Evaluation of Pharmacokinetics, Safety, Tolerance, and Activity of Combination of Zalcitabine and Zidovudine in Stable, Zidovudine-Treated Pediatric Patients with Human Immunodeficiency Virus Infection

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A double-blind phase II trial compared zalcitabine (0.03 mg/kg/day) in combination with zidovudine (720 mg/m²/day) and zidovudine monotherapy in 250 clinically stable, previously zidovudine-treated, human immunodeficiency virus–infected children. The combination was well-tolerated except for an increased incidence of neutropenia (14%) compared with that in children receiving monotherapy (5%). No differences were noted for time to first AIDS-defining illness or death, neuropsychologic status, or weight Z scores. In patients in the combination arm, the CD4 cell count decline was slower (13% per year) than in patients receiving monotherapy (25% per year) ($P = .03$), and quantitative peripheral blood mononuclear cell virus load remained lower at all time points ($P = .08$). Deaths were fewer in patients receiving combination therapy (4) compared with those in patients receiving monotherapy (10) ($P = .083$). Thus, administration of zidovudine with zalcitabine to children with prior zidovudine treatment did not result in a significant increase in toxicity compared with that resulting from zidovudine monotherapy and demonstrated improvement in immunologic and virologic surrogate markers.

Antiretroviral therapy with zidovudine has been shown to produce improvement in neurodevelopmental deficits, weight gain, and CD4 cell numbers in children with human immunodeficiency virus (HIV) infection [1, 2]. Until recently, zidovudine has been the standard first-line drug recommended for initiating antiretroviral therapy in children. The beneficial effects of zidovudine, however, are time-limited because of frequent emergence of viral resistance to the drug [3]. Children receiving zidovudine monotherapy whose HIV isolates developed in vitro resistance to zidovudine showed greater clinical deterioration compared with children whose virus isolates retained zidovudine susceptibility [4, 5]. Another major limitation of zidovudine has been its hematologic toxicity, which requires frequent monitoring and dose modifications.

Alternative approaches for primary treatment of HIV infection involving combinations of antiretroviral drugs are currently being explored, with the goal of minimizing drug toxicities and maximizing antiretroviral activities. AIDS Clinical Trials Group (ACTG) protocol 152, a study to evaluate zidovudine in combination with didanosine in symptomatic, previously untreated HIV-infected children, showed that both didanosine monotherapy and zidovudine plus didanosine combination therapy had better safety and efficacy than did zidovudine monotherapy and did not significantly differ from each other [6]. The present study was not an efficacy study and was designed to evaluate zidovudine in combination with zalcitabine (dideoxycytidine) in clinically stable children who had previously been treated with zidovudine. Zalcitabine, a nucleoside analogue reverse transcriptase inhibitor, is highly effective in decreasing HIV-1 replication and appears to have additive, synergistic, or both effects in vitro when tested in combination with zidovudine [7, 8]. In adults, the use of zalcitabine in combination with zidovudine has shown promising results in slowing immunologic decline and reducing virus burden [9–11]. Such benefits were noted for zidovudine-naïve
adults [9, 10] and a subset of those previously treated for 
≥6 months [11]. A more recent study [10] indicated that the
sustained decline in plasma HIV RNA copies was best achieved 
with a zidovudine plus zalcitabine combination rather than with 
either zidovudine monotherapy or zidovudine plus didanosine.
These studies suggest that the combination of zidovudine and 
zalcitabine may be useful for treating HIV-infected adults, 
particularly those who have not received previous antiretroviral 
therapy.

In children, experience with use of zalcitabine has been lim-
ited. In an earlier pilot study, zalcitabine was found to be safe 
but not associated with significant antiretroviral activity 
[12]. A phase II study of monotherapy in children with ad-
vanced HIV disease found zalcitabine to be safe and associated 
with decline in p24 antigen level at 12 weeks compared with 
that at baseline [12a]. The present study compared the safety, 
tolerance, and pharmacokinetics of zidovudine monotherapy 
with the combination of zidovudine plus zalcitabine and was 
performed in zidovudine-exposed children.

Methods

Study Design and Entry Criteria

ACTG 190 was a randomized, double-blind study of zidovudine 
monotherapy versus a combination of zidovudine and zalcitabine 
in HIV-infected children and was conducted at 50 clinical centers 
across the United States and Puerto Rico. Children between 3 
months and 12 years of age who had laboratory-confirmed HIV 
infection and whose condition had remained stable while the child 
received a zidovudine dose of ≥120 mg/m² every 6 h or 480 
mg/m²/day for ≥6 weeks were eligible for the study. The study 
patients were stratified at entry according to whether the duration 
of prior zidovudine therapy was ≤52 or >52 weeks.

Children were excluded if they had history of intolerance to 
zidovudine or toxicity resulting from zidovudine or of disease 
progression while being treated with zidovudine. Other exclusion 
criteria included allergy to zalcitabine, intractable vomiting attrib-
uted to zidovudine, or laboratory abnormalities within 2 weeks 
before enrollment consisting of total bilirubin >3 times the upper 
limit of normal, serum aspartate aminotransferase concentration
>10 times the upper limit of normal, serum creatinine >1.5 
mg/dL, white blood cell count of <1500 cells/mm³, absolute neu-
rophil count of <750 cells/mm³, hematocrit <24% (or hemoglo-
bin <8 g/dL), and pancreatic amylase >2 times the upper limit 
of normal. Children were excluded if they had clinical myositis, 
history of symptomatic pancreatitis, peripheral neuropathy, abnor-
mal nerve conduction velocity, presence of cardiomypathy, or 
active malignancy requiring chemotherapy.

Pubertal females were required to undergo a serum test to ex-
clude pregnancy. Treatment with intravenous or intramuscular 
immunoglobulin, hyperalimentation, dietary supplements, and hema-
topoietic agents including granulocyte colony-stimulating factor 
or erythropoietin were permitted. Age-appropriate immunizations 
and Pneumocystis carinii pneumonia prophylaxis, given in accor-
dance with the then current US Public Health Service guidelines 
[13], were strongly recommended in all children. Use of intrave-
nous pentamidine was permitted on a case-by-case basis with fre-
quent monitoring of serum amylase concentrations. Use of drugs 
with potential to cause nephrotoxicity, hepatotoxicity, bone mar-
row toxicity, or peripheral neuropathy were not permitted, except 
under circumstances requiring approval by the study chair. Antiret-
roviral agents other than zidovudine were not permitted within 6 
weeks of start of treatment. Abnormal clinical events and labora-
tory values were graded from 1 to 4 for management of toxicities 
arising from the use of study medications.

Treatment Protocol

Children were assigned in double-blind fashion to receive either 
zidovudine (Burroughs Wellcome, Research Triangle Park, NC; 
currently Glaxo-Wellcome) at 240 mg/m²/dose and zalcitabine 
(Hoffmann-LaRoche, Nutley, NJ) at 0.01 mg/kg/dose or zidovu-
dine at 240 mg/m²/dose and a zalcitabine placebo. Both medica-
tions and placebo were given orally every 8 h in liquid form. Each 
child was evaluated by medical history and physical examinations 
before, at the time of protocol entry, and every 28 days thereafter.
At these visits, subjects were evaluated for intercurrent illness, 
medications, and hospitalizations. Blood was drawn for complete 
blood cell count, chemistry determinations, and periodic immuno-
logic and virologic evaluations. Neurologic evaluation was per-
formed at enrollment and every 8 weeks subsequently. Routine 
testing for nerve conduction velocity for children 3–36 months of 
age was optional by site but was strongly recommended for all 
children who developed clinical signs or symptoms of peripheral 
neuropathy during the study. Chest radiograph, computed tomogra-
phy or magnetic resonance imaging of the brain, retinal examina-
tion, and echocardiogram were obtained at study enrollment and 
at the end of the study. Neuropsychologic evaluation was done at 
entry and at end of study for all children and during the study at 
16-week intervals for children aged 3–30 months and every 32 
weeks for children aged >30 months. The battery of neuropsycho-
logic tests administered depended on the age of the child. For this 
report we have restricted analysis to the mental index for the 
Bayley scales of infant development (age 3–30 months) [14], the 
general cognitive index for the McCarthy scales of children’s abili-
ties (age 30 months to 6 years) [15], and the full-scaled score for the 
Wechsler intelligence score for children—revised (age 6–16 
years) [16].

Population pharmacokinetic studies were done for all children. 
The first 10 children in each arm had timed pharmacokinetic stud-
ies after supervised drug administration.

Definition of Primary and Secondary Outcomes

The primary objectives of the study were to determine pharma-
cokinetics, safety, and tolerance of zidovudine and zalcitabine 
when given in combination to clinically stable, zidovudine-treated 
children. Secondary objectives were to compare the two treatment 
arms for effect on disease progression as determined by clinical, 
virologic, and immunologic determinations. The sample size of 
this study was not intended to have adequate power to compare 
efficacy of the two treatment arms and had an 80% chance of 
detecting a ≥4-fold decrease in the clinical event rate. Such a
beginning with 10 patient peripheral blood mononuclear cells arm) or duplicate in a 24-well tissue culture plate using six 5-fold dilutions, globulin (43 in the zidovudine arm and 51 in the combination of virus load as determined by quantitative HIV cell culture in study. There was no significant difference in use of immuno- tact. Virologic measure of treatment effect was based on evaluation ventricular dysfunction that did not disqualify entry into the confirmation, clinical records, death certificate, and physician con-been included in the analysis. One other child had minimal left growth velocity for age and sex at greater than the third percentile dine plus zalcitabine therapy and 123 patients receiving zidovu-

Failure to thrive was defined as failure to gain weight at a weight 1992 and February 1994, with 127 patients receiving zidovu-

Statistical Methods

Baseline characteristics were compared between treatment arms using the Wilcoxon rank sum test for nongrouped variables and \( \chi^2 \) tests for grouped variables. The comparison of the time to level 3 or 4 toxicity by treatment was the primary statistical analysis. Cox models were used to compare the time to level 3 or 4 toxicity by treatment controlling for baseline (log) CD4 cell count [22]. The frequency of severe and life-threatening toxicity was compared using an exact Kruskal-Wallis test [23]. Time to event vari- ables, including time to disease progression and time to level 3 or 4 toxicities, were compared between treatments using the log-rank test. Longitudinal changes in CD4 cell counts were analyzed using a mixed model analysis of variance for this analysis [24]; CD4 cell counts were transformed by use of the logarithm, and the transformed value was used as the dependent variable. We assumed that the log CD4 cell count for each patient had a linear trajectory with a random intercept and slope. We also assumed that the mean intercept and the mean slope depended on the treatment. Thus, the fixed effects in the model were treatment (intercept), time (mean slope), and time \( \times \) treatment (difference in mean slopes by treat- ment) and baseline age (in years). The random effect was patient, and time \( \times \) patient. We also tested for an interaction between time, treatment, and prior zidovudine use. Changes in log CD4 cell counts are interpreted as percentage of change, which they closely approximate. Since changes in weight and neuropsychologic measures over time were not linear, they were analyzed using a Wei-Johnson test [25]. Weights were transformed into an age-standardized Z score using Epi Info, version 5 (CDC, Atlanta). The effects of stratification factors on weight changes were analyzed similarly.

Results

Study Population

A total of 250 patients were enrolled between 4 December 1992 and February 1994, with 127 patients receiving zidovu-

The frequency of severe and life-threatening toxicity was compared using an exact Kruskal-Wallis test [23]. Time to event vari- ables, including time to disease progression and time to level 3 or 4 toxicities, were compared between treatments using the log-rank test. Longitudinal changes in CD4 cell counts were analyzed using a mixed model analysis of variance for this analysis [24]; CD4 cell counts were transformed by use of the logarithm, and the transformed value was used as the dependent variable. We assumed that the log CD4 cell count for each patient had a linear trajectory with a random intercept and slope. We also assumed that the mean intercept and the mean slope depended on the treatment. Thus, the fixed effects in the model were treatment (intercept), time (mean slope), and time \( \times \) treatment (difference in mean slopes by treat- ment) and baseline age (in years). The random effect was patient, and time \( \times \) patient. We also tested for an interaction between time, treatment, and prior zidovudine use. Changes in log CD4 cell counts are interpreted as percentage of change, which they closely approximate. Since changes in weight and neuropsychologic measures over time were not linear, they were analyzed using a Wei-Johnson test [25]. Weights were transformed into an age-standardized Z score using Epi Info, version 5 (CDC, Atlanta). The effects of stratification factors on weight changes were analyzed similarly.

Safety and tolerance. For assessing laboratory toxicity, hema-
tology and chemistry values were graded in levels from 0 to 4. These levels were used for all analyses of laboratory-measured parameters and were equivalent to the grades used for adverse experiences reporting requirements in most cases. For hemoglobin, neutrophils, aspartate aminotransferase, bilirubin, total amylase, lipase, blood urea nitrogen, and creatinine, the levels corresponded to those used for dose modification purposes in this study. Time to first level 3 or 4 toxicity was evaluated. Baseline abnormalities that persisted during follow-up were not counted as drug-related toxicities. Patients who progressed from level 3 at baseline to level 4 at follow-up, and patients who for the first time developed a level 3 or 4 toxicity after treatment dispensation, were counted as new toxicities.

Disease progression. Clinical criteria of disease progression were development of opportunistic infection, failure to thrive, neuropsychologic or neurologic deterioration (or both), or death. Failure to thrive was defined as failure to gain weight at a weight growth velocity for age and sex at greater than the third percentile on the growth velocity charts [19] after the first 24 weeks of study and was confirmed by repeat measurement after 1 month. Failure to thrive was attributed to disease progression only if nutritional intervention was unsuccessful for the child and if the child had no intercurrent illness responsible for the poor weight gain. Neuropsychologic deterioration was based on a drop of \( \geq 1 \) standard deviation within the same test instrument at a scheduled testing period. Sources of information for causes of deaths included pathologic confirmation, clinical records, death certificate, and physician contact. Virologic measure of treatment effect was based on evaluation of virus load as determined by quantitative HIV cell culture in duplicate in a 24-well tissue culture plate using six 5-fold dilutions, beginning with \( 10^6 \) patient peripheral blood mononuclear cells (PBMC) [20]. Each dilution of patient cells was cocultured with \( 10^6 \) phytohemagglutinin-stimulated HIV-seronegative donor PMBC for 14 days. The supernatant from each coculture was tested for the expression of HIV-1 p24 antigen by the standard HIV p24 EIA. Immunologic response was measured by change in CD4 T cell count. The virologic and immunologic tests were performed at ACTG-certified laboratories [20, 21].
Table 1. Selected baseline characteristics of study population.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>Zidovudine plus zalcitabine</th>
<th>Zidovudine</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. enrolled</td>
<td>250</td>
<td>127</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>5.18</td>
<td>4.68</td>
<td>5.65</td>
<td>.16</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.37 ± 3.23</td>
<td>5.08 ± 3.25</td>
<td>5.67 ± 3.21</td>
<td></td>
</tr>
<tr>
<td>Follow-up, weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median length</td>
<td>66.29</td>
<td>67</td>
<td>66.14</td>
<td></td>
</tr>
<tr>
<td>Mean length ± SD</td>
<td>61.16 ± 21.95</td>
<td>62.3 ± 22.06</td>
<td>60 ± 21.87</td>
<td></td>
</tr>
<tr>
<td>Sex, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td>.9090</td>
</tr>
<tr>
<td>Male</td>
<td>119 (48)</td>
<td>60 (47)</td>
<td>59 (48)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>131 (52)</td>
<td>67 (53)</td>
<td>64 (52)</td>
<td></td>
</tr>
<tr>
<td>Race, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>33 (13)</td>
<td>19 (15)</td>
<td>14 (11)</td>
<td>.4780</td>
</tr>
<tr>
<td>Black</td>
<td>109 (44)</td>
<td>55 (43)</td>
<td>54 (44)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>101 (40)</td>
<td>50 (39)</td>
<td>51 (41)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>1 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alaskan</td>
<td>1</td>
<td>1 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 (2)</td>
<td>1 (1)</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td>Previous zidovudine use, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤52 weeks</td>
<td>95 (38)</td>
<td>50 (39)</td>
<td>45 (37)</td>
<td>.6500</td>
</tr>
<tr>
<td>&gt;52 weeks</td>
<td>155 (62)</td>
<td>77 (61)</td>
<td>78 (63)</td>
<td></td>
</tr>
<tr>
<td>CD4 cells/mm³</td>
<td></td>
<td></td>
<td></td>
<td>.03</td>
</tr>
<tr>
<td>Median</td>
<td>655.25</td>
<td>697.5</td>
<td>607</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>778.7</td>
<td>870.56</td>
<td>683.85</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>617.27</td>
<td>700.3</td>
<td>503.34</td>
<td></td>
</tr>
</tbody>
</table>

698/mm³, whereas patients receiving zidovudine monotherapy had a median CD4 cell count of 607/mm³ (P = .03).

Pharmacokinetic Results

Overall, 1034 measurements of zidovudine and 518 of zalcitabine serum levels are included in this evaluation. The mean peak concentration for zalcitabine was 0.027 ± 0.020 µmol/L and occurred most frequently (56%) at the collection time of 1.5–2.5 h after dosing. The zidovudine peak concentration averaged 7.18 ± 3.93 µmol/L and occurred most frequently (90%) at the collection time of 0.5–1 h after dosing. The population AUCs for zalcitabine and zidovudine were 0.11 ± 0.04 µmol/h/L and 13.3 ± 5.4 µmol/h/L, respectively. Zidovudine levels were similar in the treatment groups, suggesting that zalcitabine did not alter zidovudine pharmacokinetics. Age did not appear to affect the pharmacokinetics of zidovudine or zalcitabine once the child’s weight or body surface area had been taken into account. For patients with more intensive pharmacokinetic evaluation, peak zalcitabine levels at week 4 were 1.02 times the levels seen after the first study dose, demonstrating no significant drug accumulation with repeated doses. Population pharmacokinetic analysis also indicated that zalcitabine apparent clearance (and AUC) were similar after the first and multiple doses.

Toxicsity

Treatment groups showed no difference in time to first biochemical or hematologic toxicity (any) (P = .47). The percentage of patients who experienced biochemical or hematologic toxicities of levels 3 and 4 were, respectively, 0.12 and 0.07 for patients receiving zidovudine and 0.12 and 0.1 for patients receiving the combination. Table 2 summarizes results for all patients who developed level 3 or 4 toxicities after treatment dispensation. There was no significant difference between treatment groups in the time to first level 3 or 4 biochemical toxicity after treatment dispensation (P = .22). However, there was a significant difference between treatment groups in time to first hematologic toxicity (P = .049); this was largely due to more frequent development of neutropenia in patients receiving combination therapy. Eighteen patients receiving combination therapy and 5 receiving monotherapy experienced level 3 or 4 neutropenia. The difference in time to development of neutropenia was also significant (P = .008) and remained significant (P = .006) when controlling for whether or not the patient was receiving trimethoprim-sulfamethoxazole, a drug known to be associated with bone marrow suppression and neutropenia. The time to first grade 3 or 4 clinical toxicities was not significantly different between treatments (P = .59). The percentages of patients experiencing level 3 or 4 clinical toxicities in the zidovudine arm were 0.14 and 0.03 and in the combination arm...
Table 2. Number of patients experiencing level 3 or 4 toxicities for first time after dispensation of protocol medications.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Zidovudine plus zalcitabine combination</th>
<th>Zidovudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>127</td>
<td>123</td>
</tr>
<tr>
<td>Abnormal laboratory value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase (&gt;2.0 ULN)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Lipase (&gt;2.5 ULN)</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Bilirubin (&gt;3.0 ULN)</td>
<td>1 (1)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Alanine aminotransferase (&gt;10 ULN)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Aspartate amino transferase (&gt;5.0 ULN)</td>
<td>3 (3)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>Alkaline phosphatase (&gt;2.0 ULN)</td>
<td>10 (2)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Creatinine (&gt;1.2 mg/dL)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Calcium (12.0 mg/dL)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>CPK (&gt;2 ULN)</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin (&lt;7.0 g/dL)</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Neutrophils (&lt;400/mm³)</td>
<td>18 (13)</td>
<td>5 (5)*</td>
</tr>
<tr>
<td>Platelets (&lt;50,000/mm³)</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Increased MCV (&gt;100)</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Clinical toxicities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatitis</td>
<td>1 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>0</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Irritability/mental status change</td>
<td>3 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>2 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Limb pain</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>2 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Cough</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (no. requiring dose modification). Parentheses in first column indicate value constituting at least level 3 toxicity. ULN, times upper limit of normal; CPK, creatinine phosphokinase; MCV, mean corpuscular volume.

* P = .049 vs. group receiving combination therapy.

were 0.13 and 0.008, respectively. Toxicities requiring dose reduction for the first time are shown in table 2. Dose reduction depended on the type of drug toxicity, and therefore, dose reduction was independent and not performed concurrently for both study drugs in the combination therapy arm. There was no significant difference between treatments in time to first dose-modifying toxicity (P = .11).

Clinical Criteria for Disease Progression

Survival. As of 30 January 1995, 14 patients had died (table 3). Four of these were in the combination therapy arm (zidovudine plus zalcitabine) and 10 were in the zidovudine monotherapy arm (log-rank test, P = .083). For children who died, the duration of study drug treatment varied between 2 and 15 months (median, 7 months) for children in the zidovudine monotherapy arm and 1 and 13 months (median, 2.5 months) for children in the combination arm. Only 5 children were receiving study drugs at the time of their deaths (2 in the combination arm and 3 in the monotherapy arm). There was also no difference in the two treatments noted when the model (Cox proportional hazards [22]) included the baseline log CD4 cell values (P = .23).

Three deaths in patients in the zidovudine monotherapy arm were attributed to hepatic failure. In 2 children, there was evidence of chronic hepatitis, and findings were consistent with cytomegalovirus hepatitis in 1. Liver biopsy in the third child showed fatty infiltration without active hepatitis.

The 2 children who had baseline cardiac involvement on study entry had worsening of cardiomyopathy while receiving study drugs (both were randomized to the zidovudine plus zalcitabine combination).

Table 3. Patient deaths in study of combination therapy versus zidovudine monotherapy in children.

<table>
<thead>
<tr>
<th>Treatment group, no.</th>
<th>Age (years), sex</th>
<th>Week</th>
<th>Primary cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine monotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1*</td>
<td>2.1, F</td>
<td>62</td>
<td>Chronic diarrhea, electrolyte imbalance</td>
</tr>
<tr>
<td>2'</td>
<td>1.2, F</td>
<td>22</td>
<td>Respiratory arrest at home, cardiomyopathy</td>
</tr>
<tr>
<td>3*</td>
<td>5.4, M</td>
<td>48</td>
<td>Respiratory and renal failure</td>
</tr>
<tr>
<td>4'</td>
<td>0.4, F</td>
<td>24</td>
<td>Chronic hepatitis, liver failure</td>
</tr>
<tr>
<td>5'</td>
<td>2.8, F</td>
<td>48</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>6'</td>
<td>1.3, F</td>
<td>14</td>
<td>Hepatitis, gastrointestinal bleeding</td>
</tr>
<tr>
<td>7*</td>
<td>8.6, F</td>
<td>16</td>
<td>Fatty degeneration of liver, liver failure</td>
</tr>
<tr>
<td>8'</td>
<td>6.3, F</td>
<td>69</td>
<td>Varicella zoster virus encephalopathy, failure to thrive</td>
</tr>
<tr>
<td>9'</td>
<td>6.8, F</td>
<td>52</td>
<td>Sepsis</td>
</tr>
<tr>
<td>10*</td>
<td>2.3, M</td>
<td>45</td>
<td>Pneumocystis carinii pneumonia (presumptive), adult respiratory distress syndrome</td>
</tr>
<tr>
<td>Zidovudine plus zalcitabine combination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11*</td>
<td>1.4, F</td>
<td>36</td>
<td>Severe cardiomyopathy, respiratory distress</td>
</tr>
<tr>
<td>12'</td>
<td>1.2, M</td>
<td>11</td>
<td>Pneumonia, respiratory failure</td>
</tr>
<tr>
<td>13'</td>
<td>1.8, M</td>
<td>54</td>
<td>Encephalopathy, progressive disseminated cytomegalovirus infection (eyes)</td>
</tr>
<tr>
<td>14'</td>
<td>4.3, M</td>
<td>58</td>
<td>Sepsis, streptococcal pneumonia, splenectomy</td>
</tr>
</tbody>
</table>

* Enrolled in study, not receiving protocol treatment.
1 Enrolled in study, receiving protocol treatment.
2 Removed from study.
zalcitabine combination). One child died, and cardiomyopathy probably contributed to it.

**Opportunistic and serious infections.** By use of the criteria defined by the CDC and the NIAID Opportunistic Infection Committee for AIDS-defining opportunistic infections and HIV-related diagnoses [26], a total of 9 patients (3 receiving zidovudine plus zalcitabine and 6 receiving zidovudine) had a new and serious opportunistic infection (table 4). The time to first serious opportunistic infection or death is shown in figure 1, and the log-rank test indicates no significant difference between treatment arms (13 vs. 7 events, $P = .16$).

**Physical growth.** For this report, the analysis of physical growth and development was focused only on weight data. Figure 2 shows mean change in weight Z score from baseline by treatment. There was no significant difference (Wei-Johnson, $P = .61$). There was an appearance of improved weight Z score in patients in the combination arm after week 64 of therapy, but the numbers are too small to make definitive conclusions. The decrease in sample sizes in the later weeks is mostly due to differences in dates of patient entry into the study.

**Neurodevelopmental status.** A total of 237 patients had valid neuropsychologic assessment at baseline. The Bayley scaled mental index and the McCarthy cognitive scaled index are directly comparable, and hence these cohorts of patients were pooled together. There was not a significant difference between changes in the Bayley mental index and McCarthy general cognitive index when the Wei-Johnson test was used ($P = .19$), nor were there differences in the Wechsler intelligence score for children full scale score ($P = .41$). The children enrolled in the study showed remarkable stability in neurodevelopmental status, and no child met the study end point on the basis of decline in developmental scores.

**Table 4.** Number of patients who experienced new opportunistic infection or AIDS-defining illness for first time after dispensation of protocol treatment.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Zidovudine plus zalcitabine combination</th>
<th>Zidovudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumocystis carinii pneumonia</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cryptosporidium infection</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Candidiasis (esophageal)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mycobacterium avium-intracellularie infection (colonic biopsy)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mycobacterium gordonae infection</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cytomegalovirus (colitis)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Herpes zoster virus encephalopathy</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>HIV wasting syndrome</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

**Figure 1.** Probability of development of AIDS-defining illness or death in patients receiving zidovudine alone (ZDV) or combined zalcitabine and zidovudine therapy (ZDV/ddC). $P = .18$, log-rank comparison between treatments.

**Laboratory Markers for Disease Progression**

For study of drug activity, the following parameters were examined: CD4 cell count, serum p24 antigen level, and quantitative viral cultures in PBMC.

**CD4 cell count.** Figure 3 shows change in mean log CD4 cell count against time enrolled in the study. The log transformation normalizes the CD4 cell count distribution. The rate of decline in CD4 cell counts was significantly slower in patients randomized to zidovudine plus zalcitabine compared with the rate of decline in those randomized to zidovudine ($P = .03$). The parameters used to estimate this decline can be interpreted as the rate of change in CD4 cells expressed as a percentage of the original value. The rate of decline was 13% per year for patients receiving the drug combination compared with 25% per year for patients receiving the single agent.

**Quantitative HIV-1 culture.** Analysis of virologic data was restricted to 205 patients for whom baseline quantitative viral culture data were available. The mean change in log10 infectious units per million (IUPM) was compared between arms. Tests were conducted to see if there was a difference in treatment results for patients stratified by duration of previous ongoing
zidovudine therapy of >6 weeks but ≤52 weeks versus >52 weeks.

The results of virologic data showed that while log_{10} IUPM at study entry was similar in patients in the combination therapy and the monotherapy arms, a greater decline was observed in patients in the combination therapy arm (figure 4). At all time points, the virus load was lower in patients in the combination therapy arm, although statistical significance was not achieved (Wei-Johnson test for change in log_{10} IUPM over time by treatment arm, \( P = .08 \)). There was no difference in virologic response to combination therapy on the basis of length of prior zidovudine therapy.

**Duration of Prior Zidovudine Treatment**

The mixed model approach was used to test whether the stratification factor of duration of prior zidovudine treatment (≤52 or >52 weeks) affected the results. This effect (time \( \times \) treatment \( \times \) stratification factor) was significant for mean log changes in CD4 cell counts but not for weight Z score. Among patients in the ≤52-weeks stratum, the yearly decrease in CD4 cell counts was 34.8% for patients receiving zidovudine and 10.2% for patients receiving combination therapy, while for patients in the >52-weeks stratum, the rates were 19.5% and 14.6%, respectively. This interaction between prior zidovudine use and the effect of treatment was significant (\( P = .011 \)).

**Discussion**

This is the first study to evaluate safety and toxicity of zalcitabine given in combination with zidovudine in HIV-infected children. The results indicate that the combination of zidovudine and zalcitabine is safe and lacking in significant toxicity. In comparison to zidovudine monotherapy, the combination therapy was associated with a significant slowing in CD4 cell decline. It should be noted, however, that patients receiving combination therapy had slightly but significantly higher baseline CD4 cell counts. Although unlikely, the possibility exists that this difference influenced the slope of CD4 cell decline. The study population had been previously treated with zidovudine for variable periods. The effect of study therapy on the decline in CD4 cell count was better in the group
who were receiving combination therapy and who had \( \leq 52 \) weeks of prior zidovudine therapy. The CD4 cell counts in the two treatment arms, when analyzed on the basis of duration of prior antiretroviral therapies at baseline, were not significantly different. Collectively, the findings suggest that combination treatment was associated with a treatment benefit in terms of CD4 cell decline.

In the present study, the peak levels of zidovudine and zalcitabine were greater than the levels associated with in vitro HIV-1 suppression [27]. Results from this preliminary analysis of population pharmacokinetics for zalcitabine are consistent with data from previous studies in children [12, 28]. The observed mean peak concentration of 0.027 \( \mu \text{mol/L} \) was lower than the concentration of 0.056 \( \mu \text{mol/L} \) expected on the basis of previous published data [12] but was higher than that (0.022 \( \mu \text{mol/L} \)) reported by Chadwick et al. [28]. Population AUC for zalcitabine was somewhat higher than previously reported in children but lower than that in adults [12, 28, 29]. These differences likely stem from differences in study design and pharmacokinetic methodologies.

Zidovudine in the present study was used at a relatively high dose (720 mg/m\(^2\)/day) administered three times daily; this dosage was based on several adult studies that have shown treatment effect on p24 antigen levels and CD4 cell counts with thrice-daily dosing. The observed peak concentrations of zidovudine were somewhat lower than expected [30], but this could have resulted from infrequent sampling, such that possibly the “true” peak value was missed. The mean population AUC of zidovudine was close to the predicted value [30], suggesting that the change in the dose regimen did not affect zidovudine pharmacokinetics. Simulation with the mean pharmacokinetic parameters indicated that switching dosing from every 6 to every 8 h increased the percentage of time zidovudine levels were below 1 \( \mu \text{mol/L} \) from \( \approx 30\% \) to 40\%. The low frequency (<5%) of multiple nondetectable drug level measurements in individual patients suggests that the patients were compliant with their drug regimens.

A major goal in developing combination therapies is to reduce toxicity associated with each drug and to improve antiretroviral activity. Zidovudine and zalcitabine each have distinct
toxicity profiles and clearance mechanisms. Unlike zidovudine, which is glucuronidated by the liver and kidneys, zalcitabine is cleared mainly by the kidneys, with 75% of the parent drug being recovered in the urine obtained [31, 32]. In adults with advanced HIV disease and no prior zidovudine experience, zalcitabine has been well-tolerated when used in combination with different doses of zidovudine [9].

In children, although prior experience has been limited, zalcitabine has not been associated with major toxicities. In adults, the major dose-limiting toxicity of zalcitabine is a painful sensorimotor peripheral neuropathy; this toxic effect occurs at doses of ≥0.06 mg/kg/day in adults and is noted at 8–14 weeks after treatment initiation [33]. In the present study, only 2 children were diagnosed with peripheral neuropathy (grades 3 and 4); both were receiving the combination of zidovudine and zalcitabine and both recovered after temporary treatment discontinuation in 1 child and dose reduction in the other. The experience with the use of zalcitabine in two other studies in children was similar [12, 12a]. In an adult study similar in design to ours, the frequency of severe peripheral neuropathy was not different in any of the treatment groups of zidovudine, zalcitabine, or zidovudine plus zalcitabine, although moderate or worse neuropathy was more commonly seen in patients receiving zalcitabine [11]. We conclude that at the dosage of zalcitabine used in this study, the occurrence of clinically significant peripheral neuropathy is infrequent.

Hematologic toxicity has been a major dose-limiting toxicity for zidovudine; however, concurrent use of granulocyte colony-stimulating factor has enhanced our ability to treat children with potentially myelosuppressive drugs, including zidovudine [34]. Zalcitabine has not been shown to be myelosuppressive in previous studies. In the present study, an increased incidence of neutropenia was noted in patients in the combination arm, although no child was required to have study drugs discontinued permanently, and the toxicity could be controlled by temporary suspension of study drugs and reintroduction at a reduced dose. Other common side effects attributed to zalcitabine include skin rashes and stomatitis, but these effects were not seen in any of the children in the present study.

Figure 4. Mean change in virus load in patients receiving combination therapy with zidovudine plus zalcitabine (ZDV/ddc) and in patients receiving zidovudine monotherapy (ZDV). Only marginal difference between treatments (trt) was seen (P = .08). Vertical bars represent SEs.
One child died of liver failure in the absence of evidence of active hepatitis on histopathologic examination. This child was randomized to zidovudine monotherapy but had not been receiving therapy for 2 weeks at the time of death. These findings are of interest because of reports of HIV-infected adults whose condition is relatively stable and who developed sudden severe hepatomegaly and macrovesicular steatosis along with metabolic acidosis [35]. It has been speculated that these findings in adults might be related to antiretroviral therapy, HIV, or another as-yet- unidentified agent.

Two children who were randomized to combination therapy had underlying cardiac disease at entry, with subsequent worsening of their cardiac function during the study; cardiomyopathy was considered to be the immediate cause of death in 1 child. Both children discontinued study drugs with worsening of cardiac disease, and the child who died had not been receiving therapy for 18 weeks. The role of antiretroviral therapy in both cases is unclear; the contribution of either drug to the development of cardiac abnormality must remain speculative, as the study was not placebo-controlled and the underlying etiology of the cardiac problem was not established. Cardiac involvement in HIV-infected children may result from underlying infection with HIV, another infection, cardiotoxic drugs, or nutritional deficiencies. Zidovudine may cause mitochondrial damage, and skeletal myopathy and has been associated with cardiomyopathy in children [36]. In adults, cardiac dysfunction has been described with the use of zidovudine, didanosine, and zalcitabine [37], with resolution of abnormalities upon discontinuation of drugs. It is possible that continued zidovudine therapy in these children with some underlying cardiac dysfunction was associated with worsening cardiac disease; the role of zalcitabine is unclear. In children who develop cardiomyopathy, discontinuation of zidovudine and use of alternative therapies should be considered.

An important consideration in drug treatment trials is whether either drug influences the development of resistance to the other drug and what effect it has on virus burden as well as on CD4 cell counts. In the present study, the rate of decline in CD4 cell counts of 13% for patients randomized to the zidovudine plus zalcitabine arm was significantly different from the 25% decline shown for patients randomized to the zidovudine arm (P = .03). The virus load measurement showed that the children receiving combination therapy showed a greater magnitude of decline starting from within the first 10 weeks of therapy. This difference was maintained throughout the treatment course, although it did not achieve statistical significance. Since CD4 cell count and virus load are major prognostic indicators in HIV infection, it is reasonable to conclude that combination therapy with zalcitabine offers advantages over zidovudine monotherapy both with respect to preservation of CD4 cell counts and reducing virus burden. It must be pointed out, however, that the children receiving the combination therapy started out with a significantly higher baseline CD4 cell count, which could be partially responsible for the better immunologic and virologic response in this group. Failure to achieve statistical significance for virus load reduction may be attributed to the nature of the study population, which was clinically stable and zidovudine-experienced at the start of therapy. This conclusion is supported by the observation made in adult studies that virus load reduction was more pronounced in zidovudine-naive subjects treated with zidovudine plus zalcitabine than in zidovudine-experienced patients [38].

The clinical activity of the combination therapy, as judged by the effect on measures such as the weight Z score or the neurodevelopmental score, showed that there was no difference in weight (P = .61) or neurodevelopmental status between patients in either treatment arm. This was not unexpected, as children enrolled into the study were carefully chosen as being stable while receiving zidovudine for varying lengths of time, as judged by attainment of optimum weight and neurodevelopmental status. Thus, this was a select group of patients who were unlikely to manifest significant gains in areas of weight or neurodevelopment.

The present study was not designed to examine efficacy. On the basis of end points of survival and opportunistic infections, the trial would have had to be continued for 5 years (versus 52 weeks) and to have accrued 1000 patients (versus 250 patients in the present study) in order to detect a hazard ratio of 1.7. It is to be noted, however, that even in this limited study, more deaths occurred in the zidovudine monotherapy arm (10) than in the combination arm (4). The incidence of AIDS-defining illnesses in patients in the combination arm was also half that in patients in the monotherapy arm, although the difference in mortality and AIDS-defining events in patients in the two arms was not statistically significant.

In summary, the combination of zidovudine and zalcitabine was safe and had an acceptable toxicity profile in children who had received prior zidovudine therapy. At the dose used, the frequency of occurrence of peripheral neuropathy was low and could be safely monitored with careful clinical examination. Clinical parameters of growth and neurodevelopmental scores were unaffected in this clinically stable population. Even though this trial was not designed to evaluate efficacy, the combination therapy was associated with significantly improved stability of CD4 cell counts, fewer deaths, and a trend toward lower virus load. The results of this study show that the combination therapy is safe and lacking significant toxicity regardless of duration of prior zidovudine treatment. These findings suggest that this combination may be more beneficial than zidovudine monotherapy in HIV-infected children.

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agement and Prema Shah for her help in analyzing the neuropsychologic data.

Study Group Members

The following members of the Pediatric ACTG Group participated in this trial. Albert Einstein College of Medicine: Arye Rubinstein, Larry J. Bernstein, and Jenny Shlo佐berg; Bellevue: William Borkowsky, Mona Rigaud, Carol Zuckerman, and Nagamah Deygo; Bronx Lebanon Hospital Center: Andrew Wiznia, Joanna Dobroszycki, Aileen Steiner, and Tracey Barnett; Chicago Children’s Memorial Hospital: Ellen Chadwick, Daniel Johnson, and Deborah Fonken; Children’s Hospital (University of Colorado, Denver): Myron J. Levin, Elizabeth McFarland, Carol Salbenblatt, and Margaret McCarthy; Children’s Hospital of Boston: Kenneth McIntosh, Andrea Rubin-Hale, Kelly Knox-Burke, and Nancy Karthas; Children’s Hospital of Michigan: Duane Harrison, Ellen C. Moore, and Charnell Cromer; Children’s Hospital of New Jersey: George D. McSherry, Edward M. Connor, James M. Oleske, and Nancy Hutcheon (St. Joseph’s Hospital and Medical Center); Children’s Hospital of Philadelphia: Richard Rutstein, Bret Rudy, Deborah Schaible, and Carol Maniglia; Children’s Hospital & Medical Center, Seattle: Ann Melvin, Sandra Burchett (Boston Children’s Hospital), Kathleen Mohan, and Jody Carpenter; Children’s National Medical Center, Washington, DC: Tamara Rakus, Maadhavi Ellaurie, Judy Hoppe, and Susan Zamer; Cumbria Presbybyterian Medical Center: Anne Gershon, Jane Pitt, Kathleen Shea, and Lorriadas Drucker; Columbus Children’s Hospital: Michael T. Brady, Jane Hunkler, Charon Callaway, and Kathalin Koranyi; Duke University: Catherine Wilfert, Ross E. McKinney, Megan Valentine, and Je‒n Hurwitz; Emory University: Steven Nesheim, Mary K. Sawyer, Judy C. Sarver, and Lynn Meadows; Harlem Hospital: Elaine J. Abrams, Susan Champion, Ricardo Urbano, and Jacqueline Brinney; Hoffmann-LaRoche, Inc: Robert Dennin, Amy Lim, and Aileen Ward; Howard University: Sohail Rana, Helga Finke, Annaouarn Jayam-Trout, and Shirley Wilson; Lincoln Hospital Center: Kiran Shah, Shudhanami Rao, Nanette C. Villarica, and Anna Cintron; Los Angeles County—University of California Medical Center: Andrea Kovacs, Margaret Khoury, and Janice Ono; Metropolitan Hospital Center: Mahrukh Bajmi, Sarla Inandar, Karen Novita, and Dianne Stumpf-Koster; Mount Sinai Medical Center: David Hodes and Leslie Rhone; North Shore University Hospital: Zenaida Tagupa and Aida Matias; Ramon Ruiz Arnau University Hospital: Washington; DC: Edward Garcia-Trias, Rosaura Aguayo, and Leticia Diaz; San Juan City Hospital: Maria T. Carrer, Milagros Gonzales, and Lylibeth Perez; Schneider Children’s Hospital: Vincent Bonagura, Susan J. Schuval, Constance Colter, and Roz Rosenthal; SUNY Health Science Center-Brooklyn: Hamid Moalloom and Savina Wiltshire; SUNY Health Science Center-Syracuse: Leonard B. Weiner, Coleen N. Cunningham, Kathie A. Contello, and Kim P. Kirkwood; Tulane University: Sara Bienvenu, James Pramberg, Margarita Silio, and Russell B. Van Dyke; University of Alabama: Marilyn Crain, Michael F. Cooney, Linda J. Jones, and Patricia Brien-Berkow; University of California, Los Angeles: Paul Krogstad, Victor Israele ( Cedars-Sinai Medical Center), Audra Deveikis (Memorial Medical Center, Long Beach), and Margaret Keller (Habor-UCLA Medical Center); University of California, San Diego: Stephen A. Spector, Wayne M. Dankner, Mary Caffery, and Lisa Stangl; University of California, San Francisco: Diane W. Wara, Alejandro Dorenboum, Ann Petru (Children’s Hospital Oakland), and Estrella B. Manio; University of Maryland: Robert Livingston (Johns Hopkins University), Nancy Hutton (Johns Hopkins University), Peter Vink, and Debra Houck; University of Massachusetts: John L. Sullivan, Katherine Luzuriaga, Barbara W. Stechenberg (Baystate Medical Center), and James Robinson (University of Connecticut Health Science Center); University of Miami: Gwendolyn B. Scott, Charles D. Mitchell, Janet Gourley, and Caridad Mendoza; University of Puerto Rico: Licette Flores, Lisette Lugo, Belinda Beau-champ, and Carmen Rivera; University of Rochester: Frank Gigli-otti, Barbra Murante, Lisa Frenkel, and Cynthia Kelley (Children’s Hospital-Buffalo); Yale University: Warren A. Andiman and Sos-tena G. Romano.

References


