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Incidence and Characteristics of Hypersensitivity Reactions to PEG-asparaginase Observed in 6136 Children With Acute Lymphoblastic Leukemia Enrolled in the AIEOP-BFM ALL 2009 Study Protocol

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ABSTRACT

The incidence of hypersensitivity reactions (HSRs) to PEG-asparaginase (PEG-ASNase) was evaluated in 6136 children with ALL enrolled in the AIEOP-BFM ALL 2009 study. Patients with B-cell precursor-acute lymphoblastic leukemia (BCP-ALL) were stratified as standard-risk/medium-risk (MR)/high-risk (HR) and those with T-ALL as non-High/HR. PEG-ASNase was administered intravenously at 2500 IU/sqm/dose. All patients received 2 PEG-ASNase doses in induction; thereafter non-HR versus HR patients received 1 versus 6 PEG-ASNase doses, respectively. After the single regular dose of PEG-ASNase at the beginning of delayed intensification, BCP-ALL-MR patients were randomized to receive 9 additional PEG-ASNase doses every 2 weeks (experimental arm [EA]) versus none (standard arm [SA]); HR patients were randomized to receive, in consolidation, 4 weekly PEG-ASNase doses (EA) versus none (SA). The HSR cumulative incidence (CI) was estimated adjusting for competing risks. An HSR occurred in 472 of 6136 (7.7%) patients. T-non- HR/BCP-Standard-Risk, BCP-MR-SA, BCP-MR-EA, HR-SA and HR-EA patients had 1-year-CI-HSR (±SE) rates of 5.2% (0.5), 5.2% (0.5), 4.0% (0.8), 20.2% (1.2), and 6.4% (1.3), respectively. The randomized intensification of PEG-ASNase did not significantly impact on HSR incidence in BCP-MR patients (1-y-CI-HSR 3.8% [0.8] versus 3.2% [0.6] in MR-EA versus MR-SA; $P = 0.55$), while impacted significantly in HR patients (1-y-CI-HSR 6.4% [1.3] versus 17.9% [1.8] in HR-EA and HR-SA, respectively; $P < 0.001$). The CI-HSR was comparable among non-HR groups and was not increased by a substantial intensification of PEG-ASNase in the BCP-MR-EA group whilst it was markedly higher in HR-SA than in HR-EA patients, suggesting that, in such a chemotherapy context, a continuous exposure to PEG-ASNase reduces the risk of developing an HSR.

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INTRODUCTION

Due to its ability to kill leukemic blasts by depleting the serum from asparagine, asparaginase (ASNase) has become a mainstay of the chemotherapy treatment for childhood acute lymphoblastic leukemia (ALL) and has contributed to the improved outcomes observed over the last decades.[1–6]

Hypersensitivity reactions (HSRs) represent the most relevant limitation to the completion of any ASNase product treatment plan in the context of study protocols for ALL.[2–6] Several observations have shown that the ASNase schedule planned in the treatment protocol is critical in fully exploiting its potential therapeutic effects leading to better outcomes,[7–9] and the PEG-ASNase product has progressively replaced the native *Escherichia coli* ASNase product in several front-line protocols, trusting that a reduced incidence of HSR should favor a better treatment compliance.[2] Patients with HSRs to PEG-ASNase usually may continue the planned ASNase treatment with the second-line product derived from the *Erwinia chrysanthemi* (*E. chrysanthemi*) strain. Treatment with any ASNase products may be also interrupted due to several additional ASNase-associated side effects and complications and this kind of treatment truncation has been associated with worse outcomes[8–11]; also shortages of the *E. chrysanthemi* product have occurred recently and have sometimes challenged the possibility to complete the *E. coli*-based ASNase treatments.[8–11] For these reasons, the reduction of the native *E. coli* ASNase associated-HSR rates has been considered a valuable goal to improve ASNase treatment completion and patients' outcome.

The AIEOP-BFM ALL 2009 was an international collaborative trial recruiting children and adolescents with ALL from June 2009 to February 2017. Instead of the native *E. coli* ASNase product used in the previous AIEOP-BFM ALL 2000 protocol,[12,13] the PEG-ASNase product was used in the AIEOP-BFM ALL 2009 study. In addition, 2 randomized questions on the effects of a PEG-ASNase treatment intensification were also addressed in medium-risk (MR) and high-risk (HR) patients.

The main aim of the present study was to evaluate and summarize the occurrence and the characteristics of HSRs to PEG-ASNase according to the different treatment phases, number of doses, exposures, and the different risk subgroups of the AIEOP-BFM ALL 2009 trial.

MATERIALS AND METHODS

Patients

Children aged ≥1 and <18 years old with newly diagnosed Ph$^-$ ALL in participating countries (Australia/New Zealand, Austria, Czech Republic, Germany, Israel, Italy, and Switzerland) were enrolled in the AIEOP-BFM ALL 2009 trial between June 01, 2010 and February 28, 2017. Out of 6281 patients diagnosed in this period, 145 were not eligible to the study because of the previous treatments (n = 49), treatment started according to another protocol (n = 25), underlying disease not allowing the start of the treatment according to the protocol (n = 20), ALL occurring as second neoplasm (n = 13), or other reasons (n = 38). Therefore, 6136 patients were finally considered eligible and treated in the study. Written informed consent was obtained from their parents or legal guardians. Inclusion and exclusion criteria are described in Suppl. Table S1. In this study, ALL patients were stratified as T-non-high risk (T-non-HR), B-cell precursor (BCP)-standard risk (SR), BCP-MR and HR (comprising both B and T ALL) groups according to their presenting biological/clinical features/response criteria. Stratification criteria and the primary/secondary study questions of the AIEOP-BFM ALL 2009 study are reported in Suppl. Tables S2 and S3, respectively.

Treatment overview

Treatment consisted of a multiagent BFM-based backbone chemotherapy protocol, including the following phases: protocol IA as induction and protocol IB as consolidation in all patients, 4 high-dose methotrexate cycles for all non-HR patients or 3 short highly intensive chemotherapy blocks for HR patients as intensification, and protocol II in all non-HR patients or 3× protocol III in HR patients as delayed intensification. Continuation phase was delivered until 24 months after treatment start were reached. The sequence of the different phases is reported in Suppl. Table S4. The outline of the whole protocol reporting the sequence of the different phases, with the doses of PEG-ASNase delivered in the whole protocol study including those planned in the 2 randomized studies (intensified use of PEG-ASNase) is shown in Figure 1. To better evaluate the impact of the number of drug exposures on the HSR rate, a new exposure to PEG-ASNase was defined as any additional drug administration (even 1 single dose) occurring after a time interval ≥4 weeks after the previous one, as already elsewhere proposed.[14] The details of the whole treatment schedule planned in the AIEOP-BFM ALL Study 2009 including the drugs used, the dosages, the administration routes, the cranial radiotherapy details, and the eligibility criteria for a stem cell transplantation are reported in Suppl. Tables S5A and S5B, respectively.

Standard PEG-ASNase treatment plan

All patients received the same PEG-ASNase product (Oncaspar, currently marketed by the company Laboratoires Servier, Suresnes, France but previously marketed by several additional pharmaceutical companies) as planned in the different treatment schedules of the different risk groups at the dosage of 2500 IU/sqm/dose, capped at 3750 IU, as 2-hour intravenous (IV) infusion. The first PEG-ASNase exposure (2 doses) was scheduled in all patients during induction while further doses were scheduled in T-non-HR, BCP-SR, and BCP-MR patients during the delayed intensification phase (protocol II, day 8, 1 dose), whereas HR patients received further doses during each of the 3 intensive blocks and each of the 3 protocols III (Figure 1). Based on the AIEOP-BFM ALL 2009 protocol indications, patients with an HSR clearly related to PEG-ASNase administration had to be shifted to the Erwinia c. ASNase product (Erwinase, and marketed by the company Clinigen Pharmaceuticals, Burton upon Trent, United Kingdom but previously marketed by several additional pharmaceutical companies), which was given every other day × 7 doses at the dosage of 20,000 IU/sqm/dose as IV 1-hour-long infusion to substitute for each missed PEG-ASNase dose. The decision to shift the patient to the Erwinia c. ASNase was based exclusively on the judgement of the treating physician. The details of the randomized studies embedded in the AIEOP-BFM ALL 2009 trial are reported below.

Randomized studies

Study R1 on daunorubicin

Patients with non-HR BCP-ALL with *ETV6::RUNX1*-negative ALL or *ETV6::RUNX1* status unknown and flow-cytometry minimal residual disease (MRD) in day 15 bone marrow <0.1% or with *ETV6::RUNX1*-positive ALL and MRD, measured by immunocytofluorimetry, in day 15 bone marrow <10% were randomized to receive 4 (standard arm [SA]) versus 2 (experimental arm [EA]) doses of daunorubicin in Protocol IA.

Studies R2 and RHR on PEG-ASNase

Patients with an HSR occurring in Protocol IA were not eligible for the R2 (MR patients) and RHR (HR patients) randomized studies (the details of these 2 randomized studies are given below). According to the protocol indications, patients undergoing the EA of these 2 randomized studies (R2 and RHR) on the extended use of PEG-ASNase and presenting with an HSR were not treated with Erwinia c. ASNase to complete the treatment planned in the EA of the R2 and RHR studies.

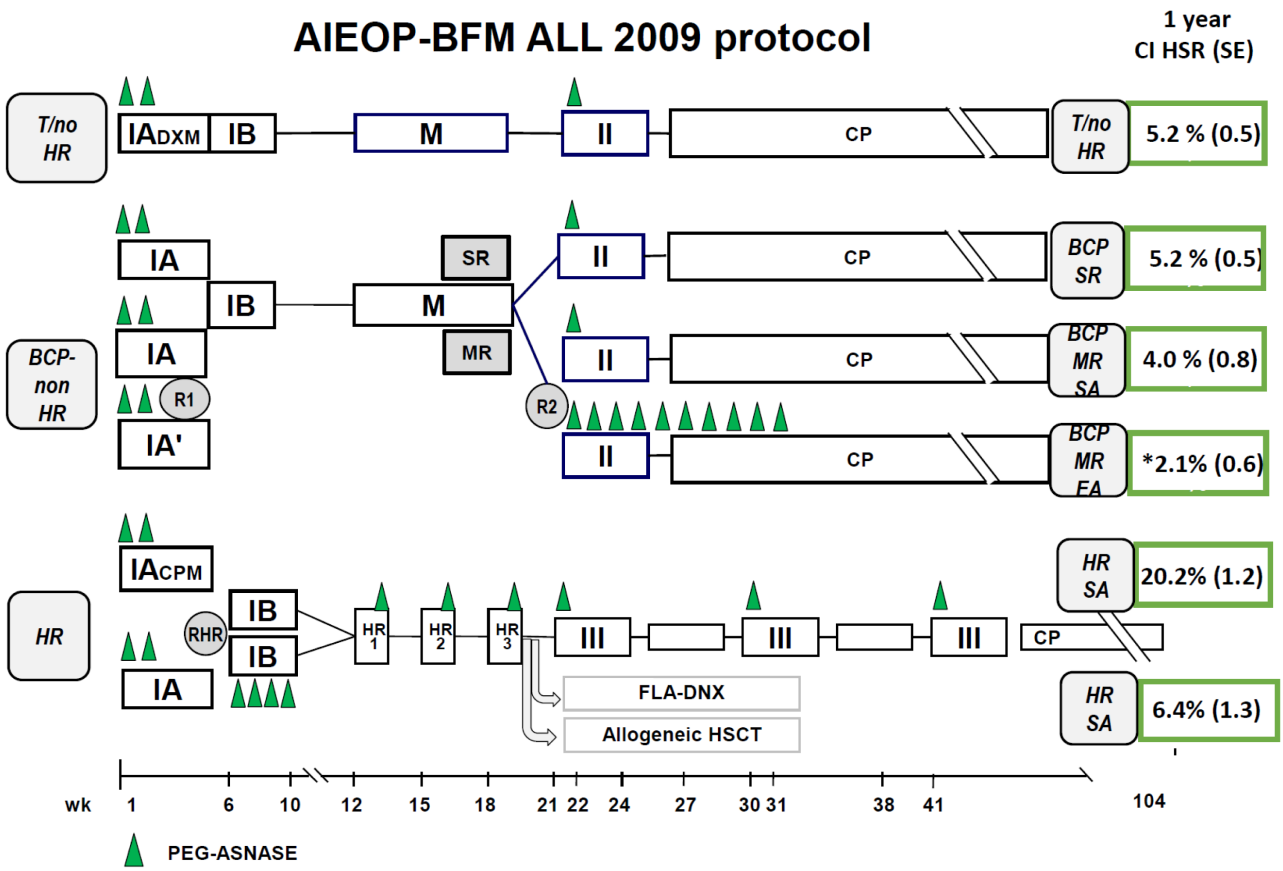

Figure 1. Outline of the AIEOP-BFM ALL 2009 study including the 3 randomized studies planned for all patients (R1), for MR patients (R2), and for HR patients (RHR). Each triangle represents 1 PEG-ASNase dose. The 1-y CI-HSR (right section of the figure) is reported for each 1 of the 6 subgroups (T-no-HR; BCP-ALL-SR; BCP-ALL-MR-SA; BCP-ALL-MR-EA; HR-SA; HR-EA). IA: Protocol IA – induction; IA': Protocol IA'- induction, including 2 doses of daunomycin (4 doses in the regular Protocol IA). IB: Protocol IB – consolidation: M: Protocol M – extracompartment therapy; Blocks: intensive polychemotherapy blocks - intensification; II and III: Protocols II and III – reinductions. *only patients receiving the 9 additional PEG-ASNase doses. BCP = B-cell precursor; CP = continuation phase; CPM = cyclophosphamide; D = dexamethasone; EA = experimental arm; FLA-DNX = block with fludarabine, cytarabine and liposomal daunomycin; HR = high risk; HSCT = hematopoietic stem cell transplantation; HSR = hypersensitivity reaction; MR = medium risk; PEG-ASNase = Peg-asparaginase; SA = standard arm; SR = standard risk.

Study R2

BCP-MR patients randomized in the R2 study received either the standard PEG-ASNase treatment, which consisted of 1 dose in reinduction (protocol II) (SA) or 1 PEG-ASNase dose plus 9 additional doses given from day 22 onwards for every 2 weeks during the reinduction (protocol II) and then continuing into the next phase (continuation) (EA). Therefore, all patients randomized to the SA or EA of the study R2 overall underwent 2 exposures to PEG-ASNase (Figure 1).

Study RHR

HR patients randomized in the RHR study received during the consolidation phase (protocol IB) either no PEG-ASNase doses (SA) or 4 weekly PEG-ASNase doses (EA) (Figure 1). Therefore, in the HR-SA, 8 PEG-ASNase doses were planned overall throughout the whole treatment journey, which corresponded to 4 different exposures to the drug, whereas in the HR-EA arm, with the 4 additional doses planned in protocol IB, 12 PEG-ASNase doses were given overall and corresponded to 3 exposures to the drug. Thus, differently from HR-SA patients, the HR-EA patients were exposed to PEG-ASNase in a more continuous way from induction through intensification and up to the reinduction.

Definition of HSR

HSRs were judged and classified exclusively by the local treating physician according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (Published: May 28, 2009; v4.03: June 14, 2010), which were in place when the protocol was started in the different countries (https://www.eortc.be/services/doc/ctc/ctcae_4.03_2010-06-14_quickreference_5x7.pdf, Immune System disorders, page 65). The HSR definitions as detailed in the CTCAE v4 are also reported in Suppl. Table S6. In brief, grade 1–2 HSR were reactions of brief duration and mild intensity and mainly characterized by skin, gastrointestinal, or mucosal/angioedema symptoms, while grade 3–4 HSR were of prolonged duration and severe up to be life-threatening and characterized by the occurrence of cardiovascular, neurologic, and respiratory symptoms. Grade 5 HSR indicated the death of the patient.

Statistical analysis

The AIEOP-BFM ALL 2009 study protocol was approved by the competent ethics committees and registered as a clinical trial at https://clinicaltrials.gov, NCT01117441, EUDRACT n. 2007-004270-43. A data and safety monitoring committee periodically supervised the conduction of the study.

HSRs were captured for the whole study cohort of 6136 AIEOP-BFM ALL 2009 patients as adverse events and were systematically and prospectively entered in the trial database. Data reported in this study consist of HSRs occurring in the overall population and in 5 main subgroups defined accounting for exposure and treatment as follows:

1. T-non-HR and BCP-ALL-SR patients, not randomized, all receiving the same standard treatment.
2. BCP-ALL-MR-SA, MR patients, randomized (R2) to receive the standard treatment.
3. BCP-ALL-MR-EA, MR patients, randomized (R2) to receive the experimental treatment.
4. HR-SA, HR patients randomized (RHR) to receive the standard treatment.
5. HR-EA, HR patients randomized (RHR) to receive the experimental treatment.

In some explorative analyses, we simply considered 2 subgroups of non-HR (points 1–3) and HR (points 4 and 5) patients. The association between HSRs and the main clinical and biological characteristics was analyzed using the χ^2 test. Event-free survival (EFS) was defined as time from diagnosis to the first event occurring among resistance, relapse, second malignant neoplasm, or death from any cause (time was censored at last follow-up if no events occurred).

The cumulative incidence (CI) curves for HSR were estimated at 1 year (since diagnosis or randomization, as appropriate) adjusting for competing risks of events, which could prevent their observation in front-line treatment (those considered in EFS). Patients who underwent hematopoietic stem cell transplantation in first CR were censored at the date of transplant. Analyses in the subgroups of randomized patients were performed according to the as treated approach and curves estimated from date of randomization. A Cox model on outcome in terms of EFS was fitted to assess the impact of HSR occurrence as a time-dependent variable, after adjusting for main characteristics at diagnosis (gender, age, immunophenotype, white blood cell count, *ETV6::RUNX1*) not involved in the stratification and for treatment received. Data analyses were performed using the SAS System (9.4). Patient follow-up data were updated as of June 2021.

RESULTS

Overall, 472 of 6136 patients developed an HSR (7.7%). Table 1 shows the demographic characteristics of the 472 patients compared with the group of patients without any HSR (n= 5664, 92.3%). As reported in Table 1, age ≥10 years, prednisone poor response, *ETV6::RUNX1* negative, and HR groups were significantly more represented in the HSR group. In Table 2, the distribution of the 472 HSRs observed in each treatment phase and by treatment received is shown in the full cohort of 6136 patients.

All patients underwent the first 2 doses in phase IA and had an HSR rate of 2.0% (N = 121/6136). The first dose of PEG-ASNase planned in protocol II (dose 3) was common to all 4729 non-HR patients with an overall HSR rate of 2.9%: 74 (3.1%) HSRs occurred among the 2376 T-non-HR/BCP-SR patients; 48 (2.8%) HSR occurred among the 1700 patients treated as MR-SA (therefore including those who were not randomized but who received 1 PEG-ASNase dose only as standard treatment); 13 (2%) and 12 (1.8%) out of the 653 patients of the MR-EA presented with an HSR either at the first or during the additional nine PEG-ASNase doses, respectively (with a very low CI-HSR [SE] of 2.1% [0.6] for those receiving the 9 additional doses, as shown in Figure 1). Most of these 653 patients (n = 411, 63%) were able to receive all the additional 9 PEG-ASNase doses as planned in the EA randomized schedule, with a mean number of 7.9 doses. In the group of 364 HR-EA patients, 15 (4.1%) had an HSR during phase IB, most of them being able (n = 306, 84%) to receive all the 4 PEG-ASNase doses planned in the EA, with a mean number of 3.7 doses. Thereafter, during the 3 blocks, only 7 of these patients (1.9%) experienced an HSR.

In contrast, 175 (16.8%) of 1043 patients treated within the HR-SA (this number includes those patients not randomized and receiving the standard treatment, therefore without additional PEG-ASNase doses in protocol IB) presented with an HSR during the subsequent intensification phase including blocks HR1, HR2, and HR3, with the majority of HSRs occurring during the HR2 block (EA versus SA: HR1 0.3% versus 5.7%; HR2 1.1% versus 9.7%; HR3: 0.5% versus 1.5%, respectively).

The HSR rate was very similar and almost negligible when the 3 protocols III were administered as reinduction treatment either to the HR-EA or to the HR-SA groups (0.3% or 0.1%, respectively). Interestingly, the HSR rates observed among non-HR patients receiving, as delayed intensification, the protocol II (only considering the single PEG-ASNase dose given on day 8 therefore after 1 week of dexamethasone coverage) were 3.1%, 2.8%, and 2.0% in the T-non-HR+BCP-SR, MR-SA, and MR-EA, respectively, and higher than those observed in patients receiving the 3 protocols III (1 single PEG-ASNase dose given on day 1 of each protocol III therefore without any dexamethasone coverage) with HSR rates being altogether 1.6% and 0.8% in the HR-SA and HR-EA groups, respectively.

Finally, in Table 2, data on the clinical severity pattern of the HSRs based on the CTCAE-v4 criteria showed that out of 465 episodes 7 were of unknown severity, 311 (66.8%) were mild (grade 1–2), and 154 (33.2%) were severe (grade 3–4). In Figure 1, the 1-year CIs of HSR observed from diagnosis (except for patients belonging to the BCP-ALL MR EA for whom the CI-HSR is reported limitedly to the administration period of the 9 additional PEG-ASNase doses) are summarized for the different risk groups of patients classified as in Table 2. In Figure 2, panel A represents the estimated CI from diagnosis of the whole study cohort, with a 1-year value of 7.7% (0.3); in BCP-MR randomized patients (panel B), the CI of HSR was not significantly different with a 1-year value of 3.8 (0.8)% versus 3.2% (0.6) in the MR-EA versus MR-SA ($P = 0.55$); in HR randomized patients (panel C), the CI of HSR was significantly different between the 2 arms, with a 1-year value of 6.4% (1.3) versus 17.9% (1.8) for patients treated in the HR-EA and HR-SA arms, respectively ($P < 0.001$). Interestingly in the HR-SA, the HSR rate observed after the Block 2 was almost twice (9.7%) in respect of that observed after the block 1 (5.7%). A very similar phenomenon was observed in the HR group of the previously conducted BFM-ALL 2000 protocol, where the native *E. coli* ASNase product was used, with HSR rates of 41.6% and 18.9% after the blocks 2 and 1, respectively.[15]

The occurrence of a HSR did not have a significant impact on the overall EFS of patients presenting with an HSR when evaluated with a Cox model (with HSR as a time-dependent covariate and adjusting for relevant variables), both in non-HR and HR patients: the estimated hazard ratio was 0.90 (95% CI, 0.60-1.35; *P*-value, 0.61) and 1.00 (95% CI, 0.76-1.32; *P*-value, 0.98) for patients who did or did not develop an HSR to the PEG-ASNase product in the non-HR and HR groups, respectively.

The Suppl. Table S6 shows that of the 472 patients showing an HSR, 408 (86.4%) continued with the second-line ASNase product (Erwinia c. ASNase); of the 64 patients who did not continue with the Erwinia c. product, 31 had various legitimate reasons to stop the treatment (including 12 patients belonging to the RMR because, by protocol indications, they were not allowed to receive the Erwinia c. ASNase product), while 24 continued with PEG-ASNase (no clear reasons were reported in the database for this re-exposure to PEG-ASNase) and in 9 patients the information was not available. The ASNase treatment schedule planned in the protocol was completed with the use of the Erwinia c. product by 287 of 408 (60.8%) patients, while 121 did not complete it because of a further HSR (this time to the Erwinia c. ASNASE product [n = 109]) or for other reasons (n = 12). The EFS of patients treated with the Erwinia c. ASNase and presenting or not a further HSR, this time to the Erwinia c. ASNase product, is shown in Suppl. Figure S1

Table 1

Comparison of the Demographic Characteristics of the 6136 Patients, Presenting With or Without an HSR, Enrolled in the AIEOP-BFM ALL 2009 Protocol[a,b]

	Patients With HSR		Patients Without HSR		Total
	N	%	N	%	N
Total	**472**	***7.7***	**5664**	***92.3***	**6136**
Gender					
Male	286	*8.1*	3254	*91.9*	3540
Female	186	*7.2*	2410	*92.8*	2596
$P = 0.18$					
Age					
1–5 y	206	*5.9*	3257	*94.1*	3463
6–9 y	98	*8.3*	1078	*91.7*	1176
10–14 y	105	*10.6*	889	*89.4*	994
15–17 y	63	*12.5*	440	*87.5*	503
$P < 0.001$					
WBC					
<20	307	*7.6*	3730	*92.4*	4037
20–100	118	*8.2*	1322	*91.8*	1440
≥100	47	*7.1*	610	*92.9*	657
Not known	0		2		2
$P = 0.66$					
CNS involved					
CNS1/2	447	*7.8*	5320	*92.2*	5767
CNS3	7	*5.0*	134	*95.0*	141
Not known	18		210		228
$P = 0.22$					
PDN response					
Good	400	*7.3*	5093	*92.7*	5493
Poor	66	*12.0*	482	*88.0*	548
Death/not known	6		89		95
$P < 0.001$					
Immunophenotype					
B-lineage	394	*7.5*	4847	*92.5*	5241
T-lineage	77	*8.8*	795	*91.2*	872
Other/not known	1		22		23
$P = 0.18$					
ETV6::RUNX1					
Positive	68	*5.1*	1255	*94.9*	1323
Negative	394	*8.3*	4343	*91.7*	4737
Not known	10		66		76
$P < 0.001$					
Final risk					
T-non-HR	28	*6.0*	442	*94.0*	470
B-SR	95	*5.0*	1811	*95.0*	1906
B-MR	115	*5.0*	2238	*95.0*	2353
HR	234	*16.6*	1173	*83.4*	1407
$P < 0.001$					

[a]Subdivided according to the occurrence or not of an HSR (row percentages and *P*-value according to χ^2 test for association are reported).

[b]Values in italic indicate the percentages calculated on the patients' numbers reported in each line of the two columns.

CNS = central nervous system; HR = high risk; HSR = hypersensitivity reaction; MR = intermediate risk; PDN = prednisone; SR = standard risk; WBC = white blood cell; y = years.

(no significant impact on outcome both in non-HR and in HR patients [panels A and B, respectively] was observed). Patients unable to complete the planned ASNase treatment because of additional reasons out of HSR to the Erwinia C. product were not included in the above-mentioned EFS analysis.

Additional toxicities usually associated with the use of PEG-ASP were also registered in the AIEOP-BFM ALL 2009 database; even if toxicities beyond HSR were not the focus of this report, the 2-year CI of pancreatitis, thrombosis, and diabetes was 3.8% (0.2), 5.7% (0.3), and 3.9%, respectively.

The Suppl. Table S7 shows the impact of the delay of PEG-ASNase dose 2 as planned on day 26 of protocol IA on the risk of developing a subsequent HSR. Administration dates of both the PEG-ASNase doses 1 and 2 were available in 5479 of 6136 patients. A higher risk of developing an HSR to the second PEG-ASNase dose was seen with a time interval between the 2 doses longer than the planned 14 days. Patients with an interval of >15 days (n = 434) presented with 40 HSR (9.2%) compared with 34 of 4971 with an interval of ≤15 days (0.7%; $P < 0.001$).

Comparison with the CI-HSR observed in the BFM ALL 2000 protocol

With the aim to better understand the differences in terms of 1-year CI-HSR (SE) observed when the native *E. coli* ASNase was given IV as first-line treatment within a very similar BFM ALL backbone (and therefore very similar to the AIEOP-BFM ALL 2009 protocol), we also report here the CI-HSR (SE) for

Table 2

Distribution of the HSR Observed in the AIEOP-BFM ALL 2009 Study Cohort of 6136 Patients[a]

		Total		T-no-HR + BCP-SR		MR-SA		MR-EA		HR-SA		HR-EA	
	Number of PEG-ASNase doses	N	%	N	%	N	%	N	%	N	%	N	%
Patients		6136		2376		1700[b]		653		1043[b]		364	
HSR to PEG-ASNase		472	*7.7*	123	*5.2*	89	*5.2*	26	*4.0*	211	*20.2*	23	*6.3*
PHASE													
IA	2	121	*2.0*	46	*1.9*	41[c]	*2.4*[c]	1[c]	*0.2*[c]	33[d]	*3.2*[d]	0	
IB	0 or 4	20	*0.3*	3[e]	*<0.1*	0		0		2[e]	*0.2*	15	*4.1*
P-II # 1	1	135	*2.2*	74	*3.1*	48	*2.8*	13	*2.0*	–		–	
P-II MR-EA	3	10	*0.2*	–		–		10	*1.5*	–		–	
MT MR-EA	6	2	*<0.1*	–		–		2	*0.3*	–		–	
HR1	1	59	*1.0*	–		–		–		58	*5.6*	1	*0.3*
HR2	1	105	*1.7*	–		–		–		101	*9.7*	4	*1.1*
HR3	1	18	*0.3*	–		–		–		16	*1.5*	2	*0.5*
P-III1	1	0		–		–		–		0		0	
P-III2	1	1	*<0.1*	–		–		–		0		1	*0.3*
P-III3	1	1	*<0.1*	–		–		–		1	*0.1*	0	
CTCAE grade													
Grade 1		49	*10.5*	14	*11.7*	10	*11.2*	4	*16.0*	16	*7.7*	5	*21.7*
Grade 2		262	*56.3*	71	*59.1*	58	*65.2*	11	*44.0*	113	*54.3*	9	*39.1*
Grade 3		138	*29.7*	32	*26.7*	21	*23.6*	9	*36.0*	68	*32.7*	8	*34.8*
Grade 4		16	*3.5*	3	*2.5*	0		1	*4.0*	11	*5.3*	1	*4.4*
Not known		7		3				1		3			

[a]In the upper part of the table HSR frequencies and rates (italic values) observed overall and in each treatment phase of the protocol by risk-group and by exposure to PEG-ASNase were calculated on the number of HSR indicated in the first line of the table for the following subgroups: T-no-HR and BCP-ALL-SR; MR according to standard (MR-SA) or experimental (MR-EA) PEG-ASNase treatment received; HR according to standard (HR-SA) or experimental (HR-EA) PEG-ASNase treatment received. CTCAE-v4 graded HSR are reported by treatment phase and patients' groups in the lower part of the table. For this part of the table the percentages were calculated on the number of HSR indicated in the second line of the table.

[b]These numbers include patients either randomized or not who received the standards PEG-ASNase treatment.

[c]All 42 MR patients with HSR in Phase IA are reported under the standard treatment (MR-SA) as they were not eligible to be randomized to RMR (except for one patient who was randomized against protocol indications); however, the percentage of HSR should be calculated on the totality of MR patients, that is, 42/2353 (1.8%).

[d]All 33 HR patients with HSR in Phase IA are reported under the standard treatment (HR-SA) as they were not eligible to be randomized to RHR; however, the percentage of HSR should be calculated on the totality of HR patients, that is, 33/1407 (2.3%).

[e]For these patients the second dose of PEG-ASNase in phase IA was postponed to phase IB, due to the occurrence of a toxic event in phase IA.

BCP = B-cell precursor ALL; EA = experimental arm; HR = high risk; HSR = hypersensitivity reaction; MR = medium risk; MT = maintenance; SA = Standard treatment arm; SR = standard risk.

the overall BFM ALL 2000 population (42.3% [1.5]) and for the SR, MR, and HR group (40.6% [2.5], 37.0% [1.9] and 65.2% [3.8], respectively).[15]

DISCUSSION

One of the major limitations ingrained with the administration of any ASNase preparation lies in its immunological characteristics; in fact, ASNase is a foreign protein for the human body and, after one or multiple exposures, it may induce the production of anti-ASNase antibodies with the possibility of clinically overt or silent HSR.[4,16,17] Factors influencing the onset of HSR or of silent inactivation are well known: the type of ASNase, the number of previous ALL treatment lines, the dosing schedule, the administration route, the concomitant medications, and the number of exposures.[2–9] Due to the above-mentioned variables, the incidence of such phenomena has been reported to widely span from 1% to 67%.[2,4,18,19] This type of toxicity puts the patients at risk of clinically severe complications that may lead to ASNase treatment truncation and worse outcomes.[3,6–9] More recently, also the phenomenon called allergic-like reaction, especially occurring during the IV infusion of any ASNase products, makes it even more difficult to discriminate between real and false HSR.[16–19] To this end, a therapeutic drug monitoring of serum ASNase activity levels is currently considered an indispensable tool to reliably evaluate the silent inactivation phenomenon and to help distinguishing real HSR from allergic-like reactions.[18,19]

In this study, we have reported the incidence of clinically overt HSR occurring in the frame of the AIEOP-BFM ALL 2009 trial wherein the PEG-ASNase product was used and substituted for the native *E. coli* ASNase product, which had been used as first-line product in the previous AIEOP-BFM ALL 2000 trial.[12,13] We have also evaluated and reported the outcome of patients who could or could not complete the PEG-ASNase treatment plan because of the occurrence (or not) of an HSR either to the PEG-ASNase or to the Erwinia c. ASNase products.

Due to the relevance of the phenomena HSR and silent inactivation, the AIEOP-BFM ALL 2009 trial was carefully monitored with an ad hoc clinical and pharmacological surveillance program; such a detailed monitoring program was planned because of the underlying change in the ASNase product in respect of the previous AIEOP-BFM ALL 2000 protocol (where the native *E. coli* ASNase product was used) and of the scientific interest in gaining additional clinical and pharmacological insights from the delivery of the 2 randomized studies on the intensified use of PEG-ASNase in MR and HR patients. Some of the findings of this extensive pharmacological monitoring program have been already reported[20–22] but as it represents a complex task, a more comprehensive evaluation of the pharmacological aspects and of their clinical consequences have not yet been completed and reported.

In a recent meta-analysis, the incidence of HSR was lower in protocols using PEG-ASNase as the first-line treatment compared with the findings reported for *E. coli* ASNase or PEG-ASNase as second-line after *E. coli* ASNase.[14] Postinduction phase, a higher number of PEG-ASNase-free intervals, and initiation of PEG-ASNase in postinduction phases were found to be the risk factors for HSR. Similar findings have been observed in the BFM-ALL 2000 protocol wherein the native *E. coli* ASNase product was used as first-line ASNase product (see the results section for more details) by using the same

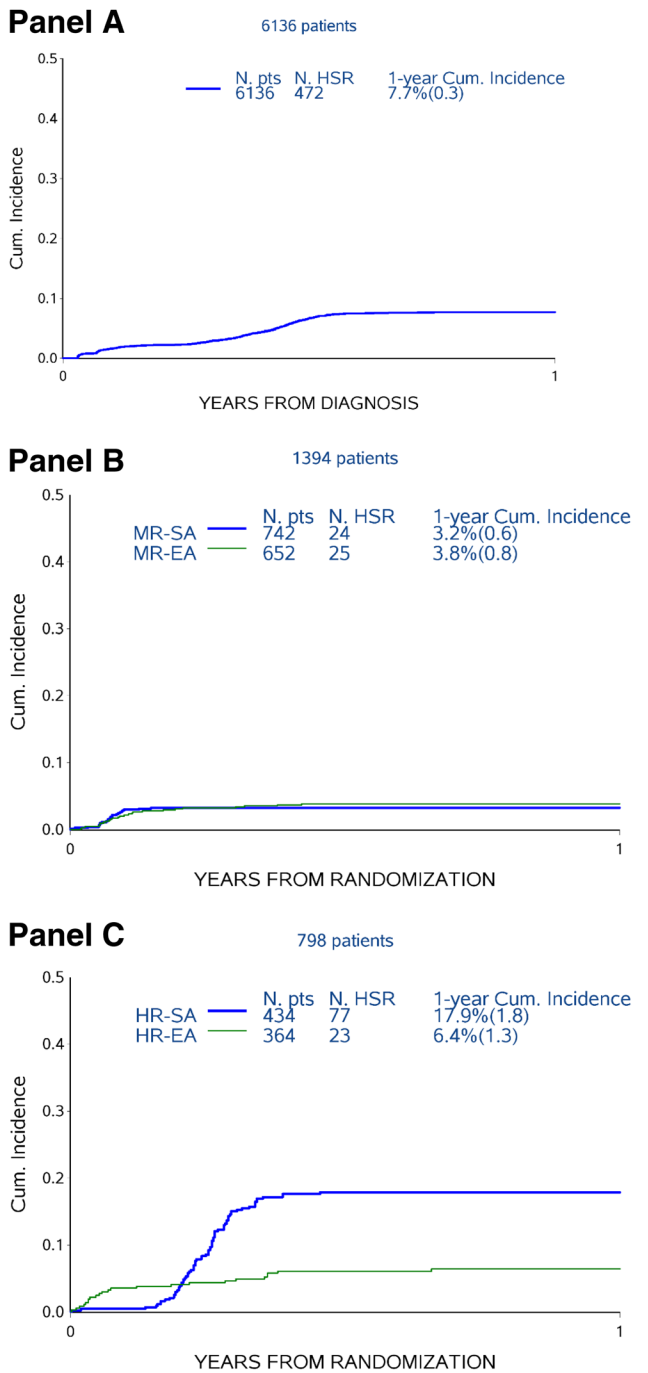

Figure 2. CI-HSR (SE) curves in different groups of patients treated within the AIEOP-BFM ALL 2009 protocol: in (A) since the diagnosis in the full cohort; in (B) since the date of randomization in BCP-MR patients included in the R2 randomized study (patients who had an HSR during induction IA and were randomized against protocol indications are excluded here; ie, 4 patients in MR-SA and 1 patient in MR-EA); in (C) since the date of randomization in HR patients included in the RHR randomized study (as treated analyses). Patients who had an HSR during induction IA and were randomized against protocol indications are excluded here (2 patients in HR-SA). Note: the y-axis covers the probability range 0.0–0.5. Cum. Incidence = cumulative incidence; EA = experimental arm; HR = high risk; HSR (SE) = hypersensitivity reaction (standard error); MR = medium risk; SA = standard arm.

administration route (IV) used in the AIEOP-BFM ALL 2009 study. The findings related to the cohort of the 6136 patients enrolled and treated with the PEG-ASNase product in the present report (AIEOP-BFM ALL 2009) show that the HSR rate was much lower (7.7%) than in the BFM-ALL 2000 protocol and 2% in the induction phase IA (administered in all patients) and 5.2%, 5.2%, 4.0%, 20.2%, and 6.3% in T-non-HR+BCP-SR, MR-SA, MR-EA, HR-SA, and HR-EA subgroups, respectively (Table 2).

The 7.7% HSR rate observed in the AIEOP-BFM ALL 2009 study is overall rather low especially when the high number of PEG-ASNase doses planned in the study are considered. In fact, this study also includes the results of the 2 randomized studies R2 and RHR where an extended and protracted use of PEG-ASNase was planned. Among the most recent studies conducted in this field with PEG-ASNase as first-line ASNase product, it is worth to recall the HSR rates/CI-HSR reported in various international groups such as in the NOPHO 2008 (1000 IU/sqm, IM, HSR rate 18.9% in the HR, 13% in the SR/MR),[23] in the DFCI 05-001 (2500 IU/sqm, IV, 12%,[24] in the COG AALL 0331 and 0232 [2500 IU/sqm, IV or IM, 9.3% and 18.1%, respectively]),[8] in the SJCRH Total XVI (2500 or 3500 IU/sqm, IV, cumulatively 18%),[25] and in the UKALL 2003 (1000 IU/sqm, IM, 4 doses <1%, 12 doses 12%).[26] Because the administration route may have an impact on the HSR rate,[4,5,8,9] we also compared our results with those reported by Burke et al,[27] where severe (grade ≥ 3) PEG-ASNase-associated HSR were significantly less frequent with the IV infusion as compared with those observed with the intramuscular route. Our findings related to the use of PEG-ASNase administered IV are overall like those reported in that experience.[27]

Looking at the different phases, patients' subgroups and number of doses and exposures to PEG-ASNase, the main findings of this study focused on HSR associated with the use of front-line PEG-ASNase can be summarized as follows:

- The overall incidence of HSR in the whole cohort of 6136 children with ALL enrolled in the AIEOP-BM ALL 2009 protocol was low.
- In induction (phase IA), a higher risk of developing an HSR was associated with a time interval between doses 1 and 2 longer than the planned 14 days (Suppl. Table S7), suggesting that even in phase IA (first exposure) longer intervals between doses associated with a immunological response in the host and with a higher risk of HSR.
- Patients receiving 2 PEG-ASNase doses in induction and further exposed to 1 PEG-ASNase dose in delayed intensification (protocol II) and stratified as T-non-HR and BCP-SR or MR-SA presented with a quite low overall HSR rate (5.2%).
- The group of BCP-MR-EA patients who received 9 additional doses (every 2 weeks) during protocol II and the continuation phase (therefore during the same second exposure to PEG-ASNase) had an HSR rate of 4.0% (CI-HSR [SE] of only 2.1% [0.6] when considering only the phase where the 9 additional doses were delivered), therefore very similar to that observed in BCP-MR-SA, thus suggesting that even after a very prolonged interval between the first 2 exposures, the HSR rate is quite similar whenever 1 or 10 doses are administered (Table 2 and Figure 1).
- In patients belonging to the HR-SA (receiving overall 8 PEG-ASNase doses over 4 exposures) it was observed the highest HSR rate (20.2%) among those observed in the above-mentioned subgroups (Table 2 and Figure 1). In this group of patients, no PEG-ASNase dose was given in protocol IB with the vast majority of HSR occurring during the intensification phase and during the HR2 block (9.7%). Interestingly, this latter finding is very similar to the one reported with the use of the native *E. coli* ASNase product in the BFM-ALL 2000 study (see the results section for more details).[15] Conversely, patients randomized to receive the HR-EA (overall 12 PEG-ASNase doses and 3 exposures with a clearly more continuous dosing) showed an overall HSR rate of 6.3% (with a negligible 1.9% observed during

the Intensification phase), therefore very similar to the HSR rate observed in patients of all non-HR subgroups. These findings suggest that a continuous exposure to several PEG-ASNase doses overall reduces the risk of developing an HSR, most probably because of a reduced pattern of antibodies production usually associated with a higher number of exposures.[14] Of note, as pointed out in the previous bullet-point, this phenomenon was not observed in SR/MR patients even if the second exposure that occurred several months after the first one; this phenomenon is somewhat difficult to explain but could be associated with the different chemotherapy background planned for the different risk groups; in particular, after the phase IA, non-HR patients consecutively underwent the consolidation (phase IB) and the extra-compartment therapy phase (protocol M, a 56-days long phase consisting of continuous oral 6-mercaptopurine and 4 IV high-dose-methotrexate courses) and most probably benefited also from this treatment sequence in terms of reduced anti-ASNase antibodies production; this phenomenon did not occur in HR-SA patients who, after the phase IB, received 3 intensification blocks characterized by an intensive multiagent chemotherapy schedule, each including 1 PEG-ASNase dose, and followed by long intervals caused by the subsequent neutropenia phase.

- The quite similar HSR rates observed among non-HR patients receiving, as reinduction, 1 protocol II (1 PEG-ASNase dose after 1 week of Dexamethasone coverage) and among the HR patients receiving, as reinduction, 3 protocols III (1 PEG-ASNase dose, without dexamethasone coverage) suggest that in such a therapeutic context, the preemptive administration (1-week long) of a potent corticosteroid as dexamethasone does not prevent HSRs. These phenomena seem hard to be explained and deserve further biological investigations.
- Two outcome analyses were performed in this study: the first on the groups of patients who developed or not an HSR to PEG-ASNase (no significant differences) suggesting that the possibility to successfully replace with the second-line product the planned PEG-ASNase treatment in the 287 patients (60.8% of the initial 408) most likely compensated for the incomplete PEG-ASNase treatment; the second on the subgroups of patients (subdivided in HR and non-HR) able or not able to complete the Erwinia c. ASNase substitutive treatment exclusively because of a further HSR to this latter product (no significant differences); patients belonging to the MR-EA were excluded from this analysis for obvious reasons. Regarding this second analysis, the very small numbers (patients were subdivided in HR and non-HR groups) made the comparison somewhat more difficult. In addition, and differently from many other similar studies we included in the analysis exclusively those presenting with a further HSR while usually also patients with silent inactivation, pancreatitis, thrombosis, or Erwinia c. unavailability were considered.[24,25]

Based on the results of the present study, we conclude that the overall HSR rate observed in the AIEOP-BFM ALL 2009 study adopting as front-line preparation the PEG-ASNase product represented a very successful strategy in significantly reducing the HSR rate in respect of the AIEOP-BFM ALL 2000 study (see the results section for more details). The main finding of the present study shows that even when several exposures to PEG-ASNase are planned, a reduced time interval between the various doses and exposures and a more continuous scheduling of the treatment lowers the risk of HSR occurrence. In addition, the adoption of intensified PEG-ASNase schedules as planned in the R2 and RHR studies was feasible and was not associated with a significant increase of the HSR rates in the EA, with a high number of patients able to conclude the PEG-ASNase treatment originally planned in the study protocol. Furthermore, in the AIEOP-BFM-ALL 2009 protocol, the additional clinical complications usually associated with the use of ASNase were fully in the expected range.

Whether the adoption of PEG-ASNase in the AIEOP-BFM ALL 2009 instead of the native *E. coli* ASNase product used in the previous AIEOP-BFM ALL 2000 study and whether the PEG-ASNase treatment intensification planned in the 2 randomized studies in MR and HR groups will translate in outcome benefits is a matter of ongoing investigations.

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AUTHOR CONTRIBUTIONS

CR, AM, MGV, AB, VC, GC, MS, and JB did study design and data analysis. LV, AA, CR, AM, AB, VC, GC, MS, SE, FN, DB, and JS contributed to patient enrollment and sample collection. MGV, DS, and MZ did statistical analysis. CR, AM, MS, MGV, DS, MZ, VC, JB, CLK, and AvS wrote the article. CR, AM, VC, MS, MZ, MGV, and JB did supervision.

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