

***Editor's Note:** In the April issue of the journal, Volume 26, Number 4, Avramis and colleagues presented data to show that in a selected group of patients with Acute Lymphoblastic Leukemia being treated on a CCG trial that the presence of silent antibodies (that is clinically undetectable) potentially rendering the therapy ineffective is present in a significant number of patients (1). Given the importance of the enzyme in treatment of patients with ALL, as evidenced by finding that a lack of enzyme activity may correlate with outcome, questions such as "how best give the enzyme?" "how to monitor its effect?" and "how to prevent the development of antibodies?" seem crucial both to the patient as well as the physician-scientist evaluating the efficacy of the treatment. That is it would seem important to know why therapy failed or why was it toxic as we move towards maximizing/individualizing therapy. John Holcenberg is an acknowledged expert both in the use of enzymes as drugs and in the pharmacology of anti-cancer agents. Rather than having me pose questions and raise issues about how to incorporate Asparaginase in treatment plans, I simply asked Dr. Holcenberg to prepare a commentary for this issue. The results are more informative and the opinion more authoritative than mine.*

Optimal Asparaginase Therapy

John Holcenberg, MD

It's been over 35 years since a bacterial asparaginase was first used for the treatment of acute lymphoblastic leukemia (ALL).¹ Nevertheless, we still don't know the best way to administer these foreign proteins to achieve optimal effect and to minimize allergic reactions. The paper by Avramis and colleagues² provides some important clues. Recent studies by several laboratories have shown that treated patients can develop a silent allergy where circulating antibodies neutralize the asparaginase activity or cause rapid clearance of the protein but do not cause clinical allergic symptoms. The paper reports a very high incidence of antibodies to asparaginase in children with higher-risk ALL treated on Children's Oncology Group Study 1961 first with native *E. coli* asparaginase and then with pegylated-asparaginase (pegaspargase). An interim outcome analysis of 280 patients found a similar hazard ratio of relapse for those who had no clinical allergy and those with clinical allergy who were subsequently switched to a non-cross-reacting *Erwinia* asparaginase. In contrast, the hazard ratio for relapse was significantly higher in the 29% of these patients who developed silent antibodies. Because these antibodies neutralized the asparaginase activity, these children received a shorter duration of effective asparaginase treatment. Since no clinical allergy was seen, they did not receive *Erwinia* asparaginase. This suggests that monitoring for silent antibodies or loss of asparaginase activity could prompt a change to *Erwinia* asparaginase and thereby improve outcome.

The duration of effective asparaginase treatment also appears to be important in two studies from the Dana-Farber ALL Consortium.^{3,4} In protocol 91-01, 12% of children received 25 weeks or less of the 30 weeks planned for asparaginase intensification therapy because of side effects or allergic reactions. These children had a significantly worse 5-year event-free-survival than those who tolerated more than 26 weeks (73% versus 90% respectively). They also showed that older children tolerated asparaginase worse than younger ones. Protocol 95-01 compared weekly doses of 20,000 IU/m² of *Erwinia* and *E. coli* asparaginase during a 20 week intensification phase. *Erwinia* asparaginase has a shorter half-life than *E. coli* asparaginase so its duration of asparaginase activity would have been shorter.

The 3-year event-free survival was significantly shorter for children treated with Erwinia- compared with those treated with E. coli asparaginase (81% versus 92% respectively).

Asparaginase treatment has been prolonged by three dosing schemes: repeat induction regimens called delayed intensification first developed by the German BFM Consortium, intensification phases like those of the Dana-Farber ALL Consortium and consolidation schemes using methotrexate followed by asparaginase first proposed by Capizzi.⁵ A Children's Oncology Group study showed that combination of intensified delayed intensification and the Capizzi regimen improved event-free survival in children with slow response to induction therapy.⁶ It is not clear which of these schemes is best but all share the problems of clinical allergic reactions, silent allergy and adverse events.

HOW CAN THESE PROBLEMS BE MINIMIZED?

First the asparaginase should be given concurrently with strong immunosuppressive drugs. Most induction and delayed intensification regimens use concurrent high dose steroids in addition to an anthracyclines in delayed intensification and some induction schemes. This appears to be adequate immunosuppression at least in standard risk children since in Children's Oncology Group study 1962 allergic reactions were rare with either native- or pegylated-asparaginase. Neutralizing antibodies developed in some children treated with native asparaginase but none developed in children treated only with pegaspargase.⁷ In higher-risk or older children, this degree of immunosuppression may not be sufficient with native E. coli asparaginase. In Children's Oncology Group study 1961, 28% of patients had allergic reactions. They had received 9 doses of native asparaginase during induction. Most allergic reactions occurred after the second dose of pegaspargase on day 42 of consolidation or the next dose of pegaspargase about 2 weeks later. The native asparaginase probably sensitized the patients to develop antibodies that cross-reacted with pegaspargase. The paper in the April issue showed that over 60% of the tested patients on this study developed circulating antibodies.² Concurrent chemotherapy included 1 g/m² of cyclophosphamide and standard doses of cytosine arabinoside, vincristine and 6-mercaptopurine. The Dana-Farber Consortium protocols give pulses of high dose steroids every 3 weeks during the asparaginase intensification. Future studies in higher-risk and older children and adolescents should consider using pegaspargase for both induction and later phases of treatment and consider steroid pulses during the consolidation and interim maintenance phases when asparaginase is administered.

Second, the lowest effective dose of asparaginase should be used. High doses of E. coli asparaginase cause partial depletion of circulating glutamine. It is possible that some of the

adverse effects of asparaginase like progressive asthenia may be due to glutamine depletion. European investigators are evaluating intravenous dosing with 1000 IU/m² of pegaspargase rather than their standard of 2500 IU/m².⁸ The current Dana-Farber ALL Consortium protocol 00-01 is studying the asparaginase activity, adverse events, and antibody formation in patients randomized to 25,000 IU/m² native E. coli asparaginase per week for 30 weeks versus individualized doses to maintain the nadir asparaginase activity above 0.1 IU/ml. The results of these studies should greatly help in the design of more optimal asparaginase use.

Third, intravenous dosing of asparaginase theoretically may be less immunogenic than intramuscular dosing. The Europeans routinely use IV dosing while this route is rarely used in the US. Pegaspargase has been approved for IV and IM dosing in the US. Children's Oncology Group has proposed further study of the safety of IV dosing with monitoring for silent antibodies.

Finally, clinical laboratories should be encouraged to routinely test for asparaginase activity. The Nessler's assay for ammonia and other asparaginase assays should be simple to automate. These assays would be much easier to do and interpret than antibody or asparagine levels. Patients should be monitored for asparaginase activity periodically during their treatment to insure that the nadir levels are at least 0.03–0.1 IU/ml. Those who have very low or undetectable levels could be switched to Erwinia asparaginase.

REFERENCES

1. Hill JM, Roberts J, Loeb E, et al. L-asparaginase therapy for leukemia and other malignant neoplasms. Remission in human leukemia. *JAMA*. 1967; 202:882–888.
2. Panosyan EH, Seibel NL, Martin-Argon S, et al. Asparaginase antibody and asparaginase activity in children with higher-risk acute lymphoblastic leukemia. *J. pediatr Hematol Oncol*. 2004.
3. Silverman LB, Gelber RG, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana Farber Consortium protocol 91-01. *Blood*. 2001;97:1211–1218.
4. Silverman LB, Levy DE, Dalton VK, et al. Erwinia L-asparaginase is less toxic and less effective than E. coli L-asparaginase in children with newly diagnosed acute lymphoblastic leukemia (abstract 1554). *Proc Am Soc Clin Oncol*. 2002;21:389a.
5. Lobel JS, O'Brien RT, McIntosh S, et al. Methotrexate and asparaginase combination chemotherapy in refractory acute lymphoblastic leukemia of childhood. *Cancer*. 1979;43:1089–1094.
6. Nachman JB, Sather HN, Sensel MG, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. *N Engl J Med*. 1998;338:1663–1671.
7. Avramis VI, Sencer S, Periclou AP, et al. A randomized comparison of native E. coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood*. 2002;99:1986–1994.
8. Appel IM, Pinheiro JPV, den Boer ML, et al. Lack of asparagine depletion in the cerebrospinal fluid after one intravenous dose of PEG-asparaginase: a window study at initial diagnosis of childhood ALL. *Leukemia*. 2003; 17:2254–2256.