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Safety profile of a single pegylated asparaginase (PEG-ASP) dose in remission induction for acute lymphoblastic leukemia (ALL)

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The incorporation of pegylated asparaginase (PEG-ASP) in pediatric and adult acute lymphoblastic leukemia (ALL) protocols remains a worldwide therapeutic approach. However the safety profile remains a challenge, and herein we report the toxicity of an intravenous single dose of 1000 IU/m2 PEG-ASP administered in remission induction for adult ALL patients. Thirty-two patients at median diagnostic age of 32 years (median of 19-65) were included in this analysis. Most patients had B-cell lymphoblastic leukemia (n=26; 78%) and 81% of cases were <55 years at study entry. 75% of patients had <30x109/l leukocyte count at diagnosis and median follow-up was 14 months (range 0.8-69). All grade 3/4 adverse events (AEs) after PEG-ASP administration were observed in 24 patients (75%). The most common grade 3/4 AEs were: decreased fibrinogen (58%), increased bilirubin (31%) and increased GGTP (27%). Clinical manifestations related to PEG-ASP were seen in 9 patients and included: abdominal pain (n=6), thrombosis (n=2), diarrhea (n=1) and pancreatitis (n=1). The median time from PEG-ASP administration to first toxic symptoms was 7 days (range 1-19), and there were also 4 (13%) early induction deaths. All deaths were observed in ≥50-year-old patients after a median of 5 days following PEG-ASP (range 1-9). Three of these four patients had massive obesity. While all expired patients had grade 4 neutropenia and thrombocytopenia at the time of death, sepsis was not present. Administration of PEG-ASP in induction remission for ALL patients resulted in a significant, but mostly reversible hepatotoxicity. This PEG-ASP treatment should be administered with caution for older, obese patients.

Key words: acute lymphoblastic leukemia, asparaginase, pegylated asparaginase, toxicity, induction, remission

L-Asparaginase remains a key component of treatment protocols in adult and pediatric ALL patients [1, 2]. Native (unmodified) asparaginase is extracted from *Escherichia coli*. The major limitation of its use relates to allergic reactions resulting from antibody production. Pegylated Escherichia coli asparaginase (PEG-ASP) has a longer half-life and is potentially less immunogenic than the native preparation. Moreover, the administration of PEG-ASP causing a prolonged depletion of plasma asparagine has demonstrated anti-leukemic efficacy similar to that of *Escherichia coli* asparaginase [1, 3]. The toxicity of PEG-ASP seems to be identical to that in L-ASP except for hypersensitivity reactions which are reported 2–3 times more common in the latter [1]. The toxicity data, including grade 3/4 side effects of PEG-ASP administration in older adults with ALL, are conflicting [4, 5].

Herein, we report the safety profile of intravenous single dose of PEG-ASP administered during remission induction in adult ALL patients.

Patients and methods

Induction treatment. All eligible patients were between 18 and 65 years old and had newly diagnosed B- or T-cell acute lymphoblastic leukemia (ALL). They were recruited between 2011 and 2016 and gave written informed consent before the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Patients received treatment according to protocols adopted by the Polish Adult Leukemia Group: ALL6. The treatment stratification was based on patient's age at diagnosis (<55 years and ≥55 years) and the presence of Philadelphia (Ph) chromosome and/or BCR-ABL transcript. According to ALL6 protocol, the Pegylated *Escherichia-coli*-derived L-asparaginase (PEG-ASP) was administered only to patients with

undetectable Ph chromosome and/or BCR-ABL. There was no difference in induction protocols between patients with B- and T-cell ALL.

The treatment protocol. For Ph(–) patients <55 years treatment was; 5-day pre-treatment with prednisone 60 mg/m^2 (40 mg/m^2 for ≥40 and <55 years), then continued on days (D) 1–28 followed by induction with vincristine 1.4 mg/m^2 and daunorubicin 50mg/m^2 (≥40 years, 40 mg/m^2) on D1, D8, D15 and D22. PEG-ASP at 1000 IU/m^2 was given on D13. Intrathecal liposomal cytarabine (Depocyte, Mundipharma) at 50mg was administered twice during induction: in the pre-treatment phase and on D10.

For ALL patients ≥55 years, the pre-treatment included dexamethasone 10mg/m^2 for 5 days continued on D1–D7 and D15–D21 followed by induction with vincristine 1.4 mg/m^2 and daunorubicin 30 mg/m^2 on D1, D8, D15 and D22. PEG-ASP at 1000 IU/m^2 was administered on D10. Intrathecal Depocyte at 50mg was given in the pre-treatment phase and on D10.

All patients received antibacterial and antifungal prophylaxis throughout the induction. Granulocyte colony-stimulating factor (G-CSF) was recommended for all neutropenic patients. Antithrombotic prophylaxis was not routinely used and fresh frozen plasma (FFP) was recommended for patients with abnormal clotting tests or bleeding; antithrombin replacement was inaccessible in our center.

Table 1. Patients characteristics at study entry.

Characteristic	N=32
Age; years, (median; range)	32 (19–65)
<55 years (%)	26 (81)
≥55 years (%)	6 (19)
Sex; male/female	17/15
Lineage B-cell/T-cell	26/6
Wbc (×10^9/l); (median; range)	9.1 (0.98–392.8)
<30 (%)	24 (75)
30–99.9 (%)	5 (16)
>100 (%)	3 (9)
Hgb (g/dl); (median; range)	9.5 (5.6–14.3)
Plt (×10^9/l); (median; range)	52 (3.0–279)
Blasts cells in PB (%); (median; range)	45 (0–100)
Blasts cells in BM (%); (median; range)	87 (20–100)
Cytogenetics (%)	
t(4;11)	3 (9)
complex karyotype	2 (6)
normal karyotype	7 (22)
failed	20 (63)
Past thrombotic event (%)	1 (3)
History of alcohol abuse (%)	3 (9)
History of allergy (%)	3 (9)

Legend: Hgb=hemoglobin; Plt=platelets; Wbc=white blood cells

Toxicity assessment. The Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 assessed toxicity. The Summary of Product Characteristics was used for laboratory and clinical events related to PEG-ASP administration. PEG-ASP antibody and serum asparaginase activity were not measured.

Statistics. The probability of overall survival was calculated by Kaplan-Meier estimate. Induction-related death was defined as any death during induction not primarily related to progressive ALL. All computations were performed with StatSoft Poland analysis software (version 10.0).

Results

Patient characteristics. Thirty-two patients at median age of 32-year at diagnosis (range 19–65) were included in this retrospective analysis. Most patients had B-cell lymphoblastic leukemia (n=26; 78%) and 81% of cases were <55 years at study entry. Cytogenetic results were obtained in 12 patients (37%); and normal diploid karyotype was the most common finding (22%). Most patients (n=19; 63%) failed karyotyping and 75% of patients had leukocyte count <30×10^9/l at diagnosis. The median follow-up was 14 months (range 0.8–69). Antithrombin (AT) concentration was normal in 17 studied patients (median 106%; range: 79–141). The median prednisone dose or equivalent administered before PEG-ASP was 1750mg (range 210–2600), and median total dose of PEG-ASP in induction was 1800 IU (range 1400–2200). Patient study characteristics are listed in Table 1.

Laboratory and clinical findings after PEG-ASP. All adverse event grades (AE) were present in studied patients, but the following clinical manifestations likely related to PEG-ASP were seen in 9 patients: abdominal pain (n=6), thrombosis (n=2), diarrhea (n=1) and pancreatitis (n=1). Median time from PEG-ASP dose to first symptoms was 7 days (range 1–19).

The following grade 3/4 AEs were observed in 24 patients after PEG-ASP administration (75%): fibrinogen decrease (58%), bilirubin increase (31%) and GGTP increase (27%). AT was measured in 12 patients after PEG-ASP and it was reduced in 8 (median 54%; range 28–118). The blood investigation details are in Table 2.

There were 4 (13%) early induction deaths among those receiving PEG-ASP. All deaths were observed in ≥50-year-old patients at diagnosis after median of 5 days after PEG-ASP (range 1–9). Three out of 4 patients had massive obesity (BMI>40) and all expired patients had grade 4 neutropenia and thrombocytopenia at the time of death. They also had slightly elevated CRP levels (median 10.5 g/l; range: 6.16–29.3) but bacterial/fungal cultures remained negative. (Table 3).

Patients surviving PEG-ASP had at least one grade 3/4 AEs. Induction chemotherapy was discontinued in 4 patients and the subsequent dose of daunorubicin and vincristine was delayed in the remaining 3 cases. Nine patients received

anticoagulants (low molecular weight heparin) for thrombosis (n=2) or as prophylaxis (n=7), and the median transfused fresh frozen plasma units was 8 (range 3–17).

Complete remission rate and survival. The complete remission (CR) rate for the entire cohort after induction was 81% (n=26), and there were the 4 patients with early deaths and 2 cases with incomplete induction treatment due to complications. One of those latter achieved CR after a second induction and the other remained primary resistant.

At the last follow-up, 18 of the 26 patients with first induction CR are still alive (69%). All those patients underwent autologous or allogeneic stem cell transplantation depending on risk factors. The remaining 8 patients died of disease relapse (n=6) or due to infectious complications after allogeneic stem cell transplantation (n=2). The median survival is 37 months and the 2-year probability of overall survival is 78%. The flow chart showing response rates and disease outcome in 32 enrolled ALL patients is shown in Figure 1.

Discussion

Despite the challenging toxicity profile, incorporation of PEG-ASP in pediatric and adult ALL protocols remains a worldwide therapeutic standard [1, 3, 4] Therefore, our ALL regimen included a single administration of PEG-ASP at a dose of 1000 IU/m^2 (median of 1800 IU) and involved only patients with undetectable Philadelphia chromosome. We observed 4 induction deaths (13%) and these were likely related to PEG-ASP use. All deaths were in patients >50 years at diagnosis and occurred at a median 5 days after PEG-ASP. This induction death rate was similar to that determined in other study groups [4]. In contrast to the UKALL14 study [4] where bacterial sepsis was the main cause of death, our expired patients had negative bacterial and fungal cultures. Moreover, CRP was only slightly elevated at the time of death. However, the concomitant presence of grade 3/4 PEG-ASP-related hepatotoxicity was demonstrated in 3 of the 4 patients who died. Similar findings were also demonstrated in the UKALL14 study; PEG-ASP associated toxicity was present in 8 of 16 induction deaths and grade 3/4 hyperbilirubinemia was the most common adverse effect.

In a more recent analysis, 122 newly diagnosed standard-risk ALL patients received *E. coli*-asparaginase or PEG-ASP induction according to MRC UKALL12 E2993 protocol. There was no difference in demographic and laboratory parameters at study entry, and OS rates were also comparable. 3 deaths occurred in the PEG-ASP cohort compared to no fatal events in the *E. coli*-asparaginase group. However, no treatment-related mortality events were considered related to asparaginase use.

The most common grade 3/4 PEG-ASP-related toxicities were hyperfibrinogenemia (23%), hypoalbuminemia (21%) and hypertriglyceridemia (15%). Grade 3 and 4 hyperbilirubinemia and transaminitis were demonstrated in 13% of patients. Severe pancreatitis and deep vein thrombosis (DVT) occurred in 6%, and 2% treated subjects, respectively. However, it is important that the incidence and severity of adverse events did not differ between PEG-ASP and *E. coli*-asparaginase treatments [6]. Hepatotoxicity was

Table 2. Laboratory adverse reactions reported with PEG-ASP therapy in Study Product Characteristics.

Event term/No of tested patients	AE grade 1–2 (No/%)	AE grade 3–4 (No/%)
Liver function		
Bilirubin increased (29)	15 (52)	9 (31)
ALT increased (30)	15 (50)	3 (10)
AST increased (29)	6 (21)	2 (7)
FA increased (22)	5 (23)	3 (14)
GGTP increased (26)	8 (31)	7 (27)
Pancreas function		
Amylase increased (15)	1(7)	1(7)
Coagulation tests		
APTT prolongation (32)	18 (56)	1 (3)
PT prolongation (26)	3 (12)	0
Fibrinogen decreased (29)	8 (28)	17 (58)

Legend: ALT= alanine aminotransferase; APTT=activated partial thrombin time; ASP=aspartate aminotransferase; GGTP= gamma glutamyl transpeptidase; FA=alkaline phosphatase; PT=prothrombin time

Table 3. Summary of deaths during induction.

Patient initials	Age (years)	Sex	Co-morbidities	Total PEG-ASP dose (IU)	Days from PEG-ASP dose	AE grade 3-4 post PEG-ASP	Clinical manifestations
SD	58	M	hypertension; hyperuricemia (BMI=24)	1650	6	bilirubin, GGTP, fibrinogen	Intracranial hemorrhage
MB	59	F	hypertension, diabetes, obesity (BMI=40)	2000	1	none	Abdominal pain, diarrhea, shock
IS	52	F	hypertension, diabetes, obesity (BMI=53), history of uterus cancer	2200	9	bilirubin, fibrinogen	Abdominal pain, hepatic-renal syndrome, shock
ZK	61	M	hypertension, obesity (BMI=47), psoriasis	2000	4	Bilirubin, GGTP	Abdominal pain, fever, shock

Legend: BMI= body mass index; GGTP= gamma glutamyl transpeptidase, M=male; F=female

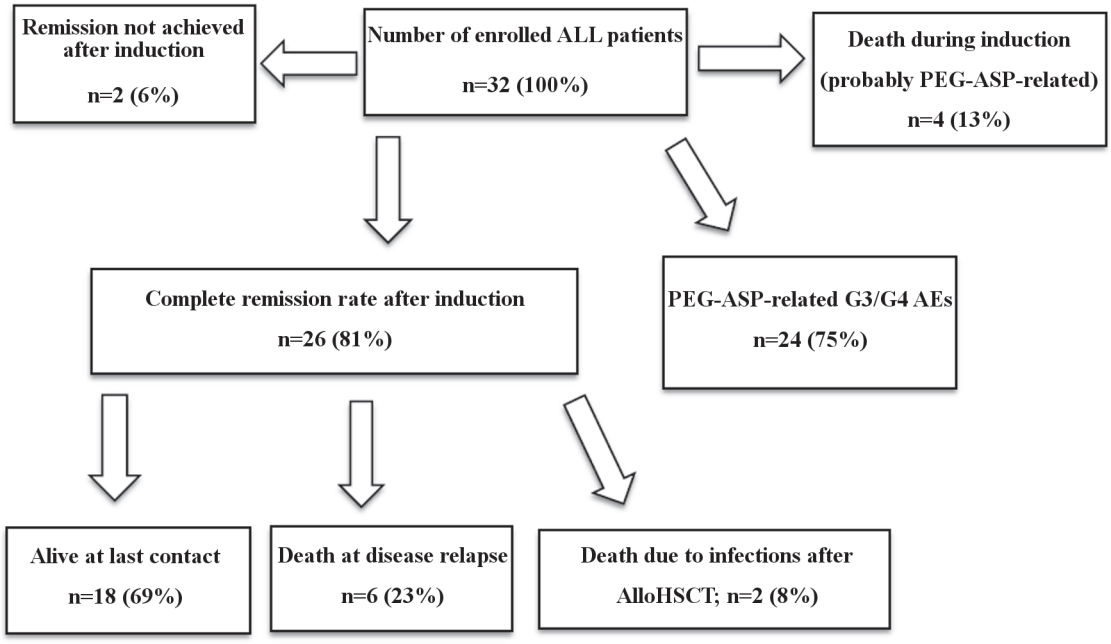

Figure 1. Flow chart of 32 enrolled patients with acute lymphoblastic leukemia. AEs=adverse events; ALL=acute lymphoblastic leukemia; AlloHSCT=allogeneic stem cell transplantation; PEG-ASP: pegylated asparaginase

also the most frequent PEG-ASP-related toxicity in a recent UKALL14 study [4]. In addition, 36% treated patients developed grade 3/4 liver dysfunction and elevated levels of serum bilirubin (23%) and alkaline phosphatase (20%) were the most common. Age >40 years and BMI were the only factors associated with the occurrence of grade 3/4 hepatotoxicity.

Hepatotoxicity was also the most common finding in our study and pancreatitis and DVT were rarely observed. Grade 3/4 hyperbilirubinemia (31%), increased gamma glutamyl transpeptidase (27%) and decreased fibrinogen (58%) were the most frequent abnormalities, and pancreas dysfunction and thrombotic episodes were only sporadic.

Several risk factors which may predict the occurrence of PEG-ASP-related hepatotoxicity in ALL patients have recently been identified. These include: body surface area >2 m^2, albumin concentration <3 mg/dl and platelet count <50×10^9/l. It is noteworthy that the 30-day mortality was significantly higher for patients who developed hepatotoxicity than in those without liver damage [7]. In contrast to above-mentioned studies, only single patients developed grade 3/4 PEG-ASP-related toxicities in an analysis reported by Douer et al.; where hyperbilirubinemia and transaminitis were found in 8% and 12% of patients, respectively. Although decreased fibrinogen and antithrombin concentrations were not associated with bleeding events, cryoprecipitate was prophylactically administered [6]. This supports our findings that no bleeding events were observed in our patient population despite severe hyperfibrinogenemia and decreased AT level after PEG-ASP in some tested patients. The occurrence of liver toxicity during remission induction determines subsequent on-time delivery of chemotherapy. In fact, chemotherapy was discontinued or delayed in 7 patients in our study and this agrees with other reports [4,6]. However, it should be noted that most PEG-ASP toxic effects are manageable and reversible.

While the patomechanism of hepatotoxicity of asparaginase is not fully understood, it has been suggested that asparaginase itself is not hepatotoxic, and that this result could be partly due to contamination by bacterial endotoxin. Other implicated factors include co-infection, co-morbidities and concomitant use of other hepatotoxic agents [8].

In the treatment of ALL patients, one must be aware of possible overlapping toxicity of PEG-ASP and other agents administered in remission induction. In our study, the induction deaths happened early (median of 5 days) after single administration of PEG-ASP dose (D10 or D13 depending on age group). This toxicity overlaps with the anthracycline dose given twice before and twice after PEG-ASP (D1, D8, D15 and D22). This finding agrees with the UKALL14 study where most deaths were early, and observed after a single PEG-ASP dose on Day 4. However, most of those fatal events were caused by septic shock [4], and in contrast, although our patient cohort exhibited grade 4 neutropenia and thrombocytopenia, none of the patients who died had infection symptoms at the time of their death.

In the light of the current knowledge, it is difficult to draw definite conclusions about PEG-ASP toxicity overlap with other agents used in ALL induction. However, this appears to

be partly due to different PEG-ASP and anthracycline dose and schedules throughout the studies and the variations in patient characteristics at study entry. Moreover, some reports included ALL with detectable Philadelphia chromosome, and the patients still received imatinib which is widely known to be hepatotoxic [4, 5, 9].

Studies also confirm that the risk of PEG-ASP-related toxicity is greatest during the first treatment cycle; most likely due to a high level of tumor-related circulating cytokines and the concomitant use of steroids [10].

The UKALL14 study identified >40 years of age as the predictor of induction death and liver-toxicity [4], and this is supported in other studies [11]. Further, omitting PEG-ASP and reducing the daunorubicin dose in the ALL treatment protocol for older patients resulted in decreased induction death [4].

Aldoss et al. report that BMI is another significant factor associated with hepatotoxicity in the UKALL14 study [4]. Elevated BMI and obesity were strongly associated with the occurrence of grade 3/4 hyperbilirubinemia, transaminitis and hypertriglyceridemia, but not with hyperfibrinogenemia [10]. These results support our findings where the four patients who died were over 50 years of age, and 3 of these had massive obesity.

The association between obesity and hepatotoxicity requires clarification. It was found in animal models that the presence of hepatic steatosis predisposes to PEG-ASP-related hepatotoxicity [12].

In conclusion, herein we demonstrated that PEG-ASP administered in induction remission for ALL patients resulted in significant, but mostly reversible, liver-toxicity and this caused a delay in subsequent chemotherapy. While the combination of PEG-ASP with other myelo-suppressive agents may have been responsible for the overlapping toxicity, PEG-ASP treatment should be administered with caution in older, obese patients with acute lymphoblastic leukemia.

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