Challenges of starting treatment protocols for acute lymphoblastic leukaemia in a low-income setting – the Blantyre experience.

Chagaluka G [1]*, Schwalbe EC [2,4]*, Chakumatha E [1], Carey P [3], O'Brien S [4], Moorman AV [4], Barretta E [4], Rogers ES [4], Dunsmure A [4], Israels T [5], Molyneux EM [1]**Bailey S [6]**

*= contribution

** = contribution

Authors affiliations.

1. Department of Paediatrics, Queen Elizabeth Central Hospital and College of Medicine, Blantyre, Malawi,
2. Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, UK
3. Department of Haematology, Royal Victoria Infirmary, Newcastle upon Tyne, UK
4. Wolfson Childhood Cancer Centre, Translation and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK
5. Department of Paediatric Oncology and Academy Outreach, Princess Maxima Centre for Pediatric Oncology, Utrecht, The Netherlands
6. Great North Childrens Hospital and University of Newcastle, Newcastle upon Tyne, UK

Correspondence: Professor Simon Bailey,
Sir James Spence Institute of Child Health, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne, NE1 4LP, UK. e-mail: simon.bailey@ncl.ac.uk

The Event Free Survival (PFS) at 5 years of children treated for Acute Lymphoblastic Leukaemia (ALL) in High Income Countries (HIC) is > 80% (1, 2), and increasingly refined risk stratification has ensured that the intensity of treatment given maximises cure rates and minimises toxicity. Supportive care and health infrastructure are a vital part of this advancement in care, but in Low Income Countries (LIC) such as Malawi, a similar range of chemotherapeutic agents and supportive care is not available. In addition, the lack of timely laboratory diagnostic testing, the inability of families to access basic care, the long course of treatment and the existence of co-morbidities in many of the children has meant that treatment of acute lymphoblastic leukaemia is extremely challenging (3).

In 2010, the first treatment protocol for ALL in Malawi was introduced at the Queen Elizabeth Central Hospital (QECH) in Blantyre (4). This first protocol to treat ALL in Malawi was mindful of the prevailing infrastructure and set realistic goals of inducing remission in 50% of children and to assess toxicity. Based on outcomes for ALL protocol 1, incremental refinements to this first protocol were introduced in subsequent protocols, numbered 2 and 3, with the aim to improve both overall and EFS (Details of treatment are shown in Supplementary Figure 1). This letter reports the results of ALL treatment over the past 9 years at the QECH, as well as describing the difficulties in establishing and maintaining a treatment programme for children with haematological malignancies in a low-income setting. The QECH is a large, public, government referral hospital in Blantyre, Malawi. The paediatric oncology unit is a 23-bedded unit housed within the paediatric department which receives about 320 new patients a year. All children < 16 years of age with a suspected cancer diagnosis are admitted to the oncology ward; all children with a confirmed diagnosis of ALL were included in this study. The entire cost of treatment and hospital stay is free of charge to the families and is approximately £1000 (without travel costs) per patient, paid by a combination of governmental health budget money and charities. Despite some travel refunds and food provided in hospital, there are considerable out of pocket costs including food en route or any accommodation that may be necessary. Informed consent was obtained from the patients/guardians prior to commencement of treatment and all three ALL treatment protocols were approved by the local ethics committee.

Initial investigations and details of the treatment protocols as well as details of the statistical analyses can be found in the supplementary material.

Forty-two children (December 2009 – December 2012) were treated on the ALL 1 protocol (all based on intention to treat), 10 (January – July 2013) on ALL 2 and 145 on ALL 3 (August 2013 - October 2019). Since ALL 1 has been previously reported (4) and ALL 2 was an interim protocol with only 10 patients enrolled, we confined further analysis to ALL 3 patients. The clinical characteristics of the cohort and outcomes can be seen in Table 1. Of the 101 patients completing 4 weeks of treatment, 97 appeared to be in remission on morphological examination of their bone marrow at week 5. Eleven patients (8%) died before treatment could be started, 37 (26%) died in induction, 13 (9%) died immediately post intensification prior to count recovery and 74 (38%) at other time points. Of the patients dying during induction, 14 (38%) were NCI low-risk (age< 10 years and WBC < 50 x 10^9/L) and 23 (62 %) high-risk, which is the same proportion as the overall cohort. It was difficult to ascertain the cause of death during induction, although in 10 (27%) of the children, sepsis was the major cause and 2 (5%) disease the major cause; the remainder were likely to be due to a combination of these factors. In the absence of routine electrolytes, the incidence of tumour lysis was not known. Of the 87 children dying after count recovery following week 5 of treatment, 61 (70%) were presumed to have died due to disease recurrence and 26 (30%) of presumed infection, some of whom had community-acquired infections. Seventy-two children (48%) completed intensification (Supplementary Figure 2). Sixteen patients (11%) failed to complete treatment for non-medical reasons. Contributing reasons were financial issues, the stress of being away from home responsibilities and the lack of easy access to return journeys. Of the 129 remaining patients, 26 (18%) were still alive when last seen, with FU times ranging from 29 to 1492 days (median 622 days). Nineteen patients are known to have relapsed (83-1349 days after treatment began, median 490 days), 5 of whom were alive when last seen. For the total cohort of 145 patients treated with ALL protocol 3, event free survival (EFS) at 24 months was 27 %, (95% CI 19-39), demonstrating a significant improvement in EFS compared to protocols 1 (n=42; EFS 10%, 95%CI 3-29) and 2 (n=11; EFS 10%, 95%CI 2-66, overall p=0.03).

Retrospective genetic analysis of a portion of the cohort is described in the supplementary material.

Setting up an ALL treatment programme in a LIC is a challenging endeavour, during which many factors have to be considered. In contrast to HIC, patients typically present late and with significant co-morbidities in LIC treatment centres. This was clearly demonstrated in our study, with the initial very high death rate during induction therapy (Protocol 1, 14/42 (33%)) which, although now reduced due to improved supportive care (Protocol 3, 38/145 (26%)), is still unacceptably high.

The diagnosis of ALL is challenging in LIC. We developed a remote pathology service which provided some level of diagnostics in which blood and bone marrow films are examined within 24-48 hours, as described in the supplementary material (5). Treatment was often commenced with the prednisolone pre-phase on the basis of clinical suspicion, whilst waiting for the blood films to be reported in Newcastle. Flow cytometry and genetic analysis are not available routinely in Malawi, and so meaningful stratification remains challenging. However, a full blood analyser was been installed in the paediatric department in 2018, which allows more timely blood counts to be performed.

Supportive care is vital in treating children with ALL. Antibiotics should be given promptly, hydration used with close monitoring and allopurinol given to help prevent the nephrotoxic effects of tumour lysis. Granulocyte colony stimulating factor is not routinely available and was used when available in a small number of carefully selected children post intensification. The nurse to patient ratio is much reduced in LIC (6) so close attention to each individual child is not easily possible. The recommendation is 1 nurse to 5 patients in LIC (6) although in the Blantyre unit this is usually 1:10, with a lower staffing ratio at night and the weekends. The number of medical staff is also reduced, there is one consultant (GC), one registrar (on 6 months rotation), one intern (for a 1-month rotation and often 'post take' or off) as well as 1 clinical officer (EC). These are areas which also need to be addressed to improve the service.

There is a relatively high incidence of pre-treatment deaths (N=11; 8%). Also, the provision of maintenance treatment was compromised by the inability to acquire a rapid blood count to adjust doses of 6-mercaptopurine and methotrexate. Parents and family members often have very few resources for travel to and from the hospital for prolonged treatment (the children travel up to 300 miles, often by infrequent and relatively expensive public transport or minibus), and the need to return for febrile episodes is very challenging to achieve. It is not surprising that failure to complete treatment was 11%, given these difficulties. A large amount of effort and expense has been and continues to be made to establish the outcome of the children and ascertain whether they have had any untoward events; this is vital in assessing the effectiveness of the treatment schedule.

The challenges in setting up an ALL treatment programme in LIC are many and vary according to specific local circumstances. The commitment at QECH to providing care for children with ALL despite limited resources demonstrates how much can be achieved. It is hoped that the lessons learned from the Blantyre experience can be used in other similarly resourced units planning to start treatment for children with ALL. (Table 2)

Conflict of Interest

The authors have no conflict of interests to declare.

Figure and table legends.

Table 1. Clinical characteristics of children treated on ALL protocol 3 (n=149).

Table 2. Summary of essential factors in setting up a treatment protocol for ALL in a low-income setting.

References.

1. Richards S, Burrett J, Hann I, Chessells J, Hill F, Bailey C. Improved survival with early intensification: combined results from the Medical Research Council childhood ALL randomised trials, UKALL X and UKALL XI. Medical Research Council Working Party on Childhood Leukaemia. Leukemia. 1998;12:1031-1036.

2. Mitchell C, Payne J, Wade R, Vora A, Kinsey S, Richards S, Eden T. The impact of risk stratification by early bone-marrow response in childhood lymphoblastic leukaemia: results from the United Kingdom Medical Research Council trial ALL97 and ALL97/99. Br J Haematol. 2009;146:424-436.
3. Molyneux E, Scanlan T, Chagaluka G, Renner L. Haematological cancers in African children: progress and challenges. Br J Haematol. 2017;177:971-978.
4. Chagaluka G, Carey P, Banda K, Schwab C, Chilton L, Schwalbe E, Skinner R, Israels T, Moorman A, Molyneux E, Bailey S. Treating childhood acute lymphoblastic leukemia in Malawi. Haematologica. 2013;98:e1-3.
5. Carey P, Fudzulani R, Scholfield D, Chagaluka G, Tomoka T, Liombe G, Banda K, Wadehra V, Samarasinghe S, Molyneux EM, Bailey S. Remote and rapid pathological diagnosis in a resource challenged unit. J Clin Pathol. 2014;67:540-543.
6. Day S, Hollis R, Challinor J, Bevilacqua G, Bosomprah E, SIOP PODCNWG. Baseline standards for paediatric oncology nursing care in low to middle income countries: position statement of the SIOP PODC Nursing Working Group. Lancet Oncol. 2014;15:681-682.

Acknowledgments.

We would like to thank the children and families of children with acute lymphoblastic leukaemia in Blantyre who deal with their disease and its consequences with remarkable dignity and fortitude. We would also like to thank the staff who tirelessly and with compassion look after the children and their families during long periods in hospital. We would also like to thank the World Child Cancer and Children with Cancer in Malawi charities that support the work in the unit in Blantyre.

Author contributions.

George Chagaluka: Study design, data collection, data interpretation, writing. Ed Schwalbe: Data analysis and data interpretation, writing, figures. Eunice Chakumatha: Data collection Peter Carey: Data collection, data analysis, study design. Stephen O'Brien: Data analysis, writing. Anthony Moorman. Data collection, genetic analysis, data analysis, figures, writing. Angela Dunsmure: Data collection, data analysis, figures. Emilio Barretta: genetic analysis. Ellie Rogers: genetic analysis. Trijn Israels: Data collection, study design. Elizabeth Molyneux: Study design, data collection, data interpretation, writing. Simon Bailey: Study design, data collection, data analysis, data interpretation, writing, figures.

Malawi ALL Protocol		3	Deaths by treatment stage by year (% of patients entering each stage)		
			Death pre treatment	Death during induction	Death post-intensification
Number of patients		145			
Median age (range)		8 (0.5-18)			
Sex (Male:Female)		85:60			
Year of diagnosis	2013	13	2 (15)	6 (55)	1 (20)
	2014	24	1 (4)	7 (30)	2 (13)
	2015	25	2 (8)	7 (30)	2 (13)
	2016	38	1 (3)	9 (24)	1 (4)
	2017	23	4 (17)	3 (16)	5 (31)
	2018	20	1 (5)	5 (26)	2 (14)
	2019	2			
Median follow up (years)		0.51			
HIV status	Negative	131			
	Positive	1			
Nutrition	Poor	45			
	Fair	63			
	Good	27			
Median White Blood Cell count (range)		35 (0-540)			
Median Haemoglobin (range)		5.6 (1.8-13.6)			

Median Platelets (range)		28 (0-595)
Median Liver size (range)		0.5 (0-12)
Median Spleen size (range)		3 (0-25)
Lymphadenopathy	No	67
	Yes	77
Bone Pain	No	101
	Yes	43
Pyrexia	No	31
	Yes	113
Median length of history (range)		30 (2-360)
Median number of blood transfusions (range)		1 (0-22)
24 month event free survival percentage(95% CI)		27 (19-39)
24 month overall survival percentage (95% CI)		20 (14-29)

Univariable analysis	**Hazard Ratio**	**Lower 95% CI**	**Upper 95% CI**	**p**
Sex (female vs male)	1.56	1.01	2.40	0.04
No. of blood transfusion prior to admission	1.09	1.00	1.20	0.04

Multivariable analysis				
Sex (female vs male)	1.65	1.07	2.54	0.02
No. of blood transfusion prior to admission	1.11	1.02	1.2	0.02

Table 3.

Essential factors in setting up a treatment protocol for ALL in a low-income setting
• Treatment protocols should be simple and easy to follow • It is better to start with a less toxic protocol and gradually increase the intensity of treatment as staff become more confident in administering the protocol and are able to prevent or manage side effects • Timely diagnosis is critical to good outcome • The protocol can only be as intense as the supportive services of the hospital make possible. • The financial burdens of treatment and transport to hospital for families must be relieved to make completion of treatment feasible.

Challenges of starting treatment protocols for acute lymphoblastic leukaemia in a low-income setting – the Blantyre experience.

Supplementary material.

Methods.

Initial examination and investigations of all children with a clinical diagnosis of ALL included weight, height and mid-arm circumference. Laboratory investigations included a full blood count, rapid HIV test, malaria test, stool and urine microscopy. Under ketamine sedation, a bone marrow aspirate (BMA) and cerebrospinal fluid (CSF) sample were taken. Slides of peripheral blood smears, BMA and CSF (cytospin was not available) were stained using Rapidiff stain kit (Clinical Service Laboratory, QECH, Cheshire, UK) and photographs of the slides, taken with a Jenoptik camera (CT 13 with Prog Res Mac Capture Pro software) mounted on an Olympus microscope (CX31), were transmitted electronically for diagnostic confirmation in Newcastle, UK by an experienced haematologist (1). Initial intrathecal chemotherapy was given at the time of the diagnostic lumbar puncture. No immunocytochemistry or flow cytometry was performed on any of the specimens due to the lack of a flow cytometer or immunological stains. Bone marrow samples were collected and shipped to the UK for retrospective genetic analysis, but the results were not available to influence treatment. Patient samples were screened by fluorescent in situ hybridisation (FISH) using commercially available probes for *ETV6-RUNX1*, *BCR-ABL1* and *KMT2A* gene rearrangements as previously described (2). In addition, DNA was extracted and copy number alterations assessed for eight loci (*IKZF1*, *CDKN2A/B*, *PAX5*, *BTG1*, *ETV6*, *EBF1*, *RB1* and the *PAR1* region) using Multiplex Ligation-dependent Probe Amplification (MLPA), using the SALSA MLPA P335 kit (ALL-IKZF1, version C1) as previously described (3, 4).

Treatment details.

Children were treated from December 2009 until December 2012 on the Blantyre ALL 1 protocol (42 children), from December 2012 until July 2013 on Blantyre ALL 2 (10 children) and from August 2013 until October 2019 on ALL 3 (145 children) (supplementary Figure 1). Children diagnosed from December 2009 until December 2018 were included in this report.

The treatment protocols were developed in a risk adaptive manner after detailed consultations between the Blantyre and Newcastle teams, taking into account local conditions, supportive care and patient logistics. ALL 1 consisted of induction chemotherapy with prednisolone (40 mg/m^2 per day in 2 divided doses) for 28 days with a week's tapering from day 29-35, vincristine (1.5 mg/m^2) weekly x 5, a single dose of cyclophosphamide at 300 mg/m^2 on day 1 and intrathecal methotrexate on day 1, 7 and 29. There were 20 weeks of maintenance with 5 days of prednisolone (40 mg/m^2 per day in 2 divided doses), following the 4 weekly intravenous vincristine (1.5 mg/m^2) and Intrathecal methotrexate. Daily oral 6 mercaptopurine (60 mg/m^2 /day) was continued throughout the maintenance period. In ALL 2 the same protocol was used but a prednisolone pre phase of one week was added (40 mg/m^2 per day in 2 divided doses) - this was an interim protocol until ALL 3 was finalised. In ALL 3, cyclophosphamide was replaced by native asparaginase (various manufacturers, dependent on hospital supplies available) given at a dose of 6000 IU/m^2 intramuscularly on alternate days, starting on day + 4, for 9 doses. In addition, after 8 weeks of interim maintenance (as described above), an intensification block (week 14) consisting of doxorubicin 45 mg/m^2) day 1 + 2, cytarabine 100 mg/m^2 per dose bd x 10 doses, etoposide 100 mg/m^2 daily x 5 days, vincristine (1.5 mg/m^2 day 1), prednisolone 40 mg/m^2 per day in 2 divided doses for 5 days and intrathecal methotrexate (day 1) was given. This was followed on count recovery by maintenance therapy as previously described (intrathecal methotrexate given every 12 weeks) up to a total of 104 weeks. Children were kept as inpatients for induction and intensification until their blood counts recovered. Monthly blood counts were performed although the results took a number of days and hence 6-mercaptopurine doses were adjusted a month in arrears. Cotrimoxazole prophylaxis, intermittent antimalarial prophylaxis and a bed net were given to all children.

Statistical analysis.

All statistical analyses were performed using R v3.6.2. Overall survival (OS) was defined as the time from date of diagnosis to death or date of last follow-up. Event-free survival (EFS) was defined as the time from diagnosis to first event (progression or relapse) or date of last follow-up. Patients dying of other causes were censored at time of death. Using the *survival* package v3.1-8, Kaplan-Meier curves were constructed, and patient groups compared with log-rank

tests. For univariable and multivariable Cox models, the proportionality assumption was tested using scaled Schoenfeld residuals. Missing data were assumed to be missing completely at random. For multivariable analysis, variables with greater than 10% missing values were removed from further consideration. Variables with fewer than 10% missing values were imputed 5 times using the *aregImpute* function from the *rms* package, v5.1-4. The modal value was selected for categorical variables and the mean value calculated for continuous data. Using forward likelihood ratio testing, multivariable models were constructed and limited to two variables because of cohort size.

Description of Additional Clinical Characteristics.

The median age at presentation was 8.0 years (6 months – 18 years), the median weight was 19.9 kg (3.4-47 kg), 85 were male (58 %) and 60 were female (42 %). Forty-five children (31%) had poor nutrition at diagnosis, 63 fair nutrition (43%), 27 (19%) good nutrition and 10 (7%) were unknown. This grading system is a subjective clinical judgement by the admitting doctor and is used for all children in the paediatric department. There was 1 child who was HIV positive, 13 whose HIV status was unknown and the remaining 131 children were HIV negative.

The median presenting white blood cell count (WBC) was 35 x 10^9/L (0 to 540 x 10^9/L), median platelet count 28 x 10^9/L (0-595 x 10^9/L) and haemoglobin 5.6 g/dL (1.8 – 13.6 g/dL).

The cause of death was thought to be disease in 84 children (79% of deaths), the majority of the remainder was unable to be determined, although sepsis was thought to be the major contributor. Seventy-eight (73%) patients died in hospital and 23 (21%) at home. The place of death was unknown in 6 patients (6%) (Figure 3).

There was no significant difference in OS between cohorts (Protocol 1, OS 9%, 95% CI 3-25; Protocol 2, OS 10%,95% CI 2-66; Protocol 3 OS 20%, 95% CI 14-29, overall p=0.10). (Figure 4)

Three of these children left the ward within the induction treatment period, 8 at last contact were alive and disease free (range of FU - 7 to 862 days, median 287 days), 2 alive with disease

and 6 had died of their disease. Of these patients, 2 re-presented to the hospital with confirmed relapse.

Univariable Cox proportional hazards models of EFS demonstrated that female sex was associated with a worse survival (HR=1.56, 95%CI 1.01-2.40, p=0.04). The number of blood transfusions received prior to admission was also associated with a worse survival (for each additional blood transfusion, HR=1.09, 95%CI 1.00-1.20, p=0.04). In multi-variable analysis, sex and number of blood transfusions prior to admission were independently prognostic (Supplementary table 1).

Retrospective Genetic Analysis.

Among the 145 patients treated on ALL-3, FISH and MLPA analyses were attempted on 37 samples from 30 patients. Unfortunately, the failure rate for FISH was high (54/111, 48%) but much lower for MLPA (3/37, 8%). FISH for *ETV6-RUNX1* was successful for 16 patients and two (13%) patients were positive (3 and 4 years old) with one additional case showing extra copies of *RUNX1* indicative of tetrasomy 21. Further centromere FISH showed gains of chromosome X and 14 consistent with high hyperdiploidy. MLPA analysis was successful for 27 patients and 13 (48%) patients harboured ≥1 CNA. The genes located on the short arm of chromosome 9 were deleted in 12/27 (44%) patients: *CDKN2A/B* deletion (n=5), *PAX5* deletion (n=5) and co-deleted (n=2). Patients with *CDKN2A/B/PAX5* had a female predominance among deletions (8 females; 4 males), high WCC (mean $71x10^9$/L) and poor outcome (11/12 died). Other deletions were rarer: *ETV6* (n=2), *EBF1* (n=1), *RB1* (n=1). None of the patients had deletions affecting *IKZF1*, *BTG1*, or the PAR1 region. The frequency of *ETV6-RUNX1* fusion in HIC is ~25% (2)) with the possible exception of a lower frequency (~12%) among Hispanics (5). The frequency of *ETV6-RUNX1* fusion in our previous report (5) was 1/9 and in this study was 2/16 giving an overall estimated frequency of 12%. This is the first study to screen patients from Malawi for the common CNAs associated with ALL. Nearly half the tested patients harboured a deletion of *CDKN2A/B* and/or *PAX5* which are both located on the short arm of chromosome 9. This frequency is marginally higher than the 30-35% reported in UK cohorts(3). Nonetheless it is noteworthy that the 9p deletion patients in this cohort appear to be associated, like in the UK, with poor risk features – high WCC and

poor outcome(6). Therefore, risk stratification of patients in Malawi, while challenging, should remain an achievable goal.

Supplementary Figures.

Supplementary Figure 1. Outline of treatment protocols for children with acute lymphoblastic leukaemia at the Queen Elizabeth Central Hospital in Blantyre. ALL 1 ran from December 2009 until December 2012, ALL 2 from December 2012 until July 2013 and ALL 3 from August 2013. The weeks corresponding to the maintenance therapy are examples and continued for a total of 24 weeks for ALL1, 36 weeks for ALL 2 and 102 weeks for ALL3.

Supplementary Figure 2. Bar plot showing the number of deaths at different stages per year diagnosed in the treatment protocol for children treated or intended to be treated on the ALL 3 protocol.

Supplementary Figure 3. Flow chart showing clinical course of patients on the ALL3 protocol.

Supplementary Figure 4. Event free (A) and overall survival (B) of Malawi ALL patients stratified by treatment protocol 1-3. P values from log-rank tests are shown.

Supplementary tables.

Supplementary Table 1. Identification of univariable and multivariable prognostic survival markers for children treated on the ALL 3 protocol. For each covariate, Cox models are summarised, showing hazard ratio, 95% confidence intervals and p values from Wald tests.

Supplementary References.

1. Carey P, Fudzulani R, Scholfield D, Chagaluka G, Tomoka T, Liombe G, Banda K, Wadehra V, Samarasinghe S, Molyneux EM, Bailey S. Remote and rapid pathological diagnosis in a resource challenged unit. J Clin Pathol. 2014;67:540-543.
2. Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, Vora A, Mitchell CD, Harrison CJ. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol. 2010;11:429-438.
3. Moorman AV, Enshaei A, Schwab C, Wade R, Chilton L, Elliott A, Richardson S, Hancock J, Kinsey SE, Mitchell CD, Goulden N, Vora A, Harrison CJ. A novel integrated cytogenetic and genomic classification refines risk stratification in pediatric acute lymphoblastic leukemia. Blood. 2014;124:1434-1444.
4. Eden OB, Harrison G, Richards S, Lilleyman JS, Bailey CC, Chessells JM, Hann IM, Hill FG, Gibson BE. Long-term follow-up of the United Kingdom Medical Research Council protocols for childhood acute lymphoblastic leukaemia, 1980-1997. Medical Research Council Childhood Leukaemia Working Party. Leukemia. 2000;14:2307-2320.
5. Aldrich MC, Zhang L, Wiemels JL, Ma X, Loh ML, Metayer C, Selvin S, Feusner J, Smith MT, Buffler PA. Cytogenetics of Hispanic and White children with acute lymphoblastic leukemia in California. Cancer Epidemiol Biomarkers Prev. 2006;15:578-581.
6. Sulong S, Moorman AV, Irving JA, Strefford JC, Konn ZJ, Case MC, Minto L, Barber KE, Parker H, Wright SL, Stewart AR, Bailey S, Bown NP, Hall AG, Harrison CJ. A comprehensive analysis of the CDKN2A gene in childhood acute lymphoblastic leukemia reveals genomic deletion, copy number neutral loss of heterozygosity, and association with specific cytogenetic subgroups. Blood. 2009;113:100-107.

Supplementary Table 1.

Univariable analysis

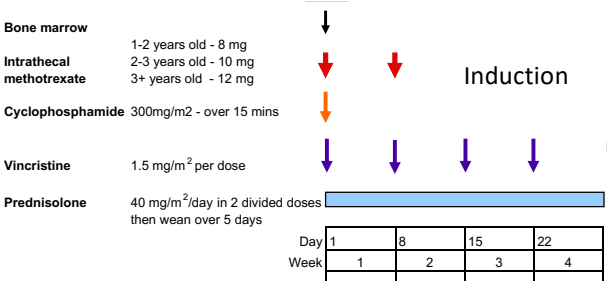
Variable	Hazard Ratio	Lower 95%CI	Upper 95%CI	p value
Sex (female vs male)	1.56	1.01	2.40	0.04
No. of blood transfusion prior to admission	1.09	1.00	1.20	0.04
HIV status	7.41	0.99	55.63	0.05
Temperature	0.80	0.64	1.02	0.07
Age (years)	1.05	0.99	1.11	0.09
Place of death (hospital versus home)	0.66	0.40	1.09	0.10
Pallor	1.60	0.83	3.10	0.16
Weight (kg)	1.01	0.99	1.04	0.24
Nutrition (versus malnourished)	0.78	0.48	1.26	0.31
BonePain	0.80	0.50	1.27	0.34
Length of history (days)	1.00	0.99	1.00	0.37
History of bleeding	0.80	0.48	1.34	0.40
Haemoglobin	0.96	0.87	1.06	0.42
Lymphadenopathy	0.87	0.56	1.33	0.51
History of Fever	0.86	0.53	1.38	0.52
Failure to complete treatment	0.82	0.40	1.71	0.60
Neutrophil count	1.00	0.97	1.03	0.78
Platelet count	1.00	1.00	1.00	0.83
Spleen size (cm)	1.00	0.96	1.05	0.85
Liver size (cm)	0.99	0.91	1.09	0.88
White blood cell count	1.00	1.00	1.00	0.95

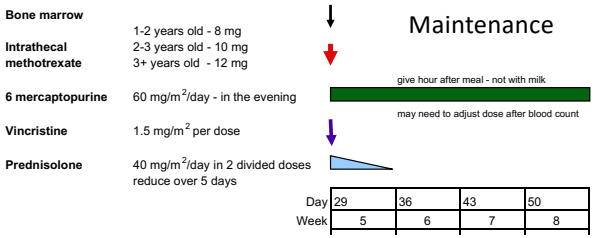
Multivariable analysis

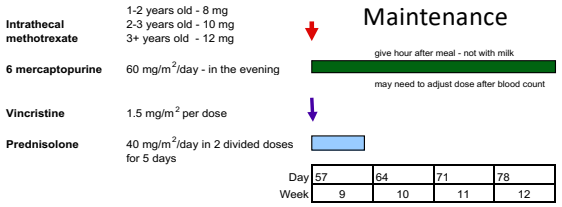
Variable				
Sex (female vs male)	1.65	1.07	2.54	0.02
No. of blood transfusion prior to admission	1.11	1.02	1.20	0.02

Figure 1.

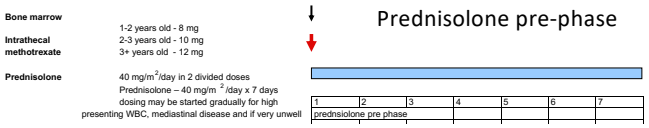
ALL 1 – 24 weeks total

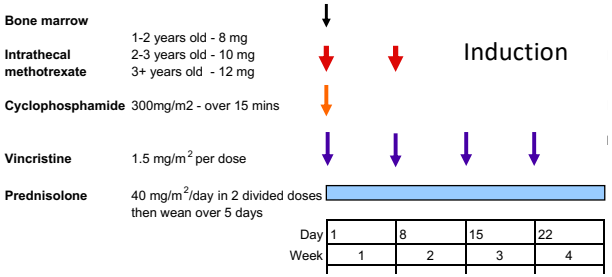


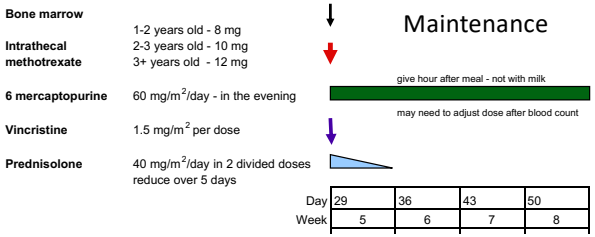


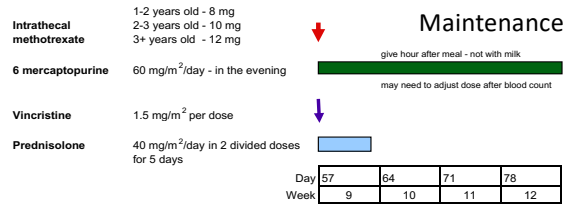


ALL 2 – 36 weeks total

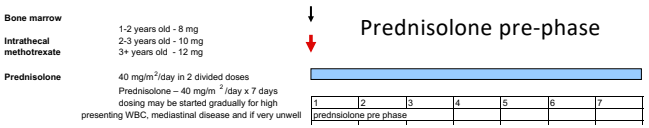


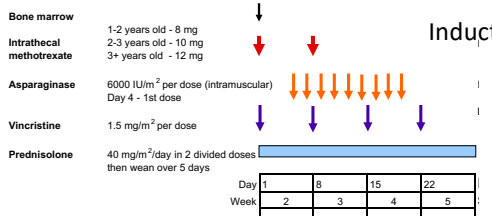


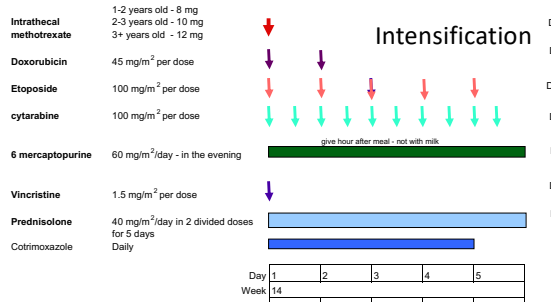




ALL 3 – 102 weeks total







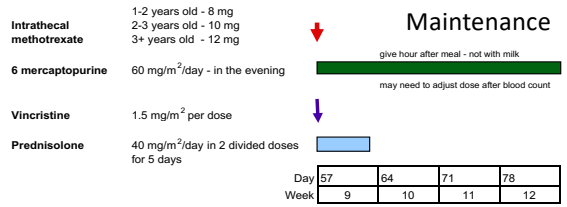


Figure 2.

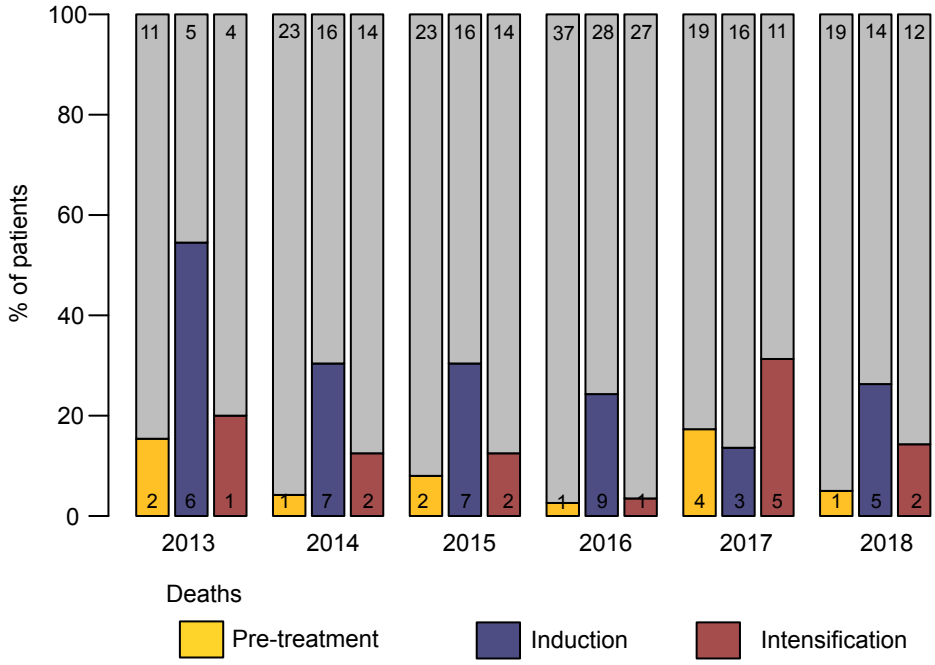
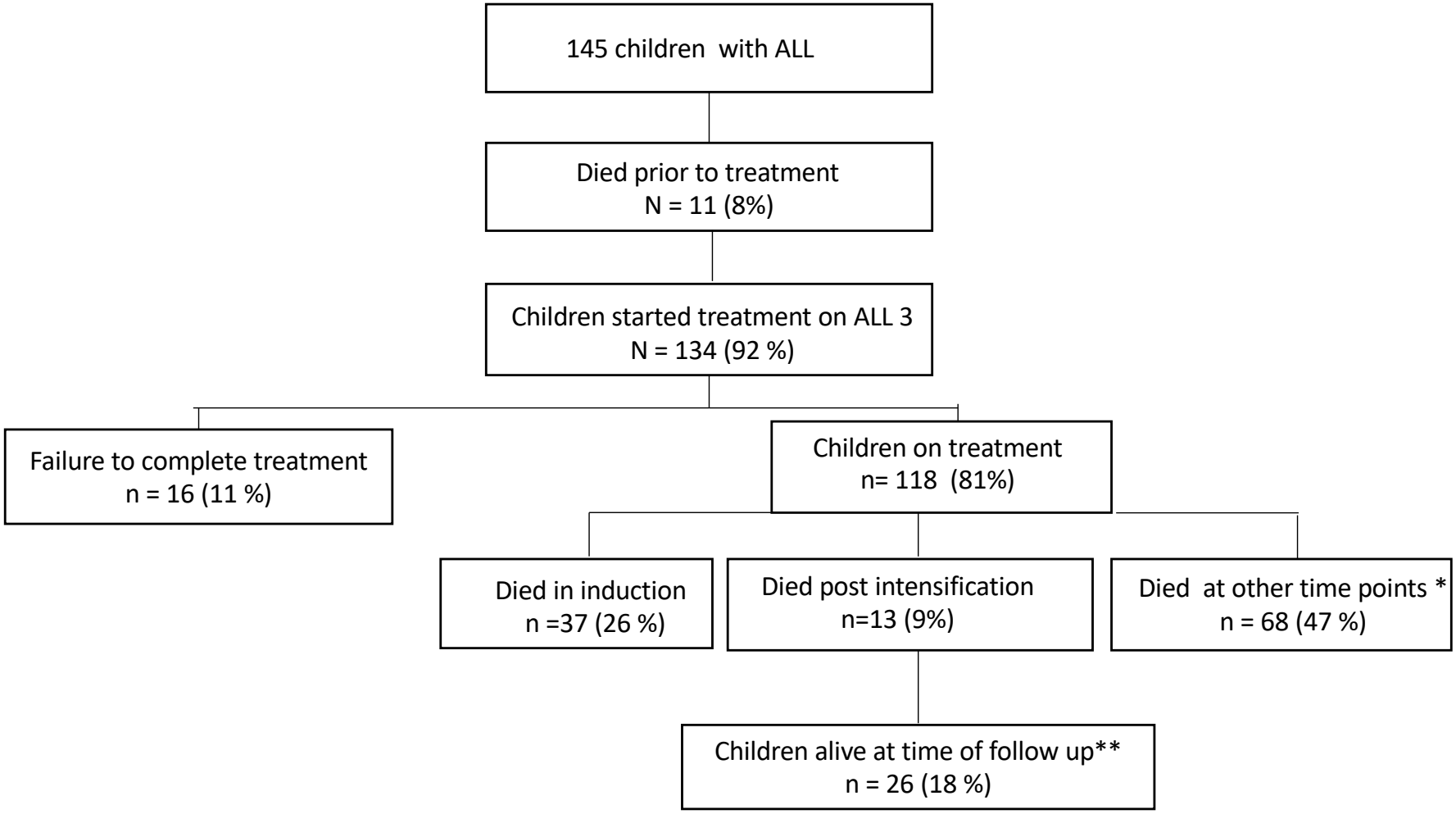

100
80
60
40
20
0
% of patients
11
5
4
23
16
14
23
16
14
37
28
27
19
16
11
19
14
12
2
6
1
1
7
2
2
7
2
1
9
1
4
3
5
1
5
2
2013
2014
2015
2016
2017
2018
Deaths
Pre-treatment
Induction
Intensification

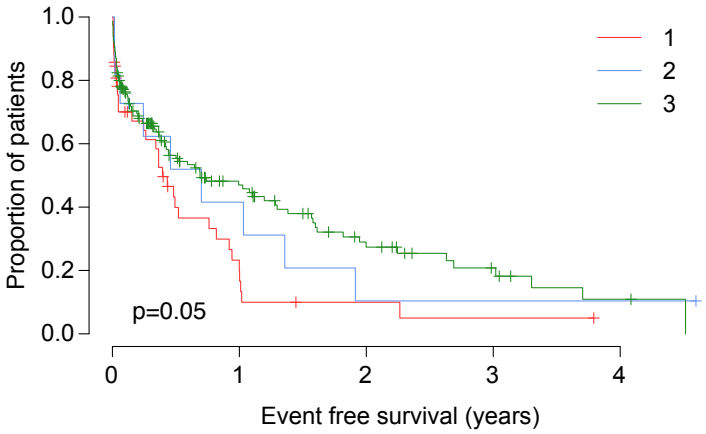
Figure 3.



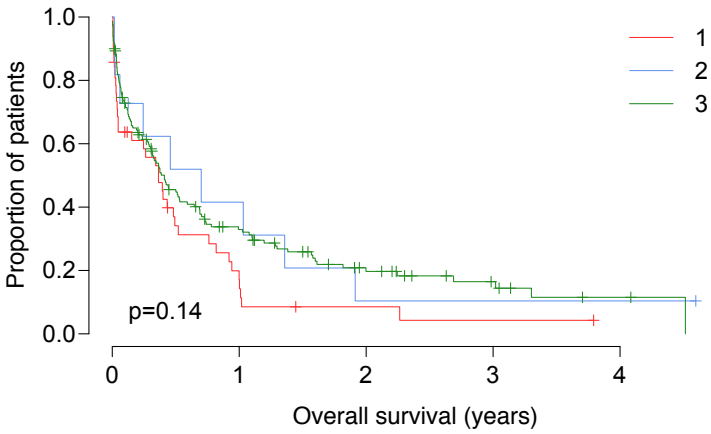
* including 6 who failed to complete treatment

** including 10 who failed to complete treatment

A


1.0
0.8
0.6
0.4
0.2
0.0
Proportion of patients
1
2
3
p=0.05
0
1
2
3
4
Event free survival (years)

B


1.0
0.8
0.6
0.4
0.2
0.0
Proportion of patients
1
2
3
p=0.14
0
1
2
3
4
Overall survival (years)