

**A phase 2 study of L-asparaginase encapsulated in erythrocytes
in elderly patients with Philadelphia chromosome negative acute
lymphoblastic leukemia:**

The GRASPALL/GRAALL-SA2-2008 Study

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ABSTRACT

Purpose : The GRASPALL/GRAALL-SA2-2008 phase II trial evaluated the safety and efficacy of L-asparaginase encapsulated within erythrocytes (GRASPA[®]) in patients ≥ 55 years with Philadelphia chromosome-negative acute lymphoblastic leukemia.

Findings : Thirty patients received escalating doses of GRASPA[®] on day 3 and 6 of induction phases 1 and 2. The primary efficacy endpoint was asparagine depletion $< 2 \mu\text{mol/l}$ for at least 7 days. This was reached in 85% and 71% of patients with 100 and 150 IU/kg respectively but not with 50 IU/kg. Grade 3/4 infection, hypertransaminasemia, hyperbilirubinemia and deep vein thrombosis occurred in 77%, 20%, 7% and 7% of patients respectively. No allergic reaction or clinical pancreatitis was observed despite 17% of Grade 3/4 lipase elevation. Anti-asparaginase antibodies were detected in 50% of patients and related to a reduction in the duration of asparagine depletion during induction phase 2 without decrease of encapsulated L-asparaginase activity. Complete remission rate was 70%. With a median follow-up of 42 months, median overall survival was 15.8 and 9.7 months, in the 100 and 150 IU/kg cohorts respectively.

Conclusions : In conclusion, the addition of GRASPA[®], especially at the 100 IU/kg dose level, is feasible in elderly patients without excessive toxicity and associated with durable asparagine depletion. (clinicaltrials.gov identifier NCT01523782).

1. Introduction

The prognosis of older patients with Philadelphia (Ph) chromosome-negative acute lymphoblastic leukemia (ALL) is poor with 2-year overall survival (OS) ranging from 20% to 39% and less than 30% long-term survival [1-6]. New drugs or new approaches are clearly needed in this difficult-to-treat patient population. In a previous study, we have shown that either continuous infusion or encapsulation of anthracyclines in polyethylene-glycol liposomes failed to improve OS [7]. Asparaginase is an enzyme that deaminase serum asparagine, depleting it from ALL lymphoblasts. Several large randomised pediatric ALL trials reported that higher cumulative doses of asparaginase result in significantly improved outcome [8-12]. In young adults, the addition of L-asparaginase has been associated with increased disease-free survival (DFS)[13] and was related with effective asparagine depletion [14]. Multiple doses of intravenous pegaspargase[15] or intramuscular native L-asparaginase[16] can be added safely in young adults. Recently, native L-asparaginase has been added to elderly ALL induction therapy in three British [3], German [17] and Spanish [2] studies but increased toxicity has been observed in these 3 cohorts, almost precluding its use in the elderly ALL population.

Encapsulation of drugs in erythrocytes has many theoretical advantages such as biocompatibility and residence-time enhancement [18]. It is possible to encapsulate large molecules in fully functional erythrocytes in order to treat ALL[19-21]. L-asparaginase encapsulated in erythrocytes has been shown to enhance half-life of the drug and to reduce the occurrence of side effects [22-24]. To evaluate the benefit of adding this new L-asparaginase formulation to standard ALL therapy in older patients, we conducted an open-label, non-randomised phase IIa dose-finding study, designed to assess the maximum tolerated dose (MTD) and the asparagine depletion of L-asparaginase encapsulated in erythrocytes (GRASPA[®] - ERYTECH Pharma, France) during first-line treatment in elderly patients with

Ph-negative ALL aged 55 years or more. Three doses of GRASPA[®] (50, 100 and 150 IU/kg) were investigated, based on the results of a previous phase I/II study [22].

2. Design and methods

2.1. Patients

Untreated patients aged 55 years or more with non-Burkitt's, Ph- and/or BCR-ABL-negative ALL were enrolled in this GRASPALL/GRAALL-SA2-2008 study. Patients with a left ventricular ejection fraction <50%, severe renal or liver dysfunction, HIV infection, or psychiatric disease were not included. Diagnostic lumbar puncture and bone marrow morphology, immunophenotyping, cytogenetics and *BCR-ABL* molecular testing were performed in all patients. In accordance with the Declaration of Helsinki, the study was approved by the Ethics Committee (CPPOuestII Angers, ID2008/23). All patients provided written informed consent. From March 2009 to October 2010, 30 patients from 20 centers were enrolled in the study.

2.2. Treatments

Two GRASPA[®] infusions were added to the consensus treatment protocol (Fig 1) for older patients with ALL defined by the European Working Group for Adult ALL (EWALL) and called "EWALL backbone"[25]. Induction comprised 2 successive phases. Escalated GRASPA[®] doses [26][27] were infused on day (D)3 of induction phase 1 and on D6 of induction phase 2. Response to induction was evaluated after haematological recovery following the second induction phase. Salvage therapy was left to investigators' discretion. Antithrombin (AT) and Fibrinogen (Fg) replacement by Fresh-Frozen-Plasma (FFP) or Fg concentrates were recommended below 60% and 0.5 g/L respectively. Pneumocystis prophylaxis was recommended from induction without other bacterial or fungal prophylaxis.

2.3. Study design

The experimental dose-escalation design aimed to establish the maximum tolerated dose (MTD) by a Bayesian approach. This methodology based on known *a priori* distribution, probability and *a posteriori* distribution, allows an update of the information as the trial progresses. Cohort of 3 patients started with a GRASPA[®] dose of 50 IU/kg. Toxicities were validated by an independent Data and Safety Monitoring Board (DSMB). The procedure was repeated until the maximum tolerated and effective dose was identified. A number between 9 and 30 patients was estimated adequate for this protocol. The trial was stopped as soon as the information obtained was deemed to be sufficient [28]. *A priori* probability distributions of toxicity and efficacy were defined according to previous preclinical and clinical information.

The primary endpoint was a composite of efficacy and toxicity criteria. The efficacy criterion was defined as plasmatic asparagine level $\leq 2 \mu\text{M}$ [22] over a period of at least 7 days after administration of GRASPA[®] during the first induction phase.

Limiting toxicity was defined as grade 2, 3 or 4 symptomatic pancreatic toxicity, grade 3 or 4 hepatic, grade 3-4 allergic toxicity or deep cerebral vein thrombosis known to be potentially related to L-asparaginase administration as well as any bone marrow hypoplasia lasting more than 30 days after the final dose of chemotherapy or any other grade 4 toxicity according to the CTCAEv3.0 scale. AT was monitored daily from D1 to D8 then on D10 and once a week for both induction cycles. Fg levels were monitored on D1, D3 then weekly during the 2 inductions.

Secondary endpoints were the global tolerability of GRASPA[®], its immunogenicity, evolution of encapsulated L-asparaginase activities, changes in asparagine levels and determination of the GRASPA[®] dose/asparagine depletion-duration relationship. Asparagine depletion was also

evaluated in the cerebrospinal fluid (CSF) on D2 and D9 of induction phases. Other secondary endpoints included complete remission (CR) rate, molecular Ig/TCR minimal residual disease (MRD)[29] at the end of induction 1 and 2, failure, death in first CR, relapse, event-free survival (EFS), Disease-Free-Survival (DFS) and overall survival (OS). Outcome data were updated on October 15th 2013.

2.4. Biological sampling and processing

Samples were collected 15 minutes before and 1, 7 and 14 days after GRASPA[®] administration and at the end of induction. For asparagine plasma and CSF evaluation, whole blood and CSF were rapidly placed after harvesting in iced-water and centrifuged (4°C, 1000 G, 15 minutes). The supernatant was immediately precipitated by sulfosalicylic acid, stored at -20°C and analysed by mass spectrometry in less than 72 hours [30]. Samples for free plasmatic and encapsulated L-asparaginase measurement [31] were stored at -20°C (CRS Mannheim Grünstadt, Deutschland). The immunogenicity of GRASPA[®] was assessed by a specific ELISA(Cemo, choisy-le-Roi, France)[32].

2.5. Statistical methods

The primary endpoint used a statistical Bayesian model “with memory”[28] consisting of spin-offs between 2 variables, i.e. probabilities of treatment-related toxicity and efficacy [33]. These probabilities were updated for each cohort using available data. The decision regarding the next dose to be administered to the subsequent cohort was based on these probabilities (adaptative model) and on DSMB recommendations. The procedure was repeated until the maximum tolerated and effective dose was identified, or the total number of patients was reached. The primary endpoint was analysed using R[®] software Version 2.9.1. Other endpoints were analysed using SAS[®] software Version 9.1. Descriptive analyses of secondary endpoints were performed. Statistical tests were exploratory, two-sided, with an α -significance level of 5%. Continuous variables were compared with the Mann-Whitney U test. Qualitative variables were compared using a Chi-square test or Fisher's exact test. Survival data were estimated using the Kaplan-Meier method [34], and compared via the log-rank test [35].

3. Results

3.1. Patient characteristics

Three, 13 and 14 patients were included at the 50, 100 and 150 dose levels, respectively. The median age was 67 years (range 59-77); 17% of patients had a WBC count $>30 \times 10^9/L$, none had CNS involvement. Twenty-six patients had B-cell precursor (BCP)-ALL and 4 had T lineage (T)-ALL. Poor-risk cytogenetics were observed in 10 patients including 3 t(4;11), 4 hypodiploidy-near triploid and 3 complex karyotypes (≥ 5 unrelated abnormalities). Steroids sensitivity, defined as $<1 \times 10^9/L$ peripheral blood blasts, was observed in 83% (25/30) of patients after the steroid pre-phase. No differences in baseline clinical and laboratory characteristics were observed across the 3 dose level groups (Table I).

3.2. Toxicity

Main adverse events are listed in Table II. Overall, 4/30 and 2/24 patients died during induction phase 1 and 2 respectively, resulting in an induction death rate of 20% (6/30). Induction deaths were related to infection (4 invasive fungal infections (IFI), 1 severe uncontrolled Gram-negative sepsis at D2 of induction 1, associated with confusion at D6 without CT scan abnormality, low AT level at D10 and pancreatitis at D16) and tumour lysis syndrome with septic multi-organ failure and coma that occurred in a refractory patient 26 days after a single GRASPA[®] infusion.

No limiting toxicity was observed at the lowest dosage. In the 100 IU/kg and 150 IU/kg cohort, dose-limiting toxicity (DLT) was observed in 2 (15%) and 5 (36%) patients respectively, suggesting that the 100 IU/kg dose has the best safety/efficacy profile for these elderly patients. Main DLT included 4 grade 3 transaminase elevation (2 in the 100 IU/kg and 2 in the 150 IU/kg cohort), 1 stroke during induction 2 after aspirin interruption in the presence of severe atheromatous carotid stenosis and 2 biological pancreatitis related to GRASPA[®] (grade 3 and 4) without clinical symptoms. The confusion described previously was observed 4 days after a single GRASPA[®] infusion in a patient who received concomitant psychotropic medication. A causal relationship with hyperammonemia can not be excluded. These DLTs were at least partly related to intercurrent events and not only to GRASPA[®] infusions in 5 out of 7 patients.

Infectious complications occurred in 77% of patients during induction 1 and were mostly IFI and/or Gram-negative infection but were less frequent during induction 2 (17% of patients).

Other adverse events observed during induction included coagulation disorders, hepatic and pancreatic toxicities.

Venous and arterial thrombosis occurred in 13% and 4% of patients during induction 1 and 2, respectively. None of these thrombosis occurred when AT level was lower than 60%. Two venous thromboses (7%) were related to central venous access and occurred on D12 and 21 of induction 1. Arterial thrombosis included 2 cerebral arterial thromboses, occurring on D28 of induction 1 and D14 of induction 2, related to cerebral IFI and to interruption of aspirin in a patient with severe atheroma. In addition, 1 patient experienced toe necrosis associated with hyperglycemia during induction 1.

During induction 1, the median AT nadir was 67% (range 26 – 95). AT deficiencies below 60% and 50% were observed in 41% and 17% of patients respectively and were more frequent at higher GRASPA[®] doses. AT nadir occurred before D8, between D8 and D14, D15 and D21 or after D22 in 31%, 28%, 17% and 24% of patients respectively. AT infusion was performed in 37% (11/30) of patients but 3 of them received AT substitution while AT was between 69 and 71%. Fg levels below 1g/l were not observed in this patient cohort. However, 3 patients received FFP or Fg concentrates during severe sepsis. None of them experienced thrombosis.

During induction 2, 2 out of 24 patients (8%) had an AT level below 60% or received AT infusion. The first patient received AT infusion after arterial thrombosis with an AT level of 62%, the second one received 4 AT concentrates, 2 FFP and 1 Fg concentrate during fatal severe sepsis.

Hepatic and pancreatic toxicities were observed only during induction 1 with 20% (6/30) transaminase elevation, 7% (2/30) hyperbilirubinemia and 17% (5/30) amylase or lipase elevation without any clinical pancreatitis. Grade 3 amylase/lipase elevation occurred 2 and 7 days before or on the day of GRASPA[®] infusion in 3 patients.

GRASPA[®] administration was followed by biological pancreatitis in 3 patients of the 150 IU/kg cohort, 7, 11 and 23 days after infusion, one of them also presenting biological pancreatitis before the GRASPA[®] infusion. Hypoalbuminemia (< 30g/l) was observed in 23% (7/30) of patients. Haematopoietic toxicity is detailed in Table III. Myelosuppression during induction 1 was more pronounced than during induction 2.

Consolidation including free L-asparaginase was performed in 19/21 CR patients, 2 patients did not receive the planned consolidation because of patient choice or poor general condition. Consolidation tolerability was acceptable. High-dose methotrexate courses were complicated by transient acute renal deficiency in 3/19 (16%), 1/15 (7%) and 1/12 (8%) consolidation phases 1, 3 and 5 respectively. Gram-negative bacteria septicaemia occurred in 6/46 (13%) high-dose methotrexate courses and 6/40 (15%) high-dose cytarabine courses. Interestingly 5/19 (26%) patients exhibited a native L-asparaginase allergic reaction during consolidation phase 1 while 1 patient presented a native L-asparaginase allergic reaction during consolidation 5.

3.3. Efficacy : Asparagine depletion, asparaginase activity and anti-L-asparaginase antibodies

With respect to the primary efficacy endpoint, asparagine depletion ($< 2 \mu\text{mol/l}$) for at least 7 days after GRASPA[®] infusion was reached in 0%, 85% and 71% of patients with 50 IU/kg, 100 IU/kg and 150 IU/kg, respectively with no statistical difference. Two weeks after GRASPA[®] infusion, asparagine was still depleted in 4/13 (31%) and 1/13 (8%) of patients treated with 100 IU/kg and 150 IU/kg GRASPA[®] respectively, while 3 weeks after infusion, it was fully depleted only in 1/11 (9%) and 0/12 (0%) of these patients, respectively. Despite the absence of clinical signs of allergy in all patients after the first and second infusions of GRASPA[®], anti-L-asparaginase antibodies were observed in 3/28 (11%), 5/17 (29%), and 11/22 (50%) of the patients evaluated at the end of induction 1, the beginning and the end of induction 2 respectively, with no obvious difference between GRASPA[®] dosages (Table II). During induction 2, full asparagine depletion 7 days after GRASPA[®] infusion was observed in 9/13 patients (69%) without anti-L-asparaginase antibodies but only in 2/11 (18%) patients with anti-L-asparaginase antibodies. In addition, the duration of asparagine depletion was shorter during induction 2 in the 7 patients with anti-L-asparaginase antibodies (6.8 days, range 4-10.5) than in the 12 patients without anti-L-asparaginase antibodies (11.6 days range 1.9-22)(Fig 2). More precisely, asparagine depletion continued for 4 days ($n = 2$) vs 11.4 days ($n = 7$) and 7.9 days ($n = 5$) vs 13.8 days ($n = 4$) in patients with and without anti-L-asparaginase antibodies in the 100 and 150 IU/kg cohort respectively.

However, encapsulated L-asparaginase activity was similar in patients with and without anti-L-asparaginase antibodies (Fig 3). The encapsulated L-asparaginase activity reached a median of 1440 and 1516 IU/L 24 hours after GRASPA[®] infusion but was still high with a median of 525 IU/L and 618 IU/L before the second infusion, 3 to 4 weeks after the first infusion, in patients with and without anti-L asparaginase antibodies respectively. In contrast, median free asparaginase activity was below the level of detection (2.5 IU/L) at day 3, before GRASPA[®] infusion, then increase to a median of 65 IU/L 24 hours after infusion then 5.1 IU/L 7 days after infusion and returned to level below the level of detection 14 and 21 days after infusion.

All 5 patients with a clinical allergic reaction to native L-asparaginase during the first consolidation cycle had anti-L-asparaginase antibodies at the end of induction 2. In addition, 1 patient with anti-L-asparaginase antibodies at the end of induction who failed to achieve CR had an allergic reaction to native L-asparaginase and then to Erwinase administered as part of a second-line treatment. However, 5/11 patients with anti-L-asparaginase antibodies at the end of induction 2 received native asparaginase during consolidation without any clinical allergic reaction.

3.4 Asparagine depletion in CSF

In the CSF, the mean asparagine concentration was $7.30 \pm 3.18 \mu\text{mol/L}$ on D2 before GRASPA[®] infusion. It was undetectable ($< 2 \mu\text{mol/L}$) 6 days after the first GRASPA[®] infusion during induction 1 and 3 days after the second GRASPA[®] infusion during induction 2 in 17/24 (71%) and 13/18 (72%) of patients, respectively. Joint analysis of induction 1 and 2 results highlighted a trend towards a correlation between plasmatic and CSF asparagine depletion (Fisher $p \approx 0.10$) with 22/38 (58%) patients with asparagine depletion in both CSF and plasma and 5/38 (13%) without depletion in plasma and CSF. On D9 of induction 2, asparagine CSF depletion was observed in 89% of 9 patients without anti-L-asparaginase antibodies and in 44% of patients with anti-L-asparaginase antibodies (Fisher $p = 0.13$).

3.5. Outcome

After two induction phases, the overall CR rate was 70% (21 patients), 20% (6 patients) died during induction and 10% (3 patients) failed to achieve CR (Table IV). Ig/TCR molecular MRD evaluated after induction 1 (MRD1) and induction 2 (MRD2) was below 10^{-3} in 44% and 79% of patients respectively. Relapses occurred in 14 patients, 8 during consolidation, 5 during maintenance therapy and 1 after the end of maintenance. Two of them received allogeneic stem cell transplantation after the second and fourth consolidation cycle but relapsed 11 and 4 months after transplant and were not censored. With a median follow-up of 42.2 months, median overall survival was 11.8 months in the entire population [95% confidence interval (CI), 8.2-27.9], more precisely 15.8 months [95%CI, 8.2-NA] and 9.7 months [95%CI, 5.8-NA] in the 100 and 150 IU/kg cohort, respectively (Fig 4). Median EFS and DFS were 7.8 [95%CI, 4.1-14.4] and 13.4 months [95%CI, 8.9-NA], respectively, for the entire population. At 2 years, OS, EFS and DFS were estimated at 30% [95%CI, 17.4-51.8%], 23.2% [95%CI, 12.2-44.6%] and 33.3% [95%CI, 18.2-61%], respectively. The slightly better results observed in the 100 IU/kg cohort compared to the 150 IU/kg cohort are related to a tendency for more toxicities, death rate and treatment interruptions rather (Table IV) than to different distribution of poor risk factors (Table I) or the occurrence of anti-asparaginase antibodies (Table II).

3.6. Prognostic factors

A prognostic analysis was performed for CR achievement and OS. In univariate analysis, none of the characteristics evaluated (age ≥ 65 or 70, BCP or T-ALL, WBC > 30 G/l, cytogenetic, albumin ≤ 35 g/l, creatinine < 120 μ mol/L, corticosteroid sensitivity, asparagine depletion longer than 7 days, anti-L-asparaginase antibodies) was predictive of CR achievement. Corticosteroid sensitivity and creatinine < 120 μ mol/L were significantly associated with improved overall survival (median OS 15.6 vs. 5.3 months, $P = 0.013$ and 16.1 vs. 1.1 months $P = 0.001$ respectively). MRD1 $< 10^{-3}$ at the end of induction 1 appeared to be also associated with improved OS (40.4 vs. 9.3 months, $P = 0.028$). In contrast, age, WBC, performance status > 1 , albumin ≤ 35 g/l, BCP or T-ALL, poor cytogenetics, MRD level at the end of induction 2, duration of asparagine depletion, L-asparaginase encapsulated activity or anti-L-asparaginase antibodies were not predictive of OS.

4. Discussion

The aim of this trial was to evaluate the MTD and the asparagine depletion induced by GRASPA[®] during first-line treatment in elderly patients with Ph-negative ALL and if the encapsulation of asparaginase into erythrocytes allowed the inclusion of L-asparaginase in induction chemotherapy in this population.

The patient characteristics were very similar to those reported in our previous study in elderly ALL subjects using pegylated-liposomal doxorubicin[7] and probably highly representative of this population: median age of 67 years, 30% of patients with cardiac history, rare T-cell ALL and hyperleucocytosis (13% and 17% respectively), 30% with poor risk cytogenetic profile and 17% with steroid resistance.

The overall toxicity of the 2 induction phases was acceptable. Myelosuppression was more severe than in the GRALLSA1 trial evaluating VAD/CVAD with or without liposomal doxorubicin but median duration of neutropenia seemed to be similar to the 24 days reported in the 28 patients described by Ribera et al[36] and treated with the same EWALL backbone without L-asparaginase. Infectious, metabolic and haematologic toxicities were more intense during induction phase 1 compared to induction phase 2 despite infusion of the same dosage of GRASPA[®]. This suggests that tolerability depends more on bone marrow leukemic involvement and associated drugs (anthracyclines, vincristine and high dose steroids versus cyclophosphamide and cytarabine) than on L-asparaginase. However, toxicity occurred more

frequently in patients receiving 150 IU/kg than 100 IU/kg. Infectious toxicity was the most common adverse event. Grade 3/4 infection was recorded in 77% of cases in induction 1, similar to the 61% rate observed in the Spanish study [36] but more frequent than in our VAD/CVAD study [7]. The use of dexamethasone might have increased the rate of infection, especially IFI [37]. Antimicrobial prophylaxis including antibiotics and antifungal therapy should probably be recommended in subsequent trials. Venous thrombosis occurred in 7% of patients which is similar to the rate of thrombosis reported in adult ALL induction [38-40]. AT decreased below 60% in 41% of patients during induction 1 which is similar to the 51% rate recorded in 214 younger adults treated with 6 infusions of L-asparaginase (7500 U/m²) in the CAPELAL trial [38]. During induction 2, where GRASPA[®] was not combined with steroids, only 8% of patients had AT levels below 60% despite GRASPA[®] infusion. CNS toxicity was infrequent; only 1 confusion occurring 3 days after GRASPA[®] infusion in a septic patient receiving psychotropic agents. Biological pancreatitis was noted in 17% of patients without any clinical pancreatitis and it should be pointed out that 2 of the 5 cases occurred before GRASPA[®] infusion. Finally, in contrast to the MRC-UKALLXII/ECOG2993 [3] trial and the GMALL 06/99 and 07/2003 [17] previous trials evaluating native L-asparaginase during induction in older patients, hepatic toxicity was acceptable in our study with only 20% hypertransaminasemia and 7% hyperbilirubinemia.

No clinical allergic reaction was observed after GRASPA[®] infusions. However, anti-L-asparaginase antibodies were observed in half of the patients assessed at the end of induction 2 without any obvious difference across GRASPA[®] dosages. The frequency of the appearance of these L-asparaginase antibodies is similar to the 61% reported in patients treated with native asparaginase [41] but higher than the 2% [42] to 36% [43] reported in naïve patients treated with Peg-asparaginase without previous native asparaginase exposition. The appearance of anti-L-asparaginase antibodies may be attributed to the presence of residual non-entrapped L-asparaginase in GRASPA[®]. When patients with anti-L-asparaginase antibodies were subsequently re-challenged with native L-asparaginase during consolidations courses or after relapse, clinical signs of hypersensitivity were observed in only 50% of them. Anti-L-asparaginase antibody occurrence was associated to a lower proportion of patients with full asparagine depletion during induction 2 (69% vs. 18% of patients) and with a 50% reduction in the duration of asparagine depletion. Encapsulated L-asparaginase activity was much higher for 4 weeks after infusion (median 618 IU/L) than the 30 IU/L[14,44] or 100 IU/L[42] observed with native asparaginase and significantly associated with better survival. Moreover, anti-L-asparaginase antibodies did not decrease encapsulated L-asparaginase activity, probably because of the protection of the red-cell membrane.

Asparagine depletion was considered as our primary endpoint at the beginning of the study in 2009 with a depletion threshold lower than the 3 µM associated with a higher remission rate in relapse children[45]. It has been recently suggested that measuring asparaginase activity levels is preferred over measuring asparagine levels to monitor the efficacy of asparaginase therapy [46] because, if not properly handled, asparagine is very rapidly degraded *ex vivo* in the tube by asparaginase leading to false low asparagine levels [47]. Overall, although observed toxicities are induced by a combination of drugs included in the EWALL backbone and GRASPA[®] or by infectious complications, addition of GRASPA[®] to the backbone is feasible without increasing toxicity in elderly ALL patients. Based on tolerability and asparagine depletion, 100 IU/kg seems to be the optimal dosage combined with the EWALL backbone.

With regards to the efficacy of the EWALL backbone associated for the first time with this new L-asparaginase formulation, the CR rate was 70% with a 10% failure rate and a 20% induction death rate. This induction death rate seems to be slightly higher than the one reported in pilot

trials of the EWALL backbone without asparaginase but these 2 pilot trials involved selected and non-consecutive patients in a few centres [25,48]. Compared to the few other studies including L-asparaginase during induction, our induction death rate is lower than in the Pethema ALL96 trial (36%), which population was similar to ours. In contrast, our induction deaths rate seems similar to that reported in the MRC UKALLXII/ECOG2993 (18%)[3] or the SWOG 9400 (18%)[49] studies, but our population is at least 10 years older than theirs. The analysis for prognostic factors being predictive of CR achievement is negative, a common result if the CR rate is high. Correlation between steroid sensitivity and improved OS is well-known in younger adults [50] but has not been described in older patients with ALL. The fact that high creatinine level is significantly associated with decreased OS is new, and particularly relevant for this elderly ALL population. The OS correlated also with the quality of the response evaluated by MRD1, but not with MRD2, probably due to the small number of patients. The quality of the molecular response seems to be improved in patients who received 100 UI/kg GRASPA®.

However, overall survival remained disappointing and addition of GRASPA® infusion during consolidation should be evaluated as addition of asparaginase during consolidation seems to improve the outcome of elderly ALL patients [51,52] as well as adults ALL[16]. Whether the anti-L-asparaginase antibodies are associated with further allergic reactions and decreased efficacy in case of further GRASPA® infusion is under investigation in a clinical trial involving up to 10 sequential GRASPA® infusions (NCT01518517).

In conclusion, the administration of GRASPA® in combination with the induction chemotherapy regimen designed by the EWALL group is feasible in older ALL patient during induction phases, especially at 100 UI/kg, without obvious increased toxicity. Comparison studies of GRASPA® versus other asparaginase formulations are needed to confirm the benefit in elderly patients of this new drug product.

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Table I: Patients characteristics.

	50 IU/kg	100 IU/kg	150 IU/kg	Total cohort
Number of patients	3	13	14	30
Median age, years(range)	68 (65-72)	65 (59-77)	66 (61-77)	67 (59-77)
Sex (M/F)	1/2	7/6	6/8	14/16
ECOG performance status (0/1/2)	3/0/0	3/7/3	3/8/3	9/15/6
HCT-CI (0/1/2/3/4/5/6/7/8), median	0/0/0/0/1/0/1/0/1, 6	3/4/2/1/3/0/0/0/0, 1	3/2/3/1/1/3/0/0/1, 2	6/6/5/2/5/3/1/0/2, 2
CIRS-G median (range)	7 (3-8)	7 (5-9)	9 (4-13)	7 (3-13)
Prior arterial hypertension (n)	1	10	6	17
Prior cancer history (n)	2	2	2	6
CNS involvement (n)	0	0	0	0
Fever at diagnosis (n)	1	3	1	5
Median WBC x 10 ⁹ /L (range)	6.5 (1.9-48.9)	2.9 (0.8-33.9)	2.5 (1.5-119)	3.7 (0.8-119)
WBC > 30 x 10 ⁹ /L (n)	1	1	3	5
Serum albumin ≤ 35g/L n = 24	1	5	3	9
Serum creatinine μmol/L median (range), n > 120	100 (86-128), 1	75 (60-168), 2	91 (56-150), 3	80 (56-168), 6
Immunophenotype BCP-ALL/T-ALL (n)	3/0	11/2	12/2	26/4
Cytogenetics (standard/poor) (n)	1/2	11/2	8/6	20/10
Median prephase duration days (range)	6 (4-8)	6 (4-10)	7 (4-11)	6 (4-11)
Corticosteroid resistance (n)	0	2	3	5

CNS: central nervous system; WBC: white blood cell count; Hb: hemoglobin; LDH: lactate dehydrogenase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; AP: alkaline phosphatase; BCP: B-cell precursor; CIRS-G: Cumulative Illness Rating Scale for Geriatrics; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index

Table II: non-haematologic adverse events.

	50 IU/kg	100 IU/kg	150 IU/kg	Total cohort
Number of patients	3	13	14	30
Overall incidence of Grade 3/4 infections n patients with at least 1 infection (%)	2 (67%)	12 (92%)	9 (64%)	23 (77%)
Pulmonary aspergillosis/zygomycosis/candidemia, n	1 (33%)	3 (23%)	3 (14%)	7 (23%)
Gram Positive Cocci bacteriemia (GPB), n	1 (33%)	3 (23%)	3 (14%)	7 (23%)
Gram Negative Bacille bacteriemia (GNB), n	1 (33%)	1 (8%)	3 (21%)	5 (17%)
Other infectious complications	1 (33%)	7 (54%)	4 (29%)	12 (40%)
Other events during induction phase 1				
Anaphylaxis reaction	0	0	0	0
Thrombosis	1 (33%)	1 (8%)	2 (14%)	4 (13%)
AT decreased < 60% / AT replacement	1 (33%) / 2 (66%)	4/12 (33%) / 3 (23%)	7 (50%) / 6 (43%)	12/29 (41%) / 11(37%)
Fibrinogen supplementation	0	1 (8%)	2 (14%)	3 (10%)
Pancreatic enzyme > 2 N (grade 3)	0	2 (15%)	3 (21%)	5 (17%)
Albumine < 30 g/l	1	1 (8%)	5 (36%)	7 (23%)
Transamins increase (> 5 N) (grade 3)	1 (33%)	2 (15%)	3 (21%)	6 (20%)
Hyperbilirubinemia > 3N	0	1 (8%)	1 (7%)	2 (7%)
Encephalopathy	0	0	1	1 (3%)
Cardiac event	1	0	1	2 (7%)
Induction phase 2				
Overall incidence of Grade 3/4 infections n patients with at least 1 infection (%)	0/2	3/13	1/9	4/24 (17%)
Invasive Pulmonary aspergillosis, n	0	1 (8%)	1 (11%)	2 (8%)
Gram Negative bacteriemia, n *	0	2 (15%)	1 (11%)	3 (13%)
Other events during induction phase 2				
Anaphylaxis reaction	0	0	0	0
Anti-L-asparaginase antibodies end induction 1	0/2	2/13	1/13	3/28
Anti-L-asparaginase antibodies end induction 2	1/2	5/11	5/9	11/22
Thrombosis	0	0	1 (11%)	1 (4%)
AT decrease < 60% or AT replacement	0	1 (11%)	1 (11%)	2 (8%)
Fibrinogen supplementation	0	1 (11%)	0	1 (4%)
Hyperbilirubinemia > 3N	0	2 (15%)	0	2 (8%)
Encephalopathy	0	1 (11%)	0	1 (4%)
Acute renal deficiency	0	2 (15%)	1 (11%)	3 (13%)

n: number of patients, AT antithrombin, * Patients presented only 1 event therefore the numbers of event are similar to the numbers of patients except for BGN septicemia (2 patients experimented 2 BGN septicemia)

Table III : Haematological toxicities

	50 IU/kg	100 IU/kg	150 IU/kg	Total cohort	range
	3	13	14	30	
During Induction phase 1	median [min-max]	median [min-max]	median [min-max]	median	
Neutrophils <0.5 x 10 ⁹ /L (days)	23 [19-29]	18,5 [10-37]	17,5 [10-26]	19	10-37
GCSF infusion (days)	22 [22-29]	18 [7-37]	15 [6-25]	17	6-37
RBC Units transfused	7 [6-10]	8 [2-16]	8 [2-16]	8	2-16
Platelets Units transfused	7 [2-17]	3,5 [0-10]	6,5 [0-11]	6	0-17
Thrombopenia <20 x 10 ⁹ /L (days)	22,5 [21-24]	17 [1-37]	5 [2-16]	12	1-37
During Induction phase 2					
Neutrophils <0.5 x 10 ⁹ /L (days)	8 [8-8]	7 [1-20]	12,5 [2-21]	8	1-21
GCSF infusion (days)	11 [11-11]	10 [3-19]	10 [3-26]	10	3-26
RBC Units transfused	1 [0-2]	6 [2-9]	6 [3-8]	6	0-9
Platelets Units transfused	1 [0-2]	2 [0-18]	3 [0-6]	2	0-18
Thrombopenia <20 x 10 ⁹ /L (days)	3 [3-3]	4 [0-15]	6 [2-19]	4	0-19
During Consolidation cycles 1 and 2					
Neutrophils <0.5 x 10 ⁹ /L (days)	.	4 [1-14]	7 [1-14]	4	1-14
GCSF infusion (days)	2,5 [1-4]	4,5 [1-17]	7 [1-11]	4	1-17
RBC Units transfused	0 [0-0]	2 [0-7]	2 [0-7]	2	0-7
Platelets Units transfused	0 [0-0]	0 [0-5]	0 [0-5]	0	0-5
Thrombopenia <20 x 10 ⁹ /L (days)	.	3 [1-9]	7 [3-17]	4	1-17

Table IV: Treatment results according to GRASPA dosing.

	50 IU/kg	100 IU/kg	150 IU/kg	Total cohort
Patients (n)	3	13	14	30
CR rate after 2 induction cycles, n (%)	2 (66%)	10 (77%)	9 (64%)	21 (70%)
Refractory ALL after induction, n (%)	0	1 (8%)	2 (14%)	3 (10%)
Induction death, n (%)	1 (33%)	2 (15%)	3 (21%)	6 (20%)
Patients in CR without induction phase 2 because of toxicities*	0	0	2 (14%)	2 (7%)
MRD1 < 10 ⁻³ after induction phase 1	0/2	5/9 (56%)	2/5 (40%)	7/16 (44%)
MRD2 < 10 ⁻³ after induction phase 2	2/2	6/8 (75%)	3/4 (75%)	11/14 (79%)
Death in first CR, n	0	0	1	1
Relapse, n	1/2	8/10	5/9	14/21 (67%)
OS at 2 years (95% Confidence Interval)	33.3% (6.7-100%)	23.1 (8.5-62.3)	35.7% (17.6-72.1)	30%(17.4-51.8)
DFS at 2 years (95% Confidence Interval)	50% (12.5-100%)	30% (12.6-77.3)	33.3% (13.2-84)	33.3% (18.2-61.0)
EFS at 2 years (95% Confidence Interval)	33.3% (6.7-100)	23.1% (8.6-62.2)	21.4% (7.9-58.4)	23.2% (12.2-44.6)

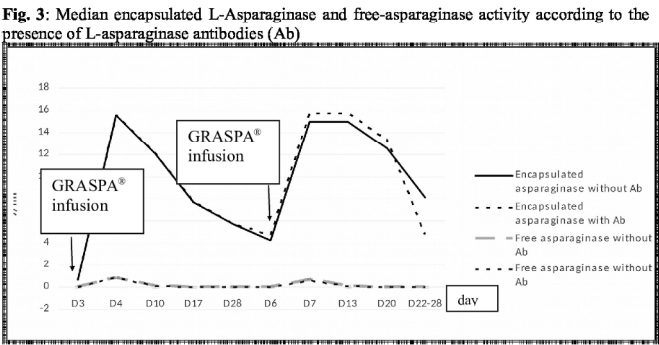
CR: complete remission; EFS: event-free survival; OS: overall survival; CI confidence interval; DFS: Disease Free Survival

*: 2 patients did not receive induction phase 2 and consolidations because of toes ischemia complicated by gram negative cellulitis (patient still in CR) and gastro-intestinal bleeding (deceased 3 months later without relapse) respectively;

Fig. 1: GRASPALL/GRAALL-SA2-2008 protocol

Prephase		
Dexamethasone	10 mg/m ²	D-5/-10 à D-1
Methotrexate	15 mg IT	D-5
Induction 1 (3 to 4 weeks)		
Dexamethasone	10 mg/m ²	D1-D2, D8-D11
Vincristine	1 mg	D1, D8
Idarubicine	10 mg	D1-D2, D8-D9
GRASPA®	50, 100, 150 IU/kg	D3
Methotrexate	15 mg IT	D2, D9
Cytarabine	40 mg IT	D2, D9
Methylprednisolone	40 mg IT	D2, D9
GCSF	150 µg/m ²	daily from Neutrophil < 1 G/l until recovery
Induction 2 (3 to 4 weeks) initiated between D21 and D28 of phase 1 as soon as a neutrophil > 0.5 G/L, regardless of platelet count		
Cyclophosphamide	300 mg/m ²	D1-D3
Cytarabine	60 mg/m ²	D2-D5, D9-D12
GRASPA®	50, 100, 150 IU/kg	D6
Methotrexate	15 mg IT	D2, D9
Cytarabine	40 mg IT	D2, D9
Methylprednisolone	40 mg IT	D2, D9
GCSF	150 µg/m ²	daily from Neutrophil < 1 G/l until recovery
Consolidation (24 weeks : 6 x 4 weeks alternating cycles)		
Cycle 1, 3 and 5		
Methotrexate	1,000 mg/m ²	D1 (500 mg/m ² if ≥ 70 years)
E Coli L-Asparaginase	10,000 UI/m ²	D2 (5,000 UI/m ² if ≥ 70 years)
GCSF	150 µg/m ²	D6-recovery
Cycle 2, 4 and 6		
Cytarabine	1,000 mg/m ² /12h	D1, D3, D5 (500 mg/m ² /12h if ≥ 70 years)
GCSF	150 µg/m ²	D6-recovery
Methotrexate	15 mg IT	D1 of cycle 1 and 3
Cytarabine	40 mg IT	D1 of cycle 1 and 3
Methylprednisolone	40 mg IT	D1 of cycle 1 and 3
Cranial irradiation	18 Grays in 2 weeks before maintenance	
Maintenance		
6 mercaptopurine	60 mg/m ² /day	up to 2 years
methotrexate	25 mg/m ² /week	up to 2 years
every 2 months during 6 months then every 3 months the last year		
Dexamethasone	40 mg	D1-D2
Vincristine	1 mg	D1

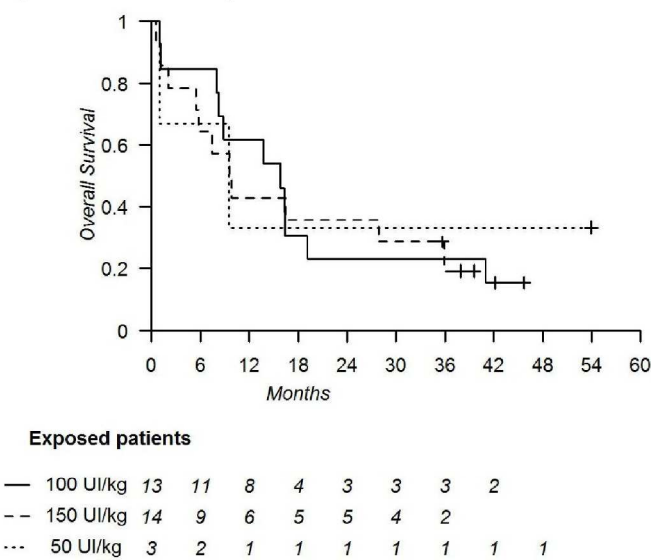
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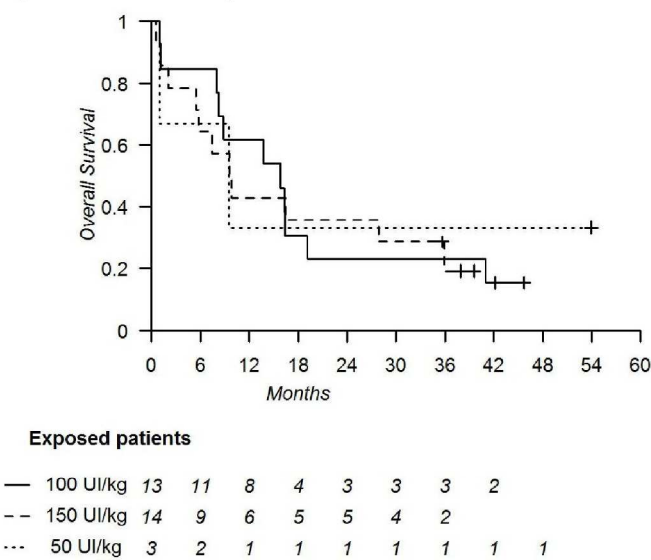
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Fig. 4: Overall survival according to GRASPA® doses



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Fig. 4: Overall survival according to GRASPA® doses



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