Erythrocyte encapsulated L-asparaginase (GRASPA) in acute leukemia

Xavier Thomas^{*1} & Caroline Le Jeune¹

Practice points

- L-asparaginase represents a major drug in the treatment of acute lymphoblastic leukemia, but the occurrence of major adverse effects often leads to early 'on study' withdrawal of the enzyme.
- Red blood cells, used as biocompatible and biodegradable vehicles for drugs, have been shown to protect L-asparaginase, enhance its half-life and reduce the occurrence of adverse events.
- GRASPA is a novel platform of red blood cell encapsulation of L-asparaginase. Plasmatic asparagine is actively pumped through the erythrocyte membrane into the intracellular compartment where it is cleaved by the entrapped L-asparaginase producing aspartate and ammonia. The enzyme is protected from reactions which would result from a systemic exposure, thus reducing the occurrence of immunological adverse events.
- Erytech Pharma (Lyon, France) is currently developing GRASPA for the treatment of acute lymphoblastic leukemia and older patients with acute myeloid leukemia.

L-asparaginase, an enzyme originally derived from *Escherichia coli*, represents a major drug in the treatment of acute lymphoblastic leukemia. However, the occurrence of major adverse effects often leads to early withdrawal of the enzyme. Main side effects include immune-allergic reactions, coagulopathy, pancreatitis and hepatic disorders. Novel asparaginase formulations and alternative sources have been developed to address this issue, but the results were not totally satisfactory. L-asparaginase loaded red blood cells (RBCs; GRASPA) represent a new asparaginase presentation with reduced immunological adverse reactions. RBCs protect L-asparaginase, enhance its half-life and reduce the occurrence of adverse events. We reviewed the history, biology and clinical experiences with L-asparaginase, and the characteristics and first clinical experiences with GRASPA in the treatment of acute leukemia.

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One of the primary drugs used in the treatment of acute lymphoblastic leukemia (ALL) is L-asparaginase. Asparaginase is an enzyme that catalyzes the hydrolysis of the amino acid asparagine to aspartic acid thereby depleting levels in the serum. Asparagine is a nonessential amino acid for normal tissues, but is essential for leukemia cells that cannot synthesize it and rely on extracellular asparagine from the serum. Asparagine is an important element for synthesis of proteins, DNA and RNA. Historically, Kidd observed regression of transplanted lymphomas after administration of serum from guinea pig to rodent in 1953 [1]. The tumor-inhibitory effect was confirmed in the 1960s by John D Broome to be due to L-asparaginase [2], and isoforms of L-asparaginase were isolated [3]. Later, the EC-2 enzyme from *Escherichia coli* showed optimal characteristics [4], and the enzyme was purified

KEYWORDS

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- chemotherapy
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- L-asparaginase • safety profile • targeted therapy

¹Hospices Civils de Lyon, Hematology Department, Lyon-Sud Hospital, Bât.1G, 165 chemin du Grand Revoyet, 69495 Pierre-Bénite, France

^{*}Author for correspondence: Tel.: +33 478862235; Fax: +33 472678880; xavier.thomas@chu-lyon.fr

for clinical studies [5]. After confirmation of efficacy in animal models [6], the first clinical uses of L-asparaginase showing transient improvement were reported after 1966 [7–9]. At the end of the 1960s, cellular asparagine synthetase was associated with resistance to L-asparaginase [10].

Over the last decades, advances in the treatment of childhood ALL have led to a significant improvement of outcomes with long-term survival in almost 90% of cases [11]. Pediatric studies have supported the use of asparaginase and asparaginase dose intensification as major factors for this improved outcome [12–15]. Asparaginase is currently used during induction, consolidation and maintenance phases of pediatric ALL therapy. Less improvements were observed in adult ALL with generally long-term disease-free survival of less than 50% [11,16]. Retrospective comparisons showed better outcome for adolescents treated with pediatric-based regimens compared with those treated with adult-based regimens [17–21]. The intensive use of asparaginase in pediatric regimens was one of the points differing among adults and children regimens. Although severe toxicity was more frequent in adults than in children [22,23], due to a decreased clearance of enzyme and therefore an increased incidence of severe adverse events, recent studies using pediatric-based regimens in young adult patients involving asparaginase dose intensification have significantly improved the outcome in this adult population [24–26]. This has resulted in renewed interest in asparaginase therapy and its subsequent toxicities in adult ALL, and started again interest in the potential benefit of asparaginase therapy in acute myeloid leukemia (AML). Because of the occurrence of major adverse effects, novel asparaginase formulations, such as *Erwinia chrysanthemi* L-asparaginase [27] and pegylated *Escherichia coli* L-asparaginase [28], were developed. Another new formulation, erythrocyte encapsulated L-asparaginase (GRASPA® [Globule Rouge ASPAraginase], Erytech) has recently demonstrated efficacy and a favorable safety profile. In this review of the literature, the first results published with GRASPA in the treatment of acute leukemia are reported, after summarizing the history, biology, pharmacology and current status of L-asparaginase in the treatment of acute leukemia.

Mechanism of action of L-asparaginase

Unlike normal cells, leukemia cells have very low levels of asparagine synthetase [29]. They are not able to synthesize asparagine and are

dependent on extracellular asparagine, which is present in serum, for their proliferation and survival [30]. Asparagine synthetase expression is regulated in part by asparagine synthetase promoter DNA CpG island methylation, methyl-CpG-binding protein level, and core histone tail acetylation and methylation [31]. Hypermethylation of the promoter inhibits asparagine synthetase gene expression. Normal cells are not dependent on extracellular asparagine due to their ability to produce asparagine from L-glutamine by the action of asparagine synthetase. In leukemia cells, L-asparaginase depletes the circulating pool of L-asparagine, which in turn inhibits protein synthesis and causes cell cycle arrest in the G1 phase and ultimately apoptosis. L-asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonia (Figure 1). Glutamine can rescue asparagine-deprived cells by serving as a substrate of asparagine synthetase to resynthesize asparagine via a transamidation reaction. Glutamine reduction is therefore also necessary for antileukemic activity against asparagine synthetase-positive cells [32,33], whereas asparagine synthetase-negative cells have been reported to be hypersensitive to asparagine depletion alone [33,34]. Both *Escherichia coli* and *Erwinia chrysanthemi* L-asparaginases possess glutaminase activity. Concentrations of asparagine in serum are on the order of 50 µM, whereas those of glutamine are on the order of 500 µM [35]. Optimal asparaginase activity depends on pH. The glutaminase activity of L-asparaginase has been shown to correlate with a clinical efficacy in refractory ALL patients [36]. Reduction of both asparagine and glutamine concentrations by L-asparaginase has been associated with inhibition of mTOR and activation of autophagy [37]. Inhibition of mTOR leads to inhibition of downstream events which suppresses synthesis of ribosomal proteins at the mRNA translation level [38]. This affects leukemia cells and contributes to L-asparaginase antileukemic activity. In fact, a rationale has been suggested for combination therapy with L-asparaginase and hydroxychloroquine – an inhibitor of autophagy [39].

Current asparaginase formulations

Three asparaginase formulations are widely used against ALL: native *Escherichia coli* asparaginase (Elspar [Lundbeck], Kidrolase [Jazz Pharmaceuticals, Inc., Dublin, Ireland], Crasnitin [Bayer AG, Leverkusen, Germany],

Figure 1. L-asparaginase converts plasmatic L-asparagine into L-aspartate plus ammonia. Leukemia cells are deficient in asparagine synthetase. L-asparagine deprivation, due to L-asparaginase activity, leads to asparagine starvation impairing protein biosynthesis in leukemia cells and then leading to apoptosis through cellular dysfunction.

Leunase [Kyowa Hakko Kirin, Tokyo, Japan], Asparaginase medac [Kyowa Hakko]), its pegylated form (Oncaspar [Sigma-Tau Pharmaceuticals, Inc., MD, USA], pegaspar-gase) and *Erwinia chrysanthemi* asparaginase (Erwinaze, Erwinase [Jazz Pharmaceuticals, Inc.]). Pharmacologic characteristics and usual administered doses of these asparaginase products are reported in **Table 1** [40]. The antileukemic activity of asparaginases depends upon the rate of clearance of the asparaginase enzyme, its pharmacological factors, the development of anti-asparaginase antibodies, the potential development of resistance and the increased contribution of asparagine either from *de novo* biosynthesis or the input from the nutrient intake [40–43]. The therapeutic benefits of asparaginase in adults are less certain than in children because of the potential occurrence of severe drug toxicities. This was confirmed in a study comparing the incidence of grade 3 to 4 toxicities according to age, which showed that older patients suffered greater incidence of liver and pancreas toxicities [22].

• Native *Escherichia coli* asparaginase

L-asparaginase purified from *Escherichia coli* has been widely used in clinical trials. The enzyme has a molecular weight of 133,000–141,000 daltons and is composed of four subunits. The specific activity of purified

enzyme is 300–400 $\mu\text{M}/\text{mn}/\text{mg}$ of protein, the isoelectric point lies between pH 4.6 and 5.5, and the K_m for asparagines is usually 1×10^{-5} [44]. The *Escherichia coli* enzyme contains 321 amino acids in each subunit. It is highly specific for L-asparagine as substrates. Complete asparagine depletion after treatment by native L-asparaginase has been described in more than 90% of cases in childhood ALL. Cytotoxicity correlates well with inhibition of protein synthesis. The mechanism of cell death may be the activation of programmed cell death or apoptosis. However, there are disparities in terms of half-life and efficacy between the various products [45,46]. L-asparaginase activity is detectable in the bloodstream for 1–3 weeks after large single doses, and L-asparagine is not measurable in serum for one week or longer [47]. Despite a poor penetration in the cerebrospinal fluid, an antileukemic effect is exerted in this sanctuary. The drug is not excreted; it is removed from the circulation by being taken up by the reticuloendothelial system [47]. Complete asparagine depletion in serum has been reported with induction doses of 10000 IU/m², 5000 IU/m² and even to 2500 IU/m² administered at 3-day intervals in order to reduce toxicity [48].

• Polyethylene-glycol-asparaginase

Polyethylene-glycol (PEG)-asparaginase is a modified form of L-asparaginase characterized by the covalent conjugation of *Escherichia coli*

Table 1. Pharmacologic characteristics and usual administered doses of current asparaginase products.

Formulation	Elimination half-life	Asparagine depletion (days) [†]	Anti-asparaginase antibody (%) [‡]	Doses
Native <i>Escherichia coli</i> asparaginase	26–30 h	14–23	45–75	6000 IU/m ² × 3/week
Polyethylene-glycol-asparaginase	5.5–7 days	26–34	5–18	2000–2500 IU/m ² every 2–4 weeks
<i>Erwinia chrysanthemi</i> asparaginase	16 h	7–15	30–50	6000 IU/m ² /d × 10, then 3 doses weekly, or 30000 IU/m ² /d × 10

[†]Days after dose administration.
[‡]Percentage of patients.

asparaginase to monomethoxypolyethylene glycol resulting in significant pharmacokinetic variations as compared with the native *Escherichia coli* formulations [49,50]. PEG-asparaginase has the same chemical properties as native *Escherichia coli* asparaginase. The elimination half-life of PEG-asparaginase is five-times longer than the native *Escherichia coli* preparations and nine-times longer than *Erwinia chrysanthemi* asparaginase (Table 1). This leads to a decreased number of injections needed to achieve therapeutic efficacy in naive patients [51]. Maximum amino acid depletion and peak enzyme activity levels are obtained within 5 days after intramuscular administration [41]. Intravenous administration achieves rapid peak levels and avoids painful intramuscular injections [52]. A dose-dependent reduction of cerebro-spinal fluid asparagine levels was observed after PEG-asparaginase administration, although asparaginase does not penetrate in the cerebrospinal fluid [53]. PEG-asparaginase also demonstrated L-glutaminase activity as found in native *Escherichia coli* L-asparaginase. In pediatric patients, because of the greater incidence of allergic reactions to native asparaginase, PEG-asparaginase is currently used as first-line treatment in most countries. In adults, adverse events can be exacerbated with use of the PEG-asparaginase relative to native asparaginase because of its longer elimination half-life. PEG-asparaginase was implicated as being ‘definitively or probably’ involved in 11 of 18 induction deaths in the UKALL 14 clinical trial [54]. PEG-asparaginase antigen does not cross-react immediately with the antibody against native asparaginase but in time it will cross react. Patients treated with PEG-asparaginase have shown more rapid clearance of lymphoblasts from day 7 and day 14 bone marrow aspirates and more prolonged asparaginase activity than those treated with native asparaginase [41]. High-titer antibodies were associated with low asparaginase activity in the native arm, but not in the PEG-asparaginase arm.

• ***Erwinia chrysanthemi* asparaginase**

Erwinia chrysanthemi asparaginase is a valid second- or third-line therapy. It has been used to treat patients having allergy to *Escherichia coli* asparaginases [55]. At a similar dosage, its activity, and therefore its efficacy, were significantly lower than observed with *Escherichia coli* asparaginase, while the incidence of toxicity was lower [56,57]. Asparagine depletion after a second exposure was also lower than that observed in patients treated with native L-asparaginase [58,59]. This led to higher doses and an increased frequency of treatment when using *Erwinia* as replacement for *Escherichia coli* asparaginase. The reason for different dosages and dose schedule of *Erwinia* being necessary to achieve same clinical efficacy is related to the shorter elimination half-life of *Erwinia* relative to the *Escherichia coli* preparation. Intravenous administration showed higher asparaginase efficacy as compared with intramuscular administration [60]. With regards to the formation of neutralizing antibodies, there were no significant differences among both routes of administration [61].

A pegylated recombinant *Erwinia* asparaginase (mPEG-r-crisantaspase) has recently undergone Phase 1 evaluation (NCT015515124) [62]. First results showed it less immunogenic and more potent than Erwinase® at depleting plasma L-asparagine with a markedly increased half-life.

Limitations of L-asparaginase administration

• **Hypersensitivity**

Bacterial proteins can induce immunologic reactions. Allergic reactions are primarily due to anti-asparaginase antibody in the circulation [41,42]. Asparaginase hypersensitivity reactions may range from itching, rash and bronchospasm to severe allergic reactions and anaphylaxis [43]. Most reactions occur within one to several hours after administration. About 60% of patients will present hypersensitivity reactions during treatment with *Escherichia coli*

L-asparaginase. PEG-asparaginase can also show hypersensitivity reactions. The incidence of skin reactions is higher after intramuscular administration than after intravenous administration. Hypersensitivity reactions are generally observed during the post-induction phase. Indeed there is a delay for generation of immune response [43]. One notable challenge is that allergic symptoms can be masked by corticosteroid therapy during the induction phase; despite the absence of allergic reaction, the presence of asparaginase antibodies can reduce the drug efficacy. Patients who develop hypersensitivity reactions to L-asparaginase have a decreased half-life for the enzyme [63,64]. Hypersensitivity reactions require changing to another form of asparaginase.

• Coagulation disorders

L-asparaginase can lead to coagulation disorders due to deficiency of inhibitor of serine protease proteins: antithrombin and α_1 -antitrypsin. Coagulation disorders are not higher with native L-asparaginase than with PEG-asparaginase [65]. Coagulation disorders are due to the effect of the drug on protein synthesis, reduction in antithrombin, fibrinogen, plasminogen and factors IX and X with prolongation of thromboplastin exposure [66]. L-asparaginase also leads to protein C and S deficiency, which increases thrombin level and the risk of bleeding and thrombosis [67].

Other toxicities

L-asparaginase treatment is associated with liver toxicity with histological presence of macro- and microvesicular liver steatosis [68,69]. The mechanism involves glutamine deficiency, decreased hepatic protein synthesis and impairment of beta-oxidation in mitochondria [68,70]. L-asparaginase can also induce pancreatic toxicity. Synthesis inhibition of amylase and lipase, regulated by asparagine, can lead to severe pancreatitis [71]. L-asparaginase can decrease insulin synthesis and induce diabetes. Reduction of the levels of asparagine and glutamine in cerebral tissue can be responsible of central nervous system adverse events such as hallucination, drowsiness and amnesia. Reversible encephalopathy syndrome has also been described. Approximately two-third of patients receiving L-asparaginase experience nausea, vomiting and chills as an immediate reaction. The only well-established drug interaction is its ability to terminate methotrexate action [72]. The antagonism of L-asparaginase given before

methotrexate is possibly the result of inhibition of protein synthesis, or the inhibition of methotrexate polyglutamylation, with decreased retention of methotrexate by leukemic cells.

• Resistance to L-asparaginase

Clinical resistance to L-asparaginase is due to L-asparaginase instability or proteolysis in the blood, production of anti-asparaginase antibodies, stromal production of asparagine and/or induction of malignant cell asparagine synthetase. The production of anti-asparaginase antibodies causes premature clearance of drug (as well as allergic reactions), leading to failure of asparagine depletion after subsequent administration of L-asparaginase [73]. Cross-reactivity was observed between pegylated and native *Escherichia coli* L-asparaginase antibodies. Resistance may also be related to a high level of cellular asparagine synthetase [74–79] which has been shown to be higher in T-lineage ALL than in B-lineage ALL [80]. Another mechanism of resistance is represented by the increased expression of asparagine synthetase by mesenchymal cells of the bone marrow microenvironment [80] – a mechanism that could contribute to the development of minimal residual disease in ALL patients [81].

• Drug monitoring for asparaginase

Effective asparaginase therapy results in sustained and prolonged asparagine depletion. Asparaginase enzyme activity >0.1 IU/ml (and possibly >0.05 IU/ml) is considered a reasonable threshold for maintaining asparagine depletion, which has been suggested to be $0.1 \mu\text{M}$ [82–84]. However, studies suggested that adult patients might have a lower bodily clearance rate for asparaginase as compared with children [85,86]. This results in increased drug levels and might explain the greater incidence of toxicities in the adult population. A randomization between a fixed dose of $25,000 \text{ IU/m}^2/\text{week}$ of native asparaginase and pharmacokinetically-guided individualized dosing based on nadir serum asparaginase enzyme activity measured every 3 weeks showed better outcome for patients receiving the individualized arm. Unexpectedly, these patients with improved responses received lower median asparaginase dose ($17,500 \text{ IU/m}^2$ vs $25,000 \text{ IU/m}^2$). The explanation for the improved outcome observed was due to identification of patients with silent hypersensitivity or silent inactivation on the individualized dosing arm in real-time and the immediate change

to *Erwinia* asparaginase. Toxicities were not different among these two groups [87]. Overall these results suggest that prospectively monitoring for the silent inactivation and changing asparaginase preparation may improve outcome. Asparaginase activity during first-line therapy with native *Escherichia coli* asparaginase and second-line therapy with PEG-asparaginase was inversely related to anti-*Escherichia coli* asparaginase antibody levels [88]. In the presence of moderate antibody levels (6.25–200 AU/ml), the switch from native to PEG-asparaginase resulted in a significant increase of asparaginase activity. *Erwinia* asparaginase was found to be the best asparaginase alternative, if antibody levels against *Escherichia coli* asparaginase antibody exceed 200 AU/ml. In case of silent allergy, patients receive a shorter duration of effective asparaginase treatment. Monitoring for silent anti-asparaginase antibodies or loss of asparaginase activity could provide a better understanding of asparaginases and thereby improve outcome. Analyses demonstrated also that patients with higher than 1 μ M of cerebrospinal fluid asparagine levels during the asparaginase treatment were more likely to have isolated central nervous system relapse later [53].

• Erythrocyte encapsulated L-asparaginase

In order to overcome the limitations of current asparaginase formulations, modified preparations of L-asparaginase have been proposed [89]. In an attempt to develop a nonimmunogenic delivery system, two delivery mechanisms are currently being explored: nanoparticle encapsulated [90] and RBC encapsulated L-asparaginase [91]. The use of RBC as a delivery system was first explored in 1979. Erythrocytes can be used as a circulating microbio reactor. The advantage of RBC as carrier is its biodegradability, general circulation throughout the whole body and lack of toxic product formation and immunogenicity. Asparagine is able to diffuse freely into erythrocytes from an external medium [92]. The first attempts to load RBC with L-asparaginase were conducted by Updike *et al.* [93] and by Kravtsoff *et al.* [94] in the 1980s and 1990s.

The half-life of free asparaginase is about 26 h [41], and that of PEG-asparaginase is extended to approximately 5 days [95]. Erythrocytes, by contrast, have an average lifespan of 120 days. RBC-encapsulated L-asparaginase has indeed been designed to increase

bioavailability and half-life of asparaginase, and therefore prolong its action in the blood circulation without any loss of specific activity. Therapy using RBC encapsulated enzymes can maintain therapeutic blood levels, reduce the dosage and frequency of drug administration and prevent the need for expensive chemical modification [96]. The enzyme-loaded RBC depletes plasma asparagine and glutamine levels, but is protected from circulating proteolytic enzymes [97]. Several methods including drug-induced endocytosis [98], electroporation [99], use of the membrane-translocating low molecular weight protamine [100] and hypo-osmotic methods [93] have been used to encapsulate drugs into erythrocytes. Reversible hypotonic dialysis is the most controlled and reproducible method. With this process, human RBCs can be loaded with 116 ± 15 IU of L-asparaginase per milliliter of red cells [96]. The resulting product allows transport of L-asparagine through the RBC membrane where L-asparaginase hydrolyzes it. Asparagine levels inside the erythrocytes are typically two- to three-fold higher than in plasma due to active transport of asparagine via a nitrogen-enriched amino acid transporter into erythrocytes [101]. Due to the RBC membrane, L-asparaginase is theoretically protected from proteolytic catabolism as well as from neutralizing antibodies, resulting in an increased half-life and a reduction in hypersensitivity reactions [96].

GRASPA (Erytech, Lyon, France) is a novel platform of RBC encapsulation of L-asparaginase that incorporates the aforementioned features (Figure 2). L-asparaginase encapsulated in erythrocytes is manufactured with compatible leucocyte reduced packed RBC prepared and delivered by a blood bank according to current approved practices. Patient pretransfusion status include validated erythrocyte phenotype and ABO grouping and an irregular antibody screening test performed less than 72 h prior to GRASPA transfusion. The encapsulation technique, reversible hypotonic lysis and resealing of the erythrocytes, is a controlled technique enabling asparaginase to be encapsulated in a safe, reproducible, controlled, automated manner. There is an extracellular fraction of asparaginase contained within the bag, as a consequence of the liberation of asparaginase during the natural hemolysis of RBCs. However, the contribution of nonencapsulated L-asparaginase to the depletion of plasma asparagine is limited. Pharmacokinetics of L-asparaginase loaded

Figure 2. Mechanism of action of GRASPA.

in RBCs (based on asparaginase activity) has demonstrated a half-life of about 1 month [102]. Toxicities with GRASPA were thrombocytopenia and anemia similar to those observed with naked L-asparaginase. No allergic reactions were observed, although all patients developed anti-asparaginase antibodies.

GRASPA in ALL

• The GRASPALL 2005-01 randomized trial

This Phase 1/2 multicenter randomized controlled trial was the first study with GRASPA in children and adult with ALL refractory or in first relapse. Duration of asparagine depletion was investigated following three doses of GRASPA. Eighteen patients received GRASPA (six patients at 50 IU/kg, six patients at 100 IU/kg and six patients at 150 IU/kg) and six patients were assigned to the control group with treatment by the native *Escherichia coli* L-asparaginase. Regarding asparagine depletion, one single injection of GRASPA at 150 IU/kg provided the same results as the intravenous administration of $8 \times 10\,000$ IU/m² of native L-asparaginase. The safety profile of GRASPA showed a reduction in the number and severity of allergic reactions and a trend towards fewer coagulation disorders when compared with *Escherichia coli* L-asparaginase. The other expected side effects were comparable among

the two L-asparaginase presentations. There were no differences among the three doses of GRASPA in terms of toxicity [102].

• The GRASPALL/GRAALL-SA2-2008 study

The prognosis of older patients with ALL is particularly poor with 2-year overall survival (OS) ranging from 20% to 39%, and long-term survival in only 30% of cases [103]. Here, native L-asparaginase has shown increased toxicity, almost precluding its use in this patient population [104,105]. In this setting, L-asparaginase encapsulated in erythrocytes may have theoretical advantages. The GRASPALL/GRAALL-SA2-2008 Phase 2 trial evaluated the safety and efficacy of GRASPA in patients ≥ 55 years with Philadelphia (Ph) chromosome-negative ALL. Thirty patients received escalating doses of GRASPA on days 3 and 6 of induction Phases 1 and 2. Asparagine depletion < 2 $\mu\text{mol/l}$ for at least 7 days was achieved in 85% and 71% of patients with 100 and 150 IU/kg, respectively, but not with 50 IU/kg. No allergic reaction or clinical pancreatitis was observed. Main grade 3/4 side effects included: infections (77% of cases), increased transaminase levels (20%), increased bilirubinemia (7%) and deep vein thrombosis (7%). Anti-asparaginase antibodies were detected in 50% of cases. They were

associated with a decreased duration of asparagine depletion. Complete remission (CR) was achieved in 70% of patients. With a median follow-up of 42 months the median OS was 15.8 months and 9.7 months for the 100 IU/kg and the 150 IU/kg cohorts, respectively [106].

• **The GRASPALL 2009-06 Phase 2/3 randomized trial**

The GRASPALL 2009-06 trial is a multi-centre, open randomized, Phase 2/3 study evaluating efficacy and safety of erythrocytes encapsulating L-asparaginase versus native L-asparaginase in combination with standard polychemotherapy (COOPRALL regimen) in adult and child patients with first recurrence of Ph chromosome-negative ALL with or without known hypersensitivity to L-asparaginase (Figure 3). Eighty patients (1–55 years of age) were registered. Patients with no prior history of allergy were randomized between GRASPA (150 IU/kg; 26 patients) and native L-asparaginase (10 000 IU/m²; 28 patients). Patients

with a prior history of allergy received GRASPA (26 patients). The primary end points were the duration of greater than 100 IU/l asparaginase activity and the incidence of asparaginase hypersensitivity during the induction phase. In the randomized trial, GRASPA significantly reduced the incidence of hypersensitivity (0% vs 43%; $p < 0.001$). The efficacy criterion was defined as plasmatic asparagines level $\leq 2 \mu\text{M}$. Asparaginase activity was maintained for 25.5 days and 9.8 days in the GRASPA and native L-asparaginase groups, respectively ($p < 0.001$). The proportion of patients achieving minimal residual disease (MRD) negativity was 35 and 25%, respectively. CR rates were 65 and 39% ($p = 0.02$), and allogeneic stem cell transplantation in responding patients can be performed in 65 and 46%, respectively. The 1-year event-free survival (EFS) rate was 65% in the GRASPA arm and 49% in the native L-asparaginase arm [107]. Mean C_{max} for encapsulated L-asparaginase was typically higher in children compared with adults.

Figure 3. Schema of the GRASPALL 2009-06 Phase 2/3 randomized trial testing GRASPA versus native L-asparaginase in combination with standard polychemotherapy (COOPRALL regimen) in patients with first recurrence of Ph chromosome-negative acute lymphoblastic leukemia in adults and children with or without known hypersensitivity to L-asparaginase. Patients with no prior history of allergy were randomized between GRASPA (150 IU/kg) and native L-asparaginase (10,000 IU/m²), while patients with a prior history of allergy received systematically GRASPA.

[†]GRASPA-S: Single cohort in allergic patients.

6-MP: 6-mercaptopurine; ALL: Acute lymphoblastic leukemia; ASP: Asparaginase; mo: Month; MTX: Methotrexate; PS: Performance status.

GRASPA in acute myeloid leukemia**• L-asparaginase in the treatment of AML**

Escherichia coli L-asparaginase is effective in killing HL-60 cells by inhibiting growth, blocking protein synthesis and inducing apoptosis [108]. In animal studies, the cytotoxicity of high-dose cytarabine has been shown potentiated by sequential administration of asparaginase [109]. French–American–British (FAB) AML subtypes M1, M4 and M5 have been shown particularly sensitive to L-asparaginase [110]. They were negative for asparagine synthetase. In this study, M1 cells appeared as sensitive as ALL cells. Another study also showed that AML cells were sensitive to L-asparaginase. However, AML cells were shown sevenfold more resistant to L-asparaginase than ALL cells [111], and M5 subtype was equally sensitive as ALL. No correlation between asparagine synthetase expression and asparaginase resistance was reported in AML [108]. Several assays have been used to predict L-asparaginase sensitivity in AML including techniques for estimation of asparagine synthetase mRNA and protein levels, and blast sensitivity to L-asparaginase [110,112].

In a pilot study of 22 poor-risk AML patients, CR was achieved in 15 cases. The median duration of remission was 5 months [113]. The treatment regimen comprised high-dose cytarabine 3 g/m²/12 h for four doses, followed by intramuscular asparaginase at 6000 IU/m². The same schedule was repeated on day 8. Based on these first results, a randomized study comparing high-dose cytarabine plus L-asparaginase with high-dose cytarabine alone was performed by the Cancer and Leukemia Group B (CALGB) [114]. The CR rate was 40% in the first group, compared with 24% in the second group ($p = 0.02$). The OS was also better in patients treated with the combination of high-dose cytarabine and asparaginase 19.6 vs 15.9 weeks; $p = 0.04$). Toxicity was similar in the two treatment arms. Although induction mortality was not different among arms, there were more patients who died of aplastic marrow after high-dose cytarabine plus asparaginase than with high-dose cytarabine alone (16% vs 7%). In another study combining high-dose cytarabine with L-asparaginase in elderly AML patients, 43/125 evaluable patients (34%) achieved CR [115]. Furthermore, a synergistic effect was observed when asparaginase and cytarabine were used sequentially. In a large series of 1294 pediatric patients, high-dose cytarabine and asparaginase intensification eliminated the benefit of prolonged

maintenance therapy and improved OS [116]. Administration of cytarabine/L-asparaginase intensification shortly after remission induction was accompanied by an improved 5-year OS rate when compared with historical controls (36% vs 29%; $p < 0.02$). More recently, a combination of methotrexate and asparaginase was tested in relapsed or refractory children with AML. The treatment was repeated every 7–10 days and yielded a response in 6 out of 15 patients [117]. A retrospective study of 108 patients with relapsed or refractory AML, who were treated with high-dose cytarabine (1–3 g/m²/12 h for five doses), mitoxantrone (6 mg/m²/d for 3 days) and asparaginase (6000 IU/m² following the last dose of mitoxantrone), showed a 37% CR rate in patients ≥ 60 years and a 49% CR rate in younger adults. Median OS was 5.8 months with a 3% survival at 24 months in older patients and 13.6 months with a 40% survival at 24 months in younger patients [118].

Based on these first results, Erytech has developed L-asparaginase encapsulated in erythrocytes for the treatment of older patients with AML.

• The GRASPA-AML 2012-01 study

A multicenter, open, randomized Phase 2b trial evaluating GRASPA combined with low-dose cytarabine versus low-dose cytarabine alone in newly diagnosed AML patients (65–85 years of age) who are unfit for intensive chemotherapy is currently ongoing (NCT01810705) [119]. The primary objective is OS. The key secondary objectives are progression-free survival, CR, EFS, quality of life, pharmacokinetic pharmacodynamic profiles and immunogenicity (Figure 4). The study is currently in recruiting phase.

Discussion

The outcome of treatment for acute leukemia has improved over the years, but the challenges in this area have remained formidable. This is particularly the case for relapsed/refractory acute leukemia and for leukemia in the elderly. Standard treatment regimens in these settings have significant toxicities and often fail to improve response rate, survival duration or quality of life.

ALL has a bimodal distribution with an early peak at 4–5 years of age followed by a second gradual increase at 50 years. Advances in the treatment of ALL over the past decades have resulted in a remarkable improvement in

Figure 4. The GRASPA-AML 2012-01 study schedule. Randomization was 2:1 with a combination of low-dose cytarabine (40 mg daily per day from day 1 to day 10) with GRASPA (100 IU/kg on day 11) in the experimental arm, and LDC (40 mg daily per day from day 1 to day 10) alone in the control arm. AML: Acute myeloid leukemia; C: Cycle; LDC: Low-dose cytarabine; RANDO: Randomization.

childhood ALL [120]. In adult ALL, despite a high CR rate, most of the patients relapse with chemo-resistant disease, and only 40–50% of adult patients remain in remission at 5 years. While response is still high and can reach 50–60% in relapsed/refractory childhood ALL, the prognosis of adult patients with relapsed/refractory ALL is dismal with a 5-year OS of only 10% [121,122], demonstrating a different need for alternative treatment strategies in adults as compared with those required in the pediatric counterpart. Relapsed/refractory ALL represents a particularly challenging situation. In this setting, the ultimate goal is to proceed to allogeneic hematopoietic stem cell transplantation after achievement of a novel CR. Consequently, the development of effective and relatively nontoxic rescue therapies is needed.

AML is a disease of the elderly; the median age at diagnosis ranges between 68 and 72 years [123,124]. There is currently no consensus regarding optimal therapeutic strategy for older adults with AML. The benefit associated with intensive therapies in older patient remains questionable [125]. Due to comorbid conditions and disease features, concerns regarding toxicity have resulted in the ineligibility of many older patients with AML for intensive chemotherapy [126]. The recent availability of new drugs or drugs with new formulations that may have an improved side effect profile may offer future improvement for this patient population. Recent evidence supports the use of lower-intensity therapies in most of these patients and emphasizes the importance of tolerability and quality of life. One promising therapeutic strategy is to combine low-intensity treatments with novel agents, such as low-dose

cytarabine with gemtuzumab ozogamicin [127] or hypomethylating agents with histone deacetylase inhibitors [128].

In both ALL and AML, novel therapeutic approaches are required. Improved understanding of the specific biologic processes involved in the proliferation of leukemic cells has generated increased interest in the development and the potential use of targeted therapies. Treatments will indeed become more tailored and individualized. Conventional drug therapy is making progress as well. Older drugs have been developed under new formulations. This is the case for L-asparaginase for which new available formulations are opening a new era for this agent in the treatment of acute leukemias. L-asparaginase is unique in cancer therapy in that it acts directly at the metabolite level, rather than on DNA, RNA or protein. In this regard, it could be considered as the first targeted therapy. L-asparaginase may be given at various doses and schedules, but higher doses increase its efficacy potential. However, antibody production in response to L-asparaginase is not uncommon, resulting in rapid disappearance from plasma. Allergic reactions and silent inactivation represent the most important limitations to the prolonged use of any asparaginase product. Routine real-time monitoring can help to identify patients with silent inactivation and facilitate a switch to a different product. Attempts to reduce immunogenicity include the development of new formulations of the drug including those incorporating the enzyme into RBCs. PEG-asparaginase displays cross-reactivity with native asparaginase that may harm its therapeutic effects. *Erwinia* asparaginase does

not display cross-reactivity to *Escherichia coli*-derived products, and may be a second-line treatment option. RBC-encapsulated L-asparaginase is a molecularly targeted agent which may augment the treatment armamentarium for acute leukemia patients. It should provide improved enzyme delivery with fewer allergic reactions, decreased immune clearance and decreased proteolysis than *Escherichia coli* L-asparaginase. Although incidence of clinical thrombosis, decreased fibrinogen and antithrombin III were lower with GRASPA, there were similar frequencies of pancreatic and hepatic dysfunction. The overall reduction of adverse events with GRASPA may be due to impairment of IgE formation and/or reduced glutaminase activity by the RBC membrane barrier.

However, studies with GRASPA only compared with native L-asparaginase. PEG-asparaginase also reduced immunologic adverse effects. It seems therefore important to see whether further studies used PEG-asparaginase head-to-head against GRASPA could demonstrate differences in terms of tolerability. Both GRASPA and PEG-asparaginase have longer half-life than native L-asparaginase. Due to its short half-life, repeated administrations of native L-asparaginase are necessary to achieve a sustained activity. GRASPA, as PEG-asparaginase are then expected to decrease the number of administration for a similar effect considering depletion duration. The most frequent and severe allergic reactions have been observed after multiple doses of asparaginase. The risk of the reaction is reduced when asparaginase is given in combination with steroids or other immunosuppressive agents. PEG-asparaginase or *Erwinia* asparaginase can circumvents recurrent allergic reactions once a reaction to native asparaginase has been documented. However, the IgG antibodies against native *Escherichia coli* asparaginase can recognize the pegylated antigen and *Erwinia* asparaginase can initiate its own immune reaction and development of anti-*Erwinase* antibodies [61]. Hypersensitivity may occur with the development of specific antibodies, which may lead either to allergic reactions or to 'silent inactivation' neutralizing L-asparaginase activity. Silent allergy in which circulating antibodies neutralize asparaginase activity can result in an ineffective contribution by the drug to the antileukemic pharmacodynamics effect, without causing any clinical allergic symptoms. Encapsulation of L-asparaginase in erythrocytes

is expected to reduce these reactions and give to allergic patient the opportunity to benefit from L-asparaginase.

Conclusion

Because L-asparaginase has been shown to be essential for the treatment of ALL and has recently demonstrated a novel interest for the treatment of elderly AML patients, it is likely that GRASPA will be developed for additional indications over the coming years. In ALL, it is used in patients who have demonstrated allergic reactions with the native L-asparaginase. In AML, the rediscovery of the potential efficacy of L-asparaginase could justify the use of GRASPA for the treatment of this disease for which there is a real medical need. The use of erythrocytes as a drug carrier appears as a promising drug delivery system. The applications of this innovative formulation, particularly for clinical use, should further concern other enzymes, such as thymidine phosphorylase for mitochondrial neurogastrointestinal encephalomyopathy, adenosine deaminase for immunodeficiency, glutamine synthetase for ammonia detoxification or blood coagulation factor IX for hemophilia B [96]. Other fields of development could be anti-infective drug-loaded RBCs (interferon- α and ribavirin for treatment of hepatitis C or antibiotic-loaded erythrocytes to treat resistant infections), anthracycline-loaded erythrocytes to reduce side effects, immunomodulation with antigen-loaded RBCs or hemoglobin allosteric effector-loaded erythrocytes for oxygen release improvement in sickle cell anemia.

Disclosure

In addition to the peer-review process, with the author's consent, the manufacturer of the product discussed in this article was given the opportunity to review the manuscript for factual accuracy. Changes were made by the author at their discretion and based on scientific or editorial merit only. The author maintained full control over the manuscript, including content, wording and conclusions.

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