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Pegylated-asparaginase During Induction Therapy For Adult Acute Lymphoblastic Leukaemia: Toxicity Data From The UKALL14 Trial

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Abstract

Safety and efficacy data on pegylated asparaginase in adult acute lymphoblastic leukaemia (ALL) induction regimens is limited. The UK National Cancer Research Institute UKALL14 trial NCT01085617 prospectively evaluated the tolerability of 1000 IU/m² PEG-ASP administered on days 4 and 18 as part of a 5-drug induction regimen in adults aged 25-65 years with de-novo ALL. Median age was 46.5 years. Sixteen of 90 patients (median age 56 years) suffered treatment-related mortality during initial induction therapy. Eight of 16 died of sepsis in combination with hepatotoxicity. Age and Philadelphia (Ph) status were independent variables predicting induction death >40yrs versus <40yrs, odds ratio (OR) 18.5 (2.02-169.0), p =0.01; Ph- versus Ph+ disease, OR 13.60 (3.52 – 52.36), p <0.001. Of 74 patients who did not die, 37 (50.0%) experienced at least one grade 3/4 PEG-ASP-related adverse event, most commonly hepatotoxicity (36.5%, n=27). A single dose of PEG-ASP achieved trough therapeutic enzyme levels in 42/49 (86%) of patients tested. Whilst PEG-ASP delivered prolonged asparaginase activity in adults, it was

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Authorship Contributions

BP, AK and AKF wrote the paper. AKF designed the study, contributed data and was Chief Investigator of the trial. AK analysed data. PS, LM, SP and PP co-ordinated the study, collected data and contributed to the analysis. AD carried out the MAAT testing. CJR, TM, AMcM and DM contributed data and managed the trial. All authors contributed to writing the Ms and approved the final version.

Conflicts of interest.

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difficult to administer safely as part of the UKALL14 intensive multi-agent regimen to those over the age of 40 years. It proved extremely toxic in patients with Ph+ ALL, possibly due to interaction with imatinib.

Introduction

Depletion of extracellular asparagine by parenteral administration of the enzyme L-asparaginase is a key component of most current therapeutic strategies in ALL. In children, intensive L-asparaginase treatment, typically delivered by pegylated *Escherichia coli*-derived (PEG-ASP) improves clinical outcome^{1 2, 3 4 5, 6}, offering a longer half-life⁷ and a lower risk of anti-asparaginase antibody formation. Safety and efficacy is less well established in older adults^{8, 9} and toxicity can be substantial¹⁰ - in a phase 2 trial, failure to deliver the intended doses was closely correlated with advancing age¹¹

UKALL14 (NCT01085617) is an on-going, multicentre, phase 3 study which addresses several questions in the treatment of newly diagnosed adult ALL. A major study aim is to evaluate the addition of 2 doses of 1000 IU/m² PEG-ASP to the standard induction regimen that had been evaluated in our previous study, UKALL1212 in which non-pegylated *E coli* L-asparaginase was given at 10,000IU daily on days 17-28 of phase 1 induction. The only other change to the ‘backbone’ induction regimen between the two consecutive national trials was the addition of a steroid pre-phase and the substitution of pulsed dexamethasone for prednisolone. The aim of these changes was to make our regimen more compatible with a “paediatric-inspired” intensive approach.

The overall endpoint of the trial is EFS. However, a specific endpoint of the PEG-ASP evaluation is toxicity related to PEG-ASP. Secondary endpoints include rate of complete remission (CR), overall survival, minimal residual disease (MRD) quantitation at the end of the first phase of induction and anti-asparaginase antibody formation. Here, we report on the outcome of PEG-ASP administered during induction in the first consecutive 91 trial subjects. At this point, it was judged by the trial management group that a toxicity end-point had been reached and a change was made to the PEG-ASP trial therapy.

Methods

UKALL 14 Induction Phase 1 Treatment

Eligible patients were 25 and 65 years old with newly diagnosed ALL, irrespective of Philadelphia (Ph) chromosome status. There was no exclusion for poor organ function or performance status at diagnosis. Ethical approval was obtained from the UK National Research Ethics Committee. All patients gave written, informed consent, according to the Declaration of Helsinki. Patients received a 5-7 day pre-phase of dexamethasone 6mg/m²/day followed by 2 sequential courses of induction therapy, termed induction phase 1 and induction phase 2, respectively. Patients with precursor B lineage ALL (pre-B ALL) were randomised to receive chemotherapy alone or chemotherapy plus 4 doses of rituximab given on day (D) 3, 10 17 and 24 of PEG-ASP 1000 IU/m² on D4 and D18, daunorubicin 60mg/m² and vincristine 1.4mg/m² (2mg max) on D1,8,15 and 21, dexamethasone 10mg/m²

D1,-4, 8-11,15-18 and a single 12.5mg intrathecal methotrexate dose on D14. Patients with Ph chromosome positive (Ph+) disease received continuous oral imatinib from D1, starting at 400mg and escalating to 600mg given daily throughout induction. Anti bacterial, anti-viral and antifungal prophylaxis was mandated, but centres used local policy for choice of agents. G-CSF support was strongly recommended. Routine anti-thrombotic prophylaxis was suggested but not mandated for all patients with platelet counts $>50 \times 10^9/l$. Anticoagulation with anti-thrombin replacement was recommended in the case of thrombosis. Routine coagulation factor replacement for laboratory-detected coagulopathy was specifically discouraged.

All patients had an initial assessment of response - including MRD response by *BCR-ABL* transcript monitoring or clonal immunoglobulin/T-cell receptor gene rearrangement quantification - at the end of induction phase 1, which did not affect treatment decision. Formal assessment of response to induction therapy (outside the scope of this report) was documented after phase 2 induction,

A simplified schema of the remainder of UKALL 14 treatment is provided in Figure 1.

Causality assessment for toxicity and death

The standard CTCAE reporting system (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm) was used. Causality of events was attributed, as is standard practice, by both the local site PI and the central study clinical team. Additional detailed questionnaires and PIs narratives were received for all induction deaths allowing a more detailed analysis of individual events.

PEG-ASP Antibody Assays

Antibodies against PEG-ASP (IgG and IgE) were measured by two indirect enzyme-linked immunosorbent assays, which detected anti-PEG-ASP and non-pegylated Anti-E.Coli (non-pegylated Asparaginase). Seroconversion was reported with positivity in at least one assay with a clearly negative pre-dose sample. Anti-asparaginase antibody ratio over negative control more than 1.1 was used to define positivity.

Serum asparaginase activity by MAAT testing

PEG ASP enzyme activity was quantified in sera using the MAAT7 assay. Therapeutic enzyme levels were defined as >100 IU/L.

Statistical analysis

Induction phase 1 treatment-related death was defined as any death occurring before the start of phase 2 induction where the cause of death was not primarily attributable to progressive ALL. Logistic regression was used to examine risk potential factors for induction death and grade 3/4 adverse events. Factors with a conservative $p < 0.2$ in the univariate analysis were included in the multivariable analyses. All analyses were conducted using Stata 14.0 (Stata Corporation, College Station, TX). Non-fatal grade III-IV adverse events causally related to PEG-ASP included were those classified as probably or definitely related. All adverse events

were graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Results

Patient characteristics

Ninety-one eligible patients (from 37 centres) were enrolled onto UKALL 14 between 30th December 2010 and 19th April 2012. One patient died before starting any treatment and has been excluded from all analyses. Patient characteristics at diagnosis are summarised in Table 1. The majority (59 of 90, 66%) of patients were over 40 years of age and nearly a third (26/90, 29%) had Ph+ ALL. Most patients (92%) had performance scores of 0-1 at diagnosis. Median follow up was 36.0 months (12 days – 50.4 months).

Induction deaths

Progress through the induction therapy blocks is shown in Figure 2. Among those commencing phase 1 induction therapy (n=90) there were 18 early deaths. Two patients died of progressive ALL and the other 16 (16/90, 18%) deaths were related to induction treatment, occurring at a median time from start to induction death of 23 days (range 10-53).

The causes of induction deaths are summarised in table 2. In 12 of 16 (75%), the causes of death were most often multifactorial; sepsis together with hepatotoxicity occurred in 8 of 16, 50.0%. Neutropenic sepsis alone occurred in 3 of 16 patients, (18.8%). A causative organism was identified in 11 of the cases of sepsis, with a gram-negative bacterial infection being responsible in 8 of those. Additional causes of death were: hepatotoxicity plus bowel ischemia (n=2), acute coronary syndrome plus neutropenic sepsis (n=1), hepatotoxicity plus pancreatitis (n=1) and pulmonary haemorrhage (n=1). Nine of 11 hepatotoxicity-associated induction deaths were associated with grades 3-4 hyperbilirubinaemia. In total, half of the induction deaths were accompanied by recognised PEG-ASP toxicities (namely those already listed in the Summary of Product Characteristics (SPC) as occurring in 1:1000 or more patients, or pancreatitis; less common, but clearly recognised as being related to PEG-ASP). There was no obvious association between baseline comorbidities and induction death.

All patients received D4 PEG ASP. Sixty-four, including 5 of the 16 who suffered induction deaths, received the second dose on day 18. For patients who died during induction, the median time from last dose of PEG-ASP to death was 13 days, (range 6 – 50).

Risk factors for induction death

Table 3 shows a univariate analysis of factors predictive of induction death. Age, Ph positivity and high-risk cytogenetics were all risk factors. Patients aged over 40 years had a more than 10-fold increase in risk of death during induction with Ph+ disease had a more than 8-fold increase compared to those who were Ph- (OR: 8.65 (2.61 – 28.71), p<0.001). There was no relationship to baseline albumin levels or body mass index (BMI). The data monitoring committee also confirmed that there was no relationship to rituximab randomization arm. The multivariable analysis is also shown in table 3. Although high-risk

cytogenetics appeared to be associated with induction death this was driven by the presence of t(9;22), so this factor alone was included in the multivariable analysis. Age and Ph status remained significantly associated with induction death; in patients who were Ph-, there were no deaths in the 21 patients aged < 40 years and 5 in the 43 patients over 40 years. In patients with Ph+ ALL, 1 of 10 patients aged < 40 years died compared to 10 of 16 who were over 40 years.

Adverse events during phase 1 induction therapy

Including the patients discussed above who subsequently died, 87 of 91 patients (97%) experienced a grade 3-5 adverse event (AE) during induction phase 1; among these 46 (51%) suffered one or more recognized PEG ASP toxicities; 34 had grade 3-5 AEs indicating liver dysfunction, including 22 with raised bilirubin. Other PEG ASP toxicities included pancreatitis (n=3), intracranial hemorrhage (n=1), allergic reaction (n=3), coagulation disorder (n=4) and 6 vascular events. Thirty-seven of 74 surviving patients (50.0%) experienced at least one recognised grade 3-4 PEG-ASP-related, non-fatal toxicity summarised in Table 4. Hepatotoxicity - including biochemical markers of liver dysfunction - were the most frequent PEG-ASP related toxicity (36.5%, n=27). Venous thromboembolism (4.1%, n=3), allergic reaction (4.1%, n=3) and pancreatitis (2.7%, n=2) were all reported, but were relatively uncommon. A complete line-listing of all AE/SAE as well as the subset known to be recognized toxicities of PEG-ASP from which table 4 is derived is given in supplementary table S1. Since liver toxicity was so prominent and can be a key determinant of subsequent on-time therapy delivery, an analysis of any pre-treatment factors associated with grade 3-4 hepatotoxicity was carried out. This is shown in Table 5. Older age and BMI were the only factors, which showed a significant association (OR: 2.88 (1.62 – 15.44), p=0.005, for patients >40 years compared to those < 40 years and OR: 1.58 (1.02 – 2.44), p=0.041 for a 5 unit increase in BMI).

Asparaginase activity, anti-asparaginase antibody formation and correlation with MRD response

A trough level of asparaginase activity by MAAT testing⁷, 13 was assessed 14 days after the first (D4) PEG-ASP dose in 49 patients in whom serum was available (n=49). Therapeutic activity (enzyme level >100IU/l) was achieved in 42 of 49 (86%). The median enzyme level in those achieving therapeutic enzyme levels was 234 (range 101.5 – 602.8) as compared with 44.8IU/l (0-97.5) in those with sub therapeutic levels. There was no evidence of an association between age and achievement of therapeutic enzyme level. Molecular MRD at the end of phase 1 induction - was documented in 27 patients. Molecular remission rates did not significantly differ between those achieving therapeutic enzyme levels of PEG-ASP and those who not (14/26 compared with 3/4), suggesting that an early complete molecular response in this setting is not contingent upon therapeutic asparaginase enzyme activity. Anti PEG-ASP antibody formation at day 18 was assessed in 59 patients and at that time point there were no instances of seroconversion.

Discussion

Successful achievement of CR during induction therapy for ALL is an absolute prerequisite for long-term disease-free survival. Numerous previous studies have suggested that induction regimens typically used in children give a better outcome for teenagers and younger adults than typical ‘adult’ regimens 14–18. As a consequence, there is general acceptance of testing the outcome of increasing the intensity of non-myelosuppressive agents such as PEG-ASP in regimens used for older adults.

Within the first 91 patients enrolled, we noted significantly greater induction mortality in UKALL14 than in our previous trial, UKALL12. Induction death occurred in 16 of the first 90 (17.8%) treated patients, and 15 of the first 59 (25%) participants aged over 40 (10/29 (33%) aged over 55). The median age of the patient population enrolled in this period was 46.5 years, with 29% of patients being aged over 55 years. By comparison, the induction death rate in our previous trial, UKALL12/ECOG2993, was 6% overall (ages 15-60, median age 36)¹² and 15% in the 55 – 65 year old patients (median age 56)¹⁹. UKALL14 did not exclude any patient with poor organ function or co-morbidity at diagnosis, but we did not find any evidence of these factors playing a causal contribution to PEG-ASP-related mortality.

Analysis of the cause of each death in UKALL14 included a complete written narrative from the treating physician as well as the standard case report forms for serious adverse event collection. Bacterial sepsis was revealed as a major contributor to the deaths. However, concomitant grade 3-4 PEG ASP toxicities were very common among those suffering induction death, such that a recognised PEG-ASP toxicity was associated with half of the 16 induction deaths. It is impossible to disentangle the role of the investigational medicinal product (IMP) PEG-ASP from that of concomitant non-IMP induction agents. However, the introduction of dexamethasone instead of prednisolone was the only change that had been made to the standard backbone regimen between UKALL12 and UKALL14.

It is noteworthy that most of the induction deaths reported here occurred after an early, single (D4) dose of PEG-ASP, any toxicity of which would overlap with the myelosuppressive toxicity of anthracycline administered on days 1, 8, 15 and 22. The 60mg/m² dose of anthracycline given in the UKALL12 regimen was well tolerated, but asparaginase was not started until day 17. The earlier addition of PEG-ASP-associated liver dysfunction to subsequent myelosuppression-related sepsis may have been responsible for the overlapping toxicity. The fact that sepsis was a major contributor to the mortality supports that contention.

To date, only 2 other studies reporting the use of PEG-ASP specifically in adults have been published, both of which report a lower dose and/or different schedule of anthracycline. A phase 2 trial CALGB 9511¹¹ reported on 102 adults treated with PEG-ASP at 2000 U/m² subcutaneously, capped at 3750 U) starting on day 5 of the 5-drug induction regimen. Although the regimen was reported to be “tolerable”, bilirubin greater than 51.3 µM (3 mg/dL) occurred in 54% of patients. CR rate was only 77% but induction deaths are not separated from induction failures within the report. There was a statistically significant

difference in median age of those achieving full dose delivery and asparagine depletion - 32 years versus 48 years. In a more recent study from Douer *et al* 20 in 51 adults median age 32 years, upper age limit 57 years reported no deaths directly as a result of PEG-ASP in regimen, which included 2,000 IU/m² PEG-ASP given on day 15. However, 3 of 51 (6%) died of neutropenic sepsis during consolidation. In this case, daunorubicin was given at 60 mg/m² intravenously (IV) on days 1, 2, and 3. Grade 3 to 4 hyperbilirubinemia (1/3 patients) or transaminitis (2/3 of patients) was very common. Douer and colleagues also reported long median intervals from PEG-ASP to bilirubin recovery, which affected subsequent chemotherapy delivery. The younger median age of patients in that study, the small number receiving concurrent imatinib (N=5), a more modest anthracycline dose and different scheduling of PEG-ASP administration so as not to coincide with the maximal myelosuppression may all have contributed to the difference in toxicity.

The German Multicentre Adult ALL Group (GMALL) have reported in abstract form 21 on 1000 adult patients aged 15-55, treated on the German 07/2003 study in which a single dose of 1000 -2000U/m² PEG-ASP was administered on day 3 during induction with a reported rate of induction death rate of less than 5% and a Grade IV hyperbilirubinaemia incidence of 16% . As with the Douer study, the younger median age (35 years) of the cohort in addition to the lower dose of anthracycline (45mg/m² versus the 60mg/m² used in the current study) may underpin the differences in toxicity.

It should be noted that the French GRAAALL group also reported that advancing age resulted in a higher cumulative incidence of chemotherapy-related deaths (23% v 5%, respectively; P < .001) and deaths in first CR (22% v 5%, respectively; P < .001) during “paediatric style” therapy, even when non-pegylated asparaginsae was used. In the study reported by Hugué et al 22, 45 years of age and above was the strongest predictor of severe toxicity,

As a consequence of the toxicity reported here, the UKALL 14 protocol was amended to omit D4 PEG-ASP for those over 40 years of age. Furthermore, PEG-ASP was completely removed from induction treatment for all patients with Ph+ ALL. Additionally, due to the high level of sepsis related to profound myelosuppression, the daunorubicin dose was halved to 30mg /m² on D1, 8, 15 and 22. One year following this amendment, when an additional 302 further patients had been recruited with 244 patients were assessable for induction death, a repeat analysis showed only 6 (2.5%) phase 1 induction deaths (data not shown).

Univariate analysis demonstrated that older age and poor-risk cytogenetics - including t(9;22) - were significantly associated with induction death. On multivariable analysis, age and Ph positivity remained as independent risk factors for induction death, suggesting that the concomitant imatinib therapy only received by those with Ph+ ALL may have compounded the potential for PEG-ASP toxicity. The precise mechanism by which this might occur is not known. However, from the summary of product characteristics for imatinib, ‘increased hepatic enzymes’ is reported as common (1:10 to 1:100), hyperbilirubinaemia occurs in between 1:100 and 1:1000 patients and finally, hepatic failure is noted as ‘rare’ namely occurring in between 1:1000 and 1:10,000 cases. Hence, the potential for overlapping hepatotoxicity of PEG-ASP and imatinib is clearly present.

Half of the patients who did not experience an induction death experienced one or more grade 3-4 PEG-ASP toxicities from which they eventually recovered - most commonly, hepatotoxicity. Twenty-three percent of patients in this study experienced grade 3 or greater hyperbilirubinaemia; by definition, a limitation or exclusion to further dosing with many commonly used ALL therapeutics. An analysis of treatment delays in our previous trial, UKALL12/E2993 study showed that delays of more than 4 weeks were associated with poorer OS and EFS in patients undergoing alloHCT, but not in patients undergoing post-remission chemotherapy²². Any long-term impact of any delays engendered by toxicity during initial induction therapy in this study will become clear when the trial is completed and we can evaluate any delays in relation to our primary end-point, EFS.

Our pharmacokinetic studies indicate that a single dose of 1000 IU/m² PEG-ASP generates therapeutic enzyme levels in most patients – 86% of assessable patients had therapeutic levels of PEG-ASP activity 14 days after administration. A recent longitudinal analysis of PEG-ASP pharmacokinetics during a ‘paediatric’ regimen in adult ALL demonstrated therapeutic enzyme activity in a similar proportion of adult patients for up to 21 days²⁰. In our study, anti-asparaginase antibodies were not seen at the early time point documented.

In conclusion we have shown that PEG-ASP achieves very effective asparagine depletion without anti-asparaginase antibody formation in the majority of adult patients. However, toxicity can be substantial in older patients, making a “paediatric-inspired” regimen of the type commonly used in paediatric and young adult therapy difficult to deliver safely to those over the age of 40 years. Avoidance of overlapping toxicities and careful timing of administration will be of key importance to the more widespread use of this type of regimen in older adults.

Since remission can be induced with minimal mortality in patients with Ph+ ALL^{23, 24}, we suggest that PEG-ASP is never co-administered with imatinib during induction therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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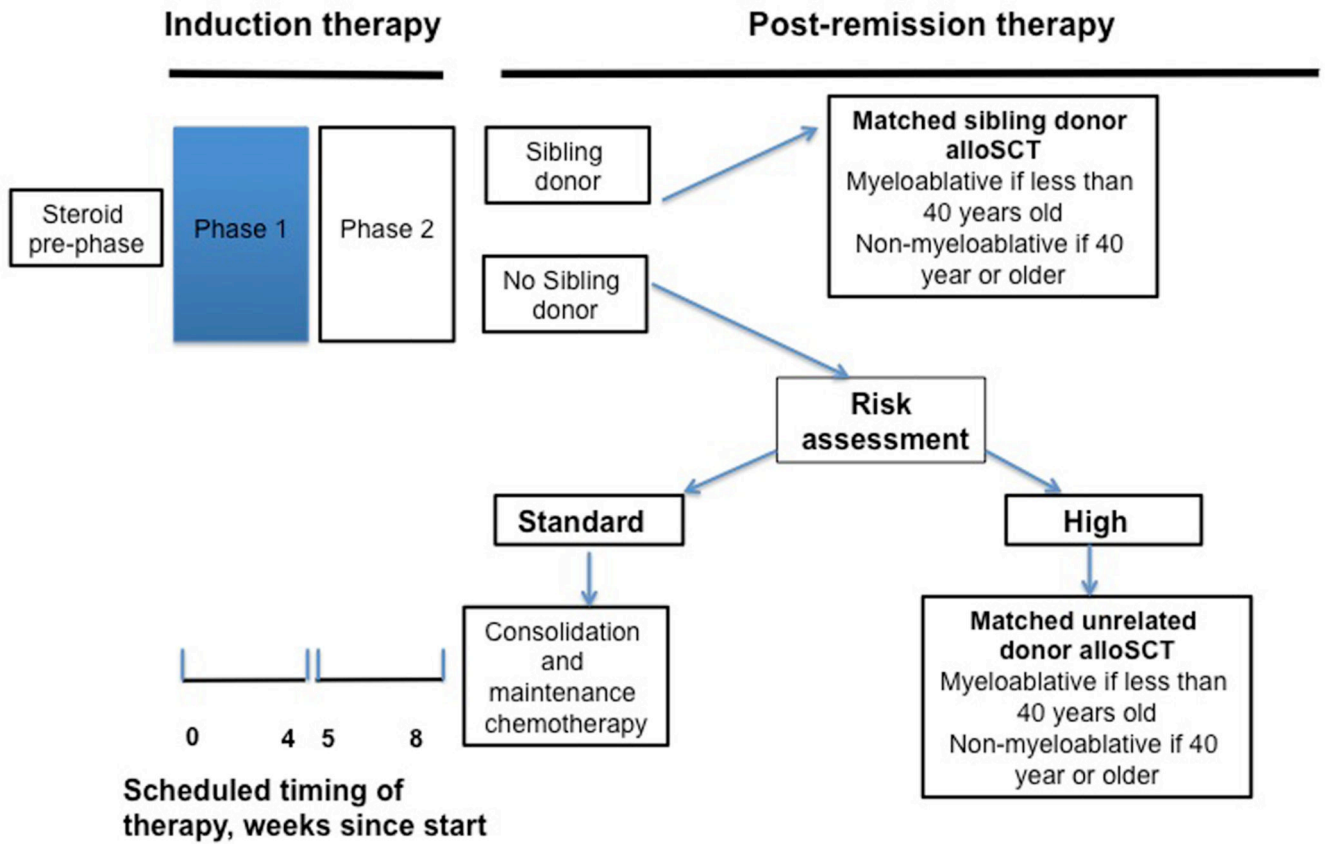


Figure 1. Schematic of UKALL 14 treatment protocol

*High risk by karyotypes: t(9;22) t(4;11), low hypodiploidy near triploidy or complex, age >40 years WBC $30 \times 10^9/L$ (pre B-ALL), $100 \times 10^9/L$ (T-ALL), Molecular Minimal Residual Disease positivity ($> 1 \times 10^{-4}$) after induction phase 2

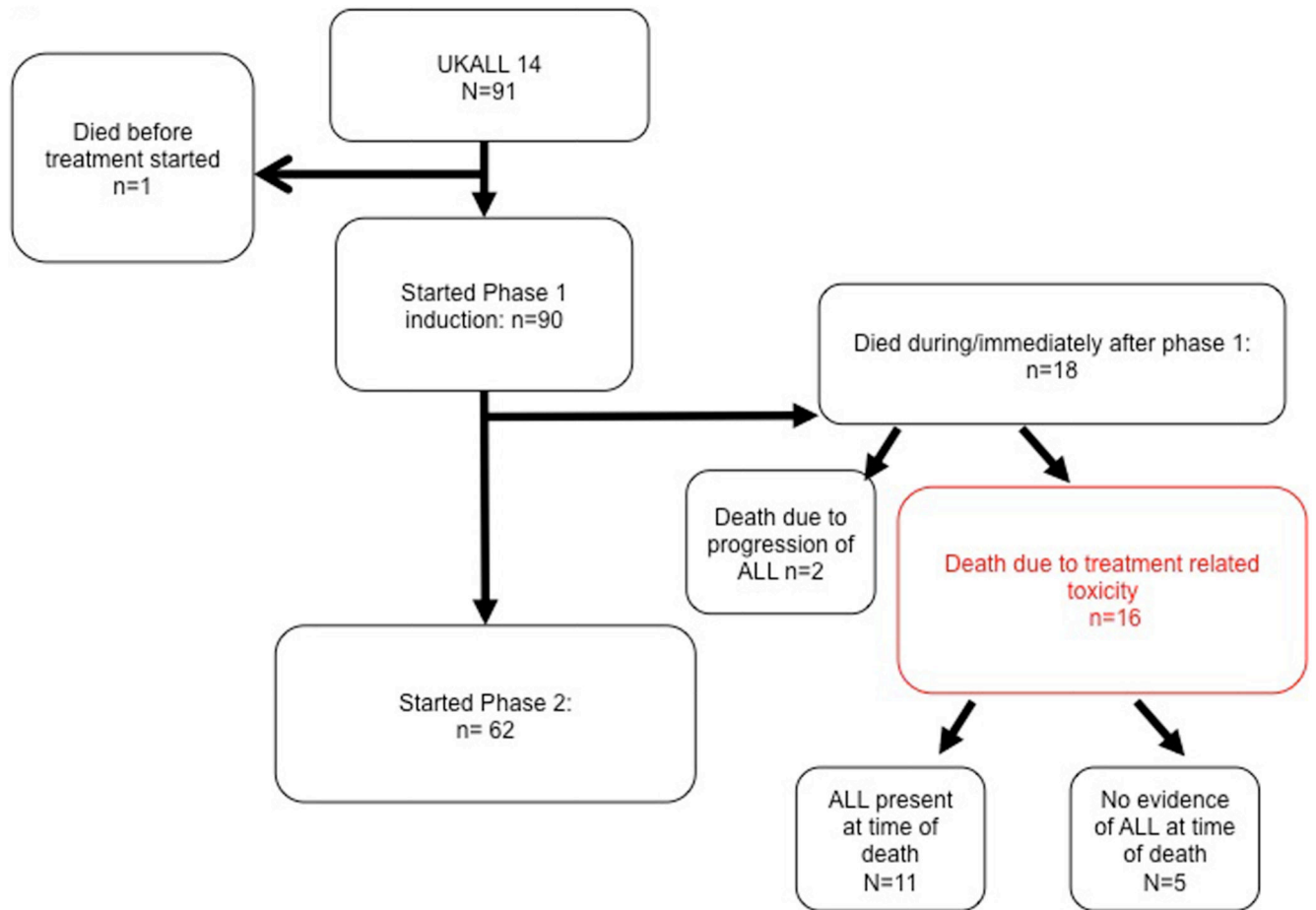


Figure 2. Flow chart of progress of the 91 patients enrolled

*Grade 4 sepsis n=4, Grade IV organ toxicity n= 4 (hepatotoxicity n= 1, pancreatitis n=1, hepatotoxicity plus neurological event n=1, hepatotoxicity plus thromboembolism together with sepsis n =1 and wrong diagnosis n=1, withdrawal of consent, n =1.

Table 1
Patient characteristics at diagnosis

Characteristic		N(%)
Lineage	B-precursor	77 (86)
	T-cell	13 (14)
Sex	Male	48 (53)
	Female	42 (47)
Age at entry (years)	Median (range)	46.50 (25 - 65)
	55	29 (32)
	41	59 (66)
Presenting WBC ($\times 10^9/l$)	Median (range)	9.26 (0.52 - 297.4)
	<30	62 (69)
	30-99.9	15 (17)
	100+	13 (14)
Cytogenetic risk status	*High risk	28 (31)
	Low risk	42 (47)
	**Unknown	20 (22)
t(9;22)	Absent	64 (71)
	Present	26 (29)
Low hypodiploidy/near triploidy	Absent	65 (72)
	Present	4 (4)
	Failed/missing	21 (23)
t(4;11)	Absent	75 (83)
	Present	7 (8)
	Failed/missing	8 (9)
Complex karyotype	Absent	64 (71)
	Present	5 (6)
	Failed/missing	21 (23)
)Performance status	0	55 (61)
	1	28 (31)
	2	5 (6)
	3	1 (1)
	Missing	1 (1)
BMI	Normal/underweight ***	31 (34)
	Overweight	25 (28)
	Obese	34 (38)

* High risk: t(9;22), t(4;11) low hypodiploidy/near triploidy or complex karyotypes

** No high risk factors and at least one risk factor is missing or failed

*** 30 in the normal range and 1 patient with a BMI of 17.

Table 2

Summary of deaths during induction

ID	Patient Details	Comorbidities	Hepatotoxicity (grade)	Neutropenic sepsis (organism)	Haemorrhage (site)	Thrombosis/Visceral ischaemia (site)	Pancreatitis	Other	PEG ASP doses	Days from last PEG-ASP dose
1003	Age 54 PH -	Liver cirrhosis	4	Not known					D4 & 18	19
1004	Age 46 PH+	None		Pseudomonas . Aeruginosa		Acute coronary Syndrome			D4	16
1011	Age 43 PH+	None	4	Escherichia.Coli				Renal Failure	D4 & 18	8
1021	Age 65 PH-	None	4	Pseudomonas . Aeruginosa		Gastrointestinal			D4	50
1026	Age 64 PH +	Depression	4	Pseudomonas . Aeruginosa					D4	20
1028	Age 38 PH +	None		Enterococcus Faecium					D4	15
1029	Age 60 PH+	IHD	4	Escherichia.Coli					D4	14
1030	Age 49 PH+	None			Pulmonary				D4	6
1039	Age 62 PH+	None	2			Small bowel			D4	10
1043	Age 63 PH-	Grade 4 renal failure	4	Klebsiella. Pneumoniae	Carotid artery puncture site				D4 & D18	43
1049	Age 56 PH+	None	4	Pseudomonas . Aeruginosa					D4	11
1057	Age: 57 PH-	None	4			Small bowel			D4	20
1061	Age: 54 PH+	Paroxysmal atrial fibrillation	3				Yes	GI perforation	D4	10
1063	Age: 62 PH+	None		Coagulase negative staph/Enterococcus Faecium					D4 & D18	12
1068	Age: 64 PH+	None	4	Respiratory Syncytial Virus				Renal Failure	D4	21
2007	Age: 55 PH-	None		Pseudomonas . Aeruginosa					D4 & D18	8

Table 3
Univariate and multivariate analysis of risk factors for induction death

Risk factor	Events/n	Univariable		Multivariable	
		Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age	40	1.00	0.03	18.50 (2.02 – 169.0)	0.01
	>40	10.23 (1.28 – 81.59)			
Gender	Male	1.00	0.77	-	-
	Female	1.18 (0.40 – 3.47)			
Baseline WBC per 10 ⁹ /l increment	16/90	1.00 (0.99 – 1.01)	0.56	-	-
Cytogenetics*	Standard risk	1.00	0.0029	-	-
	High risk	5.83 (1.20 – 28.29)			
Ph	Absent	1.00	<0.001	13.60 (3.53 – 52.36)	<0.001
	Present	8.65 (2.61 – 28.71)			
Base albumin (g/l) (10 unit increment)	16/90	1.03 (0.40 – 2.64)	0.95	-	-
Base bilirubin (µmol/L) (10 unit increment)	16/90	0.91 (0.59 – 1.42)	0.69	-	-
BMI (5 unit increment)	16/90	1.29 (0.82 – 2.02)	0.27	-	-

* analysed as no high risk factors vs. high risk Ph and high risk Ph+ gives HRs of: 1.86 (0.24 – 14.64) and 9.53 (1.86 – 49.91) respectively.

Table 4
Patients who did not die during induction: summary of the grade 3/4 AEs recognised in SPC as being related to PEG-ASP *

SOC/event term	Grade 3+ Peg Asp known AEs
	N(%)
Gastrointestinal Disorders	2 (3)
Pancreatitis	2 (3)
Hepatobiliary Disorders	3 (4)
Liver Failure	1 (1)
Liver Dysfunction	2 (3)
Immune System Disorders	3 (4)
Allergic Reaction	3 (4)
Investigations	29 (39)
Lipase Increased	1 (1)
Serum Amylase Increased	3 (4)
Alkaline Phosphatase Increased	15 (20)
Aspartate Aminotransferase Increased	4 (5)
Blood Bilirubin Increased	17 (23)
Alanine Aminotransferase Increased	10 (14)
GGT Increased	5 (7)
Metabolism And Nutrition Disorders	3 (4)
Increased Triglycerides	1 (1)
Hypoalbuminaemia	2 (3)
Nervous System Disorders	1 (1)
Intracranial Haemorrhage	1 (1)
Vascular Disorders	4 (5)
Pulmonary Embolism	1 (1)
Thromboembolic Event	3 (4)
Non CTC AE terms:	
Coagulation Disorder	3 (4)
Any liver event	27 (36)
Any Toxicity	37 (50)

* a complete line listing of Grade 3+ AE/SAE according to CTCAE criteria with assignment of causality by both site and by trial management group is provided in supplementary table S1.

Table 5
Analysis of risk factors for liver-related grade 3/4 adverse events during phase 1 induction
in patients who did not have an induction death

Risk factor		Grade 3/4 Liver AEs		
		Events/n	Odds ratio (95% CI)	p-value
Age	40	5/30	1.00	0.005
	>40	22/44	2.9 (1.62 – 15.44)	
Sex	Male	16/40	1.00	0.50
	Female	11/34	0.72 (0.28 – 1.87)	
Baseline WBC per 10x ⁹ /l increment		27/74	1.00 (0.99 – 1.01)	0.71
Cytogenetics	Standard risk	10/26	1.00	0.97
	High risk	11/29	0.98 (0.33 – 2.91)	
Ph	Absent	20/59	1.00	0.36
	Present	7/15	1.71 (0.54 -5.38)	
Base Albumin (g/l) (10 unit increment)		27/74	0.89 (0.41 – 1.92)	0.77
Base Bilirubin (µmol/L) (10 unit increment)		27/74	1.02 (0.81 – 1.30)	0.86
BMI (5 unit increment)		27/74	1.58 (1.02 – 2.44)	0.041