A Phase I Study of EZN-3042, a Novel Survivin Messenger Ribonucleic Acid (mRNA) Antagonist, Administered in Combination With Chemotherapy in Children With Relapsed Acute Lymphoblastic Leukemia (ALL): A Report From the Therapeutic Advances in Childhood Leukemia and Lymphoma (TACL) Consortium

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Summary: To address the therapeutic challenges in childhood relapsed ALL, a phase 1 study combining a survivin mRNA antagonist, EZN-3042, with reinduction chemotherapy was developed for pediatric patients with second or greater bone marrow relapses of B-lymphoblastic leukemia. EZN-3042 was administered as a single agent on days 5 and 21 and then in combination with a 4-drug reinduction platform on days 8, 15, 22, and 29. Toxicity and the biological activity of EZN-3042 were assessed. Six patients were enrolled at dose level 1 (EZN-3042 2.5 mg/kg/dose). Two dose-limiting toxicities were observed: 1 patient developed a grade 3 gastrointestinal bleeding. Downmodulation of survivin mRNA and protein were assessed after single-agent dosing and decreased expression was observed in 2 of 5 patients with sufficient material for analysis. Although some biological activity was observed, the combination of EZN-3042 with intensive reinduction chemotherapy was not tolerated at a dose that led to consistent downregulation of survivin expression. The trial was terminated following the completion of dose level 1, after further clinical development of this agent was halted.

Key Words: relapsed ALL, survivin, antisense

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B-lymphoblastic leukemia and to determine the biological activity of this agent. The first experience with a survivin antagonist in pediatric patients was terminated early because of our inability to integrate the agent with reinduction therapy due to toxicity and the lack of further pursuit of this particular agent.

MATERIALS AND METHODS

Study Population

Patients aged 1 to 21 years old with second or greater marrow relapses (M3 marrow; >25% blasts) of B-lymphoblastic leukemia with or without concomitant extramedullary disease were eligible for this study. This included patients who had undergone prior allogeneic hematopoietic stem cell transplantation, as long as they were at least 120 days from the time of transplant and had no active graft-versus-host disease. Patients with refractory leukemia after first or greater relapse and a single reinduction attempt were also eligible with a limitation of 1 patient with refractory disease in each cohort of 3 patients. Patients with mature B-ALL and Down syndrome were excluded. Institutional review boards at participating TACL centers approved the study. Informed consent was obtained from patients or from parents/legal guardians. An independent Data Safety Monitoring Committee at Children’s Hospital Los Angeles monitored study progress.

Study Design and Dose Escalation

All patients received one course of therapy as detailed in Table 1. During the 6-day prephase, the survivin antagonist, EZN-3042, was administered by 2-hour intravenous (IV) infusion on days −5 and −2 following the dose escalation schema in Table 2. Reinduction chemotherapy began on day 1 and EZN-3042 was next administered in combination with the 4-drug reinduction platform on days 8, 15, 22, and 29. The starting dose for EZN-3042 was 2.5 mg/kg/dose, which was 1 dose level below the 5 mg/kg/dose MTD (maximum tolerated dose) in an ongoing adult phase 1 trial at the time this study first opened. Patients were enrolled in groups of 3 using a standard 3 + 3 design. If the pediatric MTD was exceeded at the first dose level, then the dose of EZN-3042 was to be reduced by another level, that is, to dose level 0 (1.5 mg/kg/dose). The trial was also designed to enroll 6 additional patients at MTD, to improve the precision of estimates of the biological activity of EZN-3042.

Toxicity was graded using the CTCAE criteria, version 4.0 (http://ctep.cancer.gov). The dose-limiting toxicity (DLT) was defined as any of the following events that were at least possibly, probably, or definitely attributable to EZN-3042. Nonhematologic DLT was defined as any grade 3 or grade 4 toxicity attributable to EZN-3042 with the specific exclusion of: grade 3 nausea and vomiting; grade 3 transaminase (aspartate aminotransferase/alanine aminotransferase [AST/ALT]) elevation that returned to grade ≤1 or baseline before the next scheduled dose; grade 3 or 4 fever or infection; grade 3 or 4 electrolyte abnormalities that were transient (<24 h) and not associated with clinical sequelea; or alopecia. Hematologic DLT was defined as the absence of peripheral blood count recovery (absolute neutrophil count >500/µL and platelet count >20,000/µL) within 6 weeks of starting systemic chemotherapy (protocol day 1) by marrow aplasia, not marrow infiltration, in patients who achieved remission.

Disease Assessment

At the completion of combination therapy (day 36), a bone marrow aspirate was obtained to assess disease status. Complete blood counts were also obtained at baseline and following exposure to single-agent EZN-3042 (Table 3). Cerebral spinal fluid examination was conducted at study entry and with each scheduled dose of intrathecal chemotherapy. Minimal residual disease was determined by flow cytometry (optional participation) at the end of reinduction (day 36) in a dedicated reference laboratory at the University of Washington using the standard methodology.

Correlative Studies

To evaluate primary target engagement of EZN-3042 monotherapy, survivin mRNA and protein expression were quantitated in enriched marrow blasts before treatment was started (day −6) and on day 0 (48 h following the day −2 dose of EZN-3042) using quantitative reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent (ELISA) assays, respectively, at NYU Langone Medical Center. This time point was chosen as this was when the maximal decrease in survivin expression was observed after knockdown in preclinical studies. To optimize yield and minimize any potential artifacts associated with sample storage before shipment, fresh marrow samples were processed at local centers. Namely, leukemic blasts were purified by Ficoll-Paque extraction and were frozen in shipping media. Upon thawing, marrow samples were enriched by flow cytometry to ensure >90% blast purity. Total RNA was extracted from flow-sorted blasts using the RNeasy Mini kit (Qiagen, Valencia, CA) and 2-step RT-PCR was performed using I-Script II cDNA Synthesis kit (Bio-Rad, Hercules, CA) and Perfecta SYBR Green FastMix (Quanta Biosciences, Gaithersburg, MD). Synthesis of PCR products was monitored by the DNA Engine Opticon System (MJ Research, Waltham, MA). Data were plotted relative to β2 microglobulin expression, comparing the mRNA before and after scheduled doses of

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**TABLE 1. Details of Protocol Therapy**

<table>
<thead>
<tr>
<th>Drug and Dosage</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZN-3042 (dose assigned at study entry)</td>
<td>−5, −2, 8, 15, 22, 29</td>
</tr>
<tr>
<td>Vincristine 1.5 mg/m² IV</td>
<td>1, 8, 15, 22</td>
</tr>
<tr>
<td>Prednisone 40 mg/m²/day PO</td>
<td>1-29</td>
</tr>
<tr>
<td>Pegasparagase 2500 IU/m² IV</td>
<td>2, 9, 16, 23</td>
</tr>
<tr>
<td>Doxorubicin 60 mg/m² IV</td>
<td>1</td>
</tr>
<tr>
<td>Intrathecal cytarabine</td>
<td>0</td>
</tr>
<tr>
<td>Intrathecal methotrexate</td>
<td>15 and 36 (CNS negative)</td>
</tr>
<tr>
<td>Triple intrathecal chemotherapy</td>
<td>8, 15, 22, 29 (CNS positive)</td>
</tr>
</tbody>
</table>

CNS indicates central nervous system; IV, intravenous.

**TABLE 2. Dose Escalation Schema**

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Dose (mg/kg)</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.5</td>
<td>−5, −2, 8, 15, 22, 29</td>
</tr>
<tr>
<td>1 (starting dose)</td>
<td>2.5</td>
<td>−5, −2, 8, 15, 22, 29</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>−5, −2, 8, 15, 22, 29</td>
</tr>
<tr>
<td>3</td>
<td>6.5</td>
<td>−5, −2, 8, 15, 22, 29</td>
</tr>
</tbody>
</table>
EZN-3042 on days −5 and −2. The PCR primers used were as follows: β2 microglobulin (5'-AGTGGTGCTGGTTTCATCCATC-3' and 5'-AGTCACATGGTGTCG-3'), survivin main isoform (5'-CCACCCGCATCCTCATTACAGGCGT-3'), survivin main isoform (5'-CATGGTGCTGGTTTCATCCATC-3' and 5'-TATGTTCCTCTATGGGGGTC-3'), survivin 2B isoform (main forward and 5'-AGTGCTGGTTTCATCCATC-3'), and survivin ΔEX3 isoform (main forward and 5'-TTTCTTGGTTGGGGGTC-3'). For ELISA assays, flow-sorted blasts were lysed at a concentration of 10 million cells/mL of buffer (1 mM EDTA, 0.5% triton X-100, 6 M urea, and protease inhibitor cocktail [Roche, Basel, Switzerland] and total protein concentration determined by DC protein assay [Bio-Rad]). ELISA assays were performed according to manufacturer's protocol using Human Total Survivin DuoSet IC (DYZ467-2; R&D Systems). Data were analyzed using the MasterPlex software 4 parameter logistics (4-PL) curve fit.

**Pharmacokinetic (PK) Studies**

Blood samples were collected at the following time points to determine the PK profile of EZN-3042: 15 to 30 minutes before the first dose, at the end of the initial 2-hour infusion, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 24 hours after the end of the first infusion, at day 0 (120 h after initial infusion), and before the day 8 dose of EZN-3042 (312 h after the initial infusion).

**Statistical Considerations**

The primary endpoint for dose escalation was the occurrence of a DLT as defined above. Patients were evaluable for toxicity assessment if they terminated treatment for toxicity or intolerability, or if they experienced a DLT, or if they received 85% of the required doses of EZN-3042 and other agents without a DLT. Patients who received any prescribed combination therapy (day 0 and later) were considered evaluable for response. The primary measures of biological activity of EZN-3042 were the reduction in survivin mRNA and protein expression in leukemic blasts before treatment and after 2 doses of EZN-3042 were administered on days −5 and day −2 (baseline vs. day 0). Given the small patient number at the time of study closure, the change in survivin expression was reported using descriptive analyses.

**RESULTS**

**Patients and Accrual**

A total of 6 patients were enrolled in this study between January 31, 2011 and November 11, 2011. Patient characteristics are detailed in Table 4. Two patients had undergone prior allogeneic hematopoietic stem cell transplantation and 1 patient had refractory disease with a partial response to prior reinduction therapy at the time of study entry. One DLT was observed in the initial cohort of 3 patients, which prompted cohort expansion to 6 patients according to the study design. A second cohort of 3 patients subsequently accrued and 1 additional DLT was observed. The trial was subsequently scheduled to re-open at dose level 0, where each scheduled dose of EZN-3042 would have been decreased to 1.5 mg/kg. The trial, however, was prematurely terminated when further clinical development of this agent was stopped, before any accruals at dose level 0, a dose significantly below the MTD in the adult trial with this agent.22

**Toxicity**

Single-agent therapy with EZN-3042 on days −5 and −2 was well tolerated and no nonhematologic toxicities were reported. The most frequent grade 3 or higher nonhematologic toxicities, occurring in 3 or more patients (50%) during combination therapy (days, 1 to 36) were hepatic, metabolic, and infectious. Two DLTs occurred among the 6 patients treated on dose level 1 (2.5 mg/kg/dose). One patient developed reversible grade 3 γ-glutamyl transferase elevation on protocol day 42, 14 days after the last scheduled dose of EZN-3042. This patient also developed grade 3 AST and ALT elevation, which persisted for 10 days and then returned to prestudy baseline. Of note, this patient had grade 1 AST and ALT elevation at the time of study entry. The second DLT which was observed was grade 3 gastrointestinal bleeding on day 16. At the time of the initial onset of the bleeding, the patient had Clostridium difficile enterocolitis and was thrombocytopenic but did not have a coagulopathy. This patient was unable to complete protocol therapy because of toxicity and expired due to fungal sepsis (Candida lusitaniae).

**Response to Therapy**

Among the 5 patients assessable for response on day 36, 1 patient achieved complete remission (CR), 1 patient achieved complete remission without platelet recovery, 1 patient had stable disease, and 2 patients had progressive disease. Among the patients achieving morphologic CRs, both had detectable minimal residual disease. The 2 responding patients achieving CR and complete remission without platelet recovery remained alive in CR following stem cell transplantation with follow-up durations of 5 months and 14 months, respectively. Although response to single-agent EZN-3042 on days −5 and −2 was not formally assessed, complete blood counts were monitored

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>WBC/µL on Day −6</th>
<th>WBC/µL on Day 0</th>
<th>ABC/µL on Day −6</th>
<th>ABC/µL on Day 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9300</td>
<td>27,600</td>
<td>5022</td>
<td>24,012</td>
</tr>
<tr>
<td>2</td>
<td>20,000</td>
<td>118,340*</td>
<td>8800</td>
<td>110,056*</td>
</tr>
<tr>
<td>3</td>
<td>4510</td>
<td>12,470</td>
<td>1398</td>
<td>7856</td>
</tr>
<tr>
<td>4</td>
<td>240</td>
<td>430</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>16,070</td>
<td>25,550</td>
<td>4339</td>
<td>12,520</td>
</tr>
<tr>
<td>6</td>
<td>3000</td>
<td>3300</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Sample drawn on day 1 instead of day 0.
ABC indicates peripheral absolute blast count; WBC, white blood cell count.

**TABLE 3. WBC Count Response to Single-agent EZN-3042**
from study entry (baseline) through day 0 and no decline in WBC was observed for any of the patients (Table 3).

**Pharmacokinetics and Pharmacodynamics**

Five patients provided samples for pharmacokinetic (PK) analysis. The maximum plasma concentration of EZN-3042 at the 2.5 mg/kg dose had a median value of 7359 ng/mL (range, 3774 to 10075 ng/mL). Median half-life from the end of infusion was 0.51 hours (range, 0.29 to 0.58 h). Terminal half-life ($t_{1/2}$) was estimated to be 5.3 hours (95% confidence interval, 4.2 h-7.2 h), although this is likely an underestimate because no samples between 6 hours and 24 hours were drawn to confirm log-linear elimination. Median AUC$_{0-24}$ was 14.6 mg h/mL (range, 7.1 to 18.7 mg h/mL).

Of the 6 patients enrolled in the study, mRNA and protein analysis could be completed in 5 and 3 patients, respectively, due to limitations arising from sample collection and recovery. We saw a significant decrease in survivin transcript expression in 2 of 5 patients (patient 2 and 3, 40% and 50% decrease, respectively) where samples were available (Fig. 1A). There are multiple splice variants of the survivin gene with unique properties and function and the expression levels of survivin isoforms were also measured in the patients who showed a decrease in survivin transcript expression (patient 2 and 3). The DE3X and 2B splice variants were also downregulated but not the 2a isoform, which is not targeted by EZN-3042 (Fig. 1B), thereby confirming specific targeting of survivin transcripts by EZN-3042. Protein expression correlated with transcript expression in 3 patients, including 1 patient with downregulation, who had sufficient material for analysis (Fig. 1C).

**DISCUSSION**

This phase 1 trial was the first pediatric experience with the survivin mRNA antagonist, EZN-3042, and also the first experience combining EZN-3042 with a multiagent chemotherapy platform. Third-generation antisense compounds have changes in the chemical structure of oligonucleotides, which result in higher affinity for mRNA and higher potency in mRNA downmodulation in general and improved tissue stability, offering a potential benefit compared with earlier generation antagonists. Although EZN-3042 was well tolerated as a single agent, DLTs of transaminitis and hemorrhage were observed when it was combined with a 4-drug chemotherapy platform. Transaminitis could have resulted in part from the platform chemotherapy regimen, as the incidence of grade 3 or higher ALT elevation was 14.5% on the COG AALL01P2 study, where this platform was previously used alone; however, hepatic toxicities were the most frequently reported drug-related adverse events in the phase 1 study of EZN-3042 in adults, and antisense oligonucleotides have been shown to accumulate in the liver. The biological activity of EZN-3042 was assessed in patients in this study by measuring the downregulation of survivin mRNA and protein after single-agent exposure. We observed biological activity in 2 of 5 patients even at a low dose of EZN-3042, but higher doses could not be investigated due to the toxicity observed with combination therapy.

Our limited experience with EZN-3042 in pediatric patients with relapsed ALL showed similarities to the phase 1 trial in adults with refractory lymphomas and solid tumors, where patients also received ENZ-3042 twice...
weekly during week 1 and then weekly thereafter with or without docetaxel. The most common toxicities observed in adults were AST and ALT elevation, occurring in 42% and 38% of patients who received single-agent EZN-3042, respectively. The transaminitis was reversible, and the agent was otherwise generally well tolerated both alone and in combination with docetaxel. The best response observed with single-agent EZN-3042 (n = 24) was stable disease in 5 patients and the best response for EZN-3042 (2.5 mg/kg) in combination with docetaxel (n = 11) was a confirmed partial response in 1 patient with prostate cancer.22 Limited pharmacodynamic studies for survivin expression in hair follicles and tumor biopsies showed no consistent downmodulation of survivin mRNA or protein. Although the median half-life of ENZ-3042 was longer in adults (1.85 h) than in the small number of pediatric patients treated in our study, the implications of this finding for the dosing regimen utilized in our study are complex, as tissue half-life may be more relevant with antisense oligonucleotides than plasma half-life. Pharmacokinetic studies of antisense oligonucleotides have demonstrated rapid clearance after systemic administration, followed by accumulation in tissues, especially the liver and kidneys, with a much longer tissue half-life of several days.23,24

In summary, preclinical data have identified survivin as a key target in relapsed ALL and downregulation of survivin expression has been shown to sensitize tumor cells to conventional chemotherapy. On the basis of these observations, a phase 1 trial was developed combining EZN-3042 with a traditional chemotherapy platform for pediatric patients with relapsed ALL. Although we saw biological activity in 2 of 5 patients using a low dose of EZN-3042 administered on 2 occasions, toxicity precluded increasing the dose of this agent in this heavily pretreated group of patients. Therefore, although the study was halted prematurely when further pursuit of the agent was terminated by the industry sponsor, it is unlikely that the lower dose mandated by study design would have led to further downmodulation of survivin. Moreover, the collateral toxicity associated with treating multiply relapsed ALL potentially limits integration of novel agents. Alternative strategies, which target survivin indirectly, are presently being pursued in this population.25 Our experience in this trial both supports the paradigm of using new genomic discoveries to inform clinical trial design as well as highlighting some of the complexities of moving new agents forward in a group of patients known to experience significant toxicities with conventional agents.

ACKNOWLEDGMENT

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REFERENCES


FIGURE 1. Survivin expression following treatment with EZN-3042. Messenger RNA and protein expression analysis of patient samples before (day – 6) and after (day 0) prephase treatment with EZN-3042 is shown. Quantitative real-time-polymerase chain reaction (RT-PCR) (A and B) and enzyme-linked immunosorbent assays (ELISA) (C) were performed on bone marrow samples from patients on days – 6 and 0. mRNA expression for survivin main form (A) and survivin splice variants (B) is plotted relative to the pretreatment expression level at day – 6. Absolute protein concentration for survivin (C) on days – 6 and 0 were determined by standard curve and expressed as pg of target protein per mg of total protein. Each data point was measured in triplicate (*P<0.05 pre–post treatment).


