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REVIEW

First-line treatment of acute lymphoblastic leukemia with pegasparaginase

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Abstract: Acute lymphoblastic leukemia (ALL) accounts for almost 4000 cases annually in the United States, approximately two thirds of which are in children and adolescents. Treatment results of ALL have improved considerably in the past decade, due to an optimal stratification of patients and a rational use of different antileukemic agents among which L-asparaginase (L-ASNase) plays a fundamental role. This drug has been used in pediatric ALL chemotherapy protocols for almost 3 decades. In the 1970s and 1980s a chemically modified form of this enzyme called pegasparaginase (PEG-ASNase) was rationally synthesized to decrease immunogenicity of the enzyme and prolong its half-life. The different advantages of PEG-ASNase have been demonstrated in many clinical studies, the last of which underline the utility of this drug in front-line therapy of ALL. In this review, we discuss the pharmacological advantages and clinical potential of PEG-ASNase and its important use in first-line treatment of ALL.

Keywords: pegasparaginase, acute, lymphoblastic leukemia, pegylation

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous group of disorders that result from the clonal proliferation and expansion of malignant lymphoid cells in the bone marrow, blood and other organs.[1] This is the most common type of cancer in children and adolescents accounting for 23% to 25% of all malignant diseases.[2] Pui et al report that in the 1990s, the 5-year event-free survival (EFS) rates for childhood ALL generally ranged from 70% to 83% in developed countries with an overall cure rate of approximately 80%.[2] Unfortunately, the experience with adult ALL has been far less rewarding but treatment results have improved considerably in the past decade with an increase of complete remission (CR) rates to 85% to 90% and overall survival (OS) rates to 40% to 50%.[3] For all patients, specific treatment approaches differ but generally consist of remission-induction therapy followed by intensification (or consolidation) therapy and continuation treatment. Central nervous system (CNS)-directed therapy, given for varying lengths of time depending on the patient's risk, is administered in order to prevent meningeal progression or relapse.

L-asparaginase (L-ASNase) is an important antileukemic agent used in first-line treatment of a variety of lymphoproliferative disorders. It has been used in treatment of pediatric ALL for almost 30 years.[4–8] L-ASNase was identified as a potential antileukemic agent in 1961 when it was isolated as an antilymphoma component of guinea pig serum.[9,10]

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In 1964 the asparaginase of bacterial origin was isolated. Mashburn reported the purification of *Escherichia coli* L-asparaginase (EC-L-ASNase) and demonstrated its tumoricidal activity.[11] Currently, 3 asparaginase formulations are available in the United States: *E. coli* native L-ASNase, erwinase and pegasparaginase (PEG-ASNase). The antitumor effect of this enzyme results from the depletion of asparagine (ASN), an amino acid essential to lymphoblast leukemic cells leading to an inhibition of protein synthesis with consequent considerable cytotoxicity.

It has been also demonstrated that application of extended high dose of L-ASNase may compensate reduced leukemia control resulting from adoption of a reduced intensity chemotherapy schedule (Berlin-Frankfurt-Munster backbone) for treatment of children with standard risk ALL.[12] The role of L-ASNase as an antileukemia drug has been a matter of discussion due to the high rate of allergic reactions. The major limitation to the use of L-ASNase is dose limiting clinical hypersensitivity, which occurs in 3% to 78% of patients treated with unmodified forms of enzyme.[6–15] However, these clinical complications seem to have been solved by use of modified versions of L-ASNase: polyethylene glycol (PEG)-conjugated asparaginase (pegasparaginase) has significant pharmacological advantages over native EC-L-ASNase and also allows adequate plasma enzymatic activity and asparagine depletion,[16] and can be substituted in cases of hypersensitivity to native L-ASNase. Coupling of the enzyme to PEG was identified as a process by which the immunogenic reactions of the drug were diminished without altering its antineoplastic property.[17,18] PEGylation also increases drug stability and the retention time of the conjugates in blood, and reduces proteolysis and renal excretion, thereby allowing a reduced dosing frequency.[19] Thus, conjugating the native EC-L-ASNase molecule to PEG has provided multiple advantages such as: a definite reduction of immunogenic properties, increased nonreactivity to antibodies, a considerably longer half-life and a reduction of the number of injections for the patient. In recent years, clinical trials have established the importance of PEG-ASNase in frontline pediatric and adult ALL therapy. In this review we discuss about the advantages of PEG-ASNase and its use in first-line treatment of ALL.

Chemistry and pharmacology of the drug
Chemistry of the drug and pegylation

L-ASNase has been produced in large quantities from two bacterial species, *E. coli* and *Erwinia caratovora*. The enzyme obtained from these two sources has been found to have lowest toxicity among various similar enzymes.[20] *E. coli* produces two asparaginases, type EC-1 and EC-2.[21] The purified *E. coli* enzyme has a molecular weight of 133 to 141 kDa.[22,23] The chemical structure of all asparaginases is composed of four identical subunits, A, B, C, and D with an active site on each subunit. The molecular weight of each subunit is 22 kDa.[24] Subunits A and B and subunits C and D form extensive interactions, resulting in dimers. Each dimer has two active sites which contain an aspartate residue, but only the tetrameric form has enzymatic activity.[25,26] The specific activity of purified enzyme is between 300 and 400 µmol of substrate per minute per milligram of protein.

The process known as pegylation, which consists of conjugating biomolecules with PEG, is a well-established technology for increasing the circulating half-life of protein and liposomal pharmaceuticals. In general the advantages of PEGylation technology are increased bioavailability, increased blood circulation of the drug, optimized pharmacokinetics (see below), decreased immunogenicity and decreased frequency of administration.[19] PEGs are non-toxic, water-soluble polymers that create a shield around the pegylated drug, preserving it from renal clearance, enzymatic degradation, and recognition by cells of the immune system.[27] Pegylation of L-ASNase was developed in the 1970s and 1980s. Abuchowski et al[17] were first to successfully couple PEG to L-ASNase. PEG-ASNase is formed by the covalent conjugation of EC L-ASNase (L-asparagine amidohydrolase, type EC-2, EC 3.5.1.1) to monomethoxypolyethyleneglycol (total PEG portion molecular weight 5 kDa).[28–30] PEG-ASNase, which is formed by 4 identical subunits with interactions resulting in dimmers like EC L-ASNase, has similar chemical properties to EC L-ASNase with optimal conditions for activity at pH 7.0, and reaction temperature of 50 °C isoelectric point at pH 5.0.[30] Pegylation covers immunogenic epitopes, reducing hypersensitivity reactions associated with EC L-ASNase and allowing free access of ASN to the active sites of the enzyme while limiting EC L-ASNase's uptake by the reticuloendothelial system, protecting its antigenic determinants from immune detection.[27,31] This also delays the elimination of the enzyme by means of reticuloendothelial system, prolonging the half life of the drug.[27,29,32] In the US, PEG-ASNase is manufactured by Enzon from EC L-ASNase obtained from Merck and Co. Inc. In Europe the PEG-ASNase product is derived from the Kyowa Hakko native asparaginase protein.[33–36] Commercially available preparations for therapy are known generically as pegaspargase; the trade name is Oncaspar® (Enzon, South Plainfield, NJ, USA).

Antineoplastic action

The antineoplastic action of L-ASNase is based on the assumption that tumor cells, especially lymphatic cells, require a huge amount of ASN to maintain their rapid malignant growth. L-ASNase catalyzes the hydrolysis of L-ASN to L-aspartic acid and ammonia.[37,38]

L-ASNase has a significant effect in depletion of serum ASN and kills tumor cells by depriving them of an essential factor required for protein synthesis. Both EC L-ASNase and PEG-ASNase hydrolyze asparagine into aspartic acid and ammonia and deplete circulating asparagines from plasma, the main exogenous sources of ASN.[39–41] Leukemic cells lack sufficient asparagine synthetase compared with normal cells, so they cannot re-synthesize the asparagine *de novo*.[42] Cell cycle arrest in the G1 phase has been documented in the murine L5178Y cell line[43] and also in the MOLT-4 human T-lymphoblastoid line, resulting in apoptosis.[44]

Pharmacokinetics and pharmacodynamics

In vivo studies revealed that the half-lives of *Erwinia* and EC L-ASNase are similar, with 10 hours as mean half-life.[45] The drug seems to remain confined to the vascular space after administration. It has been detected in the pleural fluids and ascites[46] but not in cerebrospinal fluid (CSF).[47] PEG-ASNase, as well as native L-ASNase, has pharmacokinetic properties that include a monophasic half-life, a one-compartment model and a single elimination phase.[48] The peak and trough levels and the area under the curve of PEG-ASNase are proportional to the dose. The distribution volume, clearance and half-life of PEG-ASNase are not dose dependent.[49–51] The elimination half-life of PEG-ASNase is approximately 5.5 to 6 days, 5 times longer than that of EC L-ASNase and 9 times longer than that of *Erwinia* ASNase.

Comparative half-lives of different types of L-ASNase are listed in Table 1.[49,50,52] PEGylation, as shown in Table 1, confers a markedly prolonged half-life to the drug. Table 1 (last row) shows 5 patients who presented hypersensitivity reaction to EC L-ASNase with a decreased serum $T_{1/2}$ (hours) of PEG-ASNase given at 2500 IU/mq. This confirms that the pharmacokinetics of PEG-ASNase may be adversely affected by the development of antibodies directed against the ASNase portion. Modified enzyme is cleared more rapidly in patients with hypersensitivity, although the $T_{1/2}$ remains longer than that of the native enzyme.[50] Armstrong et al studied the effects of the presence of anti-PEG antibodies that are discussed below.[63] The presence of anti-PEG antibodies was highly associated with rapid clearance of PEG-ASNase.

Table 1 Pharmacokinetic studies of patients undergoing therapy with L-asparaginase

L-asparaginase preparation	No. patients	$T_{1/2}$ (h)	Dose range (IU/mq)	Ref.
E. coli	9	34 ± 8	2500–25000	50
E. coli	7	20 ± 6	–	49
E. coli[a]	5	Undetectable	25000	50
Erwinia[b]	10	16 ± 3	25000	50
Erwinia	13	6,4 ± 5	30000	52
PEG	25	357 ± 243	500–8000	49
PEG	10	138 ± 78	2500	50
PEG[c]	5	44 ± 6	2500	50

[a]Patients with previous hypersensitivity to *E. coli* L-asparaginase.
[b]Intravenous administration (all others intramuscular).
[c]Patients with previous hypersensitivity to *E. coli* L-asparaginase, treated with PEG-ASNase.

On the route of administration of PEG-ASNase, the adult and pediatric clinical trials reported[16,53,54,93,97] show that intravenous (iv) administration can be used, without higher incidence or severity of allergic or nonallergic side effects than reported for intramuscular (im) or subcutaneous administration. The iv route of administration will eliminate painful injections and achieve rapid peak levels and asparagine depletion. Following the im administration of PEG-ASNase (2500 UI/m^2) the serum levels of ASN fall by 4 days and remained depleted for around 3 weeks.[48] After iv administration PEG-ASNase deamination of ASN occurred by 2 hours after administration and was sustained for ~3 weeks (dose of 2000 UI/m^2) in adult ALL patients[53] and for ~5 weeks (dose of 2500 UI/m^2) in pediatric ALL patients.[54]

PEG-ASNase penetrates poorly into the CSF.[16,25,48] However, ASN depletion in the CSF has been documented following both EC L-ASNase and PEG-ASNase administration and may be dose-dependent.[25,48,55] The CSF ASN level is known to rebound once L-ASNase therapy is completed.[16,48]

Drug resistance

For L-ASNase and PEG-ASNase, different mechanisms of drug resistance have been recognized. A possible mechanism of resistance to L-ASNase is the development of antibodies that neutralize the enzyme.[55,67] The development of antibodies against PEG-ASNase and EC L-ASNase can reduce their activity, leading to failure of asparagine depletion after readministration of the drug.[50,53,56–58,63] PEG-ASNase has been reported to cause the development of anti L-ASNase antibodies in L-ASNase-naive patients treated with PEG-ASP, at a rate

of up to 12% in children[48,60] and 4% to 15% in adults.[53,62] The presence of circulating anti-PEG was very closely associated with rapid clearance of PEG-ASNase, which could consequentially render the treatment ineffective.[63] The IgG antibody produced can be associated with type II immune allergic reactions. Antibody development may also occur in patients who do not show clinical allergy symptoms (silent hypersensitivity).[55,56] It has been reported that prior exposure to EC L-ASNase increases the development of anti-PEG-ASNase antibodies, and patients who had developed anti-L-ASNase antibodies to L-ASNase also developed anti-PEG-ASNase antibodies in more than 65% of cases,[64] but this could be determined by the potential cross reactivity of the IgG antibodies. While the development of antibodies does seem to be correlated with diminished drug effect, the clinical significance of such antibodies is still being debated.[56,58,63]

An increase in ASN synthase activity (the enzyme responsible for the synthesis of l-asparagine in normal cells) has been noted in blasts of patients with ALL clinically resistant to the drug[64] and in murine lymphoma cells.[66,67] A concomitant increase in messenger RNA as well as enzyme levels for ASN synthase have been described in several cell lines.[68,69]

Another possible mechanism suspected by Gallagher[70] is that the pool of L-ASNase sensitive cells may produce cytokines that control the expansion of resistant cells and when sensitive cells are killed by L-ASNase, resistant cells escape from regulatory control.

In 2003 Holleman et al in 2003[71] associated the resistance to different classes of drugs like L-ASNase and prednisolone with impaired apoptosis in childhood ALL: caspase-3 or PARP [poly(ADP-ribose) polymerase] inactivation seem to be responsible for resistance to these drugs. Moreover, loss of spontaneous caspase-3 activation *in vivo* is associated with relapse in adults with ALL.[72]

Toxicity

The toxicity profile of L-ASNase and PEG-ASNase falls under two main categories, those pertaining to immunological sensitization to a foreign protein, and the adverse events related to the inhibition of protein synthesis. L-ASNase causes little bone marrow depression and usually does not affect the gastrointestinal or oral mucosa or hair follicles.

Toxic hypersensitivity reactions include cutaneous rashes, serum sickness, bronchospasm, and anaphylaxis.[73] The frequency of these reactions for EC L-ASNase ranges

from 13% to approximately 30%.[74] Data on file from the manufacturer (Rhone-Poulenc Rorer) of PEG-ASNase report in 174 patients an allergic reaction rate of 10% in previously nonhypersensitive patients and of 32% in patients hypersensitive to EC L-ASNase.[75] Desensitization protocols have been described but have not been studied in large numbers of patients.[76] The majority of patients experience evidence of hepatotoxicity with an elevation in transaminase and bilirubin levels and abnormal alkaline phosphatase levels have also been reported.[57,65] A decrease in serum albumin, fibrinogen and serum lipoprotein levels is also a manifestation of liver dysfunction. Pathologically, fatty infiltration of the liver has been noted.[76] Coagulation abnormalities such as low levels of clotting factors have been consistently demonstrated as well as deficiencies of antithrombin III, protein C and protein S.[57,77–80] Clinically significant hemorrhage due to L-ASNase therapy is very uncommon, while thrombosis of peripheral, pulmonary or central nervous system occurs in up to 10% of patients.[81] Congenital procoagulant abnormalities may contribute to the development of thrombic complications; however, the role of central venous catheters and steroid therapy, commonly used in leukemia patients, remains to be clarified.[81–83] Pancreatitis, characterized by abdominal and/or back pain, anorexia, nausea and vomiting, is a well-documented complication of L-ASNase that occurs in 10% to 16% of patients with a mortality rate between 1.8% and 4.6%.[84,85] The mechanism of L-ASNase-induced acute pancreatitis is not completely understood. Pathologically, the pancreas is hemorrhagic, suggesting an involvement of coagulation abnormalities. Neurotoxicity, which can occur with symptoms such as depression, lethargy, fatigue, somnolence, confusion, irritability and agitation, is documented in up to 25% of adult patients treated with l-asparaginase,[86] but rarely occur in children. This effect has been suggested to be a result of lack of L-ASN and l-glutamine in the brain.

A lower incidence of complications was found to be associated with PEG L-ASNase.[13,33] PEG-ASNase is generally well tolerated by the majority of the patients and grade 3 or 4 toxicity are rare. The primary toxicity of PEG-ASP is to that of immunologic reactions due to linked the exposure to the bacterial proteins. These reactions range from transient flushing or rush and urticaria (grade 1 and 2) to bronchospasm angioedema and anaphylaxis (grade 3–4).[33] Aside from hypersensitivity, the major side effects of PEG-ASNase are due to inhibition of normal protein synthesis, similar to those of native L-ASNase.

Clinical trials with PEG-ASNase

Because of low hypersensitivity and other pharmacological advantages, PEG-ASNase has been the subject of many clinical application in the last 20 years. The PEGylated enzyme has been found to be safe for most patients allergic to EC L-ASNase and the delayed plasma clearance seems to have solved the problem of the need for frequent medication, but some questions need to be readdressed. In this section we report phase I and II clinical trials of PEG-ASNase followed by a discussion of first-line therapy.

Phase I trials

Ho et al conducted a phase I dose-escalation study in which 31 adult patients received iv PEG-ASNase (dose range 500 to 8000 IU/m^2) over 1 hour fortnightly.[31,49] The mean half-life of the drug was 357 hours, suggesting the use of a 2-weekly treatment interval. Three patients developed anaphylactic reactions (one patient from a 500 dose, one from a 2000 IU/m^2, one from 4000 IU/m^2) and hyperglycemia and hepatic dysfunction were other major associated toxicities. This study has been considered the base for subsequent trials, which adopted the same range of doses in between 2000 and 2500 IIU/m^2 for clinical studies.

In another phase I study Vieira Pinheiro et al[87] infused 500 IU/m^2 of PEG-ASNase in children with relapsed ALL, reaching the goal to maintain serum L-ASNase activity (>100 IU/L) adequate for requisite depletion of L-ASN. Most of the patients responded well to this low dose for at least 1 week, suggesting that with careful drug monitoring, lower doses may be administered successfully. Taylor et al in 2001[88] conducted a phase I clinical trial and pharmacodynamic evaluation of PEG-ASNase in patients who had advanced stage solid tumors. They administered PEG-ASNase at doses of 250, 500, 1000, 1500 and 2000 IU/m^2 twice weekly, and obtained in most cases a low level of L-ASN for 14 days. L-ASN levels were extremely low for 2 weeks (except in patients receiving 250 IU/m^2), with most prolonged depletion at 2000 IU/m^2. Grade 1–2 hypersensitivity reactions were most frequently reported at the 2000 IU/m^2 dose.

Phase II trials

In 1995 Ettinger et al reported the results of a multicenter, phase II, open label clinical trial (ASP-201A) conducted with patients with recurrent ALL.[33] Twenty-one patients received a single dose of pegaspargase (2000 IU/m^2 every weeks) during an initial 14-day investigational window then followed by vincristine 1.5 mg/m^2 weekly × 3, prednisone 40 mg/m^2/day × 21, doxorubicin 40 mg/m^2 and intrathecal chemotherapy beginning on day 14. All had previously received L-ASNase . Of the 18 evaluated for response on day 14 after the window with PEG-ASNase monotherapy, 3 (17%) achieved a complete response and 1 (6%) a partial response for a combined response rate of 23%. By completion of the 35-day induction period, 78% (14 of 18) of evaluated patients achieved complete or partial remission. Five patients experienced mild urticaria and mild local allergic reactions. There was no evidence of anaphylactic problems during the treatment. The incidence of hyperglycemia and pancreatitis was less than expected from the studies with native L-ASNase.

Aguayo et al achieved a completed response rate of 22% in 22 adults patients with recurrent ALL treated with PEG-ASNase, methotrexate, vincristine and prednisone. The chemotherapy protocol consisted PEG-ASNase 2500 IU/m^2 on days 1 and 14.[89]

Douer et al treated 14 adult patients with ALL subjected to 2000 IU/m^2 on day 16 of the multi-agent protocol and obtained 93% complete response. The other agents included vincristine, prednisone and daunorubicine.[90]

Muss et al treated 21 patients with non-Hodgkin's lymphoma with 2000 mg/m^2 PEG-ASNase and the drug displayed modest activity in this heterogenous group of patients because only two partial responses were achieved.[91]

First-line treatment of ALL with PEG-ASNase

Here we review the experience with PEG-ASNase as a front-line treatment for patients with ALL. Randomized clinical trials in children[48,92] and nonrandomized trials in adults,[53,62,90,93] demonstrated that substitution of PEG-ASNase for EC L-ASNase in polychemotherapy regimens for ALL has similar efficacy. The Children's Cancer Group (CCG) carried out a randomized comparison between PEG-ASNase (2500 IU/m^2 × single dose on day 1) and native L-ASNase (6000 IU/m^2 weekly × 3 doses).[94] The overall complete response rates after 4 weeks were comparable: 98% for PEG-ASNase vs 100% for the native drug, but PEG-ASNase produced a faster rate of blast clearance than the native drug (63% vs 47% at day 7, 96% vs 83% at day 14). Another randomized trial by CCG[48] has been conducted comparing EC L-ASNase and PEG-ASNase in treatment of 118 children with newly diagnosed standard-risk ALL. PEG-ASNase was administered at a dose of 2500 IU/m^2, im on day 3 of the 4-week induction phase and on day 3 of

each of two 8-week delayed intensification phases. Native L-ASNase was administered at a dose of 6000 IU/m^2 im 3 times weekly for 9 doses during induction and for 6 doses during each delayed intensification phase. In patients who received PEG-ASNase, the number of days of asparaginase activity exceeded >0.03 IU/mL and was greater than the number of days in native enzyme-treated subjects during both the induction and delayed intensification phases of treatment. With a median follow-up of 3.2 years, the 3-year EFS rates were approximately 80% in both arms, but even if adverse events, infections, and hospitalization were similar between arms, the native arm presented a antibodies high-titer associated with low asparaginase activity. As a result of this study,[48] which demonstrated similar sustained depletion of serum ASN concentrations in patients receiving PEG-ASNase compared to those receiving EC L-ASNase, on July 24, 2006, the US Food and Drug Administration granted approval for PEG-ASNase for the first-line treatment of children with acute ALL as a component of a multiagent chemotherapy regimen.[60]

In addition to these pediatric studies on safety, tolerability and efficacy of PEG-ASNase in first-line treatment of ALL, studies focusing the administration schedule have been reported.

Silverman et al compared the use of EC L-ASNase 25,000 IU/m^2 weekly 3 × 30 doses and PEG-ASNase 2500 IU/m^2 every 2 weeks × 15 doses during intensification therapy. EFS at 5 years was 80%, 84% ± 4% for native L-ASNase and 78% ± 4% for PEG-ASNase-treated patients (p = 0.29).[92] The 5-year EFS for patients treated with less than 25 weeks of any L-ASNase vs at least 26 weeks of L-ASNase were 73% ± 7% and 90% ± 2%, respectively (p = 0.01).

The problem of the weekly or biweekly administration of PEG-ASNase has been studied also by Abshire who carried out a randomized phase II[95] comparing weekly with biweekly PEG-ASNase administration (2500 IU/m^2) during ALL reinduction. In the chemotherapy schedule were also included vincristine (1.5 mg/m^2 weekly × 4 doses), doxorubicin (60 mg/m^2 on first day), prednisone (40 mg/m^2 daily × 29 days) and intrathecal medication (biweekly × 3 doses from day 1). There was a highly significant difference (p = 0.003) in second remission rates, with 97% (69 of 71) achieving complete response in the weekly group and 82% (60 of 73) in the every 2 weeks group.

Other pharmacokinetic and pharmacodynamic studies on PEG-ASNase were carried out in the pediatric population.[16,96] The Italian group investigated the pharmacological effects of the administration of PEG-ASNase given as a first-line product in children with ALL.[16] They investigated ASP serum enzymatic activity and serum and CSF levels of ASN in 20 children with newly diagnosed ALL treated with PEG-ASNase as a first line. The drug was administered during induction at the dosage of 1000 IU/m^2 iv on days 12 and 27 and during reinduction only once at the same dosage. Among the 20 patients treated in induction serum PEG-ASNase activity was ≥100 IU/L in 18/18, 11/11 and 15/18 of the samples available on days 22, 25 and 27, respectively, and in 16/16, 12/15 and 5/8 samples available on days 36, 39 and 45, respectively. In the 15 patients treated during reinduction, serum PEG-ASNase activity ≥100 IU/L was observed in 14/15, 11/14, 6/10, and 0/12 samples available on days 11, 15, 18 and 23, respectively, after the administration of the drug. CSF asparagine levels were below the detection limit of the method only in a few patients during both induction and reinduction. They concluded that PEG-ASNase given as a first-line L-ASNase product allowed adequate plasma enzymatic activity and asparagine depletion during both exposures to the drug even if CSF asparagine depletion was inadequate.

These experiences with pediatric patients demonstrate that PEG-ASNase is safe and tolerable, with lower or similar frequency than other ASNase formulations of toxic reaction to EC L-ASNase for front-line treatment of children affected by ALL; it also has a more convenient administration schedule because of its prolonged half-life. There is no doubt that PEG-ASNase has improved treatment options for pediatric patients affected by ALL, but many questions remains especially regarding adult patients. As noted, the efficacy and toxicities of PEG-ASNase and EC L-ASNase in adult ALL have been compared only in nonrandomized trials.[53,62,90,93] An important trial on feasibility, tolerability and efficacy of IV PEG-ASNase in adult ALL was carried out by Douer et al[53] They studied pharmacodynamics and safety of IV PEG-ASNase during remission induction in 25 adult ALL administrating a single dose of 2000 IU/m^2 as part of a standard frontline induction regimen. PEG-ASNase administered iv was well tolerated with no allergic reaction or pancreatitis and a long duration of ASN depletion. The largest nonrandomized clinical trial to date investigating the beneficial effect of PEG-ASNase in adult ALL was conducted by Wetzler et al[62] The aim of the study was to explore differences in overall survival and disease-free survival of those patients who achieved ASN depletion compared with those who did not. They concluded that effective ASN depletion with PEG-ASNase as part of an intensive multiagent therapeutic regimen in ALL is feasible in adults and is associated with improved outcomes. These two studies confirm the feasibility and the efficacy of PEG-ASNase in first-line therapy also

for adult ALL, but some questions remain. As concluded by Douer, the role of dose intensification especially in patients with anti L-ASNase antibodies, and prolonged duration of PEG-ASNase administration, need further study. Similarly, further studies are needed also to clarify the effects of intensifying the dose of PEG-ASNase to achieve prolonged CSF asparagines depletion.

A question that remains to be addressed is how anti L-ASNase and PEG antibodies formation affects ALL patient outcome. The effect of neutralizing antibodies on ALL outcome is controversial. The experience of Woo et al suggests there is no effect on the outcome of overall treatment in patients with hypersensitivity to the drug,[98] but Panosyan et al showed that patients with neutralizing antibodies without signs of clinical allergy had a significantly worse outcome than patients who did not develop antibodies.[56] Fu et al suggest that alternating the various asparaginase formulations (PEG-ASNase, high dose EC L-ASNase and *Erwinia* ASNase) during the various phases of ALL therapy may help circumvent the production of neutralizing anti-L-ASNase antibodies.[32] The problem of developing anti-PEG antibodies has to be considered as a potential risk of using a PEG-conjugated drug as a front-line treatment. As said before, Armstrong et al studied how antibodies against PEG adversely affect PEG-ASNase therapy in ALL patients.[63] They tried to determine whether anti-PEG was associated with rapid clearance of PEG-ASNase in pediatric patients enrolled in the ALL Berlin-Frankfurt-Muenster 2000 studies. They concluded that the presence of anti-PEG was very closely associated with rapid clearance of PEG-ASNase, which explains similar observations with other PEG-conjugated drugs.[99] For this reason they recommend that patients should be screened for pre-existing anti-PEG and routinely monitored for the development of anti-PEG in order to find patients for whom a modified dosing strategy or use of a nonPEGylated drug would be appropriate. The role of hypersensitivity and antibody development in the efficacy of PEG-ASNase in ALL treatment needs to be further explored in prospective clinical trials.

Conclusions

Many studies have demonstrated that the conjugation of L-ASNase with PEG has achieved the purpose of providing a long-duration form of the drug with a low grade of hypersensitivity. The advent of PEG-ASNase partially overcame the large limitation of immunogenic complications associated with native versions of the drug. This agent is very

effective and for most patients needs to be administered only once every 2 weeks, as opposed to the 2 or 3 times a week for EC L-ASNase. Clearly, administering only 1 or 2 doses of a drug rather than 6 to 9 with the same anti-leukemic efficacy is considerably more convenient. Moreover the safe administration to patients with hypersensitivity to the *E. coli* drug makes it valuable for reinduction therapy in selected patients with ALL. The optimal usage of PEG-ASNase for the first-line treatment of patients affected by ALL remains to be determined precisely, especially considering the problem of dose intensification, drug resistance and development of anti PEG-ASNase antibodies. The monetary cost to patients of PEG-ASNase is greater than that of native versions, but the reduced complications of therapy make overall treatment cost considerably less than that of conventional preparations.[100,101]

In the near future it is hoped that the details for an individualized approach to therapy will be developed in order to exploit the maximum potential of this new important drug.

Disclosures

The authors report no conflicts of interest.

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