

L-asparaginase loaded red blood cells in refractory or relapsing acute lymphoblastic leukaemia in children and adults: results of the GRASPALL 2005-01 randomized trial

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Summary

L-asparaginase encapsulated within erythrocytes (GRASPA[®]) should allow serum asparagine depletion over a longer period than the native form of the enzyme, using lower doses and allowing better tolerance. The GRASPALL 2005-01 study, a multicentre randomized controlled trial, investigated three doses of GRASPA[®] for the duration of asparagine depletion in a phase I/II study in adults and children with acute lymphoblastic leukaemia (ALL) in first relapse. Between February 2006 and April 2008, 18 patients received GRASPA[®] (50 iu/kg: $n = 6$, 100 iu/kg: $n = 6$, 150 iu/kg: $n = 6$) after randomization, and six patients were assigned to the *Escherichia coli* native L-asparaginase (*E. coli* L-ASNase) control group. GRASPA[®] was effective at depleting L-asparagine. One single injection of 150 iu/kg of GRASPA[®] provided similar results to $8 \times 10\,000$ iu/m² intravenous injections of *E. coli* L-ASNase. The safety profile of GRASPA[®] showed a reduction in the number and severity of allergic reactions and a trend towards less coagulation disorders. Other expected adverse events were comparable to those observed with *E. coli* L-ASNase and there was also no difference between the three doses of GRASPA[®].

Keywords: childhood leukaemia, erythrocytes, leukaemia, new drugs for leukaemia.

Acute lymphoblastic leukaemia (ALL) is the most frequent malignant disease in childhood. The 5-year event-free survival (EFS) rate ranges from 80% to 85% (Pui *et al*, 2008). However, relapses still occur in 15–20% of cases with an outcome that remains generally unfavourable (Pui, 2003). In adults, the 5-year EFS ranges from 20% to 40% (Le *et al*, 2007) and approximately 50–60% of remitters will relapse (Raff *et al*, 2007). L-asparaginase (L-ASNase), an enzyme derived from *Escherichia coli*, has been used for the treatment of ALL since 1970, in combination with other chemotherapeutic agents (Pui, 1995). The occurrence of major side

effects often leads to early 'on study' withdrawal of the enzyme. They include mainly immuno-allergic reactions, coagulopathy, pancreatitis and hepatic disorders (Oettgen *et al*, 1970). New asparaginase formulations (pegylated forms) or alternative sources (derived from *Erwinia Chrysanthemi*) have been developed to address this issue, but the results were not completely satisfactory. Despite the efficacy of *E. coli* and *Erwinia*-derived ASNase (Duval *et al*, 2002) and the recent introduction of Peg asparaginase (Avramis *et al*, 2002), there is still opportunity for the development of new ASNase presentations.

In this setting, red blood cells (RBC), used as a biocompatible and biodegradable vehicle for drugs, have been shown to protect L-ASNase, enhance its half-life and reduce the occurrence of side effects. Preclinical data showed that this method of presentation increased asparagine depletion when compared to the native form (Kravtsoff *et al*, 1996a,b), and should be of benefit for most patients with ALL.

The GRASPALL 2005-01 trial, a phase I/II study, investigated the dose effect relationship of three doses (50, 100 and 150 iu/kg) of L-ASNase encapsulated within erythrocytes (GRASPA[®]) on the duration of serum asparagine depletion (<2 µmol/l). Tolerance, pharmacokinetics (PK), pharmacodynamics (PD) and immunogenicity of GRASPA[®] were also evaluated and compared to the native *E. coli* L-ASNase.

Patients and methods

Trial design

This was a phase I/II comparative, randomized and multi-centre (24 centres) clinical trial using four parallel groups. Patients were randomized at the time of registration. Allocation of treatment was stratified according to age to ensure a well-balanced distribution of adults and children in all groups.

Study population

Patients, aged 1–55 years, with ALL in first relapse [medullary and/or central nervous system (CNS) relapse] or refractory to induction therapy were eligible for inclusion. Patients who had previously received a bone marrow transplantation in first complete remission (CR) were also eligible. Inclusion criteria comprised no cardiac, pulmonary, renal or hepatic disorders and no serious chronic infection, or neurological dysfunctions [World Health Organization (WHO) grade > 2], except for those related to leukaemia. All patients received *E. coli* L-ASNase during first line treatment. Patients with trisomy 21 were not included. Patients with further relapse, isolated extra-medullary relapse (other than CNS), hypersensitivity to L-ASNase (WHO grade 3–4), history of pancreatitis, presence of rare and dangerous anti-erythrocyte antibodies, or history of grade 3 transfusion incidents were excluded. Pregnant or breast-feeding women were also excluded. Informed consent was obtained and signed by each patient, their parents or legal representative prior to enrolment into the study. The study was performed according to institutional guidelines and was approved by a French Ethical Committee.

Treatment

GRASPA[®] was manufactured according to an industrial automated process by ERYTech-France, Lyon, France. This cell-based medicinal product consisted of *E. coli* L-ASNase loaded into homologous RBC at a concentration of 50% and suspended in saline, adenine, glucose, mannitol (SAGM;

Grifols, Meyreuil, France). The original RBC bag was delivered by the blood bank centre (Etablissement Français du Sang, Lyon, France) as a matched ABO type and RBC leucocyte-reduced concentrate. GRASPA[®] was manufactured over a 3-h process, stored at 2–8°C and administered within 1 d of manufacture, according to regular transfusion practices. L-ASNase (issued from the same *E. coli* L-ASNase pack for the entire study) concentration was 10.8 ± 16 iu/ml of the suspension. GRASPA[®] acted as a microbioreactor with entrapment of L-ASNase into red cells, allowing intracellular cleavage of asparagine (Fig 1).

The COOPRALL schedule [a Berlin-Frankfurt-Münster-derived European Organization for Research and Treatment of Cancer-French Acute Lymphoblastic Leukaemia Group (EORTC-FRALLE) protocol] was used as backbone chemotherapy. It consisted of successive multi-agent blocks of chemotherapy. F1 and F2 blocks were used as induction treatment in patients with late relapse (first CR duration >30 months), while VANDA (dexamethasone, cytarabine, mitoxantrone, etoposide, asparaginase) block was used in those with early relapse (first CR duration ≤30 months). R2 block followed by R1 block were used for consolidation treatment, as previously reported (Domenech *et al*, 2008).

The randomization process comprised two steps. First, patients were randomly assigned either to the control group (25% of patients), with intravenous *E. coli* L-ASNase (OPI-EUSA, Limonest, France) administration at a standard dose of 10 000 iu/m² every 3 d from day 4 of the F1 block or day 6 of the VANDA block, or to the GRASPA[®] group (75% of the patients). In the GRASPA[®] group, patients were secondly assigned, in a double blind manner, to one of the three parallel groups: GRASPA 1 (50 iu/kg), GRASPA 2 (100 iu/kg) and GRASPA 3 (150 iu/kg). These doses were chosen following predictions from pharmacokinetics (PK)-pharmacodynamics (PD) modelling, according to the results obtained from a phase I study (Kravtsoff *et al*, 1996b). The first dose of GRASPA[®] was administered on day 4 (F1 block) or on day 6 (VANDA block) of the first chemotherapy cycle. Re-administration was performed at the same dose on day 6 of the R2 block when a patient fulfilled the following criteria: serum asparagine level >2 µmol/l on day 1 of the R2 block, absence of any major complications (WHO grade >3) after the first administration, and no indication of haematopoietic stem cell transplantation. If serum asparagine level was <2 µmol/l, patients were monitored as indicated in the general outline of the study. The treatment schedule is summarized in Fig 2.

Efficacy and safety variables

The primary endpoint was to determine the relationship between GRASPA[®] dose and asparagine depletion duration (lower rate: 2 µmol/l). Secondary endpoints included: tolerance (compared to that of native L-ASNase) regarding adverse events, immunogenicity of GRASPA[®] (defined by measuring anti-asparaginase antibody levels), kinetics of administered

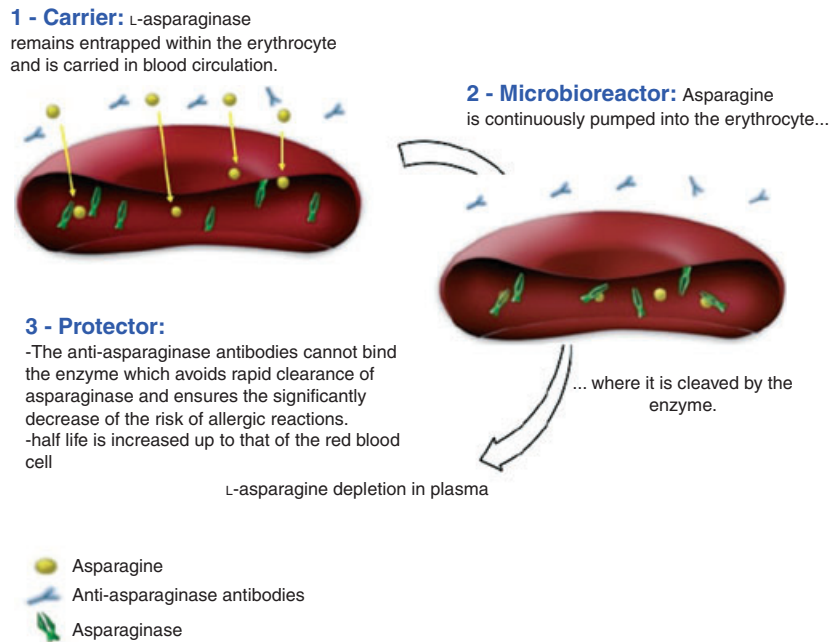


Fig 1. Mechanism of action of GRASPA.

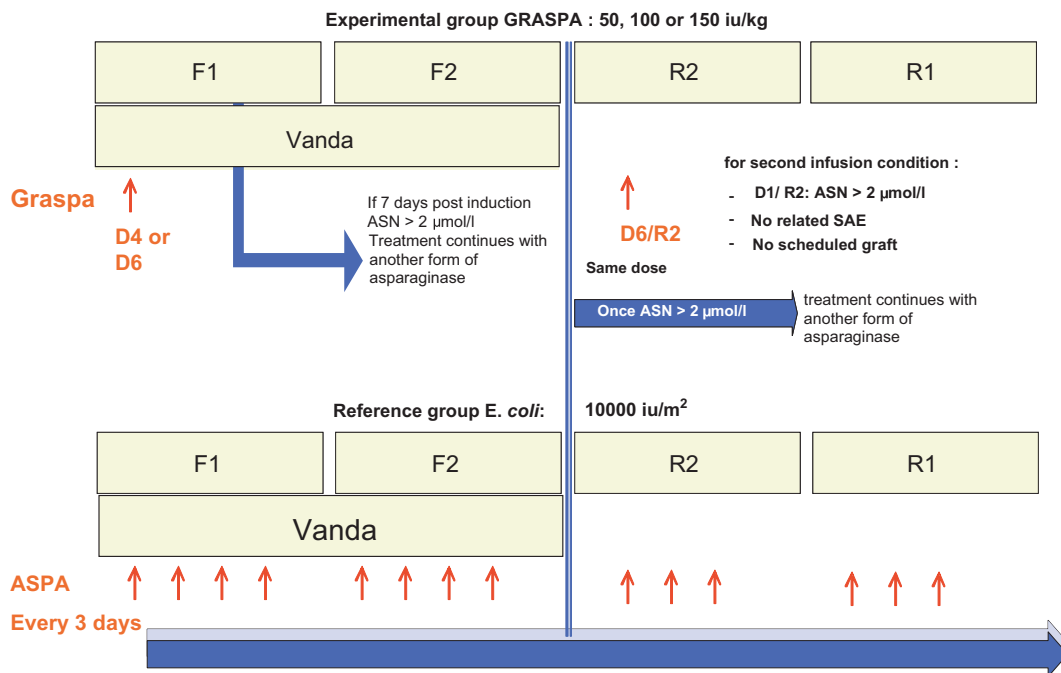


Fig 2. COOPRALL schedule. F1 and F2 blocks were administered as induction therapy for patients with late relapse and VANDA block for those with early relapse. R2 and R1 blocks were administered as consolidation therapy. ASN, asparaginase; ASPA, L-asparaginase; SAE, serious adverse events.

RBC, PK of encapsulated L-ASNase level, and PD of plasma amino-acids (asparagine, aspartic acid, glutamine, glutamate).

The primary efficacy variable was the mean of minimal depletion duration using the last PD time with asparagine value <2 $\mu\text{mol/l}$. Samples for PK and PD studies were collected at regular intervals: time 0, 15 min, 6 h, Days 1, 2, 3, 6, 12, 18,

24, 40, 50, 60, 75 and 120 and refrigerated immediately. The plasma samples were prepared by the local laboratories (centrifugation) and stored at -20°C before being sent to the central laboratory, and for the encapsulated L-ASNase, the whole blood sample was sent to the central lab. PK samples were sent within 3 d after sampling. Plasma was precipitated

by methanol and analyzed immediately by mass spectrometry or stored at -20°C . Asparagine depletion was also evaluated in the cerebrospinal fluid (CSF) at day 5 (block F1 or VANDA). Patients were monitored for 12 months: 4 months on study and 8 months of follow-up.

Patients with plasma asparagine levels $>2\text{ }\mu\text{mol/l}$ at day 6 post-transfusion were withdrawn from the study, but accounted for in the analysis. Patients presenting with a Serious Adverse Events (SAE) following the first infusion of GRASPA[®] were also withdrawn, as were those scheduled for hematopoietic stem cell transplantation shortly after R2-R1 blocks. In the control group, patients presenting SAE were also removed from the study. The investigator was then allowed to introduce another form of L-ASNase.

Statistical methods

The primary endpoint was the duration of asparagine depletion measured from the first administration of GRASPA[®] to the last PK time with asparagine value $<2\text{ }\mu\text{mol/l}$. The sample size was calculated with the assumptions that the duration of asparagine depletion ($<2\text{ }\mu\text{mol/l}$) should be 5 d for 50 iu/kg, 20 d for 100 iu/kg and 40 d for 150 iu/kg. Three patients/dose/strata (12 patients in the adult strata and 12 patients in the paediatric strata) were needed for comparing the three GRASPA groups (50, 100 and 150 iu/kg) by using an analysis of variance (ANOVA) test for each age strata with a bilateral alpha risk of 5% and a power of 80%.

Descriptive statistics were provided for baseline and response variables. Analyzes were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Regarding PK and PD data, the day of first experimental drug administration was considered as the reference date for calculations. Analyzes were made on an intention to treat principle, and populations were defined as patients receiving at least one dose of *E. coli* L-ASNase or GRASPA[®]. Regarding asparagine depletion, as spacing samples were not obtained at constant intervals during follow-up, a sensibility analysis was performed on the primary endpoint using (i) the first PK time with asparagine value $>2\text{ }\mu\text{mol/l}$ and (ii) a linear interpolation between the last PK time with asparagine value $<2\text{ }\mu\text{mol/l}$ and the following point. The individual PK parameters in the three GRASPA[®] groups concerned encapsulated L-ASNase and administered RBC.

The individual PK parameters in the control group concerned plasma L-ASNase. Maximum mean concentration (C_{max}) and terminal half-life were calculated using log-linear regression from the linear proportion of the logarithmic transformed concentration-time plot. Mean overall systemic exposure parameters (area under the curve; AUC) were also estimated.

Results

Between February 2006 and April 2008, 24 patients (12 adults and 12 children, each group containing three children and

three adults) with ALL were included in the study and divided into four groups according to treatment; each group contained three children and three adults. Eighteen patients assigned to GRASPA[®] (GRASPA 1 group: 50 iu/kg, $n = 6$; GRASPA 2 group: 100 iu/kg, $n = 6$; GRASPA 3 group: 150 iu/kg, $n = 6$), and the six patients assigned to the *E. coli* L-ASNase group, received at least one dose of the treatment. The mean ages for children and adults treated were 9.9 years (range, 4.7–16.8 years) and 30.3 years (19.3–46.8 years) in the GRASPA[®] group and 7.9 years (5.4–10.9 years) and 31.2 years (29.2–34.8 years) in the *E. coli* L-ASNase group. In the GRASPA[®] group, 10 patients presented with an early relapse and eight had a late relapse *versus* one with early relapse and five with a late relapse in the *E. coli* L-ASNase group.

Eighteen patients were randomized to the GRASPA[®] group and received their first dose of GRASPA[®] according to the protocol schedule. At the end of induction therapy, only 10 patients were eligible for a second dose of GRASPA[®]: three in the GRASPA 1 group, four in the GRASPA 2 group and three in the GRASPA 3 group. Two patients from the GRASPA 1 group were withdrawn for asparagine depletion failure at day 7 and received another ASNase (one received Peg-ASNase and one *E. coli* L-ASNase). In one case from the GRASPA 3 group, asparagine was still depleted on day 1 of the R2 block, and the patient did not receive a second injection of GRASPA[®]. Two patients were withdrawn for SAE (one for invasive pulmonary aspergillosis and one for severe hepatic WHO grade 4 cytotoxicity). Three patients had not achieved CR at the end of the induction phase.

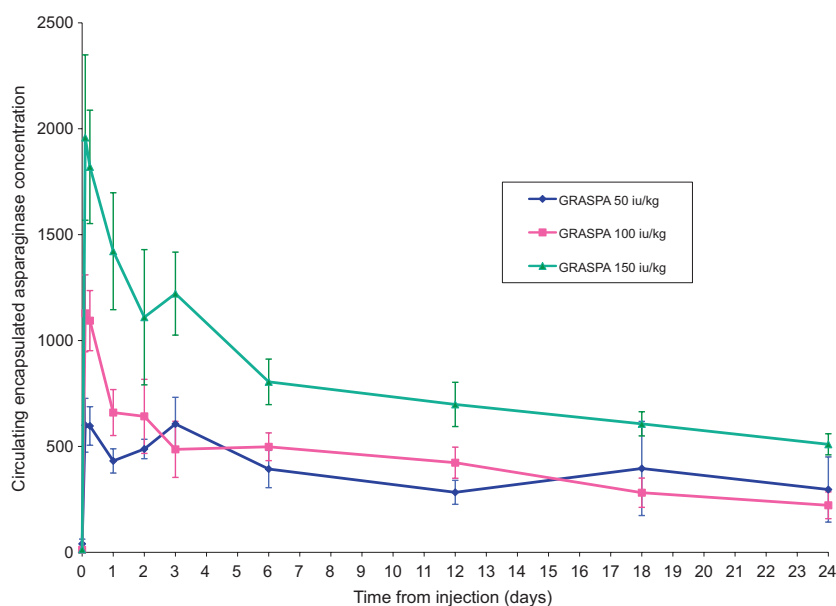
All six patients randomized in the native L-ASNase group received at least one dose ($10\,000\text{ iu/m}^2$) of treatment according to the protocol schedule. Three of them received all eight injections as planned. One patient received seven injections because of a metabolic disorder (hypertriglyceridaemia), one patient received six injections because of thrombosis and one patient received only four injections because of anaphylactic shock. Among the three patients receiving eight doses, one presented a mild allergic reaction after the last ASNase injection. Three patients were withdrawn from study after induction therapy for thrombosis, anaphylactic shock and allergic reaction. Only one of the three patients entering consolidation treatment had fully completed the L-ASNase administration schedule. One patient was withdrawn at the end of the R2 block because of transplantation and one because of allergic reaction.

Efficacy results of induction (one injection of GRASPA[®] versus eight *E. coli* L-ASNase)

Data regarding asparagine depletion are presented in Table I. The mean ($\pm$ SEM) value of circulating encapsulated ASNase concentration over time is presented for each group in Fig 3. There was no major difference between the GRASPA 1 group (50 iu/kg) and the GRASPA 2 group (100 iu/kg) with the SEM overlapping at different time points. The half-life of encapsu-

Table I. Duration of Asparagine depletion after the first injection of GRASPA®/*E. coli*.

Duration of depletion (days)	<i>E. coli</i>	Graspa 50	Graspa 100	Graspa 150
Minimum duration (last point value < 2 µmol/l)				
Mean (SD)	14.89 (8.3)	5.97 (3.3)	6.15 (0.4)	14.63 (13.3)
Median (min – max)	13.05 (6–27.2)	5.95 (2.9–12.0)	6.00 (5.9–6.9)	9.05 (5.8–40.0)
Maximum duration (first point value >2 µmol/l)				
Mean (SD)	24.42 (15.9)	11.32 (5.0)	11.93 (0.6)	22.5 (15.1)
Median (min – max)	22.00 (9–48.3)	11.95 (5.8–19.2)	11.95 (11.0–12.9)	16.00 (12.0–51.0)
Mean duration (linear interpolation between last point value <2 µmol/l and the next one)				
Mean (SD)	20.65 (11.5)	8.63 (8.6)	9.03 (0.4)	18.57 (14.2)
Median (min – max)	18.50 (7.5–37.8)	8.95 (4.4–15.6)	9.00 (8.5–45.5)	12.53 (8.9–45.5)

**Fig 3.** Encapsulated asparaginase (GRASPA®): mean (±SEM) concentration-time profiles (IU) after the first injection.

lated L-ASNase was calculated using the 18 first injections. Mean half-life of GRASPA® was 40 d. Free ASNase (<4%) was detected within the first 24 h following the administration of GRASPA® and was not detected thereafter.

Duration of asparagine depletion after one injection of GRASPA® at 150 iu/kg gave similar results to those observed after the administration of eight injections of *E. coli* L-ASNase.

Only a few samples were available for the analysis of asparagine depletion in the CSF. Asparagine was depleted in two of the three patients studied in the GRASPA® group and in three of the four patients studied in the *E. coli* L-ASNase group. All patients depleted asparagine in the CSF also had depleted serum levels.

After the 2nd injection of GRASPA®: 10 patients

The depletion duration was similarly analyzed after the second injection of GRASPA®. The mean depletion duration was

6.97 d (range, 6.2–10.45 d) for the GRASPA 1 group (50 iu/kg), 2.79 d (0.1–8.65 d) for the GRASPA 2 group (100 iu/kg) and 23.95 d (10.60–38.1 d) for the GRASPA 3 group (150 iu/kg). One patient in the GRASPA 3 group showed depleted asparagine levels in both the CSF and serum at the same time point. Three of the 10 patients receiving the second injection of GRASPA were depleted for a short time.

‘Expected’ adverse events (allergic reactions, pancreatic disorders, hepatic disorders, protein synthesis disorders and coagulation disorders) are reported in Table II. After two cycles, less allergic reactions were observed in the GRASPA® group than in the *E. coli* L-ASNase group (1/18 patients *versus* 3/6 patients, respectively). There was a trend toward less pancreatic, hepatic and coagulation/protein synthesis disorders in the GRASPA® group. More severe toxicities were observed in adults as compared to children: the two patients withdrawn from the study for SAE were adult patients. An anaphylactic shock and an acute pancreatitis were also observed in adult

Table II. Respective frequency of the L-ASNase 'expected' adverse events for *E. coli* L-ASNase and GRASPA[®] groups (according to the dose) during the first cycle.

	<i>E. coli</i> L-ASNase (<i>n</i> = 6)	GRASPA [®] (<i>n</i> = 18)	
	Patients, <i>n</i> (%)	Patients, <i>n</i> (%)	Dose
Allergic reaction	2 (33%)	0	
Grade III and IV	2	0	
Grade II	0	0	
Pancreatitis enzymes elevation	1 (17%)	3 (16%)	100
Hepatic disorder	3 (50%)	7 (38%)	50 (1), 100 (1), 150 (5)
Hypoalbuminaemia/proteinaemia	2 (33%)	0	
Coagulation disorder	4 (66%)	3† (16%)	50 (2†), 100 (1)
With clinical thrombosis	1	0	
Without clinical sign	3	3	
Fibrinogen*: (mean ± SD)	15.8 (2.5)	30.2 (3.9)	
Antithrombin III*: (mean ± SD)	634 (51)	787 (93)	

The parameters used to define the adverse events were those of the Common Terminology Criteria for Adverse Events v3.0. (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcae3.pdf). For the hepatotoxicity, we retained elevation of bilirubin and/or transaminases and reported only grade III and IV hepatotoxicity. For the hypoproteinaemia/ hypoalbuminaemia and coagulation disorders, all events (grades I–IV) were reported.

*Mean value [$\pm$ standard deviation (SD)] of the area under the curve (AUC) of Fibrinogen and Antithrombin III for *E. coli* L-ASNase and GRASPA[®] groups during the first cycle.

†Among these three patients, one of them (group 50 iu/kg) presented asparagine depletion failure and was switched to *E. coli* L-ASNase.

patients, while only one child presented a severe side effect (thrombosis).

In the *E. coli* L-ASNase group, one patient suffered an anaphylactic shock. However, anti-ASNase antibodies were not monitored. Two patients presented allergic reactions (one at the end of induction therapy and one during consolidation) and showed anti-ASNase antibodies. Only one patient had anti-ASNase antibodies at study inclusion and an asparagine depletion <8 d. The other one had anti-ASNase antibodies but showed a good asparagine depletion in both the serum and the CSF following the eight injections administered during induction therapy and the six injections given during consolidation. In the GRASPA[®] group, one patient had anti-ASNase antibodies at study inclusion and had an asparagine depletion <8 d. Only one patient from the GRASPA 1 group (50 iu/kg) presented a clinical allergic reaction (after the 2nd injection). However, this patient did not develop anti-ASNase antibodies. In the GRASPA 2 group (100 iu/kg), three patients (one child and two adults) had anti-ASNase antibodies before the 2nd injection of GRASPA[®]. Asparagine depletion was observed for only a few hours (<24 h) in two of them and the third patient was not depleted. Free ASNase was not detected in the serum of these three patients and the PK of GRASPA[®] was not modified. Other events were the same in the GRASPA[®] and the *E. coli* L-ASNase groups.

Discussion

L-ASNase has been used since 1970 in combination with other chemotherapeutic agents in the treatment of ALL. L-ASNase

activity is related to the dependency of malignant cells to asparagine, a non-essential amino acid. Extra-cellular destruction of asparagine by L-ASNase leads to leukaemic cell death. Given that the use of L-ASNase is often marked by the occurrence of major side effects, the administration of a new presentation of L-ASNase, such as its encapsulation within erythrocytes (GRASPA[®]), with a better tolerance would certainly represent an important improvement for the treatment of ALL.

Free ASNase was detected within the first 24 h following the administration of GRASPA[®] and was not detected thereafter. This confirmed the intracellular process for asparagine depletion. Nevertheless, PK of L-ASNase entrapped within RBC was long (half-life: 40 d) and similar to that observed with regular RBC, suggesting that the enzyme was not subject to degradation. In addition, asparagine depletion was maintained for 18.5 d (range, 8.9–45 d) after the administration of 150 iu/kg of GRASPA[®]. These results indicate that serum asparagine concentrations rapidly decrease after GRASPA[®] administration and remain low for a prolonged period, as it was observed with eight injections of *E. coli* L-ASNase. RBC transporting GRASPA[®] acted as microbioeffectors, pumping asparagine from plasma to the erythrocyte, in which it was cleaved by the enzyme.

Clinical hypersensitivity reactions are common features during treatment with L-ASNase. Mean clinical manifestations include generally local or generalized eruption, oedema, hypotension and potential anaphylactic shock. In contrast to what was observed in the *E. coli* L-ASNase group, no severe clinical allergic manifestations were reported in the GRASPA[®]

group. These results were in accordance with the hypothesis that ASNase entrapped within RBC can prevent the binding of antibodies directed against ASNase. Mechanisms and specific relationships between the level of antibodies, inactivation of L-ASNase, asparagine level and clinical reactions remain, however, not clear. The inactivation of ASNase has been related to the production of anti-ASNase antibodies, thereby affecting its ability to deplete asparagine. A high frequency of antibodies has been reported (70% of patients) with higher levels amongst patients who developed allergic reactions (Woo *et al*, 1998).

Three patients treated with a second injection of GRASPA® showed asparagine depletion for only a few hours. Nevertheless, erythrocyte entrapped ASNase activity was maintained, with PK data confirming that the enzyme is definitively protected inside the RBC. No clinical hypersensitivity reaction was noted in these patients but anti-ASNase antibodies were detected. Although these data are confusing, we hypothesize that a circulating factor or a drug could interact with the amino acid permeability and/or the transport of L-ASNase through the erythrocyte membrane. Preclinical investigations are ongoing to clarify this observation.

Finally, GRASPA® was associated with less coagulation disorders, when compared to *E. coli* L-ASNase, particularly regarding decreased level of antithrombin III. It has been suggested that glutaminase activity of *Escherichia coli*- or *Erwinia carotovora*-derived ASNase may be responsible for hepatic toxicities associated with a decreased antithrombin III and albumin activity (Distasio *et al*, 1982). We suggest that the reduced side effects could be related to a significantly lower depletion in glutamine with GRASPA®, as compared to that observed with *E. coli* L-ASNase (data not shown). Although glutamine deamination by ASNase may contribute to enhancing leukaemic cell apoptosis (Panosyan *et al*, 2004), a free-glutaminase ASNase from *Vibrio succinogenes* showed a potent anti-tumoural activity without severe hepatotoxicities (Distasio *et al*, 1982). Another potential explanation could be a

direct interaction between L-ASNase and plasma coagulation factors, which could increase their degradation (Kelo *et al*, 2002).

In conclusion, one single injection of 150 iu/kg of GRASPA® provided similar activity as eight injections at 10 000 iu/m² of native *E. coli* L-ASNase given to deplete asparagine in children and adults with relapsing ALL. The safety profile of GRASPA® showed a reduction of allergic reactions and a trend towards less coagulation disorders. Thus, GRASPA® appears to be a promising product with a good safety profile for the treatment of relapsing and refractory ALL. New trials with GRASPA® are currently ongoing in children and adults with ALL who previously developed allergic reactions to the native L-ASNase or to the pegylated formulation. This seems particularly important for frail patients such as elderly patients, for whom native L-ASNase is generally not included in trials because of its poor tolerance.

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Conflict of Interest

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