (18)	0.186
>1	2.0 (17)	6.5 (11)	0.025
>2	1.88 (11)	3.0 (10)	0.073
>5	7.67 (4)	8.5 (6)	0.914

analysis generated a hypothesis and showed that the number of new active MRI-evident lesions in a subgroup of patients with high-level disease activity in the valacyclovir treatment group was reduced compared with that in the placebo treatment group. The 68% reduction in new lesion formation was significant (*p* = 0.025). In addition, a clinically relevant measure was the proportion of scans free of new activity. The increase in the number of scans free of new activity favored valacyclovir over placebo (5% vs 28%, respectively; *p* < 0.001).

No differences in rates of MS relapses and EDSS scores were observed during 24 weeks of treatment. Because most MS relapses are clinically silent and clinical scoring systems are relatively insensitive, we did not expect valacyclovir to have an impact on these clinical measures in this pilot trial with a short duration and a small number of patients.

In vitro studies demonstrated the following IC$_{50}$ (median inhibitory concentration) ranges for acyclovir: 0.1 to 3.0 μM, HSV; 1.6 to 5.1 μM, varicella-zoster virus; 6.0 to 7.0 μM, EBV; and 13.3 to 111.0 μM, HHV-6.[12,21,22] The expected mean serum level of acyclovir after administration of 1 gram of valacyclovir three times daily is about 15 μM.[14,23] Thus, HSV, varicella-zoster virus, and to a lesser extent EBV may have been suppressed during valacyclovir treatment in this study. Based on in vitro studies, suppression of thymidine kinase–deficient HHV-6 would not be expected during the regimen of 1 gram of valacyclovir three times daily that was used in our study. However, in clinical studies of cytomegalovi-

rus (CMV) infection (the *UL97* gene product of CMV phosphorylates acyclovir before inhibition of CMV DNA polymerase), 8 grams of valacyclovir daily effectively suppressed CMV reactivation and prevented disease outbreaks.[24] It can be speculated that a protein kinase similar to the *UL97* gene product of CMV[25] could account for in vivo susceptibility of HHV-6 to valacyclovir.

If reactivation of one or more herpesviruses is associated with the pathogenesis of MS, then viral reactivation may correlate with clinical disease activity. Indeed, recent data suggest a correlation between clinical disease activity and EBV reactivation in MS.[4] In this case, the valacyclovir dosage of 1 gram three times daily may not be optimal, because the inhibitory effect of acyclovir against EBV is only modest.

The observation that valacyclovir treatment in a subgroup of patients with high levels of MRI-evident disease activity was associated with impaired formation of new lesions and with an increase in the number of scans free of new active lesions warrants further study. In particular, the development of new drugs that are specific to individual members of the herpesvirus family is needed to test the hypothesis that herpesvirus infection plays a role in the pathogenesis of MS. It is possible that certain patients with MS with severe disease activity will benefit from treatment with valacyclovir. Further studies should define this subgroup and provide information on the potential clinical benefit of antiviral drugs in the overall management of MS.

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