Infection in children with acute lymphoblastic leukaemia

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Infection is the commonest cause of morbidity and mortality in children undergoing treatment for acute lymphoblastic leukaemia (ALL). This population-based study aimed to describe the prevalence of microbiologically documented infections in children with ALL during different phases of treatment, and to examine associations with patient characteristics and treatment-related variables.

267 patients diagnosed with ALL aged between 1-17 years were identified from the Haematological Malignancy Research Network over a near 13 year period (2004-2017). Their microbiological samples, including blood cultures, respiratory samples and samples sent for fungal testing, were analysed across different phases of treatment.

254/267 (95.1%) patients had at least one microbiological sample taken during treatment (5,240 samples), and 230/254 (90.5%) of these had at least one positive sample (1,171 positives). Among positive samples, respiratory viral pathogens were identified across all phases of treatment. In the few positive blood culture samples, there was a predominance of Gram +ve bacteria. During induction, females, those diagnosed under the age of ten years, and those treated under more intensive regimens were more likely to have at least one positive microbiological sample. This was also the case for patients that were treated under regimen B during consolidation (intensive but relatively short), and those diagnosed under the age of ten years during delayed intensification. During maintenance, patients who lived in more deprived areas had higher rates of positive blood cultures.

To date, this is the first UK cohort of patients with ALL treated in a paediatric setting to report on microbiological history during treatment. Demonstrating that some patient groups are more at risk than others, and showing microbial differences within and between patients across the various treatment phases, this research highlights the importance of taking a longitudinal approach and indicates several key areas for future research.

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List of Abbreviations

ALL acute lymphoblastic leukaemia

AML acute myeloid leukaemia

BAL bronchoalveolar lavage

BFM Berlin-Frankfurt-Munster

CI confidence interval

CNS central nervous system

CONS coagulase-negative *Staphylococcus*

CSF cerebrospinal fluid

CT computerised tomography

CVL central venous line

EFS event free survival

ET endotracheal

FUO fever of unknown origin

HES Hospital Episode Statistics

HIE hypoxic-ischaemic encephalopathy

HLH haemophagocytic lymphohistiocytosis

HMDS Haematological Malignancy Diagnostic Service

HMRN Haematological Malignancy Research Network

HSCT haematopoietic stem cell transplant

ICD-O3 International Classification of Diseases for Oncology, 3rd Edition

IFI invasive fungal infection

IG Interim Guidelines

IMD Index of Multiple Deprivation

IQR interquartile range

IRM infection-related mortality

ISRCTN International Standard Randomised Controlled Trial Number

LBL lymphoblastic lymphoma

LRTI lower respiratory tract infection

LSOA lower super output area

MCS microscopy and culture

MDT multi-disciplinary team

MRC Medical Research Council

MRD minimal residual disease

MRIS Medical Research Information Service

MRSA Methicillin-resistant Staphylococcus aureus

N/A not applicable

NCI National Cancer Institute

NHL non-Hodgkin lymphoma

NHS National Health Service

NICE National Institute of Health and Care Excellence

NOS not otherwise specified

NPA nasopharyngeal aspirates

OR odds ratio

OS overall survival

PCR polymerase chain reaction

PET positron emission tomography

PJP *Pneumocystis jirovecii*

POSCU paediatric oncology shared care unit

RR rate ratio

RSV respiratory syncytial virus

SER slow early response

SOP standard operating procedure

TKI tyrosine kinase inhibitor

TRM treatment-related mortality

WCC white cell count

WHO World Health Organization

Chapter 1 Introduction

The outcome of childhood acute lymphoblastic leukaemia (ALL) has shown steady improvement with standard-risk groups now expecting over 90% survival at five years developed regions of the world (1–4); in these patients death is a relatively rare and devastating event. Infection has internationally been reported as the leading cause of treatment related mortality (5–10). Despite the known gravity of infection in this patient group, there is a paucity of detailed reports on microbiology (7,8,11–13). The incidence of pathogens isolated from children treated for ALL in the UK has not been described. The categorisation of patient groups more at risk of complicated infection could inform clinical practice and direct future research. Although this study cannot address all facets of the above, with this work I hoped to move some way towards gaining greater understanding about the documented infection in children with ALL during their treatment.

This chapter (Chapter 1) starts with the description of ALL; its presentation, diagnosis, risk stratification of management and outlines the treatment eras of the trials included in this thesis. The second part of the chapter presents an overview of the literature underpinning this thesis, with a particular focus on available evidence on infection predisposition and associated pathogens.

Chapter 2 describes the methodology used in this study with information on the sources of data and their analyses.

Chapter 3 presents the results; it starts with setting the scene of the study and describing the cohort of patients. It then provides an outline of the included microbiological samples through the whole treatment. Presentation of results by treatment phase follows, with detailed reports on microbiological samples in the context of treatment and patient characteristics. A final section summarises the findings with overall presentation of pathogens and their sources.

Finally, Chapter 4 discusses the findings and compares and contrasts them with other contemporaneous studies. The findings are evaluated and appraised. It also presents the limitations of this work and future directions.

1.1 Acute lymphoblastic leukaemia

ALL is an aggressive haematological malignancy with transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extra-medullary sites. It can occur at any age, although it is primarily a disease of childhood with 75% of cases occurring in patients under the age of ten years at diagnosis (Figure 1.1-1) (14).

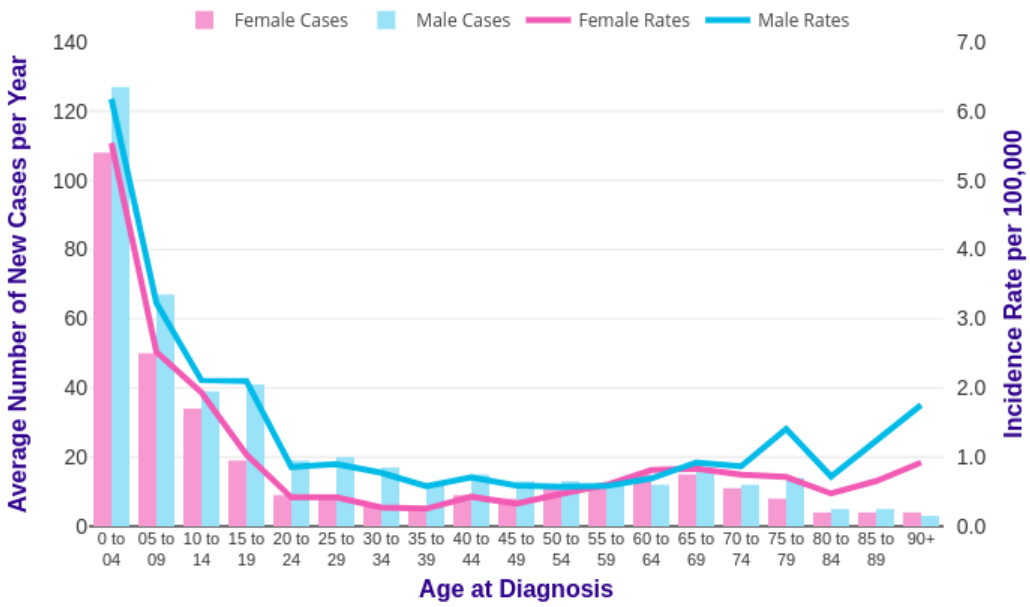

Figure 1.1-1 ALL: Average number of new cases per year and age specific incidence rates per 100,000 population, UK, 2015-2017 (15)

Around 78% of all leukaemias diagnosed in children are ALL; 85% are of B-cell lineage and 15% T-cell lineage (16). The incidence of B-ALL peaks between the ages of two to five (17,18); and it is marginally more common in males with a male to female ratio of 1.3:1 (19). The male predominance is demonstrated in Figure 1.1-1 which reflects both lineages of ALL with excess

seen in males particularly until the age of 49 years. T-ALL tends to occur at a slightly older age; the incidence in boys is four times that of girls which can explain the big difference between males and females seen in the 15-19 year old in Figure 1.1-1 (20). Only a small proportion of children (<5%) have an underlying genetic condition that predisposes to the development of ALL; these include ataxia telangiectasia, and Down syndrome which confers a 10 to 30 fold increased risk of developing ALL (21,22).

1.2 Presentation

Children with ALL present with symptoms relating to infiltration of the blasts in the bone marrow, lymphoid system and extra-medullary sites. As the volume of leukaemia blasts increases in the bone marrow, normal haematopoiesis is reduced and all haematopoietic cell lines can be affected (Figure 1.2-1). The most common presenting features of ALL are nonspecific, and making the distinction from ordinary, self-limiting diseases of childhood may be difficult. A meta-analysis of over 3,000 children from 33 studies reported that more than half of the affected children had at least one of the following five features at presentation: palpable liver, palpable spleen, pallor, fever, or bruising; 6% of children were asymptomatic at diagnosis. Common clinical findings include hepatomegaly (64%), and/or splenomegaly (61%), lymphadenopathy (50%), musculoskeletal pain (43%) and fever (60%) (23). Fever may be caused by infection or a constitutional symptom due to the disease itself. Haematological abnormalities are common; half of the patients present with anaemia and/or bleeding/bruising with around 60% having a low platelet count. The white cell count (WCC) may be low, normal, or high with half of patients presenting with a WCC of under 10 x 10^9/L and 20% with over 50 x 10^9/L. Less common manifestations include headache/other neurological findings, testicular or ocular involvement in rare cases. Central nervous system (CNS) involvement is seen in <5% of patients and is usually leptomeningeal rather than parenchymal and may present with headache, vomiting, cranial nerve involvement and/or signs of meningism.

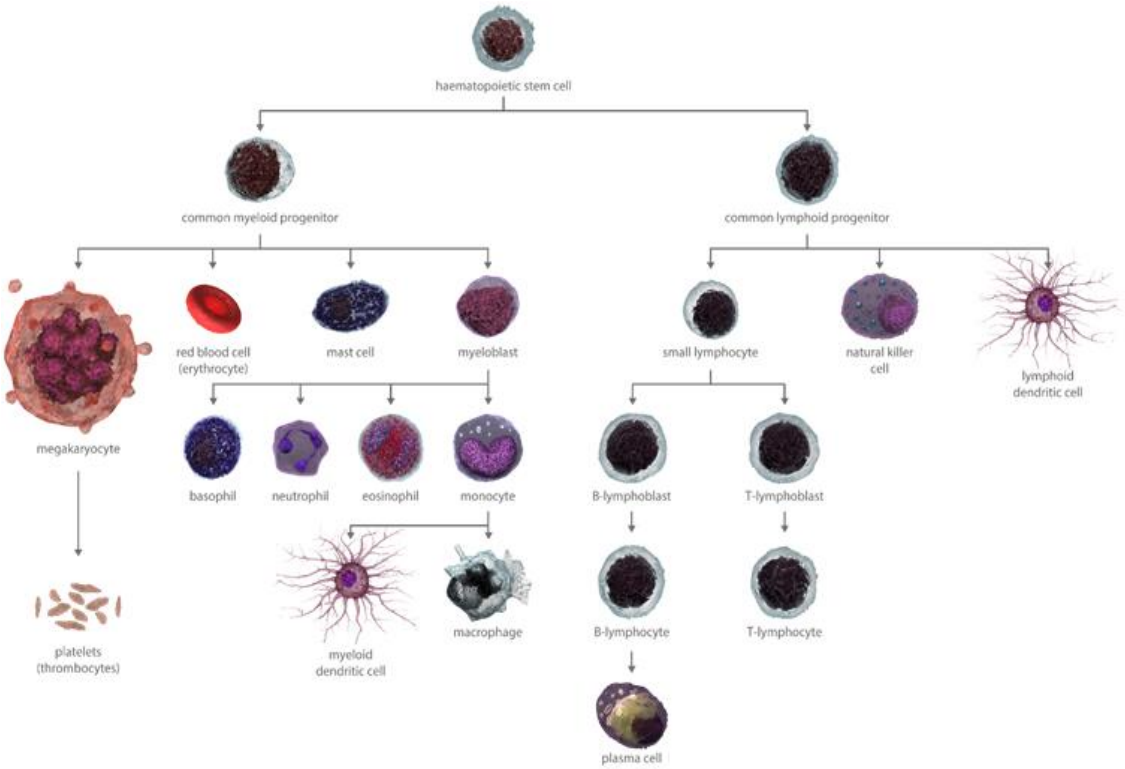

Figure 1.2-1 Normal haematopoiesis

1.3 Diagnosis

When leukaemia is first suspected, the most important initial investigations are a full blood count and blood film. Sometimes, however, the white cell count may be only slightly raised, or even low if blasts remain sequestered within the bone marrow, therefore sole reliance on the above tests for diagnosis can be misleading. Also, clearly identifiable blasts are not always present (24). Suspicious clinical context in conjunction with any abnormality of the full blood count and/or film microscopy should lead to a bone marrow aspirate which provides morphological, immunological and genetic information. In the bone marrow aspirate, most cases will have a significant infiltrate of lymphoblasts: the leukaemia cell population is usually >75%, and frequently >90% (16).

Immunophenotyping using flow cytometry identifies cell surface antigen patterns associated with particular subtypes of ALL. Precursor B-cell type is the most common, which is positive for the CD10 and CD19 cell surface

markers (25–28). Around 15% of children with ALL will have the T-cell (CD3 positive) phenotype (29). These children tend to be male and older, with more frequent presentation of higher WCC, CNS involvement and bulkier disease, including mediastinal masses. In the past, survival of children with T-ALL was inferior to children with B-ALL. However, the introduction of more intensive chemotherapy has resulted in a significant reduction in outcome differences (30,31).

Cytogenetic analysis identifies specific genetic abnormalities which have important prognostic and therapeutic implications and play a critical role in leukaemogenesis (32–35). Cytogenetic abnormalities, which are independent prognostic variables, have been incorporated in the World Health Organization (WHO) classification of ALL (Figure 1.3-1) (36); up to 70% of children diagnosed between the ages of one and ten years have good risk cytogenetics (37). Patients without specific translocations are referred to as patients with B-ALL not otherwise specified (B-ALL NOS). None of the genetic abnormalities seen in T-ALL predict outcome and are not included in treatment stratification (38).

ALL has multiple entities with distinct combinations of somatic genetic alterations which include aneuploidy (changes in chromosome number), chromosomal rearrangements, deletions, and gains of DNA and DNA sequence mutations (39,40). In 25%-30% of children with B-cell ALL, leukemic cells have high hyperdiploidy (>50 chromosomes) due to non-random chromosome gains. This subtype is associated with an excellent prognosis (41). Hypodiploidy (<44 chromosomes) occurs in 2%-3% of children with B-cell ALL and is a strong negative prognostic factor (42,43).

There are two functional classes of translocations. The first class relocates oncogenes into regulatory regions of actively transcribed genes causing dysregulated expression of an intact protein and the second juxtaposes two genes to encode a chimeric protein that functions in a different way from the proteins from which it is derived (39). An important example of this is the t(12;21)(p13;q22) translocation (ETV6-RUNX1 fusion). This translocation is

found in approximately one quarter of childhood ALL and is associated with a favourable outcome (33,44). Other important examples include the t(9;22)(q34;q11.2) translocation and chromosomal rearrangements involving the chromosome 11q23. The t(9;22) (q34;q11.2) results in formation of the Philadelphia chromosome which encodes BCR-ABL1; an activated tyrosine kinase (45). This translocation is associated with disease that is refractory to standard chemotherapy, but outcomes have improved significantly since the introduction of tyrosine kinase inhibitors (TKI) given in combination with chemotherapy (46,47). MLL gene rearrangements (involving chromosome 11q23) are the most common cytogenetic abnormalities in infant ALL and occur in 1%-2% of older children (48). Treatment outcomes of ALL with MLL gene rearrangement differ by age group, with infants having the worst outcomes (49,50). Genomic profiling and sequencing studies have identified additional subtypes of ALL, providing new insights in risk stratification of treatment. These are beyond the scope of this study, as the cytogenetic markers referred to in Chapter 3 have been outlined above and are in line with the risk stratifications used in the UKALL 2011 trial.

Finally, lumbar puncture is another essential investigation at diagnosis to identify the presence of CNS disease which may be asymptomatic. Leukaemia blast cells are identified morphologically and by flow cytometry in the cerebrospinal fluid (CSF) of some patients, mostly in the absence of neurological symptoms. Patients with CNS involvement are managed with additional specific CNS directed treatment (51).

B-cell lymphoblastic leukaemia/lymphoma, not otherwise specified (NOS)
B-cell lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
B-cell lymphoblastic leukaemia/lymphoma with hypodiploidy
B-cell lymphoblastic leukaemia/lymphoma with hyperdiploidy
B-cell lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2)[BCR-ABL1]
B-cell lymphoblastic leukaemia/lymphoma with t(v;11q23)[MLL rearranged]
B-cell lymphoblastic leukaemia/lymphoma with t(12;21)(p13;q22)[ETV6-RUNX1]
B-cell lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3)[TCF3-PBX1]
B-cell lymphoblastic leukaemia/lymphoma with t(5;14)(q31;q32)[IL3-IGH]
B-cell lymphoblastic leukaemia/lymphoma with intrachromosomal amplification of chromosome 21 (iAMP21)
B-cell lymphoblastic leukaemia/lymphoma with translocations involving tyrosine kinases or cytokine receptors (BCR-ABL1–like ALL)
T-cell lymphoblastic leukaemia/lymphomas
Early T-cell precursor lymphoblastic leukaemia

Figure 1.3-1 The 2016 revision to the WHO classification of acute lymphoblastic leukaemia

1.4 Treatment

The treatment of ALL in children is one of the success stories of modern chemotherapy. Risk-stratified treatment with combination chemotherapy, based on clinical and biologic features, has led to 5-year overall survival of around 90% in developed regions of the world (1–3).

The aim of ALL treatment is to induce and maintain disease remission. To that end, combination chemotherapy is used to kill the leukaemia cells in order that the bone marrow can return to normal haematopoiesis (17). Treatment is administered in phases of chemotherapy; induction, consolidation, interim maintenance, delayed intensification and maintenance. The treatment typically lasts for two to two-and-a-half years, apart from in the UK where the boys are treated for an extra year (three years from the start of interim

maintenance) (2,52,53). CNS directed therapy aims to prevent relapse due to sequestrated leukaemia cells in the central nervous system or cerebrospinal fluid. There are situations when allogeneic haematopoietic stem cell transplantation (HSCT) is an option for the patients at very high risk of relapse (54).

Induction comprises the first block of intensive chemotherapy; the goal being to achieve complete remission by eradicating more than 99% of the initial disease burden of leukaemia cells and allowing the bone marrow to return to normal haematopoiesis (55). For this to be achieved, highly cytotoxic combination chemotherapy is administered (56). The agents used include a steroid (prednisolone or dexamethasone), vincristine, and asparaginase and intrathecal methotrexate. High-risk patients have the addition of an anthracycline in induction.

The consolidation and interim maintenance phases are initiated when normal haematopoiesis has been restored. The goal of this part of treatment is to eradicate any remaining leukaemia cells that could cause relapse (33). Regimens used include combination chemotherapy of high-dose methotrexate with mercaptopurine and high-dose asparaginase (57,58). The risk-stratification of each individual patient dictates the intensity of their treatment, with regimen A being the least intensive and regimen C being the most intensive. Very high doses of methotrexate have been shown to improve the outcomes of patients with T-ALL (59). The use of asparaginase during the consolidation phase, and throughout the post-induction treatment, has led to improvement in survival with less morbidity (57,60,61).

Delayed intensification is a repetition of the initial induction phase of treatment, which happens a few months post remission, however for patients treated under the least intense regimen (regimen A) this is the first time they would receive an anthracycline i.e., doxorubicin. Trials have shown that intensifying treatment post remission is key to successful management of ALL (32,59,62).

The last phase, maintenance, involves mainly oral chemotherapy administered at home. During this phase, patients receive oral mercaptopurine and methotrexate with intrathecal methotrexate, with or without pulses of dexamethasone and vincristine.

1.4.1 Risk stratification and recent UK trials

Clinical, laboratory and genetic alterations combined lead to risk stratification of treatment. In the time (eras) of the UK national childhood ALL clinical trials/treatments discussed within this thesis, the independent predictors for relapse in childhood ALL were age, WCC at presentation, and immunophenotype.

At the present time, the single most important marker of prognosis is the time needed to eliminate the majority of the blasts to undetectable levels (63). Early response to treatment is predictive of the risk of relapse and is used to assign patients to subsequent risk-adapted treatment (64). Currently, the gold standard for assessment of early response to treatment is the measurement of minimal residual disease (MRD) at the end of induction. MRD refers to the number of residual leukaemia cells expressed in a percentage of normal nucleated cells in the bone marrow. This is measured at set time points in treatment. MRD has been recognised as the most powerful prognostic indicator for survival in patients with ALL, even in patients with standard-risk features at presentation (32,65,66). Patients with levels of MRD that are 0.01% or higher at the end of induction therapy (or at later time points) have a three to five times higher risk of failure of treatment and death than patients with MRD levels less than 0.01% (66,67). Intensification of therapy in these high-risk patients can improve outcomes (62,68).

UKALL 2003 (October 2003-June 2011) recruited patients with ALL aged 1-25 years (69). Risk stratification was categorised into clinical standard, intermediate, and high-risk groups based on National Cancer Institute (NCI) criteria combined with cytogenetics and early response to treatment which was assessed by bone marrow blast counts on day 8 (for NCI high-risk

patients) and day 15 (for NCI standard-risk patients) (Appendix A). MRD assessment was used for clinical standard-risk and intermediate-risk patients. Those classified as MRD low-risk were randomly assigned to receive one or two courses of delayed intensification.

The results from UKALL 2003 provided further evidence of the benefit of treatment intensification to patients defined as high-risk by MRD measured at day 29 of induction (2). UKALL 2003 also included a randomised treatment change based on MRD at day 29, with low-risk patients (undetectable MRD at the end of induction/week 11) randomly assigned to one or two courses of delayed intensification. No significant difference in event free survival (EFS) was seen between the groups, with a reduction in relapse risk resulting in a 5-year EFS of 87%, with an overall survival of 91% (2).

The subsequent UKALL 2011 trial, which is also detailed in Appendix A, was designed to improve survival and quality of survival by addressing: treatment-related mortality and morbidity, poor prognosis of CNS relapse, poor prognosis of very early marrow relapse and superior outcomes seen for young adults treated on paediatric protocols. The aim was to define whether further refinement risk stratification and treatment intensity based on MRD improved survival whilst reducing the overall treatment burden in patients aged 1-24 years diagnosed with ALL or lymphoblastic lymphoma (LBL) (T-cell non-Hodgkin Lymphoma (NHL) or Smlg-ve precursor B-NHL). At the time of diagnosis patients were stratified for treatment based on their risk of relapse. NCI defined patients with ALL as standard-risk if they were aged between 1-9 years at diagnosis, and had an initial WCC < 50 x 10^9/L (70,71). Around 60% of children with ALL fall into this group. Patients were classed as high-risk when they were aged ≥10 years and/or had WCC ≥ 50 x 10^9/L at diagnosis. Patients with T-ALL were also classed as high-risk since in the past, survival of children with T-ALL was inferior to children with B-ALL.

Cytogenetic markers were also used to guide risk stratification. The UKALL 2011 trial classified t(12;21) and high hyperdiploidy as good-risk cytogenetic markers (53). Poor-risk markers were: near haploid (24-30 chromosomes) and

low hypodiploidy (31-39 chromosomes), MLL rearrangement (and iAMP21 and t(17;19)- not described in our cohort).

All patients with T-ALL, or LBL, received a four-drug (regimen B) induction, as did patients with known high-risk cytogenetics at the start of treatment. All patients with Down syndrome received regimen A induction. In patients with CNS disease at diagnosis additional weekly intrathecal methotrexate was used until two consecutive clear samples of CSF were obtained. If CSF was clear by day 29 they continued with NCI and MRD directed therapy. In the case of persistence of CNS disease they were transferred to regimen C with MRD measured at week 14. Those that remained at high risk were taken off protocol, as were patients who failed to respond adequately to induction therapy (≥25% of blasts at day 29 or T-ALL with MRD >10%). Post induction treatment was determined by MRD in ALL, or tumour volume assessment in LBL (52).

There were originally two randomisations within UKALL 2011, the first in induction and the second in interim maintenance. The objective of the first randomisation was to reduce toxicity through the introduction of a short (14 day) course of high-dose (10mg/m^2/day) dexamethasone, as opposed to the standard 28 days of 6mg/m^2/day. Recruitment to this randomisation stopped while the trial was open (March 2017) as a futility analysis demonstrated that the trial was underpowered to show a significant improvement in reducing adverse events with short dexamethasone (52).

The second randomisation in UKALL 2011 was at the start of interim maintenance and investigated the effect on CNS relapse and quality of life in patients receiving either high-dose methotrexate without prolonged intrathecal therapy or the standard UK CNS-directed ALL therapy at the time with protracted intrathecal therapy. It also aimed to assess the effect on relapse risk and quality of life in patients receiving monthly pulses of vincristine and dexamethasone during maintenance. The methotrexate and pulses randomisation split patients into four groups, with patients being randomised to receive either high-dose methotrexate or standard interim maintenance

followed by a single delayed intensification and either maintenance with pulses or without pulses of vincristine and dexamethasone. If a patient was randomised to high-dose methotrexate therapy, they would not have subsequent intrathecal methotrexate in maintenance, but could be randomised to either pulses or no pulses. If they were randomised to standard interim maintenance within their allocated regimen, they were randomised to maintenance therapy with or without pulses, and all patients received intrathecal methotrexate. All patients in UKALL 2011 had one delayed intensification course, and intensive treatment was limited to those who were not MRD low-risk. Treatment lasted two years from the start of interim maintenance for female patients, and three years from the start of interim maintenance for male patients.

Full details of the UKALL 2003 and UKALL 2011 protocols and chemotherapeutic agents are provided in Appendix A.

1.5 Infection-related complications

In developed regions of the world, in the 10% of children who do not survive to five years, mortality tends to be driven by refractory/relapsed disease and treatment-related toxicity (72). Treatment-related morbidity and mortality in children with ALL remains unacceptably high, despite the impressive cure rates. The incidence of treatment-related mortality (TRM) in recent childhood ALL trials is reported to be between 2-4% (5,9,10). The leading cause of TRM is infection; infection-related mortality (IRM) between 1.7% and 2.3% has been reported in developed regions of the world (5–9). In the UK, the UKALL 2003 trial reported 117 treatment-related deaths out of 3,126 participants. Of these deaths, 64.1% (75/117) were attributed to infection, constituting 2.4% of all included patients (10).

Infection is not only the leading cause of TRM, but also the leading cause of treatment-related morbidity/complications, including permanent end-organ damage, contribution to chemotherapy delays and modifications, and increased exposure to antibiotics (59,73–75). More than half of children with

haematological malignancies will have at least one episode of documented infection during treatment (76). Most of the episodes of fever with suspected or documented infection, particularly at time of neutropenia, have historically led to long admissions for administration of intravenous antimicrobials. During these admissions, patients may suffer adverse effects, including antimicrobial toxicity, prolonged hospital stays, nosocomial infections, along with potential psychologic impact on the patients and financial burden to the health care system (77). The burden of recurrent exposure to broad spectrum antibiotics and repeated access to central venous lines (CVLs) increases vulnerability to additional typical and atypical infections further. Furthermore, protracted empiric and therapeutic use of broad-spectrum antibiotics may contribute to the evolution of resistant microbiologic flora and fungal superinfections. Once infections have been contracted, they can deteriorate into overwhelming infectious events that can lead to associated morbidity, along with interruptions of planned chemotherapy which can in turn increase the risk of relapse.

1.6 Review of infection in children with ALL

Infections are a major cause of morbidity and mortality in children being treated for ALL. However, limited information is available on infectious complications, since published data tend to be from studies restricted to relatively small series from single institutions and/or mostly including patients enrolled in trials. There have only been two reports to date on infections during the entirety of treatment in ALL, but unfortunately viral respiratory pathogen information is lacking (8,11). No reports have been published in the UK at the time of this thesis. Most reports have been retrospective reviews of infections in patients treated under trials and in the few instances of prospective studies, the focus has been on specific system symptomatology without exhaustive information on pathogens (7,8,12,78,79). There is a complete lack of evidence on the delays relating to infection in the treatment of children with ALL. Furthermore, the types of infections and the microorganisms isolated from different sample sites have not been described at length. The published studies to date are outlined below in chronological order.

Comprising a total of 245 infections in 59 children with ALL, the first series describing infection in children with ALL was published in the late 1990s (80). Half of the infections happened during neutropenia and 54% were microbiologically documented. High-risk patients had more bacteraemia and fever of unknown origin (FUO) than standard-risk patients. In patients with bacteraemia, there was an equal split in the Gram +ve and Gram -ve bacteria isolated from blood cultures.

In 2001, Lex and colleagues looked at infectious complications in 120 children with ALL treated in a single hospital under the COALL-05-92 trial (1992-1998) (78). Most febrile episodes in both standard- and high-risk patients occurred after treatment courses with high-dose cytarabine. Although there is mention of bacterial, fungal and viral pathogens, these were not specified nor were the sites of sampling stated, other than blood. In this series, 60% of febrile episodes were associated with clinically/microbiologically documented infection. Gram +ve pathogens had a much higher incidence than Gram -ve. Standard-risk patients at diagnosis suffered from fever significantly more frequently than high-risk patients, which the authors attributed to very low initial leukocyte counts, as well as other intrinsic factors predisposing to infection. No correlations between febrile episodes and high-risk leukaemia subtypes were found. Some patients were, however, noted to be more likely to suffer from infections than others with the same disease burden, and the authors hypothesized that individual predisposition could be contributing to the occurrence of infection.

Individual factors were also discussed in the study of Graubner et al who investigated infectious complications in 293 children with ALL (7). Contrary to the results of Rahiala et al (80) and Lex et al. (78), most infectious episodes (71%) were FUO, with only 15% microbiologically documented. The most frequent clinically documented infections were respiratory tract infections. In the microbiologically documented infections, Gram +ve bacteraemias were the most common, especially with coagulase-negative *Staphylococcus* (CONS) and viridans group streptococci. Their finding of low incidence (15%) of infectious episodes in induction was surprising, and contrasts with other

studies. Most infections were found in the intensification (48%) and re-induction (33%) phases. Chemotherapy dose-reduction resulted in a significant reduction in infectious complications. This was also reported by the Dutch Childhood Oncology Group, with reduced intensification/maintenance chemotherapy given to the good-risk childhood ALL group, resulting in a major decrease in infectious morbidity (79). The main contributors to reduced infectious morbidity were thought to be: shorter neutropenic episodes (which are known to be a major risk factor for infections), less frequently accessed CVLs, and less severely affected memory B-cells in the less intensively treated patients (81,82).

In another retrospective study among children being treated for ALL in Greece, most of the severe infections were seen during induction; but numerically most infections happened in maintenance (347/610), followed by induction (124/610) (11). The most common infection site was the respiratory tract, with most bacteraemias (75%) occurring during periods of neutropenia, and just over half of the bacteraemias happening during induction. Contrary to other contemporaneous studies, Gram -ve pathogens represented 50% of bacteria, with the most commonly isolated bacteria being *Staphylococcus* species (Gram +ve) and *Escherichia coli* (*E. coli;* Gram –ve) (81).

Febrile events during treatment for childhood leukaemia were reviewed in 252 patients treated across 16 year in a single centre in Taiwan (12). Over half of the microbiologically documented infections were at the time of neutropenia. In agreement with other studies, the respiratory tract was the most common site of infection. Younger patients (diagnosed under the age of ten years) and patients with higher-risk disease were more likely to have fever and microbiologically documented infections. Similarly, the findings of Inaba *et al*. identified young age (diagnosed under the age of ten years), intensive chemotherapy and lack of neutrophil surge after dexamethasone as factors associated with infection-related complications (8). This is the largest series from a single centre published to date, including 2,420 infection episodes in 409 children. Upper respiratory tract was the most common site of infection

(n=389), and bloodstream infections were mostly seen during induction. Female sex was associated with longer duration of neutropenia and higher frequencies of documented infections and bloodstream infections. There have been other reports suggestive of higher infection risk and higher TRM in female compared to male patients (5,10). It has been speculated that there are gender differences in both the immunologic response to infection and the toxicity post chemotherapy (83).

The largest series to date includes 1,363 children treated for ALL nationally in Poland 2012 to 2017 (13). Just over half of patients were reported to have at least one microbiologically documented infection during treatment. There was a Gram +ve predominance (60%) in the bacteraemias; the most commonly isolated microorganisms were *Staphylococcus* species (45.8%) followed by *E. coli* (17.1%). Most infections were bloodstream infections, in contrast to other studies. Similar microorganisms were also most commonly isolated in a large study in Turkey; staphylococci represented the majority of isolates (38.7%), followed by *E. coli* (12.9%) (84).

1.7 Contributors to infection in children with ALL

Many reasons have been identified as potential causes of increased infection susceptibility in children with ALL. These patients, most of whom are under the age of five years at diagnosis, have an immature immune system which is additionally burdened by an underperforming bone marrow due to disease infiltration. They are then exposed to intensive high-dose combination chemotherapy with steroids with further effects on myelo- and immuno-suppression on all aspects of their immunity - innate, adaptive, and physiological barriers.

- **Neutropenia**

The most prominent effect of chemotherapy on immunity is neutropenia, which makes patients susceptible to bacterial infection (85). A period of severe bone marrow suppression is required for successful treatment of acute leukaemia.

However, neutropenia coupled with a compromised host response makes patients vulnerable to infections from bacterial and other pathogens.

It has been known for decades that the duration of chemotherapy-induced neutropenia is regarded as the most important risk factor for infections in paediatric oncology patients (86,87), and that neutropenia is the most important contributing factor predisposing to infectious complications (8,78,80,88–97). The most frequent infection-related complication is febrile neutropenia, and is the commonest cause of unplanned admissions which often has a complicated and potentially lethal course (98,99). This significant burden of morbidity led to the creation of national guidelines on the management of febrile neutropenia, published by the National Institute for Health and Care Excellence (NICE) (100). Key recommendations include delivery of a beta-lactam monotherapy (e.g., piperacillin/tazobactam) within one hour of presenting with a temperature of ≥38°C and confirmed or suspected neutrophil count of ≤0.5 x 10^9/L. The mainstay of management of febrile neutropenia in the UK is, therefore, immediate admission to hospital and administration of intravenous broad-spectrum antibiotics in order to avoid potentially life-threatening complications. This can lead to prolonged hospital admissions as current practice is to continue with antibiotics at least until the blood cultures are reported negative, the patient is well and afebrile for at least 24 hours. This approach is the safest but has significant impact on the quality of life of children being treated for cancer. It is also not indicated in the majority of cases, as only 20-30% of patients with febrile neutropenia have a serious bacterial infection (101,102). Other causes of fever include viral or fungal infections, mucositis, drug effects or malignancy. However, all patients are treated in the same way, so recent studies have tended to focus on identifying patients who genuinely need antibiotics, as those at low risk of severe bacterial infections can be safely treated with upfront, or early step-down to, oral antibiotics (NICE guidance) (100,103–105). The stratification of management of febrile neutropenia entails a reliable upfront risk-stratification score with the potential complimentary use of biomarkers to identify the point in each episode at which intravenous antibiotics could be stopped and

discharge from hospital could be considered (104,106). These are exciting ongoing research focus points.

Fever, however, persists despite broad-spectrum antibiotic therapy in up to 40% of febrile neutropenia cases (93,107), and this group of patients remains challenging, not only due to prolonged and potentially complicated hospital stays but also due to the high risk of infection-related mortality. These patients are treated with the addition of empirical antifungal agents. In patients at increased risk for infection, treatment is started in the absence of diagnostic evidence when invasive fungal diseases are suspected clinically.

Persistent neutropenia can also postpone further chemotherapy administration, thus delaying planned treatment. Indeed, recurrent neutropenia can lead to modification of treatment intensity, thus making maximum tolerated treatment potentially inefficient. Chemotherapy has not only quantitative but also qualitative effects on the neutrophils. Impaired oxidative burst of neutrophils has been considered as a potential risk factor for infections in children with ALL, especially at the beginning of treatment (108).

- **Treatment intensity, intensive treatment phases and certain chemotherapeutic agents**

Severe myelosuppression has been seen in patients who receive multi-agent combination chemotherapy with higher dose-intensity predisposing to more (12,109) associated with infection-related complications (8,91,110,111). Moderate reduction of chemotherapy intensity may significantly reduce the rate of infectious episodes in children undergoing treatment for ALL (7,79).

Higher dose intensity in high-risk patients compared to standard-risk is associated with higher infection risk, with more infections occurring in intensive phases in each patient’s treatment. Documented infections, especially in the bloodstream, were found to be more common during intensive treatment phases, such as induction, consolidation and delayed

intensification; intensive chemotherapy increasing the frequency and duration of neutropenia (8). Two studies reported higher incidence of infectious complications in high-risk patients compared to low-risk patients during consolidation (7,11).

Infection, suspected and confirmed, is common during induction; with 30%-74% of children having at least one febrile episode (7,11,78,112,113). Infectious complications and IRM are also more frequent during induction (10). The majority of serious infections happen at the initial part of treatment (12,109). During induction, patients tend to be neutropenic and immunosuppressed and not in a consolidated leukaemia remission (88,94). In this context, the predominance of episodes of bacteraemia during induction is not surprising, as it is an intensive part of treatment (11,80,94). Fungal infections are also more common during induction, and are a significant cause of IRM (114). Younger patients, females, those with Down syndrome, and those with severe neutropenia are all at increased risk of infection-related complications during induction (5,8,83,113).

The agents most commonly associated with myelosuppression are cyclophosphamide, anthracyclines and cytarabine. Cyclophosphamide causes bone marrow suppression, especially when used in high doses (115). It is used during consolidation and delayed intensification phases of treatment of childhood ALL in the UK protocols (2,52). A higher incidence of febrile episodes was noted after high-dose cytarabine and asparaginase caused prolonged leukopenia in a retrospective review of infectious complications in children with ALL (78). Bloodstream infections were seen mostly in phases where patients received high-dose cytarabine therapy (8). Methotrexate (usually high-dose) causes mucositis and therefore increases the risk of infection (116,117). In the UK, dexamethasone is used during induction, interim maintenance and again as pulses with vincristine during maintenance. Dexamethasone can induce marrow release of granulocytes, reduce their recruitment into tissue, delay their apoptosis, and promote de-margination of granulocytes (118). It has been reported to be highly immunosuppressive due to toxicity against lymphocytes (119). Inaba et al. reported significantly

increased risk of infection in the absence of granulocyte surge after the administration of dexamethasone (8).

- **Age**

ALL studies consistently report that younger patients are more at risk of infection than older patients (diagnosed under ten years in some studies, others under five, and in one under 2.5 years) (8,12,112,120,121). This could be due to an immature immune system, as younger children may have encountered fewer infections prior to their diagnosis and have a naive immune system. Other contributing factors could include poorer understanding of personal hygiene in this group and the more frequent use of percutaneously sited lines (with external tubing) compared to implantable CVLs. Patients diagnosed under the age of ten years have been reported to have significantly longer duration of neutropenia in all phases compared to those diagnosed over the age of ten (8).

- **Sex**

Females have been found to be more at risk of infection-related complications and infection-related mortality, despite the slight male predominance of the disease overall (5,10,121). This may be due to a combination of factors. For instance, it has also been speculated that there are gender differences in both the immunologic response to infection and the toxicity post chemotherapy (83). There are reports of longer and more profound neutropenia in females compared to males, particularly after prolonged administration of steroids (5,8,10).

- **Down syndrome**

Children with Down syndrome are at increased risk of infection and infection-related mortality throughout all treatment phases (10). IRM in patients with Down syndrome is higher not only during induction, but also during the less intensive maintenance part of treatment. This suggests that these patients

would potentially benefit from increased supportive care with additional antibiotic prophylaxis. In the UK, treatment intensity was reduced in these patients (after July 2009) due to excess TRM; patients with Down syndrome were treated as standard-risk patients with increased supportive care measures including prophylactic antibiotic therapy (10,122).

- **Central venous lines**

Indwelling CVLs are a constant infection risk, as skin flora can colonise the lumens and lead to recurrent growth from blood cultures taken from the line. Implantation of a foreign material into a vessel increases the risk of line-related infections (109,123,124). The ability of certain microorganisms to produce a biofilm, and the inability of many antimicrobial agents to penetrate the biofilm makes line related infections difficult to eradicate without the removal of the infected CVL (125). It has been shown that fewer infection-related complications are seen with implantable CVLs compared to those with external tubing (126). A common route of infection in percutaneous CVLs is the migration of colonising microorganisms from the skin at the insertion site into the cutaneous line tract eventually reaching the tip of the line. In the context of intensive chemotherapy causing mucositis, the gut and skin can be colonised with microorganisms which can lead to CVL infection. When the hub of the line becomes contaminated it can cause intraluminal colonisation of the long-standing CVL. Finally, occasionally lines can be seeded with microorganisms that migrate from other locations of infection or contamination at the time of access. The spectrum of clinical significance in CVL related infections range from colonisation of the line, to CVL related local infection, to more severe complications including bacteraemia and septicaemia.

- **Loss of barrier integrity**

Mucositis is the inflammation and ulceration of the mucus membranes lining the digestive tract from the mouth to the anus. Lesions develop as a result of chemotherapeutic agents (particularly S-phase agents including cytarabine, methotrexate, and daunorubicin) attacking the rapidly dividing cells of the

gastrointestinal tract. Severity can range from mild painless tissue changes to widespread ulceration. Mucosal damage can act as a portal for normal gut flora to reach the circulation, therefore increasing the risk of infection (127,128). Patients who develop oral mucositis are particularly at risk of infection with viridans group streptococci (129).

- **Nutritional status**

Another contributing factor to the level of immunodeficiency in children with ALL is malnutrition (130). Underweight patients have longer duration of neutropenia and their immune responses can be further impaired by decreased production of immunoglobulins, complement and cytokines due to malnutrition.

1.8 Pathogens

- **Bacterial bloodstream infections**

As outlined above, bacteraemias are a common complication in children with ALL during their treatment; they are the second most common type of infection after respiratory tract infections, and range from simple febrile episodes to potentially life-threatening septic events. Most microbiologically documented infections in immunocompromised patients, particularly at times of neutropenia, arise from the patient's endogenous micro-flora, with only a small proportion being acquired from exogenous sources or as a result of environmental exposure (101,131). As such, it is often possible to anticipate the potential aetiology of an infection depending on the site where the infection started. For example infections with a cutaneous origin are likely to be caused by Staphylococcus species and other microorganisms that colonise the skin (*Bacillus* species, *Corynebacterium* species, *Candida* species). Patients with severe oral mucositis may have infections caused by viridans group streptococci, whereas those with significant intestinal mucositis may have Gram -ve and enterococcal infections (131).

A predominance of Gram +ve bacteria has been consistent in studies of children with cancer (13,78,80,95,132). This is probably the result of the widespread use of CVLs, antimicrobial prophylaxis directed primarily against Gram -ve bacilli and the frequent use of chemotherapeutic agents that can cause significant oral mucositis (133,134). Gram +ve bacteria account for 33%-81% of bloodstream infections in children with ALL (7,8,12,78,91,121). The most common Gram +ve bacteria are CONS, *Staphylococcus aureus*, and viridans group streptococci. Around half (30%-50%) of Gram +ve bloodstream infections are caused by CONS; the most common microorganism isolated from CVL associated bloodstream infections. CONS can form a bacterial biofilm on the material of the line inhibiting complete bacterial eradication with antibiotics resulting in increased risk of re-infection (8,11,80,135). CONS has low virulence and seldom causes life-threatening infections even in severely neutropenic patients. CVL related infections with CONS can often be treated with antibiotics alone, although line removal may be required if the infection recurs (136).

S. aureus is the second most commonly isolated Gram +ve species from patients with neutropenia (131). S. aureus if more virulent than other staphylococci and can cause significant morbidity and mortality including endocarditis and deep seated abscesses. Unlike CONS, CVL related infection with S. aureus almost always requires line removal (136). Of particular concern are methicillin resistant strains (MRSA) which are increasing in frequency depending on geographical and institutional factors (136).

Viridans group streptococci are major components of oral micro-flora and are also common pathogens in children with acute leukaemias, causing up to 30% of infections (77,91,137). Patients particularly at risk are those receiving high-dose chemotherapy causing severe mucosal damage which can facilitate entry of these pathogens into the bloodstream. They can cause life threatening infections including streptococcal toxic shock syndrome. They can also be associated with antimicrobial resistance.

The gastrointestinal tract is an important source of infection in patients with neutropenia, with most pathogens being enteric Gram -ve bacilli. Gram -ve organisms, although less frequent in recent years, are a major cause of morbidity for children with leukaemia. The proportion of microbiologically confirmed cases of invasive infections with Gram -ve pathogens varies widely between 18%-67% (76). Gram -ve pathogens are more often associated with severe sepsis, and infection-related mortality rates may be as high as 30% (93,138,139). In UKALL 2003, IRM secondary to bacteria that were Gram-ve was reported to be more common than those that were Gram +ve; it is therefore of paramount importance that empirical antibiotics used in treatment of infection in children with ALL have adequate efficacy against Gram -ve bacteria (10). The most frequently isolated Gram -ve pathogens in most large series are *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumoniae* (5,138,140). Despite the overall decline in frequency of Gram -ve infections, some pathogens are seen more frequently in recent years; these include *P. aeruginosa*, non-aeruginosa pseudomonas, *Acinetobacter* species and *Stenotrophomonas maltophilia* (131,141). Gram -ve microorganisms are more commonly isolated from percutaneously inserted CVL compared to surgically implanted ones (126). *Pseudomonas aeruginosa* is the most important pathogen and causes between 15%-20% of Gram -ve infections (140). It can develop resistance to antibiotics and be difficult to treat and eradicate (142). *Stenotrophomonas maltophilia* causing colonisation and/or infection has also been reported in children with haematological malignancies (143).

- **Respiratory viruses**

As outlined above, in common with all young children, the most frequent site of infection in children with ALL is the respiratory tract (7,8,11,12). Respiratory viruses are the most commonly isolated pathogens at times of respiratory tract infection signs and can present with a wide range of manifestations – from mild “common cold” symptoms to progression to lower respiratory tract infection and increased hospital admission rates (144,145). There is, however, a paucity of published evidence on the role of respiratory viral infections during treatment for ALL (144,145).

In UKALL 2003, 5/75 infection-related deaths were at the time of a respiratory viral isolate; adenovirus was found in three cases and respiratory syncytial virus (RSV) in two cases (10). Indeed, the realisation that respiratory viruses are common, and can have an unpredictable course in the immunocompromised host, has led to recent interest in investigating the gravity of respiratory viral infections in children treated for ALL (144,145). This is in line with new attempts to stratify the management of febrile neutropenia in order to reduce hospital admission. Children with ALL who develop febrile neutropenia are invariably admitted to hospital for broad spectrum antibiotics to cover the possibility of bacterial infection. Often, however, the only isolated microbiological cause in these episodes is a respiratory virus.

A prospective multi-centre study in Finland looked at the incidence of respiratory viruses in febrile children being treated for leukaemia. Of the 138 febrile episodes, 44% coincided with a respiratory viral isolate. Rhinovirus was the most commonly isolated pathogen (22%), followed by RSV (11%) and influenza A (4%) (145). Similarly, Hakim et al. reported that 43% of respiratory infection episodes in children with ALL were viral (144); influenza was the most commonly isolated virus, followed by RSV (their assay did not include rhinovirus). In patients with a viral respiratory tract infection, 61% were hospitalised with 26% experiencing a complicated course, 80% had their chemotherapy delayed and 0.7% died. These serious complications, associated with respiratory viral infections, are proof of the urgent need to analyse which factors make respiratory infections more likely to happen in the first place, potentially facilitating the development of serious adverse events.

Overall, RSV is the virus most frequently associated with lower respiratory tract infection (LRTI), which can have a complicated course and with high incidence of bacterial superinfection (146,147). Metapneumovirus can have a similar phenotype to RSV (148–150). Children with ALL are also more susceptible to influenza infection; it can have a more complicated course with prolonged symptoms and viral shedding, long hospital admissions and potential chemotherapy delays (151). Rhinovirus has been shown to have a high viral load that can persist for a while in the respiratory tract; it is therefore

commonly isolated in repeat samples from the same patients. Furthermore, there has been some evidence to suggest that viral load is correlated to severity of symptoms; in immunocompromised hosts it can be isolated in low viral load and may represent a “bystander” virus which may be repeatedly isolated in patients who have contracted it (152). Although acute respiratory infections with rhinovirus often have a self-limiting course in healthy people, in patients with haematological malignancies it can cause severe complications including pneumonia (153). The persistent shedding of rhinovirus could be due to inefficient immunological control of a single infectious episode (154,155).

- **Fungal infections**

Invasive fungal infections (IFI), although rare and difficult to diagnose, remain the most challenging infections to treat, diagnose and prevent in children with ALL (156,157) . Risk factors for IFI in children with ALL include prolonged and profound neutropenia, prolonged corticosteroid use, presence of CVL, use of broad-spectrum antibiotics, significant colonisation and environmental exposure in the non-hospital setting (158).

IFIs are more common in high-risk patients and are more frequently seen during induction (8,159). Although IFIs are associated with significant morbidity and mortality, few paediatric studies have explored their pattern during ALL treatment phases, many of which combine data from patients with non-lymphoblastic leukaemia and HSCT (160–164). These studies have reported a variable incidence of IFI in children with ALL, which ranges between 4% and 35% depending on geography, treatment era, chemotherapy protocol, risk category, and prophylaxis regimen. Increased incidence of IFI has been found in high-risk patients and during intensive phases of treatment of induction, consolidation and delayed intensification (8,164). In a recent European study, the majority of proven and probable IFIs occurred during induction, with an overall attributable mortality of 13% (165). This was confirmed in a large multi-centre study in Australia, which reported a higher

prevalence of IFI in children treated on high-risk chemotherapy protocols (159).

The landscape of fungal pathogens is constantly changing. *Candida* species are the most common, but *Aspergillus* species and other less common fungal pathogens are becoming more prevalent; the proportion of non-albicans candidaemia is increasing and non-Aspergillus moulds are emerging, which may have significant implications for prophylactic and empiric management (163). *Candida* species commonly present in the gastrointestinal tract, may produce invasive infections in the immune compromised host when mucosal barrier is disrupted, or normal gastrointestinal flora is abrogated by antimicrobial treatment (166). Repeated exposure to broad-spectrum antibiotics may lead to clearance of bacterial pathogens from a patient's microbiome with subsequent susceptibility to *Candida* infection. *Candida* may also colonise and form a biofilm in CVLs which can make antifungal treatment ineffective and cause recurrence of infection when antifungal agents are stopped (167,168). CVL removal for bloodstream infections associated with Candida is recommended (169–171).

Fungal pathogens are generally difficult to isolate, as many are angio-invasive, and are best diagnosed by isolating the microorganism from deep tissue samples. As invasive procedures are difficult in unwell patients and carry risks, indirect tests have been developed for the diagnosis of fungal infection. Detection of fungal antigens in body fluids, including Cryptococcus, Aspergillus and Candida antigens, can be clinically useful. β-D-glucan is an antigen found in a broad range of fungal microorganisms, including *Aspergillus* and *Candida* species (172,173).

In the infection related deaths reported from UKALL 2003, 12/75 were related to fungal infections; *Aspergillus* species were the most commonly isolated (8/12), followed by *Candida* species (3/12) (10). So, although fungal infections are rare, they can cause significant morbidity and mortality. Therefore, vigilance is required in early suspicion and timely initiation of diagnostic investigations and of empirical antifungal treatment.

1.9 Summary

Children treated for ALL are vulnerable to infection, which can complicate their course of treatment in many, often unpredictable, ways. There is a paucity of published data with which to characterise patients more at risk of presenting with either suspected or microbiologically documented infections. With 5-year survival at 90%, half of the children who die of ALL die secondary to treatment toxicity, of which the major cause is infection. Importantly, such deaths may be potentially preventable through identifying the characteristics of patients at increased risk of infection and optimisation of supportive care.

Detailed information on the frequency and causal pathogens of infection is needed for the risk of serious infections to be identified and lowered. Furthermore, it has become clear that some children are burdened with recurrent infections during their treatment, whereas others go through treatment with fewer complications. The reasons for this are still unknown. Hence, it is of importance to analyse which factors make children prone to infection and why some seem to suffer more than others.

Chapter 2 Methods

2.1 Aims and objectives

It is well known that infections are the most common complications of ALL chemotherapy and are the leading cause of infection-related mortality. However, comprehensive reports on the microbiological findings in children being treated for ALL are limited. Accordingly, with the aim of addressing this deficit and informing clinical practice, this research describes the microbiological samples taken from 267 children (aged 1-17 years) treated for B- or T-ALL in the UK over a period of nearly 13 years (September 2004-February 2017).

The primary objective of this research is to describe the prevalence and characteristics of microbiologically documented infections in children with ALL and to examine the relationships between infection during different phases of treatment and demographic, disease-related and treatment-related variables.

The analyses focus on all included microbiological samples taken during treatment for ALL. The samples are analysed per treatment phase, and demographic and treatment-related patient factors are examined. In the case of positive samples, information on the identification of common pathogens per sample type and site of isolation is presented.

As evident in the introductory chapters, there is paucity of population-based data on infections seen in children with ALL across all treatment phases. The importance of infections in these patients and their impact on morbidity and mortality has been previously outlined. This study addresses the problem through a combination of epidemiological methods and clinical knowledge of paediatric haematology.

2.2 Study population

The study population was sourced from the Haematological Malignancy Research Network's (HMRN) population-based patient cohort, which was established by National Health Service (NHS) clinicians and York university researchers in April 2004. Predicated on NHS infrastructures, full details of HMRN's methods and ethical approvals have been published (174,175), and are available on the study's website (www.hmrn.org). Briefly, covering a catchment population of around 4 million, patient care with the HMRN region is provided by 14 hospitals organised into five adult multi-disciplinary teams (MDTs) and a network-wide paediatric haematology and oncology service (Figure 2.2-1).

As a matter of policy, within HMRN all haematological cancers and related disorders (whether originating in the NHS or private sector, and irrespective of age, prognosis, trial entry, or treatment intent) are diagnosed and coded using the clinically meaningful most up-to-date WHO International Diseases for Oncology Classification (currently ICD-O3, 176) by haemato-pathologists working at the Haematological Malignancy Diagnostic Service (www.hmds.info) in Leeds; a single integrated haemato-pathology laboratory which provides the national model for complex diagnostic services (177).

Patients enter HMRN's cohort on the day they are diagnosed. Around seven months after the initial diagnosis, research nurses (employed by the University of York) extract a core clinical dataset from patient medical records (paper and electronic). Information collected includes blood test results, performance scores, scans (e.g., positron emission tomography (PET), computerised tomography (CT), etc) and patient-reported symptoms (Appendix C). All treatment, management and response data are collected (e.g., observation, initial and subsequent chemotherapy and radiotherapy, stem cell transplant, supportive and palliative care). Additional data linkages and abstractions are triggered either by changes in state (e.g. death, disease progression, relapse, treatment initiation) or subtype-specific data updates.

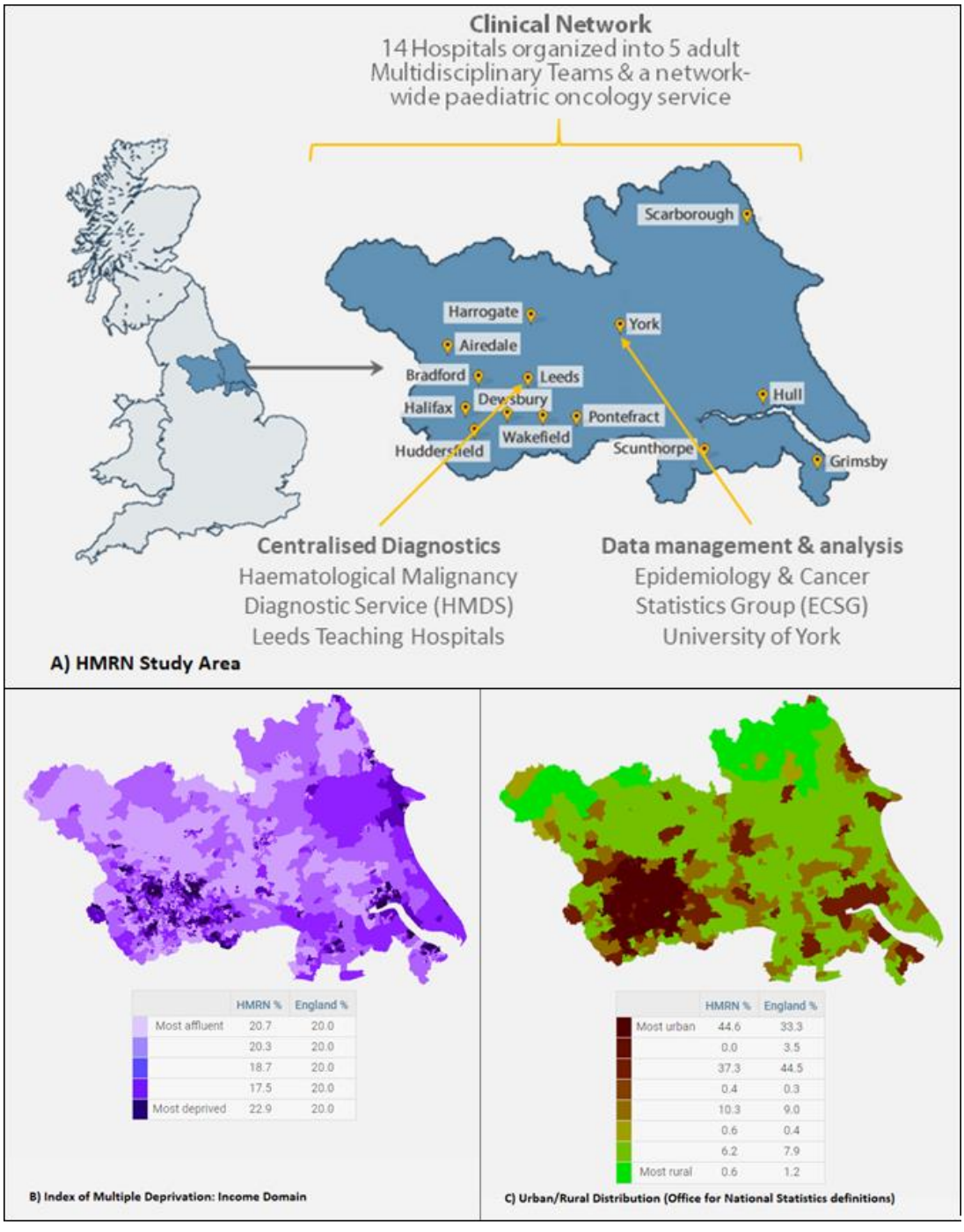

Figure 2.2-1 HMRN Study Area and Population Demographics

HMRN's cohort has Section 251 support under the NHS Act 2006 enabling it to operate in a similar way to a cancer registry: ethics approval (IRAS 289074) from Leeds West Ethics Committee, R&D approval from each NHS Trust and exemption from Section 251 of the Health & Social Care Act (CAG 20/CAG/0149). Hence, all patients diagnosed within the catchment are registered in the database, and their care pathways tracked until death, regardless of consent; and all are 'flagged' nationally for death and cancer by the Medical Research Information Service (MRIS) and linked by NHS Digital to nationwide health administrative databases. Deaths are notified monthly with information available on the causes reported in the death certificate, and linkages to cancer registrations and inpatient and outpatient Hospital Episode Statistics (HES) annually.

Within HMRN, populations and area-based measures of urban/rural status and deprivation are routinely obtained from the UK census and other national data sources (Figure 2.2-1). Based on the lower super output area (LSOA) of residence at the time of diagnosis, patients are assigned a measure of area-based deprivation, the index of multiple deprivation (IMD:income-domain) (178). The IMD can comprise information from seven domains (income, employment, health, education/skills/training, barriers to housing/services, crime, living environment) (179). HMRN uses the IMD:income-domain as relative measure of deprivation, in common with most other health researchers (174,175,178,180–183); distributing the IMD:income-domain scores of LSOAs between quintiles, with group one representing the most affluent areas and five representing the most deprived areas. Driven largely by small numbers of events, for summary purposes in this thesis, IMD data are divided in the tables into high (quintiles 1 to 3) and low (quintiles 4 and 5).

Children diagnosed with ALL in the HMRN area receive their intensive chemotherapy treatment in Leeds Children's Hospital; all patients are managed during the first four phases of treatment by this single regional paediatric haematology/oncology centre. There are a few paediatric oncology shared care units (POSCU) that provide supportive care and maintenance chemotherapy in an outpatient setting, including Hull, Calderdale, Pinderfields

and Scarborough hospitals. Patients who are geographically closer to those centres who present with episodes of suspected infections are treated there, with regular input from the tertiary centre in Leeds when the episodes of infection are uncomplicated. Patients with documented infections and/or complicated courses are transferred to Leeds Children's Hospital.

Inclusion criteria for this study:

1. Diagnosed with B- or T-lymphoblastic leukaemia/lymphoma after their 1st birthday and before their 18th birthday
2. Diagnosed between the 1st September 2004 and the 19th July 2016
3. Resident in the HMRN region at the time of their diagnosis
4. Treated primarily at Leeds Hospital under the following protocols, regardless of trial participation (the details of these protocols are described in Appendix A):
    - UKALL 2003 trial or non-trial protocol (October 2003 to June 2011)
    - Interim guidelines (July 2011 to March 2012)
    - UKALL 2011 trial or non-trial protocol (April 2012 to December 2018)

Age cut-offs were chosen to capture patients treated under the UKALL protocols in the paediatric setting. Children diagnosed under the age of one are not treated under these protocols, and those diagnosed over the age of 18 are treated in the adult haematology department. Based on the above criteria, 268 children with ALL were identified from the HMRN database. Of note, case ascertainment was performed through cross-checking of known cases in the given timeframe with the data manager for paediatric oncology/haematology. The only discrepant patients (n=4) were privately treated patients, and those who moved out of region for treatment. One patient who died prior to the start of treatment, was also excluded from the analysis.

Information on mortality was derived from the causes recorded on the death certificates. In order to classify each death as infective or non-infective in cause, an international classification system was used (184,185). The

causes of death recorded on the death certificate were also checked against the patients' medical records.

2.3 Censor dates

For each patient, time at risk was defined as the time between the first and last day of treatment under the included protocols.

The censor date for each patient was marked by one of the following events depending on which happened first:

- End of treatment: usually two years from the start of interim maintenance for female patients, and three years for male patients
- Date of starting a different treatment pathway (e.g., different treatment protocol, condition for haematopoietic stem cell transplant (HSCT)
- Date of relapse (if relapse occurred during treatment)
- Date of death (if patient died during treatment)
- End of data collection: 15th February 2017. This meant that all included patients would have at least reached the beginning of maintenance.

2.4 Additional data

Core data from HMRN (https://hmrn.org/about/data) were supplemented with additional clinical information; this included microbiological sample information, full blood count results and chemotherapy administration information, as outlined in Section 2.4.1. This additional data collection was performed manually by myself accessing the results' server which holds this aspect of the clinical records. The information I extracted included data for every microbiological sample and full blood counts from diagnosis to end of treatment, or other censoring event. This was done retrospectively tracking through each patient's treatment till their end of follow up. Patient records were accessed using their NHS number. In order to link to HMRN data, the NHS numbers were removed from the extracted data and a unique pseudonymised study identifier was used instead.

Microbiological samples are taken upon suspicion of infection with pyrexia or other clinical concerns. In the case of patients presenting with fever and neutropenia, in Leeds Children's Hospital febrile neutropenia is defined as an episode of pyrexia of ≥38°C with a neutrophil count of ≤0.5 x 10^9/L. At the time of presentation with febrile neutropenia, all lumens of the CVL (if present) would be sampled for blood cultures along with a peripheral sample within an hour of the central cultures. Broad-spectrum antibiotics were administered as soon as blood cultures were taken and within an hour of presentation with pyrexia. Respiratory samples were taken at the same time depending on presenting symptoms. Patients would then be risk-stratified based on examination and laboratory findings for stepdown to oral antibiotics after 24 hours if they remained apyrexial and were clinically well. This was only considered for patients who had completed at least four weeks of maintenance treatment. At all other treatment phases inpatient intravenous antibiotics were continued while the patient was febrile and neutropenic. Antibiotic cessation was considered in patients with negative blood cultures who remained apyrexial for at least 48 hours in the absence of clinical source of infection. For patients who remain neutropenic and febrile for 5-7 days with no microorganism growth from the blood cultures but clinical concerns of infection, Aspergillus antigen tests were considered, along with the addition of empirical antifungal therapy.

The management of fever during admission for planned chemotherapy or other reasons was similar, with blood culturing at the time of fever and initiation of broad-spectrum antibiotics. The management of non-neutropenic patients depended on the clinical findings and status of the patient. In all cases of positive blood cultures microbiology advice would have been sought regarding the duration and specific antibiotic treatment. In the cases of CVL infections, microbiology advice, status of patient and pathogen isolated would have dictated the management and potential line removal. Line locks were used alongside intravenous antibiotics in the cases of attempted line salvage. The specifics of antimicrobial treatment for each of the above episodes are not within the remit of this research. Regarding antimicrobial prophylaxis, all patients received co-trimoxazole (trimethoprim and sulphamethoxazole) as

Pneumocystis jirovecii (PJP) prophylaxis for two days a week throughout treatment.

2.4.1 Microbiological samples

Microbiological samples are used in this thesis as a proxy for the suspicion of infection. Information was collected on all included microbiological samples sent during treatment. This included blood samples, respiratory samples, and samples from other biopsy sites. Viral serology, urine and stool samples, swabs from the genitourinary and gastrointestinal tract, skin, eye, and mouth swabs were excluded. CSF samples sent for MCS or viral testing were also excluded (only the two CSF samples positive for fungal pathogens have been included).

For each microbiological sample, the following information was collected:

- Date of sample
- Clinical information on the request form (where available)
- Site of sampling
- Test that the sample was sent for (particularly relevant for respiratory samples which could have been sent for viral, fungal or bacterial testing, or all the above)
- Positivity
- Isolated microorganism(s) if positive

All patient samples were given a sample number. The sources of microbiological samples included in the study include: blood cultures; respiratory samples tested for bacterial, viral, fungal pathogens and *Pneumocystis jirovecii* (PJP); and blood and other non-upper respiratory tract samples tested for fungal pathogens (with antigen testing, microscopy and culture or other tests outlined below.

Blood samples

Blood culture samples collected from CVLs and venepuncture sites (peripheral) sent for microscopy and culture (MCS) were included. In the case of indwelling CVL, the standard operating procedure (SOP) dictated sampling of all lumens of the indwelling CVL and obtaining a sample from peripheral venepuncture within an hour of presentation with suspected infection (Appendix D). This is termed as “paired cultures” and they are very important in order to differentiate between a line associated bacteraemia, as the differential time to positivity is a main factor. If the blood culture sample taken from any indwelling line becomes positive more than two hours before the peripheral (venepuncture) sample, the bacteraemia is considered line associated. If, on the other hand, all blood cultures become positive within a two hour window of each other, the infection is not considered to be line associated.

When blood culture samples were taken from all lumens of a CVL, with a peripheral sample having been taken within an hour, the data collection counted all samples as one blood culture sample referred to as “paired cultures”. This was only the case when all these samples were negative, or when all these samples were positive for the same microorganism(s); if some were negative and some positive then they were treated as having been collected as separate samples (i.e., one sample from CVL lumen(s) and another from venepuncture marked as peripheral culture on the same date).

The techniques and technology used for the identification of pathogens in blood cultures changed over the years of the study. Initially, analytical profile index (API) was used which was then replaced by a Vitek machine in 2009 (186,187). Subsequently in 2011 the Maldi-Tof technology was introduced which is an automated reliable system of identification of pathogens (188).

Blood samples sent for the investigation of fungal infection were also included in this study and are presented under the ‘samples sent for fungal testing’ sections. These include serological tests and fungal microorganisms identified

from prolonged blood culture growth in cases where invasive fungal disease was strongly suspected. In these instances, the blood culture samples were alerted to the lab for longer incubation as fungal pathogens take longer to grow. The serological tests performed were: Aspergillus antigen testing, β-d-glucan testing (used since 2015), Candida antigen testing (which also became available in 2015), Cryptococcus antigen testing, and mycology serology. B-d-glucan is a cell wall component of a wide variety of fungal pathogens. The serological tests can be used to aid the diagnosis of invasive fungal disease but need to be interpreted with caution. They are sensitive with a good negative predictive value, i.e., good for excluding infection.

Respiratory samples

Respiratory samples sent for microscopy and culture (MCS), respiratory viral polymerase chain reaction (PCR) testing and PJP testing were included. Respiratory sample types included: nose/throat swabs, nasopharyngeal aspirates (NPA), endotracheal tube (ET) secretions, sputum, and bronchioalveolar lavage samples (BAL). BAL is the sampling of lower respiratory tract through the installation and subsequent recovery of sterile saline solution at the time of bronchoscopy. It is an important procedure which frequently assists in the diagnosis of pulmonary infection, particularly in the immunocompromised host. Due to its invasive nature, BAL sampling is reserved for unwell patients with an unclear cause of infection that involves the respiratory tract as, particularly children, patients may need assisted ventilation after the procedure. Respiratory viral samples were tested for a standard PCR viral panel of: adenovirus, parainfluenza 1-4, influenza A (H1 and H3), influenza B, rhinovirus, metapneumovirus, respiratory syncytial virus (RSV), and the panel also included *Mycoplasma* PCR (which is a bacterial pathogen).

Each respiratory sample taken relates to one sample number based on the date and the source of sample. In the case of multiple sources being sampled on the same date each sample would relate to as many sample numbers as the locations sampled that day. For example, if a patient had a respiratory

sample sent for viral PCR from their nose and a sputum sample sent on the same day, then two separate sample numbers would be created. If, however, a patient had a sample taken from their nose sent for viral PCR and for MCS on the same day, then the sample would have been collected as one sample. Each sample, therefore, could be positive for more than one pathogen of any type: viral, bacterial, fungal, PJP.

Some of the pathogens isolated from upper respiratory tract samples are likely to represent colonisation rather than be the cause acute infection and therefore their role in the clinical picture cannot be assessed. The analysis has, however, included all samples during treatment.

Other sampling sites (for fungal testing)

Samples sent for fungal testing (with MCS) from other, normally sterile, sites were included: CSF, arm skin biopsy, liver, oesophageal, lung, and renal stone tissue biopsies.

2.4.2 Full blood count results

All full blood count results available during treatment of each patient were collected, so that the presence of neutropenia at the time of a microbiological sample could be quantified. Neutropenia was defined as a neutrophil count $\leq 0.5 \times 10^9$/L.

Data included:

- Date of sample
- Total white cell count
- Absolute neutrophil count
- Absolute lymphocyte count

2.4.3 Electronic chemotherapy records (Chemocare)

Data on chemotherapy administration for each patient, along with delays in administration, were collected. Data on treatment delays and modifications

were collected until the beginning of maintenance, and from that point onwards only the start and end of maintenance, or censor date for other reasons, were recorded.

The electronic chemotherapy record used in the Leeds Children's Hospital since 2003 is "Chemocare", which records chemotherapy agents, dates of administration, and dosage. Prescription charts are generated through Chemocare, and printed and checked by the nursing staff administering the treatment. To verify that the information recorded in the system was in line with written records, chemotherapy data collected from 50 randomly selected patients using Chemocare were checked by myself and one of the paediatric pharmacists against hand-written records kept by the pharmacy team. There was complete agreement between the two sources.

Data collected from Chemocare included:

- Treatment protocol
- Trial participation
- Randomisation allocation, in the case of trial participants
- Regimen(s) of treatment
- Start and end of each phase of treatment
- Treatment delays, start and end dates, with reason (where documented)
- End of treatment date

Each patient's treatment information was collected per treatment phase, based on the date of start and end of each phase. The start dates for each phase were the actual start dates, not the expected ones based on the treatment protocol. In case of delays within, or at the end of, each phase the delay was added to the duration of the relevant phase. Hence, some phases will appear to have lasted longer than the expected number of weeks based on the protocol as they incorporate treatment delays within them. Treatment delays were mostly due to awaiting count recovery to start the next phase as

per protocol, but also due to patients being unwell, which would often be due to suspected or proven infection.

2.5 Data linkage and analysis

NHS numbers were required for additional data collection. The data collected from the sources outlined in Section 2.4 were then anonymised by removing the NHS numbers; and were replaced by the unique study identifiers in order to link to demographic, diagnostic and other data in the HMRN database.

The relationships between infection during different phases of treatment and demographic, disease-related, and treatment-related variables were examined by comparing the numbers of patients with and without samples; or any positive and no positive samples in each phase. Due to the small numbers which precluded the use of finer divisions, age and IMD were analysed as dichotomous variables. In line with the NCI risk groups, two main age groups (1-9 and ≥10 years) are used throughout the analysis. The two IMD: income-domain groupings were more affluent (quintiles 1-3) and more deprived (4-5). For febrile neutropenia, the neutrophil count was also dichotomised (cut-off of $\leq 0.5 \times 10^9$/L) as per international guidelines. . Comparisons between groups were examined using the Fisher's exact test, with $p<0.05$ being considered statistically significant. Data were analysed in STATA 16 (189).

During consolidation and maintenance where duration of treatment was very different between patient groups, the rates of patients who were sampled or who had at least one positive sample were used to make comparisons. Person-weeks at risk were calculated from the start of the phase until the first sample, or the first positive sample; or where the patient had no samples- or no positive samples-, until the start of the next phase, end of treatment or other censor date. To examine whether the rates of samples, or of positive samples, differed between patient groups, all samples, or positive samples, were counted, and person-time for these analyses was from the start of the phase until the start of the next phase, end of treatment or other censor date. Rates and 95% confidence intervals (CI) are reported per 100 person-weeks

and compared using the Mantel-Haenszel method. Further statistical analyses using advanced regression techniques (190,191) examined the risk of being sampled and the rate of sampling by patient characteristics, and are presented by phase and sample type in Appendix E.

Chapter 3 Results

3.1 Study population

The study included 268 patients with acute B- or T-lymphoblastic leukaemia diagnosed between the ages of one and 17 years between 1st September 2004 and 31st July 2016. As expected, the majority of the patients were diagnosed with B-ALL (221/268) and there was a slight male predominance (151/268) (Table 3.1-1). The median age at diagnosis was 5.3 years (IQR: 3.1-8.5), with nearly half of the patients diagnosed between the ages of one and four years. Patients with T-ALL were marginally more likely to live in more deprived areas than those with B-ALL, but the difference was not statistically significant.

Table 3.1-1 Patient and diagnostic characteristics by lineage

	Total N (%)	B-ALL N (%)	T-ALL N (%)
Total	**268**	**221**	**47**
Sex			
Female	117 (43.6)	105 (47.5)	12 (25.5)
Male	151 (56.4)	116 (52.5)	35 (74.5)
Age at diagnosis (years)			
1-2	66 (24.6)	63 (28.5)	3 (6.4)
3-4	61 (22.8)	57 (25.8)	4 (8.5)
5-9	71 (26.5)	55 (24.9)	16 (34.0)
10-17	70 (26.1)	46 (20.8)	24 (51.1)
Median (IQR)	5.3 (3.1-10.5)	4.4 (2.9-8.6)	10.5 (6.5-14.2)
Deprivation			
More affluent	140 (52.2)	121 (54.8)	19 (40.4)
More deprived	128 (47.8)	100 (45.2)	28 (59.6)
Diagnostic Subtype (ICD-O3)			
B-ALL			
- hyperdiploidy	86 (32.1)	86 (38.9)	N/A**
- NOS	80 (29.9)	80 (36.2)	N/A
- t(12;21)	44 (16.4)	44 (19.9)	N/A
- t(9;22)	6 (2.2)	6 (2.7)	N/A
- hypodiploidy	3 (1.1)	3 (1.4)	N/A
- MLL rearrangement	2 (0.7)	2 (0.9)	N/A

*NOS: Not otherwise specified **N/A: Not applicable

Patients with predisposing genetic conditions were included in the cohort: five had Down syndrome and two ataxia telangiectasia. As shown in Table 3.1-2, all seven were diagnosed with B- ALL and had a comparatively high median age of twelve years at diagnosis. The two patients with ataxia telangiectasia died during induction. Of the five patients with Down syndrome (three males and two females) three had B-ALL NOS, one had t(9;22) and one had hyperdiploidy. They were all treated under regimen A apart from two: one treated under regimen B and one under regimen C, and all but one reached the end of treatment (one reached the end of data collection while in

maintenance) and the patient with t(9;22) was moved to a different treatment protocol at the end of induction.

Table 3.1-2 Characteristics of patients with predisposing genetic conditions

	Patients
Total	7
Sex	
Female	2
Male	5
Age at diagnosis (years)	
1-9	3
10-17	4
Median (IQR)	12.1 (12.1-17.2)
Deprivation	
More affluent	5
Less affluent	2
Diagnosis	
B-ALL NOS	3
B-ALL hyperdiploidy	2
B-ALL t(9;22)	2

3.1.1 Time at risk

Time at risk (of infection) was defined as the time from the date of diagnosis to the last day of treatment under one of the included treatment protocols. The minimum time at risk was one day (death before the start of induction) and the maximum was 3.5 years, with a median of 2.3 years (IQR: 1.9-3.2). Most patients reached the end of treatment or end of data collection (227/268, 84.7%). The number of patients for each censor event is presented in Table 3.1-3.

Table 3.1-3 Events marking censor dates with number of patients affected

	Patients N (%)
Total	**268**
End of treatment	177 (66.0)
End of data collection	50 (18.6)
Death on treatment*	18 (6.7)
Relapse on treatment	9 (3.4)
HSCT**	6 (2.2)
Moved to different protocol	5 (1.9)
Only had part of treatment	2 (0.7)
HLH***	1 (0.4)

*one patient died before starting treatment **Haematopoietic stem cell transplant *** Haemophagocytic lymphohistiocytosis

3.1.2 Treatment protocols, regimens, and phases

One patient died before the start of treatment and is not included in the analysis. This means that the total number of patients who started treatment was 267.

Table 3.1-4 shows the regimen of treatment by disease subtype. All patients diagnosed with B-ALL with t(9;22) (Philadelphia chromosome positive disease) were moved to the appropriate treatment protocol when the cytogenetic results became available. Of the 142 patients treated in the UKALL 2003 era, the majority (124/142, 87.3%) were treated under the trial protocol and 16 were treated under the interim guidelines (IG) between the two trials (Table 3.1-5). Finally, 109 patients were treated under UKALL 2011 protocols, with 62 (56.8%) treated under the trial protocol.

Overall, 117 patients were allocated Regimen A treatment, 68 allocated Regimen B and 82 to Regimen C.

Table 3.1-4 Patients by regimen of treatment and diagnostic subtype

	Total	Regimen A	Regimen B	Regimen C
Total N (%)	**267**	**117 (43.8)**	**68 (25.4)**	**82 (30.8)**
B-ALL				
- hyperdiploidy	86	57 (66.3)	12 (13.9)	17 (19.8)
- NOS	80	23 (28.7)	28 (35.0)	29 (36.3)
- t(12;21)	44	29 (65.9)	11 (25.0)	4 (9.1)
- t(9;22)	6	3 (50.0)	2 (33.3)	1 (16.7)
- hypodiploidy	3	1 (33.3)	1 (33.3)	1 (33.3)
- MLL rearrangement	2	0 (0.0)	0 (0.0)	2 (100.0)
T-ALL	46	4 (8.7)	14 (30.4)	28 (60.9)

Table 3.1-5 Patients by protocol and regimen of treatment

	Total N (%)	B-ALL N (%)	T-ALL N (%)
Total	**267**	**221**	**46**
UKALL 2003	**124 (46.4)**	**98 (44.3)**	**26 (56.5)**
Regimen A	56 (21.0)	52 (23.5)	4 (8.7)
Regimen B	34 (12.7)	26 (11.8)	8 (17.4)
Regimen C	34 (12.7)	20 (9.0)	14 (30.4)
UKALL 2003 non-trial	**18 (6.7)**	**15 (6.8)**	**3 (6.5)**
Regimen A	10 (3.7)	10 (4.5)	0 (0.0)
Regimen B	8 (3.0)	5 (2.3)	3 (6.5)
Regimen C	0 (0.0)	0 (0.0)	0 (0.0)
Interim guidelines	**16 (6.0)**	**14 (6.4)**	**2 (4.3)**
Regimen A	7 (2.6)	7 (3.2)	0 (0.0)
Regimen B	3 (1.1)	3 (1.4)	0 (0.0)
Regimen C	6 (2.2)	4 (1.8)	2 (4.3)
UKALL 2011	**62 (23.2)**	**53 (24.0)**	**9 (19.6)**
Regimen A	25 (9.4)	25 (11.3)	0 (0.0)
Regimen B	11 (4.1)	10 (4.5)	1 (2.2)
Regimen C	26 (9.7)	18 (8.1)	8 (17.4)
UKALL 2011 non-trial	**47 (17.6)**	**41 (18.6)**	**6 (13.0)**
Regimen A	19 (7.1)	19 (8.6)	0 (0.0)
Regimen B	12 (4.5)	10 (4.5)	2 (4.3)
Regimen C	16 (6.0)	12 (5.4)	4 (8.7)

As mentioned in the introductory chapter, all patients aged ten years or more at diagnosis were allocated to regimen B, as they were in the high-risk group. The three patients allocated to regimen A, despite being over the age of ten years at diagnosis, had predisposing genetic conditions (two ataxia telangiectasia and one Down syndrome). The two patients with ataxia telangiectasia were treated under regimen A UKALL 2003 non-trial protocol, and both died during induction. Three of the five patients with Down syndrome were also treated under regimen A, but one of them was moved to a different treatment protocol after induction. The other two were treated under regimen

B (UKALL 2011 non-trial) and regimen C (UKALL 2003 trial) respectively due to high-risk minimal residual disease (MRD) on day 28. Both reached the end of treatment/data collection. The two patients treated under the UKALL 2003 protocol received one course of delayed intensification.

3.1.3 Deaths in the cohort

Information about deaths in the cohort is presented in Table 3.1-6 and Table 3.1-7. Eighteen patients died (18/268, 6.7% overall; 11/151, 7.2% males; 7/117, 5.9% females), which is in line with internationally reported all-cause mortality rates for childhood ALL in high-income countries (10, 26, 77, 179, 180). At 10.6% (5/47) the proportion of deaths in patients diagnosed with T-ALL was higher than in patients with B-ALL (13/221, 5.9%). Most deaths (13/18) were related to infection (Table 3.1-7). Infection-related deaths affected both sexes equally (seven males and six females). Although most deaths in females (6/7) were secondary to an infective cause, there was a near equal split between the infection and non-infection related deaths in males. The median age of patients who died due to infection was much lower than that of patients who died due to non-infective causes (median age at diagnosis of 4.2 versus 10.2 years). For all the patients, the number and type of microbiological samples in the days before death were checked. Although 13 deaths were classed as infection related, only nine patients had positive microbiological samples around the date of death (note that for the patient with disseminated varicella zoster septicaemia, the viral serology samples were not included in the data collection, hence it appears as though there were no positive microbiological samples around the date of death). The three patients flagged as “no samples” did not have any microbiological samples around the time of death.

In patients diagnosed under the age of ten years 11/13 deaths were due to infection. Similarly, ten of the deaths occurred in patients being treated under regimen A (who would have been diagnosed under the age of ten years). Only one death occurred in regimen C and it was secondary to infection. In patients treated under regimen B, 6/7 deaths occurred in patients being treated under

UKALL 2003 trial protocol (5/7 patients were randomised to have two courses of delayed intensification) (not presented in table).

Among the patients who died of any cause, median overall survival (OS) was 39 days (IQR: 17-269); as 10/18 deaths happened during induction (and one patient died before the start of treatment).

Two patients had ataxia telangiectasia, both were male, aged over ten and treated under regimen A. The first patient was diagnosed with B-ALL with t(9;22) and died with candidaemia during induction, and the second with hyperdiploidy B-ALL, and died after palliation post induction secondary to treatment related toxicity.

More information on all-cause mortality during treatment and when it occurred is presented in Section 3.1.4.

Table 3.1-6 Deaths due to non-infective causes

Diagnosis	Sex	Age Group	Regimen	Cause of death	Microbiological samples
B-ALL NOS	M	1-4	B	Upper GI bleed	Aspergillus antigen in blood: negative
B-ALL with t(12;21)	F	5-9	A	Extrapontine myelinolysis	*Candida glabrata* in blood
B-ALL with hyperdiploidy	M	≥10	A	Ataxia telangiectasia	No samples
T-ALL	M	≥10	B	ALL	No samples
T-ALL	M	≥10	B	HIE. Cardiac arrest	No samples

Table 3.1-7 Deaths due to infective causes

Diagnosis	Sex	Age Group	Regimen	Cause of death	Microbiological samples
B-ALL with hyperdiploidy	M	1-4	A	*S. aureus* septicaemia*	*S. aureus* in blood
B-ALL with hyperdiploidy	F	1-4	A	Septicaemia*	*S. aureus* in blood
B-ALL with hyperdiploidy	F	1-4	A	Infective endocarditis*	*C. albicans* in BAL
B-ALL with t(12;21)	M	1-4	A	Peritonitis	Negative blood cultures
B-ALL NOS	F	5-9	A	Necrotising typhlitis*	No samples
B-ALL with hyperdiploidy	F	5-9	A	Gram -ve Septicaemia	*E. coli* in blood
B-ALL with t(12;21)	M	1-4	B	Multi organ failure-neutropenic sepsis*	No samples
B-ALL with hyperdiploidy	F	1-4	A	Liver failure. Viral infection	Aspergillus antigen in blood: positive
B-ALL with hypodiploidy	M	1-4	B	Disseminated varicella zoster septicaemia	No samples
B-ALL with t(9;22)	M	≥10	A	Fungal septicaemia.* Ataxia telangiectasia	*C. albicans* in blood. Aspergillus antigen in blood: positive
B-ALL with hyperdiploidy	F	≥10	B	Septicaemia. Neutropenia*	*E. coli* in blood
T-ALL	M	≥10	C	Pneumonia	*Abiotrophia defectiva* in blood
T-ALL	M	5-9	B	Neutropenic colitis*	*Klebsiella pneumoniae* in blood

*Deaths during induction

3.1.4 Flow of patients within treatment phases

Figure 3.1-1 provides a visual representation of the flow of patients within the different treatment phases across all different treatment protocols and regimens. Overall, 267 patients started induction, with eight deaths during induction and two deaths shortly after. Information on the eight deaths during induction is provided in Table 3.1-7 (deaths marked by an asterisk); all but one occurred in patients diagnosed with B-ALL. All eight deaths were secondary to infection; four in the UKALL 2003 era, one under the interim guidelines (IG), two from UKALL 2011 trial protocol, and one under the UKALL 2011 non-trial protocol. One of the patients who died during

induction had ataxia telangiectasia. Both patients treated under UKALL 2011 trial who died during induction were randomised to have a short course of dexamethasone. The two deaths shortly after induction affected patients who received palliative treatment due to treatment toxicity; one of these patients also had ataxia telangiectasia.

As shown in Figure 3.1-1, 18 patients did not start consolidation treatment. Ten patients died during induction, four moved to different treatment protocols (one with Down syndrome), two started conditioning for HSCT, and two skipped the consolidation phase due to treatment-related morbidity during induction and had interim maintenance instead (one had fungal retinitis during induction and the other had previously been treated for another malignancy). Of the 249 patients who started consolidation, two died during this phase: one moved to a different treatment protocol and one started conditioning for HSCT. Both deaths during consolidation were secondary to infection. They affected males under the age of five years at diagnosis, and both were treated under UKALL 2003 trial protocol; a patient treated under regimen A who died of peritonitis with no positive microbiological samples during consolidation and a patient treated under regimen B who tested positive for Aspergillus antigen on two occasions who died due to disseminated varicella zoster septicaemia (viral serology samples were not included in this study).

Two-hundred and forty-seven patients started interim maintenance treatment (marked as interim maintenance I in Figure 3.1-1 to accommodate the patients who received two courses of interim maintenance in the UKALL 2003 era. The same is the case for delayed intensification I and II). There was one death during interim maintenance due to a gastrointestinal bleed: a male patient (aged 1-4) diagnosed with B-ALL NOS and treated under UKALL 2003 trial regimen B. Another patient (aged≥10) with B-ALL NOS treated under UKALL 2003 trial regimen C started conditioning for HSCT after initial treatment during interim maintenance.

Three patients skipped delayed intensification I: one previously treated for a solid malignancy who had a prolonged episode of complicated sepsis during

interim maintenance, another did not tolerate intensive chemotherapy, and a third who struggled with fungal retinitis at induction and also skipped consolidation. Therefore, 242 patients started delayed intensification I. There were no deaths during delayed intensification I. However, a female patient (aged 5-9) with B-ALL NOS treated under UKALL 2003 trial regimen A started conditioning for HSCT after initial treatment of delayed intensification I.

Of the 144 patients treated under the UKALL 2003 protocols, 93 started interim maintenance II, and 91 started delayed intensification II (one patient stopped treatment after interim maintenance II and another went straight to maintenance) (Figure 3.1-2). There were three deaths during delayed intensification II; 2/3 were from infective cause and affected two females with B-ALL with hyperdiploidy treated under regimen A. One death (aged 5-9) was due to Gram -ve septicaemia with *E. coli* growth in blood cultures and another (aged 1-4) was due to liver failure and viral infection (one positive Aspergillus antigen test near the time of death). The non-infective death also affected a female patient treated under regimen A.

Of the 235 patients who started maintenance, 88 received two courses of interim maintenance and delayed intensification. Five patients treated under the UKALL 2011 era had not started maintenance by the end of data collection and were, therefore, not included in the analysis of the maintenance phase. During maintenance, nine patients relapsed, one started conditioning for HSCT, one developed haemophagocytic lymphohistiocytosis (HLH) and one patient died secondary to pneumonia.

The flow of patients in the UKALL 2003 and UKALL 2011 protocols is shown in Figure 3.1-2 and Figure 3.1-3.

Section (3.2) presents information on the microbiological samples sent during all phases of treatment, including the samples taken during interim maintenance II and delayed intensification II of the patients who had those treatment phases.

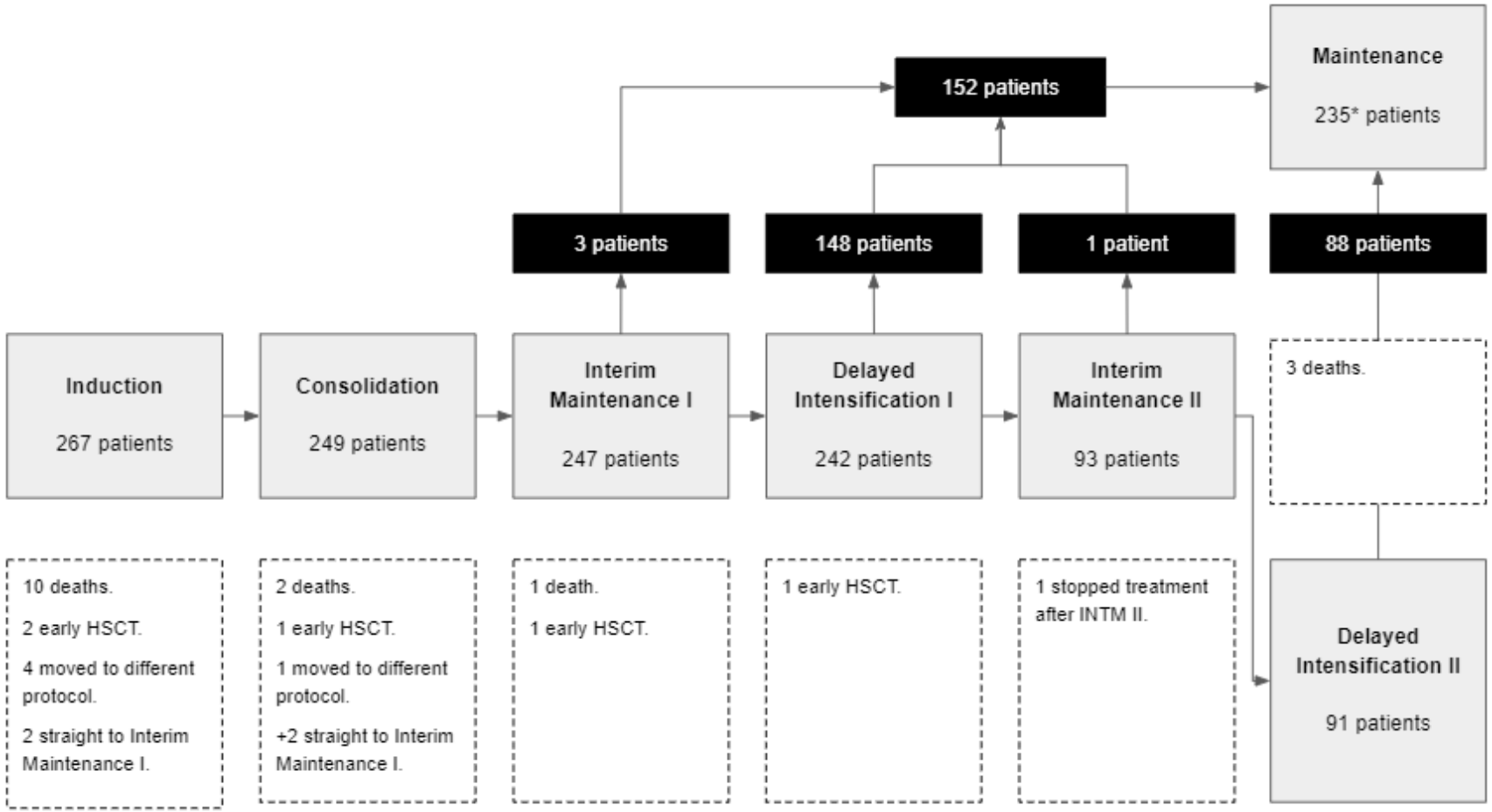

Figure 3.1-1 Patient flow during treatment under all included protocols. **The total number of patients starting each treatment phase are shaded grey; patients who did not transition to another phase are in dashed rectangles; patients who transitioned to a different phase than expected are shaded black. *Five patients had not started maintenance by the end of data collection** **(HSCT: Haematopoietic stem cell transplant. INTM II: Interim maintenance phase cycle II)**

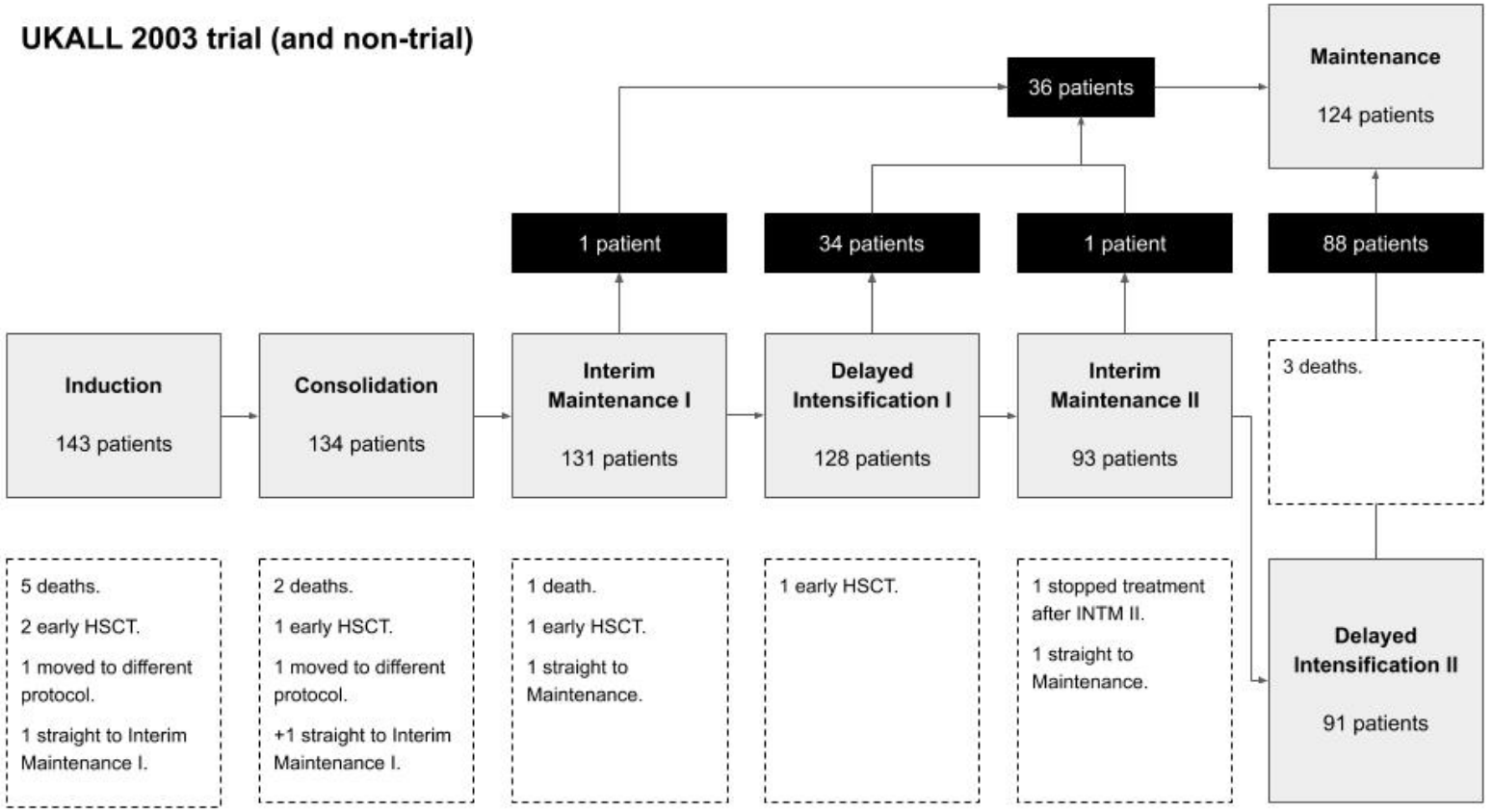

Figure 3.1-2 Patient flow during treatment under the UKALL 2003 protocols. The total number of patients starting each treatment phase are shaded grey; patients who did not transition to another phase are in dashed rectangles; patients who transitioned to a different phase than expected are shaded black.

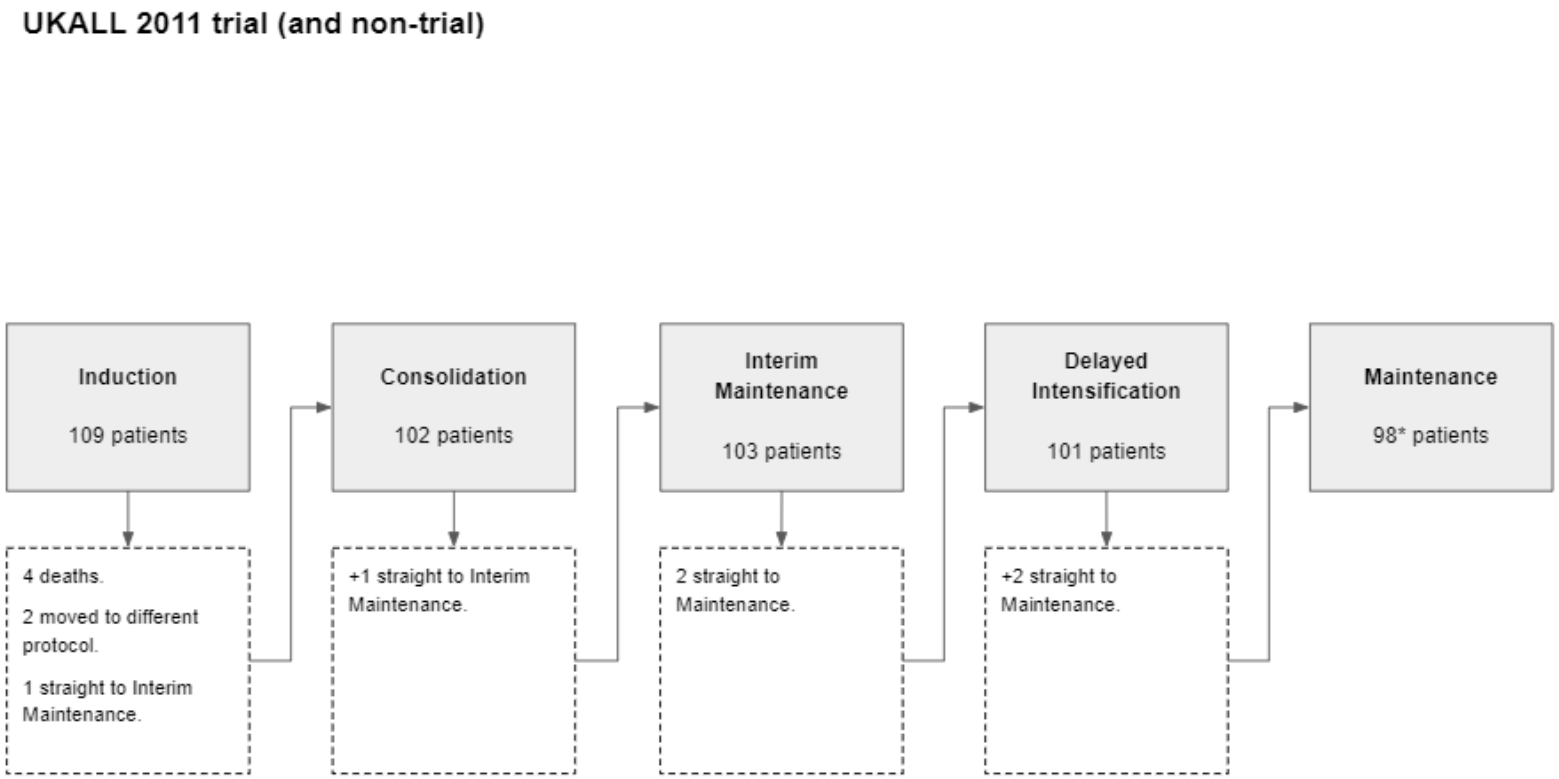

Figure 3.1-3 Patient flow during treatment under the UKALL 2011 protocols. **The total number of patients starting each treatment phase are shaded grey; patients who did not transition to another phase are in dashed rectangles; patients who transitioned to a different phase than**

3.2 Total microbiological samples and patient characteristics

During treatment, at least one microbiological sample was taken from 254 of the 267 included patients over the period of 1st September 2004 to 15th February 2017, resulting in 5,240 samples in total. There were 3,455 blood cultures, 1,401 respiratory samples and 384 (mostly blood) samples sent for fungal testing. Only 1,171 (22.3%) of the 5,240 microbiological samples were positive for at least one pathogen. 442/3455 blood cultures, 801/1401 respiratory samples and 30/384 samples sent for fungal testing. Positive microbiological samples could have one or more pathogens isolated.

The number of samples and positives by sample type per phase of treatment is shown in Figure 3.2-1. Most samples were taken during maintenance, the longest phase of treatment. It can be seen, however, that many samples and positives were taken during intensive treatment phases such as induction, consolidation and delayed intensification, despite their shorter duration. During induction, 73/430 (16.9%) blood cultures were positive compared to 10.8% of blood cultures during delayed intensification I and 11.4% during maintenance. Across all treatment phases, over half of the respiratory samples were positive. The highest positivity was seen during delayed intensification I (61.5%) followed by maintenance (59.1%). The overall positivity of samples was highest during maintenance (26.7%) followed by interim maintenance (24.4%) and then induction (23.1%).

Information on each phase is presented separately in Sections 3.3 to 3.7, this includes induction, consolidation, interim maintenance I, delayed intensification I and maintenance. A brief outline of the samples during interim maintenance II and delayed intensification II is presented in Sections 3.5.5 and 3.6.5 respectively.

Figure 3.2-2 shows the samples and positive samples per patient over time. Each patient is represented by a line on the y axis and each dot represents a sample (blue for blood cultures, red for respiratory samples and green for

fungal samples). Faded dots represent a negative sample in the relevant colour coded category and bold dots represent a positive sample. The x axis shows time in days from the start of treatment. Most of the patients completed their treatment (a longer duration for males as their treatment lasts for three years from the start of interim maintenance versus two years for girls). Overall, there are a lot more negative samples (faded dots) than positive samples (bold dots) and this is the case for all sample types. Also evident is that some patients had a lot more sampling and/or positive samples than others. There appears to be relatively more positive samples during the first few months of treatment, but for some patients this pattern of multiple samples and positives carries through their entire treatment period. Finally, samples tend to be taken in clusters, as do positive samples, possibly representing episodes of infection or suspected infection.

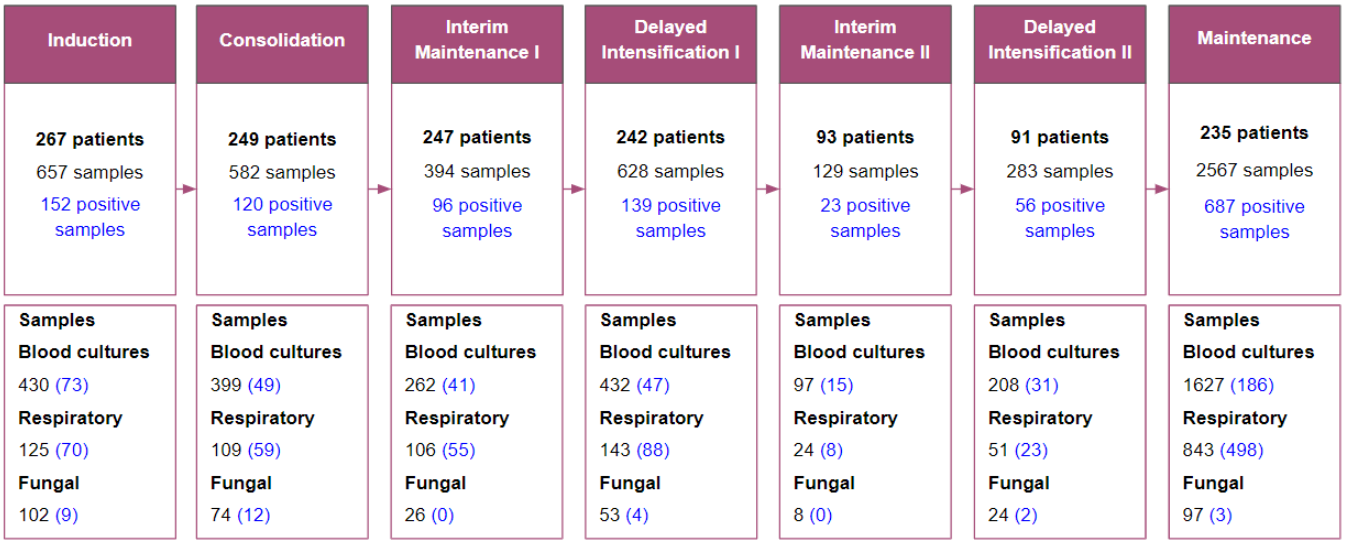

Figure 3.2-1 Patients and their samples during treatment phases

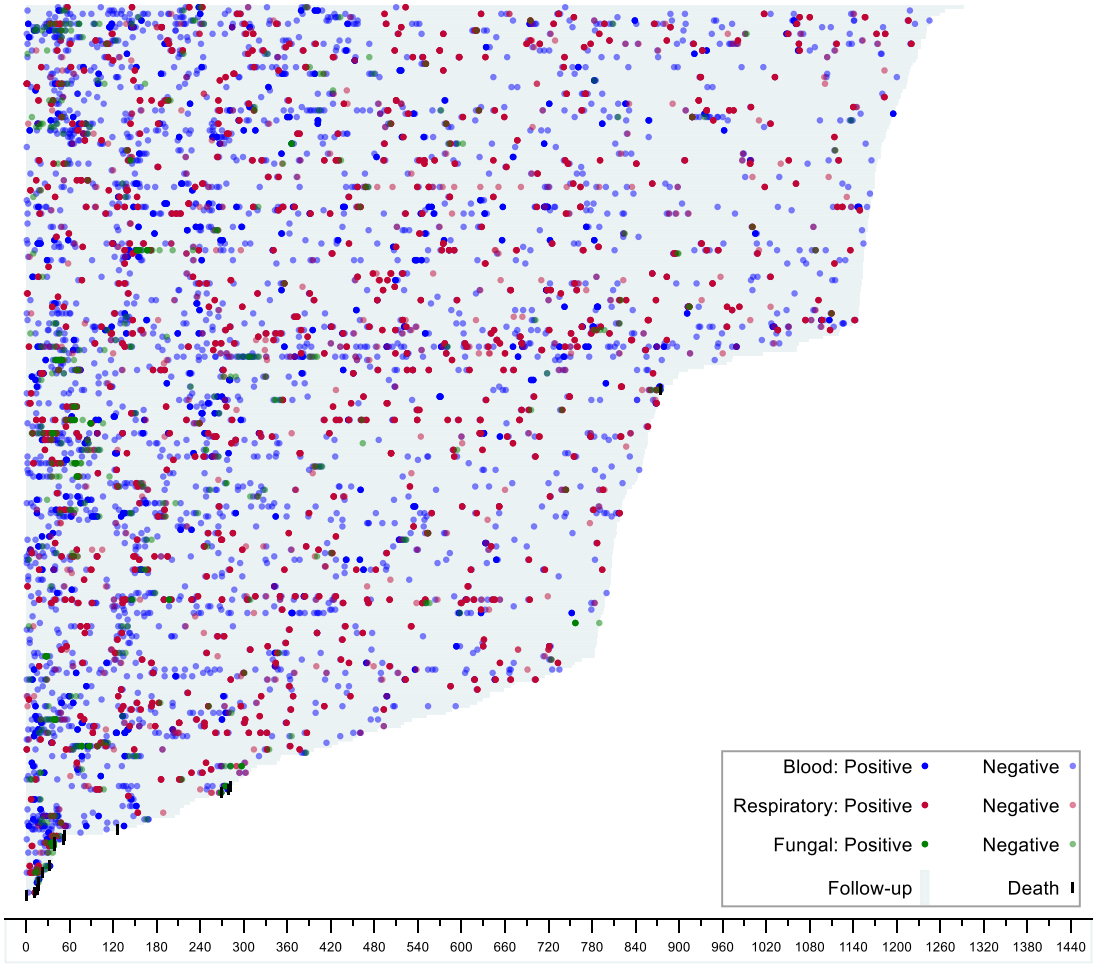

Figure 3.2-2 Samples per patient over days on treatment

The number of samples per patient ranged from 0 to 78 (median: 16, IQR: 8-25). Thirteen (4.8%) patients did not have any microbiological samples taken during treatment and their characteristics did not vary from those who did (data not presented). Only five of these reached the end of treatment without any samples (or near the end of treatment, as one patient was approaching the end of maintenance at the end of data collection). All but one of these five lived in areas with paediatric oncology shared care units (POSCU), and so were more likely to have presented to their local centre with episodes of fever or uncomplicated infection. These patients still received all inpatient chemotherapy at Leeds Children's Hospital, and therefore the absence of samples on the records from Leeds suggests that they did not have any suspected infections during those parts of treatment. Furthermore, they would have been transferred to Leeds in

the case of complicated febrile episodes. Of the remaining eight, four were moved to different treatment protocols post induction (around four weeks), two died during induction, and two received only part of the treatment under the included protocols for a duration of nine and 22 months respectively.

Similarly, the number of positive samples also varied widely; ranging 0 to 30 positive samples per patient (median: 4, IQR: 1-6). Most patients (230/254, 90.5%) had at least one positive sample during treatment. 20 of the 24 patients without positive samples reached the end of treatment or end of data collection; 3/4 deaths were due to non-infective causes. Only two patients without positive samples died during treatment (three deaths in induction and one in interim maintenance I).

3.2.1 Blood cultures

As Figure 3.2-3 shows, blood culture sampling was frequent in most patients, but certain individuals seem to have had more positive blood cultures across all treatment phases. It is also evident that more positive samples were taken in earlier treatment phases.

Overall, 3,455 blood cultures were taken from 252/267 patients during treatment; of these, only 442 (12.8%) were positive. Of the 252 patients with at least one blood culture taken during treatment, 162 (64.3%) had at least one positive blood culture.

The number of blood cultures per patient ranged from 0 to 54 (median: 10, IQR: 5-17), and the number of positive blood cultures per patient ranged from 0 to 16 samples (median: 1, IQR: 0-2). Most of the bacteria isolated in blood cultures (384/500, 76.8%) were Gram +ve, with the majority isolated from central lines (251/384, 62.0%) (Table 3.2-1). Although Gram -ve bacteria were less commonly isolated, there were proportionately more isolated during neutropenia.

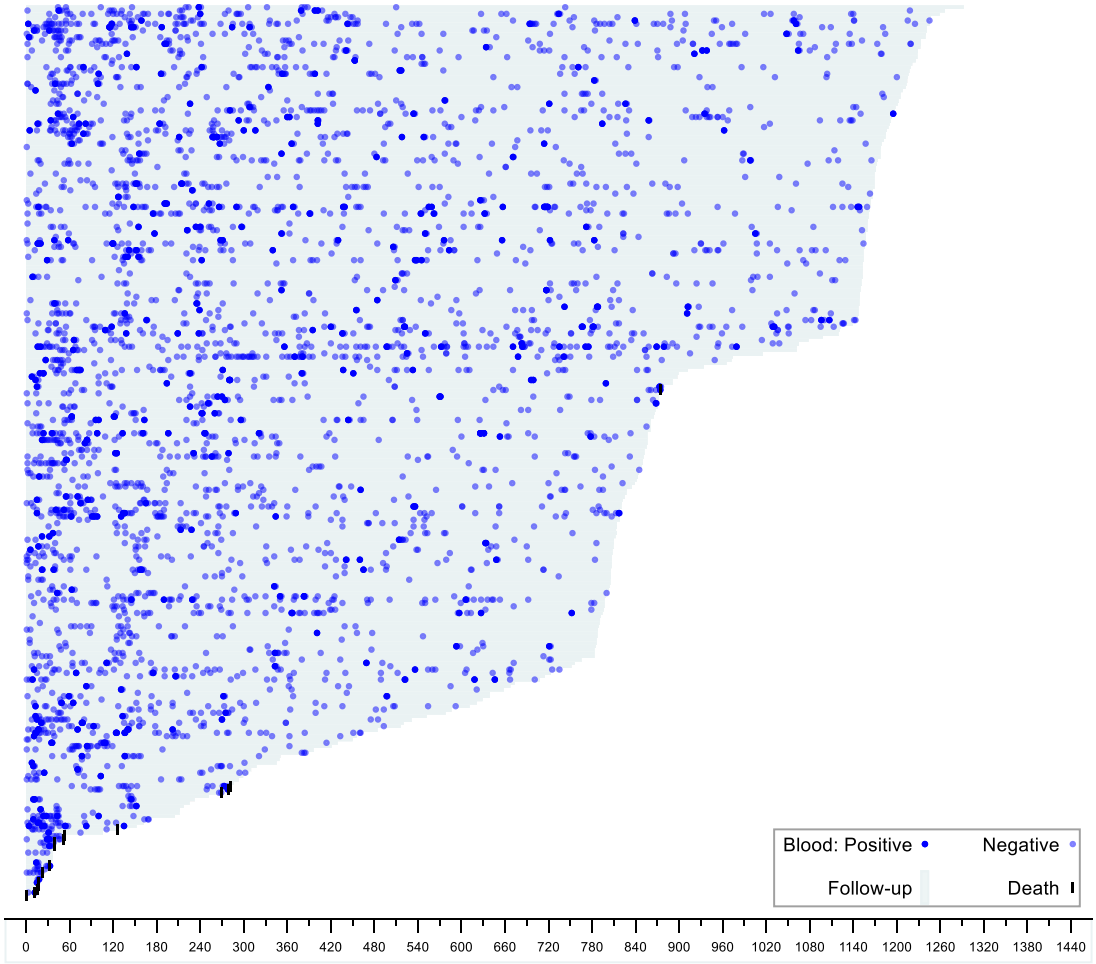

Figure 3.2-3 Blood cultures per patient over days on treatment

Overall, Gram +ve bacteria were the more frequent; 206/500 (41.2%) of the microorganisms isolated were coagulase-negative *Staphylococcus* (CONS), followed by *Streptococcus pneumoniae* (35/500), viridans-group streptococci (32/500), *E. coli* (30/500) and *Pseudomonas* species (23/500).

Table 3.2-1 Distribution of Gram category of bacteria isolated by source and neutropenia status

	Microorganisms	
	Gram -ve N (%)	**Gram +ve N (%)**
Total	**116**	**384**
Source		
Central line	72 (62.0)	251 (65.3)
Peripheral	17 (14.7)	41 (10.7)
Paired	10 (8.6)	29 (7.5)
Unknown	17 (14.7)	63 (16.5)
Neutropenia		
Yes	58 (50.0)	224 (58.3)
No	57 (49.1)	142 (36.9)
Missing	1 (0.9)	18 (4.8)

3.2.2 Respiratory samples

A total of 1,401 respiratory samples were taken. and over half of these (801/1401, 57%) were positive. As shown in Figure 3.2-4, most patients had respiratory samples taken (224/267), but most of the positives seemed to come from certain individuals who repeatedly had positive viral samples throughout treatment. The number of respiratory samples per patient ranged from 0 to 25 (median: 3; IQR: 1-7). The number of positive respiratory samples per patient varied from 0 to17 (median: 2; IQR: 0-4). Despite the high percentage of positive respiratory samples, 80 (29.0%) patients had no positive respiratory samples.

Respiratory samples were sent for viral and/or microscopy and culture (MCS) and/or testing for *Pneumocystis jirovecii*. Fungal pathogens were isolated through MCS. Some samples were taken at the same time for different types of tests. There were, therefore, 926 microorganisms isolated from the 801 positive respiratory samples (698 viruses, 80 bacteria, and 148 fungal pathogens). The most commonly isolated pathogens were viruses; rhinovirus was present in almost half the positive samples (347/698, 49.7%) followed by respiratory syncytial virus (RSV) (103/698, 14.7%).

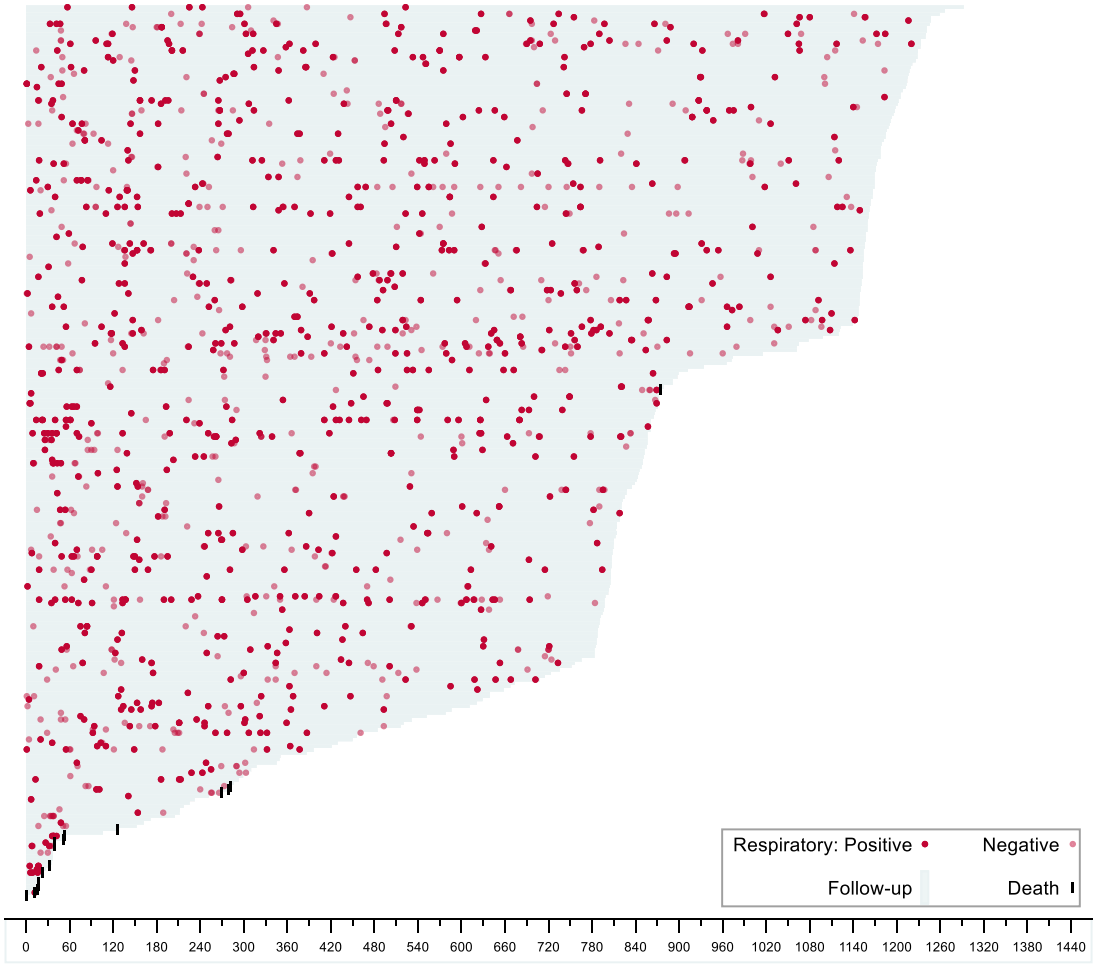

Figure 3.2-4 Respiratory samples per patient over days on treatment

3.2.3 Fungal samples from sites other than the respiratory tract

As also described in methodology, this section describes the samples sent for fungal serology testing and the blood and biopsy samples that had isolated fungal pathogens through microscopy and culture (MCS). From this point onward, the sections describing the fungal samples relate to blood and biopsy sample sites. The fungal pathogens isolated in respiratory samples are presented in the respiratory sample sections.

There were 384 samples from blood (n=373) and biopsy sites (n=11) sent for fungal testing (Table 3.2-2). These samples came from 143/267 patients

(53.5%). Only 30/384 (7.8%) samples were positive; and these came from 18 patients.

Samples were taken from around half of the patients in each diagnostic subtype, with no specific patterns seen. Only two patients diagnosed with T-ALL had a positive sample, the remaining 28 positive samples came from 16 patients diagnosed with B-ALL; six were diagnosed with B-ALL with t(12;21). There were eight patients with positive samples treated under regimen A and four treated under regimen C, with 15 and five positive samples respectively. Thirteen of the 18 patients with positive samples were female patients. Most patients with positive samples (12/18) were aged under ten years at diagnosis.

The 384 samples sent for fungal testing were mostly blood samples (n=373), plus three samples from CSF, four from lung tissue, one from an arm skin tissue, one from liver, one from oesophageal, and one from renal biopsy.

The 30 positive samples mostly represented blood samples (24/30). Seventeen were serological tests: 12 positive for *Aspergillus* antigen, two positive for *Candida* antigen, two for β-d-glucan and one for mycology serology. Seven samples had growth of fungal pathogens from MCS: three with *Candida albicans*, one with *C. tropicalis*, one with *C. glabrata*, one with *C. lusitaniae* and one with *Aspergillus fumigatus*.

The remaining six positive samples were: *C. tropicalis* isolated from one arm skin tissue biopsy, two CSF samples with isolated mycelium and *Aspergillus fumigatus*, respectively and three lung tissue samples positive for mycelium, *Aspergillus fumigatus* and *Candida parapsilosis* respectively.

Table 3.2-2 Patients and their fungal samples

	Patients			Samples	
	Total N (%)	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**267**	**143**	**18**	**384**	**30**
Diagnosis					
B-ALL	221 (82.8)	115 (80.4)	16 (88.9)	295 (76.8)	28 (93.3)
T-ALL	46 (17.2)	28 (19.6)	2 (11.1)	89 (23.2)	2 (6.7)
Sex					
Female	118 (44.2)	64 (44.7)	13 (72.2)	183 (47.6)	19 (63.3)
Male	149 (55.8)	79 (55.3)	5 (27.8)	201 (52.4)	11 (36.7)
Age at diagnosis (years)					
1-9	197 (73.8)	101 (70.6)	12 (66.7)	264 (68.7)	20 (66.7)
10-17	70 (26.2)	42 (29.4)	6 (33.3)	120 (31.3)	10 (33.3)
Regimen					
A	117 (43.8)	51 (35.6)	8 (44.4)	115 (29.9)	15 (50.0)
B	68 (25.5)	44 (30.8)	6 (33.3)	150 (39.0)	10 (33.3)
C	82 (30.7)	48 (33.6)	4 (22.3)	119 (31.1)	5 (16.7)
Deprivation					
More affluent	139 (52.1)	72 (50.0)	11 (61.1)	179 (46.6)	17 (56.6)
More deprived	128 (47.9)	71 (50.0)	7 (38.9)	205 (53.4)	13 (43.4)

The following Sections 3.3 to 3.7 present the analysis of microbiological samples per phase of treatment. The samples are initially presented in total and then by category of sample: blood culture, respiratory samples and blood/biopsy samples tested for fungal pathogens. A summary section at the end highlights the key patterns identified.

3.3 Induction

This section provides information about the microbiological samples taken during induction, examining the data in the context of the characteristics of the patients and treatment intensity.

The goal of induction is to achieve complete remission and restore normal haematopoiesis. The induction phase, although short in duration, is the first intensive block of treatment and is associated with a high risk of infection related morbidity and mortality. Patients at presentation have invariably been unwell with myelosuppression due to bone marrow infiltration and are admitted to hospital to undergo intensive combination chemotherapy. The combined effects of myelosuppression due to active disease and toxic chemotherapy make patients very vulnerable to infection. Most serious infections happen during induction treatment. Patients under the age of ten years and those treated with higher intensity (4-drug induction) have been shown to be at higher risk of infectious complications (79,81).

Figure 3.3-1 shows the treatment era of the 267 patients who started induction between 1st September 2004 and 31st July 2016: 142 (53.2%) were treated in the UKALL 2003 era, 109 (40.8%) in the UKALL 2011 era, and 16 (6.0%) under interim guidelines (IG) between the two. The length of induction chemotherapy was four weeks in the UKALL 2003 and five weeks in the UKALL 2011 era. The extra week of induction in the UKALL 2011 era was “borrowed” from consolidation, therefore the number of vincristine and intrathecal methotrexate doses were the same overall but the numbering of the weeks changed. In total, 137 (51.3%) patients were treated using regimen A, 96 (35.9%) regimen B, and 34 (12.8%) regimen C. Patients in regimen A received a three-drug induction, with dexamethasone, vincristine, peg-asparaginase and intrathecal methotrexate. Patients in regimens B and C received more intensive treatment: a four-drug induction with the addition of daunorubicin (at a higher dose in regimen C).

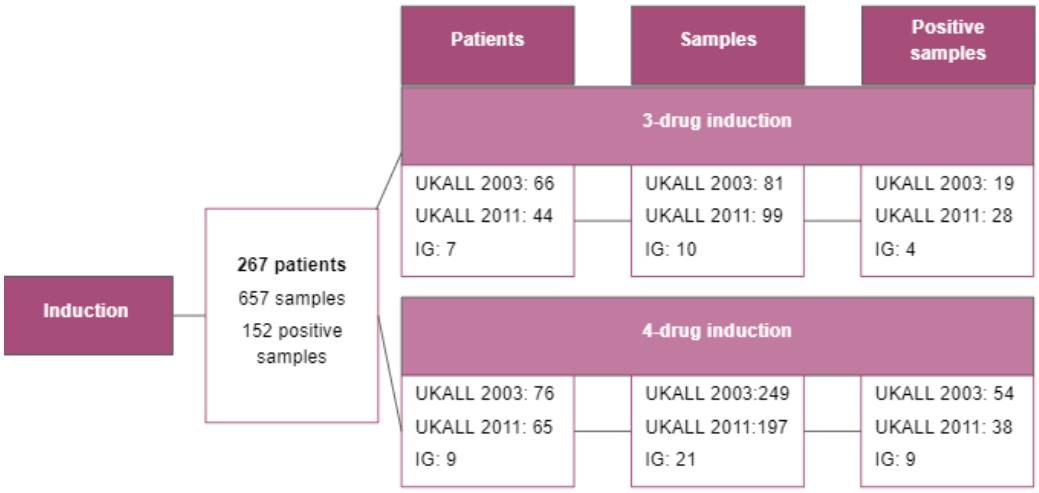

Figure 3.3-1 Patients and their samples during induction by treatment protocol and regimen

3.3.1 Microbiological samples during induction

During induction, 166 (62.2%) patients had at least one sample taken, yielding 657 microbiological samples in total (Table 3.3-1). Half (n=83) of the 166 patients had samples that were positive for at least one pathogen, but only 152/657 (23.1%) of the total samples taken were positive.

The number of samples per patient varied widely: 101 (37.8%) had none, 57 (21.4%) had one, 36 (13.5%) had two, 21 (7.9%) had three, and 52 (19.4%) had four or more (the maximum was 31). Likewise, the number of positive samples per patient also varied widely; 184 (68.9%) had none positive, 51 (19.1%) had one, 12 (4.5%) had two, 11 (4.1%) had three, and nine (3.4%) had four or more (the maximum was nine).

As expected, most patients were diagnosed with B-ALL, the most common lineage, with only a few having a rarer subtype. No significant differences were observed between the samples of patients with different diagnostic subtypes. As noted earlier (Section 3.1), seven patients had predisposing genetic conditions: five had Down syndrome and two ataxia telangiectasia (Table 3.3-1). Patients with such conditions are more susceptible to infection (122). Five of the seven

had at least one sample, and all five had at least one positive sample; the two patients without samples had Down syndrome.

The characteristics of patients who had at least one sample, or at least one positive sample during induction, did not differ greatly from those who did not (Table 3.3-1). Although no statistically significant differences were seen, a few interrelated observations may be worth noting. More patients with B-ALL (73/221, 33.0%) had positive samples compared to patients with T-ALL (10/46, 21.4%). Also, more female patients (43/118, 36.4%) had positive samples compared to males (40/149, 26.8%), and more patients diagnosed under the age of ten had samples (129/197, 65.5%) and positives (66/197, 33.5%) compared to those diagnosed at ten years or over, (37/70, 52.8%) and (17/70, 24.3%) respectively. The same was the case for patients treated with a four-drug induction having positive samples (53/150, 35.3%) compared to those treated with a three-drug regimen (30/117, 25.6%). Across all treatment eras more patients treated with a four-drug induction had at least one positive sample, but this difference was not statistically significant (Table 3.3-2). In UKALL 2003, 26/49 (53.0%) patients who received a four-drug induction had at least one positive compared to 13/35 (37.1%) who received a three-drug induction, and in UKALL 2011 the figures were 22/40 (55.0%) and 15/31 (48.4%) respectively. Although the proportions of patients with at least one sample and positive sample were similar across the two protocols, the raw numbers of total samples and positive samples were higher in patients treated under regimen A of the UKALL 2011 protocols versus those treated under regimen C whereas the opposite was seen in those treated under the UKALL 2003 protocols. This could be due to a combination of factors including better lab techniques in isolating viruses which are more common in younger children (likely to have been treated under regimen A) and differences in practices in keeping patients in the hospital during induction.

Only 28 patients with T-ALL had samples taken during induction (Table 3.3-3), and only ten of these had positive samples. Female patients diagnosed with T-ALL were more likely to have at least one positive sample compared to males with T-ALL (Fisher’s exact p=0.011). However, the numbers were very small as

only eight females diagnosed with T-ALL had at least one sample taken during induction, and six of these had at least one positive sample. Among the 221 patients diagnosed with B-ALL, those treated with a four-drug induction (n= 108) were more likely to have at least one positive sample (43/69 patients with at least one sample) compared to those treated under regimen A (30/69) (Fisher's exact p=0.045).

Table 3.3-4 distributes the samples by age at diagnosis. No differences were seen in samples taken, but differences were evident in positive samples. In patients diagnosed under the age of ten years those who had a four-drug induction were more likely to have at least one positive sample compared to those treated under regimen A (Fisher's exact p=0.022). Only 34 of the 152 positive samples came from 17 patients diagnosed at ten years or over, 26 of which were from 15 of the more intensively treated patients.

UKALL 2011 dexamethasone randomisation

In UKALL 2011, patients were randomised to receive a standard course of dexamethasone for 28 days or a shorter high-dose course for 14 days. This randomisation closed while the trial was open. In our cohort, 32 patients (17 in regimen A, four in B and 11 in C) were randomised to receive a short course of dexamethasone and 30 a standard one. No significant differences were seen in the distribution of samples and positive samples between the two groups (data not presented). However, proportionately more, 27/32 (84.4%) patients randomised to have a short dexamethasone had at least one sample taken compared to 139/235 (59.1%) patients who had standard dexamethasone. Of the 152 positive samples during induction, 29 came from 27 patients who received a short dexamethasone course: 12 blood cultures, 16 respiratory samples and one fungal sample. Of note, there were two deaths in induction under the UKALL 2011 trial, both affecting patients who were randomised to a short course of dexamethasone (due to *Staphylococcus aureus* septicaemia and endocarditis respectively).

The differences in patient characteristics and regimen of treatment are explored by microbiological sample category in Sections 3.3.2-3.3.4. Of the total 657

microbiological samples, 430 (65.4%) were blood cultures, 125 (19.0%) respiratory samples and 102 (15.6%) samples sent for fungal testing. Only 152/657 (23.1%) samples were positive: 73 (48.0%) blood cultures, 70 (46.0%) respiratory samples and nine (6.0%) samples sent for fungal testing.

Table 3.3-1 Characteristics of patients and their samples during induction

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**267**	**101**	**166**	**83**	**657**	**152**
Diagnosis						
B-ALL	221 (82.8)	83 (82.2)	138 (83.1)	73 (87.9)	545 (83)	127 (83.6)
- hyperdiploidy	86 (32.2)	29 (28.7)	57 (34.3)	28 (33.7)	226 (34.4)	58 (38.2)
- NOS	80 (29.9)	35 (34.7)	45 (27.1)	23 (27.7)	167 (25.4)	29 (19.1)
- t(12;21)	44 (16.5)	15 (14.9)	29 (17.5)	15 (18.1)	113 (17.2)	25 (16.5)
- t(9;22)	6 (2.3)	3 (2.9)	3 (1.8)	3 (3.6)	18 (2.8)	7 (4.6)
- hypodiploidy	3 (1.1)	1 (1.0)	2 (1.2)	2 (2.4)	13 (2.0)	3 (1.9)
- MLL rearrangement	2 (0.8)	0 (0.0)	2 (1.2)	2 (2.4)	8 (1.2)	5 (3.3)
T-ALL	46 (17.2)	18 (17.8)	28 (16.9)	10 (12.1)	112 (17)	25 (16.4)
Predisposing conditions	7 (2.6)	2 (1.9)	5 (3.0)	5 (6.0)	31 (4.7)	11 (7.2)
Induction regimen						
A (3-drug)	117 (43.8)	46 (45.5)	71 (42.8)	30 (36.1)	190 (28.9)	51 (33.6)
B or C (4-drug)	150 (56.2)	55 (54.5)	95 (57.2)	53 (63.9)	467 (71.1)	101 (66.4)
Sex						
Female	118 (44.2)	44 (43.6)	74 (44.6)	43 (51.8)	329 (50.1)	84 (55.3)
Male	149 (55.8)	57 (56.4)	92 (55.4)	40 (48.2)	328 (49.9)	68 (44.7)
Age at diagnosis (years)						
1-9	197 (73.8)	68 (67.3)	129 (77.7)	66 (79.5)	504 (76.7)	118 (77.6)
10-17	70 (26.2)	33 (32.7)	37 (22.3)	17 (20.5)	153 (23.3)	34 (23.4)
Deprivation						
More affluent	139 (52.1)	58 (57.4)	81 (48.8)	44 (53.0)	319 (48.6)	68 (44.7)
More deprived	128 (47.9)	43 (42.6)	85 (51.2)	39 (47.0)	338 (51.4)	84 (55.3)

Table 3.3-2 Patients and their samples during induction distributed by protocol and regimen

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**267**	**101**	**166**	**83**	**657**	**152**
UKALL 2003	**143**	**59**	**84**	**39**	**330**	**73**
A (3-drug)	66 (46.5)	31 (52.5)	35 (41.7)	13 (33.3)	81 (24.5)	19 (26.0)
B or C (4-drug)	77 (53.5)	28 (47.5)	49 (58.3)	26 (66.7)	249 (75.5)	54 (74.0)
Interim Guidelines	**16**	**5**	**11**	**7**	**31**	**13**
A (3-drug)	7 (43.7)	2 (40.0)	5 (45.4)	2 (28.6)	10 (32.3)	4 (30.8)
B or C (4-drug)	9 (56.3)	3 (60.0)	6 (54.6)	5 (71.4)	21 (67.7)	9 (69.2)
UKALL 2011	**108**	**37**	**71**	**37**	**296**	**66**
A (3-drug)	44 (41.3)	13 (35.1)	31 (43.7)	15 (40.5)	155 (52.4)	47 (71.2)
B or C (4-drug)	64 (58.7)	24 (64.9)	40 (56.3)	22 (59.5)	141 (47.6)	19 (28.8)

Table 3.3-3 Patients and their samples during induction distributed by lineage

	B-ALL					T-ALL				
	Patients			Samples		Patients			Samples	
	No samples N (%)	At least one sample N (%)	At least one positive N (%)	Total N (%)	Positives N (%)	No samples N (%)	At least one sample N (%)	At least one positive N (%)	Total N (%)	Positives N (%)
Total	**83**	**138**	**73**	**545**	**127**	**18**	**28**	**10**	**112**	**25**
Sex										
Female	39 (47.0)	66 (47.8)	37 (50.7)	279 (51.2)	70 (55.1)	5 (27.8)	8 (28.6)	6 (60.0)	50 (44.6)	14 (56.0)
Male	44 (53.0)	72 (52.2)	36 (49.3)	266 (48.8)	57 (44.9)	13 (72.2)	20 (71.4)	4 (40.0)	62 (55.4)	11 (44.0)
Age at diagnosis (years)										
1-9	60 (72.3)	114 (82.6)	60 (82.2)	430 (78.9)	102 (80.3)	8 (44.4)	15 (53.6)	6 (60.0)	74 (66.0)	16 (64.0)
10-17	23 (27.7)	24 (17.4)	13 (17.8)	115 (21.1)	25 (19.7)	10 (65.6)	13 (46.4)	4 (40.0)	38 (34.0)	9 (36.0)
Induction regimen										
A (3-drug)	44 (53.0)	69 (50.0)	30 (41.1)	187 (34.3)	51 (40.2)	2 (11.1)	2 (7.1)	0 (0.0)	3 (2.7)	0 (0.0)
B or C (4-drug)	39 (47.0)	69 (50.0)	43 (58.9)	358 (65.7)	76 (59.8)	16 (88.9)	26 (92.9)	10 (100.0)	109 (97.3)	25 (100.0)

Table 3.3-4 Patients and their samples during induction distributed by age at diagnosis

	Age at diagnosis: 1-9 years					Age at diagnosis: 10-17 years				
	Patients			Samples		Patients			Samples	
	No samples N (%)	At least one sample N (%)	At least one positive N (%)	Total N (%)	Positives N (%)	No samples N (%)	At least one sample N (%)	At least one positive N (%)	Total N (%)	Positives N (%)
Total	**68**	**129**	**66**	**504**	**118**	**33**	**37**	**17**	**153**	**34**
Diagnosis										
B-ALL	60 (88.2)	114 (88.4)	60 (90.9)	430 (85.3)	102 (86.4)	23 (69.7)	24 (64.9)	13 (76.5)	115 (75.2)	25 (73.5)
T-ALL	8 (11.8)	15 (11.6)	6 (9.1)	74 (14.7)	16 (13.6)	10 (30.3)	13 (35.1)	4 (23.5)	38 (24.8)	9 (26.5)
Sex										
Female	32 (47.1)	60 (46.5)	35 (53.0)	277 (55.0)	68 (57.6)	12 (36.4)	14 (37.8)	8 (47.1)	52 (34.0)	16 (47.1)
Male	36 (52.9)	69 (53.5)	31 (47.0)	227 (45.0)	50 (42.4)	21 (63.6)	23 (62.2)	9 (52.9)	101 (66.0)	18 (52.9)
Induction regimen										
A (3-drug)	45 (66.2)	68 (52.7)	28 (42.4)	171 (33.9)	43 (36.4)	1 (3.0)	3 (8.1)	2 (11.8)	19 (12.4)	8 (23.5)
B or C (4-drug)	23 (33.8)	61 (47.3)	38 (57.6)	333 (66.1)	75 (63.6)	32 (97.0)	34 (91.9)	15 (88.2)	134 (87.6)	26 (76.5)

3.3.2 Blood culture samples

During induction, 152/267 (56.9%) patients had at least one blood culture sample taken and around one third (n=52) of these had samples that were positive (Table 3.3-5). Of the 430 blood culture samples, 73 (16.9%) were positive for at least one bacterial microorganism.

Following the pattern seen for total samples in Section 3.3.1, the number of blood culture samples per patient varied during induction: 115 (43.1%) had none, 57 (21.4%) had one, 40 (14.9%) had two, 15 (5.6%) had three, and 40 (14.9%) had four or more (the maximum was 15). Similarly, the number of positive samples per patient also varied: 215 (80.5%) had none positive, 29 (10.9%) had one, 15 (5.6%) had two, five (1.8%) had three, and three (1.1%) had four (which was the maximum).

As seen with the total number of samples (Table 3.3-1), characteristics of patients who had at least one blood culture or at least one positive blood culture sample during induction did not differ greatly from those who did not (Table 3.3-5).

Looking at the source of blood cultures, around a third were taken from central lines (136/430), with 29 (21.3%) being positive for at least one microorganism (Table 3.3-6). 51 blood cultures were paired samples (see 2.4.1), and only six of those were positive. No source was documented in around a quarter of the blood cultures.

Table 3.3-5 Characteristics of patients and their blood culture samples during induction

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**267**	**115**	**152**	**52**	**430**	**73**
Diagnosis						
B-ALL	221 (82.8)	94 (81.7)	127 (83.6)	45 (86.5)	359 (83.5)	61 (83.6)
- hyperdiploidy	86 (32.2)	33 (28.7)	53 (34.9)	16 (30.8)	146 (34.0)	25 (34.2)
- NOS	80 (29.9)	39 (33.9)	41 (27.0)	16 (30.8)	118 (27.4)	20 (27.4)
- t(12;21)	44 (16.5)	18 (15.7)	26 (17.1)	11 (21.2)	76 (17.7)	13 (17.8)
- t(9;22)	6 (2.3)	3 (2.6)	3 (2.0)	1 (1.9)	9 (2.1)	1 (1.4)
- hypodiploidy	3 (1.1)	1 (0.9)	2 (1.3)	1 (1.9)	8 (1.9)	2 (2.7)
- MLL rearrangement	2 (0.8)	0 (0.0)	2 (1.3)	0 (0.0)	2 (0.5)	0 (0.0)
T-ALL	46 (17.2)	21 (18.3)	25 (16.4)	7 (13.5)	71 (16.5)	12 (16.4)
Sex						
Female	118 (44.2)	47 (40.9)	71 (46.7)	28 (53.8)	216 (50.2)	40 (54.8)
Male	149 (55.8)	68 (59.1)	81 (53.3)	24 (46.2)	214 (49.8)	33 (45.2)
Age at diagnosis (years)						
1-9	197 (73.8)	78 (67.8)	119 (78.3)	41 (78.8)	333 (77.4)	57 (78.0)
10-17	70 (26.2)	37 (32.2)	33 (21.7)	11 (21.2)	97 (22.6)	16 (22.0)
Induction regimen						
A (3-drug)	117 (43.8)	54 (47.0)	63 (41.4)	18 (34.6)	136 (31.6)	25 (34.2)
B or C (4-drug)	150 (56.2)	61 (53.0)	89 (58.6)	34 (65.4)	294 (68.4)	48 (65.8)
Deprivation						
More affluent	139 (52.1)	63 (54.8)	76 (50.0)	27 (51.9)	218 (50.7)	35 (47.9)
More deprived	128 (47.9)	52 (45.2)	76 (50.0)	25 (48.1)	212 (49.3)	38 (52.1)

A total of 86 bacteria were isolated from 73 positive blood cultures, of which 11 had more than one pathogen - two bacteria were isolated from nine, and three bacteria from two. Looking at the Gram category in the positive blood cultures by source (Table 3.3-7), the majority (59/86, 68.6%) were Gram +ve

and over one third of those (21/59, 35.6%) were isolated from central line cultures. Only 27 Gram -ve bacteria were isolated, of which 11 were from central line cultures and none in paired cultures. Overall, the most commonly isolated microorganisms were coagulase-negative *Staphylococcus* (CONS) isolated 22 times in 17 patients, followed by *S. aureus* (13/59) and *E. coli* (10/59) (Table 3.3-8). In the six positive paired culture samples which came from six different patients, the following bacteria were grown: two CONS, three *S. aureus* and one *Streptococcus pneumoniae*.

Two-hundred and sixty-three (39.6%) blood cultures were taken at the time of neutropenia, and 53 (20.2%) of these were positive (Table 3.3-8). Regardless of neutrophil count, Gram +ve bacteria were the most commonly isolated and CONS was the most frequently isolated microorganism. Gram -ve bacteria were, however, seen more frequently at times of neutropenia (21/27).

Table 3.3-6 Distribution of blood culture samples and positives during induction by source

	Blood cultures N (%)	**Positive blood cultures N (%)**
Total	**410**	**73**
Source		
Central line	136 (31.6)	29 (39.7)
Peripheral	131 (30.6)	21 (28.8)
Paired	51 (11.8)	6 (8.2)
Unknown	112 (26.0)	17 (23.3)

Table 3.3-7 Distribution of Gram category of bacteria isolated during induction by source and neutropenia status

	Microorganisms	
	Gram -ve N (%)	**Gram +ve N (%)**
Total	**27**	**59**
Source		
Central line	11 (40.8)	21 (35.6)
Peripheral	7 (25.9)	18 (30.5)
Paired	0 (0.0)	6 (10.2)
Unknown	9 (33.3)	14 (23.7)
Neutropenia		
Yes	21 (77.8)	42 (71.2)
No	6 (22.2)	17 (28.8)

Table 3.3-8 Isolated bacteria during induction by Gram category and neutropenia status

Bacterial microorganisms			
	Total	**Neutropenia**	**Non-neutropenia**
Total	**86**	**63**	**23**
Gram +ve bacteria			
Total	**59**	**42**	**17**
CONS	22	14	8
S. aureus	13	12	1
Viridans group streptococci	8	7	1
Enterococcus species	7	4	3
Strep. pneumoniae	3	3	0
Actinobacteria	2	0	2
MRSA	1	0	1
Streptococcus dysgalactiae	2	1	1
Brevibacillus agri	1	1	0
Gram -ve bacteria			
Total	**27**	**21**	**6**
E. coli	10	9	1
Enterobacter species	5	5	0
Pseudomonas aeruginosa	3	3	0
Bacteroides fragilis	3	0	3
Klebsiella pneumoniae	2	1	1
Moraxella catarrhalis	1	0	1
Leptrotrichia trevisanii	1	1	0
Raoultella species	1	1	0
Serratia marcescens	1	1	0

Fifty-six microorganisms were isolated in blood cultures of patients who had a four-drug induction, versus 30 in those treated under regimen A (Table 3.3-9). CONS was the most common isolate in both groups. There were more Gram -ve bacteria in more intensively treated patients (18 versus nine in patients treated under regimen A). *E. coli* was isolated in eight blood cultures

and methicillin-resistant *Staphylococcus aureus* (MRSA) in one blood culture from a patient who received a four-drug regimen.

Table 3.3-9 Isolated bacteria during induction by treatment intensity

Bacteria in blood cultures			
3-drug induction Regimen A		**4-drug induction Regimen B/C**	
Total	**30**	**Total**	**56**
CONS	9	CONS	13
S. aureus	5	*E. coli*	8
Bacteroides fragilis	3	*S. aureus*	8
Enterobacter cloacae	3	Viridans group streptococci	7
E. coli	2	*Enterococcus* species	6
Strep. pneumoniae	2	*Pseudomonas aeruginosa*	2
Enterococcus species	1	*Klebsiella pneumoniae*	2
Pseudomonas aeruginosa	1	*Enterobacter* species	2
Viridans group *Streptococcus*	1	*Streptococcus pneumoniae*	1
Strep. dysgalactiae	1	MRSA	1
Actinomyces naeslundii	1	*Moraxella catarrhalis*	1
Bacillus species	1	Actinobacteria species	1
		Leptortrichia trevisanii	1
		Raoultella species	1
		Serratia marcenscens	1
		Brevibacillus agrii	1

3.3.3 Respiratory samples

During induction, 61/267 (22.8%) patients had at least one respiratory sample, of which over two thirds (43/61, 70.5%) had at least one positive respiratory sample (Table 3.3-10). Of the total 125 respiratory samples, over half (70/125, 56.0%) were positive for at least one bacterial, viral or fungal pathogen.

Following the pattern seen for total samples (Section 3.3.1), the number of respiratory samples per patient varied. Two-hundred and six (77.2%) had

none, 34 (12.7%) had one, 12 (4.5%) had two, six (2.3%) had three, and nine (3.4%) had four or more (the maximum was eight). Similarly, the number of positive samples per patient also varied: 224 (83.9%) had none positive, 30 (11.2%) had one, five (1.9%) had two, four (1.5%) had three, and four (1.5%) had four or more (the maximum was six).

The diagnostic characteristics of patients who had at least one respiratory sample or positive sample were broadly similar to those of patients with none (Table 3.3-10). However, 48/62 (77.4%) patients with respiratory samples and 34/43 (79.0%) with positive samples were diagnosed under the age of ten.

Table 3.3-10 Characteristics of patients and their respiratory samples during induction

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**267**	**206**	**61**	**43**	**125**	**70**
Diagnosis						
B-ALL	221 (82.8)	170 (82.5)	51 (83.6)	37 (86.0)	101 (80.8)	57 (81.4)
- hyperdiploidy	86 (32.2)	61 (29.6)	25 (41.0)	19 (44.2)	47 (37.6)	32 (45.7)
- NOS	80 (29.9)	66 (32.0)	14 (23.0)	9 (20.9)	27 (21.6)	9 (12.9)
- t(12;21)	44 (16.5)	38 (18.4)	6 (9.8)	4 (9.3)	15 (12.0)	7 (10.0)
- t(9;22)	6 (2.3)	4 (1.9)	2 (3.3)	2 (4.7)	3 (2.4)	3 (4.3)
- hypodiploidy	3 (1.1)	1 (0.5)	2 (3.3)	1 (2.3)	3 (2.4)	1 (1.4)
- MLL rearrangement	2 (0.8)	0 (0.0)	2 (3.3)	2 (4.7)	6 (4.8)	5 (7.1)
T-ALL	46 (17.2)	36 (17.5)	10 (16.3)	6 (14.0)	24 (19.2)	13 (18.6)
Sex						
Female	118 (44.2)	89 (43.2)	29 (47.5)	20 (46.5)	71 (56.8)	40 (57.1)
Male	149 (55.8)	117 (56.8)	32 (52.5)	23 (53.5)	54 (43.2)	30 (42.9)
Age at diagnosis (years)						
1-9	197 (73.8)	149 (72.3)	48 (78.7)	34 (79.0)	100 (80.0)	55 (78.6)
10-17	70 (26.2)	57 (27.7)	13 (21.3)	9 (21.0)	25 (20.0)	15 (21.4)
Induction regimen						
A (3-drug)	117 (43.8)	98 (47.6)	19 (31.1)	15 (34.9)	29 (23.2)	21 (30.0)
B or C (4-drug)	150 (56.2)	108 (52.4)	42 (68.9)	28 (65.1)	96 (76.8)	49 (70.0)
Deprivation						
More affluent	139 (52.1)	111 (53.9)	28 (45.9)	19 (42.2)	51 (40.8)	28 (40.0)
More deprived	128 (47.9)	95 (46.1)	33 (54.1)	24 (57.8)	74 (59.2)	42 (60.0)

Table 3.3-11 shows respiratory samples distributed by source: 80/125 (64.0%) samples and 47/70 (67.1%) positive samples were nose/throat swabs. Thirteen samples came from nasopharyngeal aspirates (NPA) and nine of these were positive. There were ten from bronchioalveolar lavage

(BAL), four of which (from four different patients) were positive for ten pathogens in total. Of the 12 samples from endotracheal tube (ET) secretions, five pathogens were isolated from three samples (from three different patients).

Overall, 85 pathogens were isolated from 70 positive respiratory samples. Around half the pathogens (41/85) isolated were respiratory viruses and 26/41 were from nose/throat swabs (Table 3.3-12). Only 16 bacterial pathogens were isolated, mostly from nose/throat swabs. The following pathogens were isolated in the four positive BAL samples: one positive for *Enterococcus* species, *Candida albicans* and CONS, another for *C. albicans* and CONS, another for parainfluenza 1 and rhinovirus and another for parainfluenza 4, *C. albicans* and respiratory syncytial virus (RSV). Five pathogens were isolated in three positive samples from ET secretions: one positive for *C. albicans*, another for *C. albicans*, rhinovirus and *Enterococcus* and a third one had *Pseudomonas aeruginosa*.

The more commonly isolated bacteria were enterococci (Table 3.3-11). All bacterial pathogens were isolated from patients who had a four-drug induction (apart from two samples positive for *Coliform* species). The most commonly isolated virus was rhinovirus found in 24/41 (58.5%) positive respiratory viral samples, followed by RSV (8/41) and parainfluenza viruses (7/41). All but three positive samples for viral testing came from patients diagnosed under the age of ten.

Of the 28 fungal pathogens isolated, 24 were *Candida albicans,* with 19 isolated from nose/throat samples. *C. albicans* was, however, isolated from the BAL samples of three patients and ET secretions of two, which could have been potentially clinically significant. Of the three patients with fungal pathogens isolated from a BAL sample, one died during induction due to infective endocarditis. This patient was aged three years and treated under regimen A and had one BAL sample positive for parainfluenza 4, *C. albicans* and RSV.

Table 3.3-11 Distribution of respiratory samples and positives during induction by source

	Respiratory samples N (%)	Positive respiratory samples N (%)
Total	**125**	**70**
Source		
Nose/throat swab	80 (64.0)	47 (67.1)
NPA	13 (10.4)	9 (13.0)
ET secretions	12 (9.6)	5 (7.1)
BAL	10 (8.0)	4 (5.7)
Sputum	9 (7.2)	4 (5.7)
Unknown	1 (0.8)	1 (1.4)

Table 3.3-12 Distribution of microorganism type in positive respiratory samples during induction by source

Positive respiratory samples				
	Total	**Bacterial**	**Viral**	**Fungal**
Total	**85**	**16**	**41**	**28**
Source				
Nose/throat swab	26 (30.6)	9 (56.3)	26 (63.4)	19 (67.9)
NPA	9 (10.6)	0 (0.0)	9 (22.0)	0 (0.0)
ET secretions	5 (5.8)	2 (12.5)	1 (2.4)	2 (7.1)
BAL	10 (11.8)	3 (18.8)	4 (9.8)	3 (10.7)
Sputum	6 (7.0)	2 (12.5)	0 (0.0)	4 (14.3)
Unknown	1 (1.2)	0 (0.0)	1 (2.4)	0 (0.0)

Table 3.3-13 Pathogens isolated during induction by microorganism type

Bacteria		**Viruses**		**Fungal**	
Total	**16**	**Total**	**41**	**Total**	**28**
Enterococcus species	4	Rhinovirus	24	*C. albicans*	24
CONS	3	RSV	8	*C. parapsilosis*	1
Coliform species	3	Parainfluenzas	7	Yeasts	3
Staph aureus	3	Influenzas	2		
Moraxella catarhallis	1				
P. aeruginosa	1				
Strep. pneumoniae	1				

3.3.4 Fungal pathogens

At least one sample sent for fungal testing was taken from 49/267 (18.4%) patients during induction (1-9 samples per patient). The samples came from 15 patients treated under regimen A, 18 under regimen B and 16 under regimen C. Only five patients (three females and two males) had positive fungal samples: two had one positive sample, two had two, and one had three.

Overall, 102 samples were sent for fungal testing during induction; all but two of which were blood samples - one was a liver biopsy, and one was an oesophageal biopsy sample. Blood samples were mostly (91/100) tested for Aspergillus antigen, six for microscopy and culture (MCS), two for β-d-glucan and one each for Candida antigen, Cryptococcus antigen and mycology serology. Only nine blood samples were positive.

All but one of the five patients were treated under regimen A. A male patient (aged≥10) diagnosed with B-ALL with t(9;22) died secondary to fungal septicaemia. He had two positive blood samples for Aspergillus antigen and one positive blood culture for *C. albicans* within three days, and died the day after the positive blood culture. Another male patient (age group 1-4) diagnosed with B-ALL with t(12;21) was treated under regimen A and had a blood sample positive for β-d-glucan followed by a sample positive for

Candida antigen two days later. This patient had a significant delay in treatment and required treatment modification, received an interim maintenance phase after induction, and was moved to consolidation following that.

The remaining three patients with positive fungal samples at induction survived to the end of treatment and did not have any scheduling modifications. One female patient had *Aspergillus fumigatus* isolated from a blood culture (age group 1-4, treated under regimen B), another female had *C. tropicalis* isolated from two blood cultures (age group 5-9) and finally, a female patient had a blood culture positive for C. albicans (age group 1-4).

3.3.5 Summary of findings during induction

Most microbiological samples taken during induction were blood cultures (430/657), which was not surprising as patients are mostly in hospital during this phase, especially if their treatment is complicated by fever, neutropenia or clinical concerns. While in hospital, it would be less likely to contract a respiratory pathogen, especially viruses, as patients with any symptoms of cough/coryza are isolated in side rooms.

Although not statistically significant, a few observations may be of note. Proportionately more female patients were found to have positive samples during induction. This is consistent with the published data showing that females are more likely to develop profound prolonged neutropenia and associated infection related morbidity, and that they are at higher risk of infection related mortality (5,8,10). As expected, proportionately more patients diagnosed under the age of ten and those treated with a four-drug induction had positive samples. These findings are in line with other studies showing that younger patients and those more intensively treated are more likely to have infectious complications (8,12,79,81).

Around half of patients had at least one blood culture taken during induction, with one in three patients with a sample having at least one that was positive. Around two thirds of the bacteria in blood culture samples were isolated during

a time of neutropenia. Over two thirds of bacteria were Gram +ve (predominantly CONS, *S. aureus*, viridans group streptococci and enterococci) and less than a third Gram -ve (mostly *E. coli*, *Enterobacter* and *Pseudomonas aeruginosa*).

Less than one in four patients had at least one respiratory sample, with two thirds of these having at least one that was positive, the majority of whom (77.4%) were diagnosed under the age of ten years. There were four patients with positive BAL samples and three with positive ET secretions samples. These findings imply significant infection-related morbidity as BAL samples are reserved for very unwell patients with no clear source of infection (but potential concerns about respiratory source), and ET secretions are from ventilated patients in paediatric intensive care units.

Although over a hundred samples were sent for fungal testing, only nine were positive, as microbiologically documented invasive fungal diseases are often suspected but difficult to confirm (157). The high index of suspicion in patients with prolonged episodes of febrile neutropenia or other protracted complicated infective episodes is evidenced by the number of samples sent for fungal testing during induction. As presented above, one death with confirmed candidaemia was seen in our cohort. Another patient had long delays and a modification in treatment secondary to implied morbidity from fungal infection during induction.

As presented in section 3.1.3, ten patients died during induction: eight from infective causes during treatment for induction and two patients on palliative care post induction treatment.

The next section presents information about the samples of the 249 patients who started consolidation phase treatment (10 patients died, four moved to different treatment protocols, two started conditioning for haematopoietic stem cell transplant (HSCT), and two skipped the consolidation phase due to treatment-related morbidity during induction and had interim maintenance instead).

3.4 Consolidation

The goal of consolidation is to eradicate residual blast cells. This part of treatment, which is initiated after remission has been achieved, aims to kill any residual leukaemia cells that could potentially cause relapse.

Figure 3.4-1 shows the treatment eras of the 249 patients who started consolidation between 29th September 2004 and 7th September 2016: 134 (53.8%) were treated in the UKALL 2003 era, 101 (40.6%) in the UKALL 2011 era, and 14 (5.6%) under interim guidelines (IG) between the two.

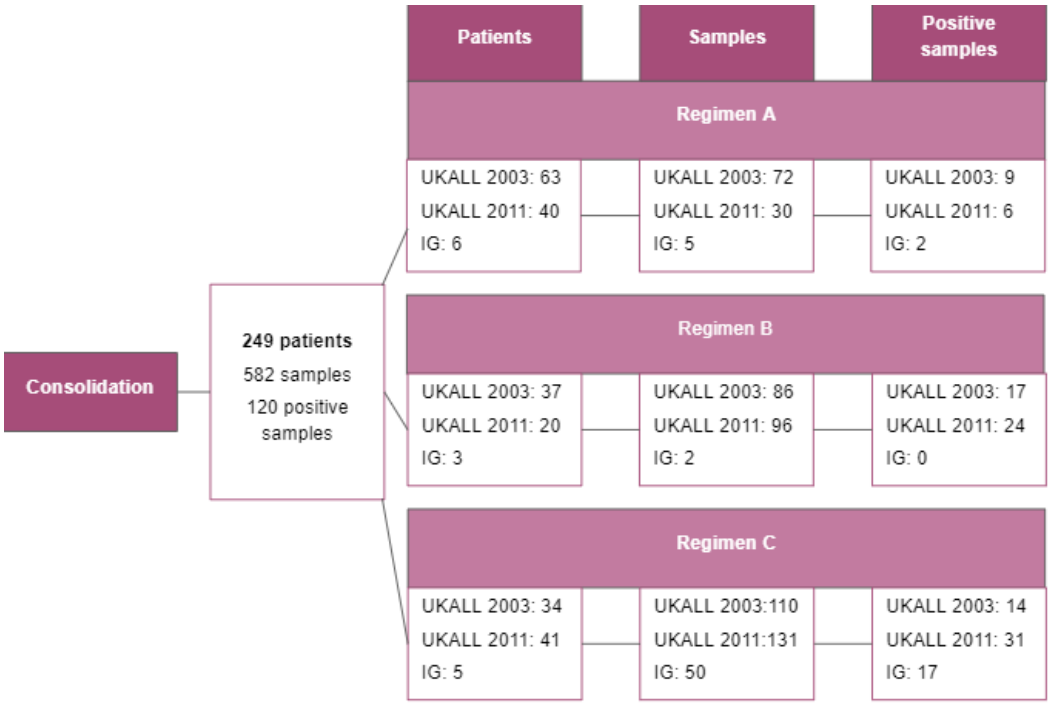

Figure 3.4-1 Patients and their samples during consolidation by treatment protocol and regimen

Consolidation length and chemotherapy agents administered varied with regimen (Figure 3.4-2). Patients treated under regimen A in the UKALL 2003 era had a four-week consolidation period with daily oral 6-mercaptopurine and weekly intrathecal methotrexate. Regimen A in the UKALL 2011 era was the same but lasted three weeks (as discussed in Section 1.4 the numbering of weeks changed and the “extra” consolidation week of UKALL 2003 was moved to induction, as patients in the UKALL 2003 era had four weeks of induction and in the UKALL 2011 era they had five). In all eras, regimen B consolidation lasted five weeks with daily oral 6-mercaptopurine for four weeks and weekly intrathecal methotrexate (four weeks in the UKALL 2003 and three in the UKALL 2011 era, again due to the re-numbering of weeks in UKALL 2011), with the addition of cyclophosphamide and cytarabine in four pulses (standard BFM consolidation). Regimen C had the longest consolidation, nine weeks in the UKALL 2003 era and ten in UKALL 2011. In regimen C, there was the addition of vincristine and peg-asparaginase to regimen B (augmented BFM consolidation). So, patients treated under regimen C had a considerably longer consolidation phase with more chemotherapy agents.

The combination of cyclophosphamide and cytarabine administered in regimens B and C has been shown to increase susceptibility to infection (8, 76). Cytarabine, in particular, can cause severe and prolonged myelosuppression and bowel toxicity and has been associated with more bloodstream infections; particularly with viridans group streptococci. In regimen C, the combination of cytarabine and peg-asparaginase causes prolonged neutropenia which increases the risk of infection, particularly bacteraemia. Patients receiving cytarabine and peg-asparaginase have been found to have a higher incidence of infection and prolonged aplasia (76). So, patients treated under regimen B, and more so those treated under regimen C, would be expected to have more microbiological samples and, more importantly, more positive samples.

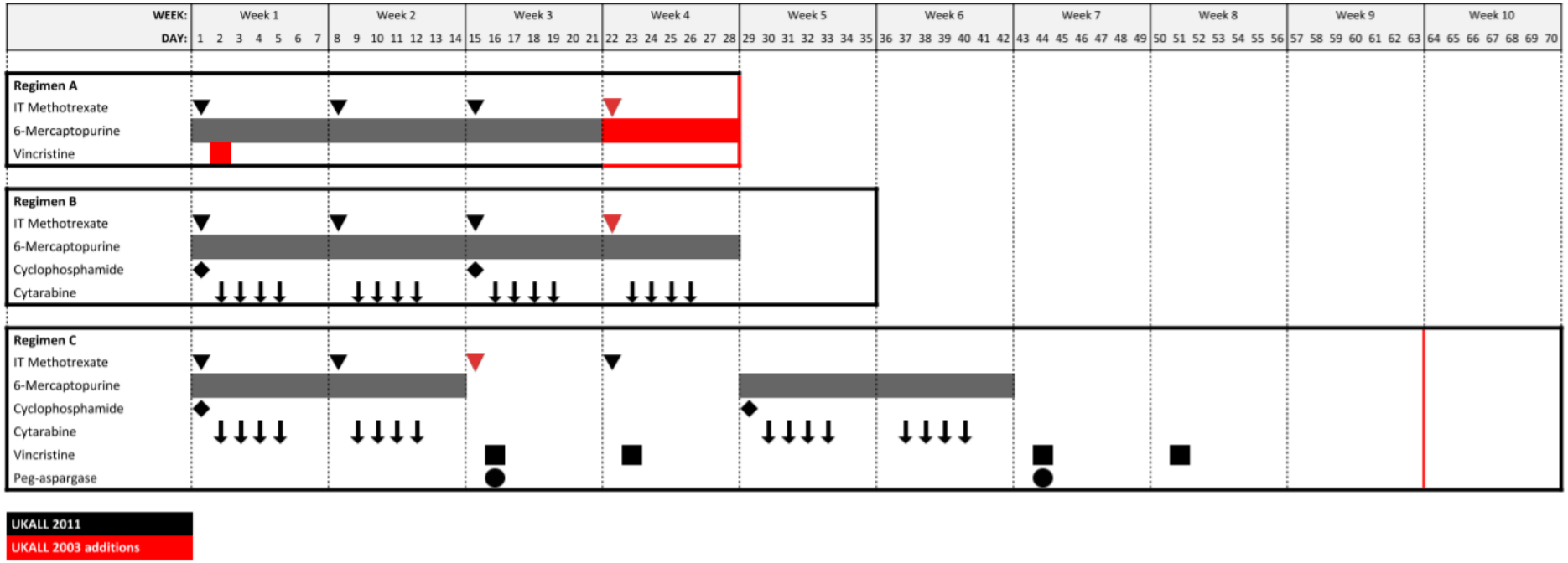

Figure 3.4-2 Consolidation chemotherapy administration by regimen. The black borders represent duration of consolidation as defined by the treatment protocol in each regimen of UKALL 2011 era. The black and grey symbols represent common administration timing of agents in both treatment eras. The red colour shows the additions in UKALL 2003 era.

3.4.1 Microbiological samples during consolidation

During consolidation, 151 (60.6%) patients had at least one sample, yielding 582 microbiological samples in total (Table 3.4-1). Just under half (n=68) of the 151 patients had samples that were positive for at least one pathogen, but only 120/582 (20.6%) samples were positive.

As seen for induction, the number of samples per patient varied widely: 98 (39.4%) had none, 42 (16.9%) had one, 30 (12.0%) had two, 21 (8.4%) had three, 20 (8.0%) had four, and 38 (15.3%) had five or more (the maximum was 18). Likewise, the number of positive samples per patient also varied: 181 (72.7%) had none positive, 43 (17.3%) had one, 12 (4.8%) had two, three (1.2%) had three, and 10 (4.0%) had four or more (the maximum was seven).

Sample rates and 95% confidence intervals per 100 person-weeks are shown in Table 3.4-1. The rate of patients with a sample was 19.1 per 100 persons-weeks (95%CI 16.3-22.4), i.e. 19 among 100 patients a week had the first of their consolidation samples taken. Five among 100 patients a week had the first sample testing positive (95%CI 4.1-6.5). No difference was observed in rates of patients sampled by age, sex or regimen, but there was a suggestion that the rate of patients with positive samples was higher in regimen B at 7.6 (95% CI 4.9-11.6) than A, at 4.3 (2.6-7.3) or C, at 4.6 (3.3-6.5) although differences were not statistically significant.

Examining the rate of all samples by regimen, it can be seen that at 52.1 (95% CI 45.1-60.2) per 100 person-weeks, the rate of all samples for regimen B was significantly higher than that of regimen A, at 31.2 (25.8-37.7: χ^2=18.2, p<0.001) and regimen C at 32.0 (28.5-35.8; χ^2=27.5, p<0.001) per 100 person-weeks. More importantly, at 11.6 (8.6-15.8) per 100 person-weeks, the rate of all positive samples was also higher in regimen B compared to regimen A at 5.0 (3.1-8.0; χ^2=9.2, p=0.002) and regimen C at 6.8 (5.3-8.7; χ^2=7.2, p=0.007) per 100 person-weeks.

The differences between patient characteristics and regimen of treatment by microbiological sample category are explored in Sections 3.4.2 to 3.4.4. Of the total 582 microbiological samples, 399 (68.6%) were blood cultures, 109 (18.7%) respiratory samples and 74 (12.7%) samples were sent for fungal testing. Only 120 samples were positive; 49 (40.8%) blood cultures, 59 (49.2%) respiratory samples and 12 (10.0%) samples sent for fungal testing.

Table 3.4-1 Person weeks and sampling rates per 100 person-weeks during consolidation, distributed by sex, age and regimen

	Patients						Samples					
	At least one sample			At least one positive sample			All samples			All positive samples		
	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate (95%CI)	N	Person-weeks	Rate(95%CI)
Total	151	791.0	19.1(16.3-22.4)	68	1318.3	5.2(4.1-6.5)	582	1606.5	36.2(33.4-39.3)	120	1606.5	7.5(6.2-8.9)
Sex												
Male	86	426.9	20.1(16.3-24.9)	37	743.4	5.0(3.6-6.9)	330	884.6	37.3(33.5-41.6)	60	884.6	6.8(5.3-8.7)
Female	65	364.2	17.8(14.0-22.8)	31	574.9	5.4(3.8-7.7)	252	721.9	34.9(30.9-39.5)	60	721.9	8.3(6.5-10.7)
Age												
1-9	111	544.9	20.4(16.9-24.5)	50	898.2	5.6(4.2-7.3)	406	1118.8	36.3(32.9-40.0)	89	1118.8	8.0(6.5-9.8)
10-17	40	246.1	16.3(11.9-22.2)	18	420.1	4.3(2.7-6.8)	176	487.7	36.1(31.1-41.8)	31	487.7	6.4(4.5-9.0)
Regimen												
A	46	251.6	18.3(13.7-24.4)	14	324.5	4.3(2.6-7.3)	107	342.9	31.2(25.8-37.7)	17	342.9	5.0(3.1-8.0)
B	39	202.1	19.3(14.1-26.4)	21	276.8	7.6(4.9-11.6)	184	353.0	52.1(45.1-60.2)	41	353.0	11.6(8.6-5.8)
C	66	337.3	19.6(15.4-24.9)	33	717.0	4.6(3.3-6.5)	291	910.6	32.0(28.5-35.8)	62	910.6	6.8(5.3-8.7)

Rates and 95% confidence intervals (CI) are shown per 100 person-weeks. Person-weeks were calculated from the start of consolidation until the first sample, or the first positive sample, to examine the rate of the first sample or the first positive sample; for all samples and all positive samples, follow-up continued from the start of consolidation until the start of interim maintenance, the next phase or death. Differences between rates were tested using the Mantel-Haenszel method; statistically significant differences were seen for the rate of all samples for regimen B compared to the rate of all samples for regimen A (χ^2=18.2, p<0.001), and for the rate of all positive samples for regimen B compared to the rate of all positive samples for regimen A (χ^2=9.2, p=0.002).

3.4.2 Blood culture samples

Around half, 143/249 (57.4%) of the patients had at least one blood culture sample; only 33/143 (23.0%) of these had samples that were positive (Table 3.4-2). Of the total 399 blood culture samples; 49 (12.3%) were positive for at least one bacterial microorganism.

Sample rates and 95% confidence intervals per 100 person-weeks are shown in Table 3.4-2. Among patients, the rate of at least one sample was 17.4 per 100 persons-weeks (95%CI 14.8-20.5) and the rate of at least one positive sample was 2.2 (95%CI 1.6-3.2). No statistically significant differences were seen for age, sex and regimen. However, as in Table 3.4-1, significant differences were evident in the rates of samples by regimen. In regimen A, of the 41 patients who had at least one sample, only one patient had one that was positive. In regimen B, 13/37 (35.1%) patients with samples had at least one that was positive, and in regimen C the proportion was 19/65 (29.7%). Again, as in Table 3.4-1, at 34.0 (95%CI 28.4-40.7) per 100 person-weeks, the rate of all samples for regimen B was significantly higher than that of regimen A (21.6, 95%CI 17.2-27.1; χ^2=9.62, p=0.002) and regimen C (22.5, 95%CI 19.6-25.8; χ^2=13.0, p<0.001). Although the rate of all positive samples was also higher in regimen B (5.4, 95%CI 3.4-8.4) compared to regimen C (3.2, 95%CI 2.2-4.6), this was not statistically significant (χ^2=3.2, p=0.072).

Table 3.4-2 Person weeks and sampling rates per 100 person-weeks for blood cultures during consolidation distributed by sex, age and regimen

	Patients						Samples					
	At least one sample			At least one positive sample			All samples			All positive samples		
	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate (95%CI)	N	Person-weeks	Rate(95%CI)
Total	143	822.0	17.4(14.8-20.5)	33	1468.3	2.2(1.6-3.2)	399	1606.5	24.8(22.5-27.4)	49	1606.5	3.1(2.3-4.0)
Sex												
Male	80	452.1	17.7(14.2-22.0)	18	820.7	2.2(1.4-3.5)	237	884.6	26.8(23.6-30.4)	26	884.6	2.9(2.0-4.3)
Female	63	369.9	17.0(13.3-21.8)	15	647.6	2.3(1.4-3.8)	162	721.9	22.4(19.2-26.2)	23	721.9	3.2(2.1-4.8)
Age												
1-9	105	559.6	18.8(15.5-22.7)	23	1003.4	2.3(1.5-3.4)	281	1118.8	25.1(22.3-28.2)	36	1118.8	3.2(2.3-4.5)
10-17	38	262.4	14.5(10.5-19.9)	10	464.9	2.2(1.2-4.0)	118	487.7	24.2(20.2-29.0)	13	487.7	2.7(1.5-4.6)
Regimen												
A	41	261.8	15.7(11.5-21.3)	1	345.1	0.3(0.04-2.1)	74	342.9	21.6(17.2-27.1)	1	342.9	0.3(0.04-2.1)
B	37	209.1	17.7(12.8-24.4)	13	318.4	4.1(2.4-7.0)	120	353.0	34.0(28.4-40.7)	19	353.0	5.4(3.4-8.4)
C	65	351.1	18.5(14.5-23.6)	19	804.8	2.4(1.5-3.7)	205	910.6	22.5(19.6-25.8)	29	910.6	3.2(2.2-4.6)

Differences between rates were tested using the Mantel-Haenszel method; statistically significant differences were seen for the rate of all blood cultures for regimen B compared to the rate of all blood cultures for regimen A (χ^2=9.62, p=0.002) and for regimen C (χ^2=13.0, p<0.001), but not for the rate of all positive blood cultures for regimen B compared to the rate of all positive blood cultures for regimen C (χ^2=3.2, p=0.072).

Looking at the source of blood cultures, over half (228/399) were taken from central lines, of which 31/228 (13.6%) were positive with at least one microorganism (Table 3.4-3 and Table 3.4-4). Of the 80 blood cultures that were paired samples (see 2.4.1), only six were positive and no source was documented for 65/399 (16.3%).

A total of 52 bacteria were isolated from 49 positive blood cultures; three samples had two bacteria. The majority of bacteria (37/52, 71.1%) were Gram +ve and 24/37 (64.9%) were isolated from central line cultures (Table 3.4-4). Coagulase-negative *Staphylococcus* (CONS) was the most common Gram +ve microorganism (21/52), followed by viridans group streptococci (8/52) (Table 3.4-5). CONS was isolated 21 times from 17 patients. Of the 16 Gram -ve bacteria isolated; 11 were isolated from central lines and three were found in paired cultures. The most commonly isolated Gram -ve microorganism was *Pseudomonas aeruginosa* (isolated five times from four patients). In the six positive paired culture samples which came from six different patients, the following bacteria were grown: two CONS, and one each of *Pseudomonas aeruginosa*, *E. coli*, viridans group *Streptococcus* and *Capnocytophaga sputinega*.

Just over half of blood cultures (213/399, 53.4%) were taken during neutropenia; 29 (13.6%) of those were positive with at least one microorganism (30 microorganisms in total) and 21/30 were Gram +ve (Table 3.4-4). The neutrophil count was not known at the time of three positive samples; one each with *Pseudomonas aeruginosa*, CONS and viridans group *Streptococcus*. Regardless of neutrophil count, the most commonly isolated bacteria were Gram +ve. The majority of isolated Gram -ve bacteria (9/15), however, were detected at a time of neutropenia.

As shown in Table 3.4-6, 31 bacteria were isolated from the blood cultures of patients treated under regimen C and 20 under regimen B. The one positive blood culture from a patient treated under regimen A had a growth of *S. aureus*. There were ten Gram -ve bacteria isolated from six patients treated under regimen C and five from four patients treated under regimen B. Twenty-

one Gram +ve bacteria were isolated from 16 patients in regimen C and nine in regimen B; CONS was the most common microorganism in both regimens. Viridans group streptococci were isolated from three samples in regimen C and two in regimen B (all from different patients). Overall, a wider range of microorganisms was isolated from the 29 patients treated under regimen C with positive samples compared to the 19 patients in regimen B (Table 3.4-6).

Table 3.4-3 Distribution of blood culture samples and positives during consolidation by source

	Blood cultures N (%)	**Positive blood cultures N (%)**
Total	**399**	**49**
Source		
Central line	228 (57.1)	31 (63.4)
Paired	80 (20.1)	6 (12.2)
Peripheral	26 (6.5)	6 (12.2)
Unknown	65 (16.3)	6 (12.2)

Table 3.4-4 Distribution of Gram category of bacteria isolated during consolidation by source and neutropenia status

	Microorganisms	
	Gram -ve N (%)	**Gram +ve N (%)**
Total	**16**	**37**
Source		
Central line	11 (68.8)	24 (64.8)
Paired	3 (18.7)	3 (8.1)
Peripheral	2 (12.5)	4 (10.8)
Unknown	0 (0.0)	6 (16.3)
Neutropenia		
Yes	9 (56.3)	21 (56.7)
No	5 (31.2)	14 (37.8)
Missing	2 (12.5)	2 (6.5)

Table 3.4-5 Isolated bacteria during consolidation by Gram category and neutropenia status

Bacterial microorganisms			
	Total	**Neutropenia**	**Non-neutropenia**
Total	**52**	**30**	**19**
Gram +ve bacteria			
Total	**37**	**21**	**14**
Microorganism			
CONS	21*	13	7
Viridans group streptococci	8*	3	4
Diptheroid species	3	3	0
S. aureus	3	1	2
Streptococcus species	1	0	1
Gemella morbillorum	1	1	0
Gram -ve bacteria			
Total	**15**	**9**	**5**
Pseudomonas aeruginosa	5*	4	0
E. coli	2	1	1
Pseudomonas species	1	0	1
Moraxella catarrhalis	1	1	0
Moraxella osloensis	1	0	1
Klebsiella oxytoca	1	0	1
Stenotrophomonas maltophilia	1	0	1
Capnocytophaga sputigena	1	1	0
Fusobacterium species	1	1	0
Sphingomonas paucimobilis	1	1	0

*Three microorganisms were isolated at time of unknown full blood count result (see text above)

Table 3.4-6 Isolated bacteria during consolidation by regimen (B and C)

Bacteria in blood cultures			
Regimen B		**Regimen C**	
Total	**20**	**Total**	**31**
Microorganism		**Microorganism**	
CONS	9	CONS	12
Pseudomonas aeruginosa	3	*Diptheroid* species	3
Viridans group streptococci	3	*Pseudomonas aeruginosa*	2
E. coli	1	Viridans group streptococci	2
S aureus	1	*Moraxella* species	2
Streptococcus species	1	*E. coli*	1
Fusobacterium species	1	*S. aureus*	1
Aerococcus viridans	1	*Klebsiella oxytoca*	1
		Pseudomonas species	1
		Aerococcus viridans	1
		Capnocytophaga sputigena	1
		Gemella morbillorum	1
		Micrococcus species	1
		Sphingomonas paucimobilis	1
		Stenotrophomonas maltophilia	1

3.4.3 Respiratory samples

During consolidation, 66/249 (26.5%) patients had at least one respiratory sample; with over two thirds (45/66) of these having at least one positive sample (Table 3.4-7). The majority of patients with respiratory samples (49/66) were diagnosed under the age of ten years. Of the 109 respiratory samples; just over half (59/109) were positive with at least one bacterial, viral or fungal pathogen.

Respiratory sample rates and 95% confidence intervals per 100 person-weeks are shown in Table 3.4-7. The rate of patients with at least one sample was 5.0 per 100 persons-weeks (95%CI 3.9-6.3) and the rate with at least one

positive sample was 3.1 (95%CI 2.3-4.2). No statistically significant differences were seen for age, sex and regimen. As in Table 3.4-1 and Table 3.4-2, significant differences were seen in sample rates for regimen B (9.6, 95%CI 6.9-13.5) compared to regimen C (5.7, 95%CI 4.4-7.5; χ^2=5.75, p=0.016). Patients treated under regimen B also had higher rates of positive samples (4.8, 95%CI 3.0-7.7) compared to those treated under regimens A (3.8, 95%CI 2.2-6.5) and C (3.2, 95%CI 2.2-4.6); but the numbers were small and the differences were not statistically significant.

Table 3.4-7 Person weeks and sampling rates per 100 person-weeks for respiratory samples during consolidation distributed by sex, age and regimen

	Patients						Samples					
	At least one sample			At least one positive sample			All samples			All positive samples		
	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate (95%CI)	N	Person-weeks	Rate(95%CI)
Total	66	1323.1	5.0(3.9-6.3)	45	1430.8	3.1(2.3-4.2)	109	1606.5	6.8(5.6-8.2)	59	1606.5	3.7(2.8-4.7)
Sex												
Male	39	729.3	5.3(3.9-7.3)	26	794.3	3.3(2.2-4.8)	62	884.6	7.0(5.5-9.0)	32	884.6	3.6(2.6-5.1)
Female	27	539.9	4.5(3.1-6.6)	19	636.5	3.0(1.9-4.7)	47	721.9	6.5(4.9-8.7)	27	721.9	3.7(2.6-5.5)
Age												
1-9	49	918.3	5.3(4.0-7.1)	36	980.3	3.7(2.6-5.1)	82	1118.8	7.3(5.9-9.1)	46	1118.8	4.1(3.1-5.5)
10-17	17	404.8	4.2(2.6-6.8)	9	450.6	2.0(1.0-3.8)	27	487.7	5.5(3.8-8.1)	13	487.7	2.7(1.5-4.6)
Regimen												
A	20	317.8	6.3(4.1-9.8)	13	328.5	4.0(2.3-6.8)	23	342.9	6.7(4.5-10.1)	13	342.9	3.8(2.2-6.5)
B	18	281.3	6.4(4.0-10.2)	12	307.7	3.9(2.2-6.9)	34	353.0	9.6(6.9-13.5)	17	353.0	4.8(3.0-7.7)
C	18	724.0	3.9(2.7-5.6)	20	794.7	2.5(1.6-3.9)	52	910.6	5.7(4.4-7.5)	29	910.6	3.2(2.2-4.6)

Rates and 95% confidence intervals (CI) are shown per 100 person-weeks. Person-weeks were calculated from the start of consolidation until the first respiratory sample, or the first positive respiratory sample, to examine the rate of the first respiratory sample or the first positive respiratory sample; for all respiratory samples and all positive respiratory samples, follow-up continued from the start of consolidation until the start of interim maintenance, the next phase or death. Differences between rates were tested using the Mantel-Haenszel method; a statistically significant difference was seen for the rate of all respiratory samples for Regimen B compared to the rate of all respiratory samples for Regimen C (χ^2=5.75, p=0.016).

There were 66 pathogens isolated from 59 positive respiratory samples; seven samples were positive for two pathogens. Over two thirds of respiratory samples (79/109) and positives (47/59) were nose/throat swabs (Table 3.4-8). Most pathogens (43/66) isolated were respiratory viruses, and the vast majority of these (39/43) were from nose/throat swabs (Table 3.4-9 and). There were four samples from bronchioalveolar lavage (BAL); two of which were positive and three samples from endotracheal tube (ET) secretions, one of which was positive.

The most commonly isolated virus was rhinovirus; found in 27/43 samples, followed by parainfluenza viruses (6/43) and respiratory syncytial virus (RSV) (5/43). The two positive BAL samples isolated *Pseudomonas aeruginosa* and RSV respectively (two different patients). Of the three samples from endotracheal tube (ET) secretions, one was positive for *Aspergillus fumigatus*. Only one patient diagnosed over ten years had a positive respiratory sample for influenza A H3, all other respiratory samples that were positive for viruses came from patients diagnosed under the age of ten years (data not presented).

Seven bacterial pathogens were isolated, most were from sputum samples where the most commonly isolated bacteria were *Enterococcus* species (3/7). Of the 16 fungal pathogens isolated, 11 were *Candida albicans*; two came from sputum samples (of the same patient four days apart) and nine from the nose/throat swabs of seven patients. There was one nose/throat swab with *Candida kefyr* and two with yeasts. Two samples were positive for *Aspergillus fumigatus* (from ET secretions of a male patient with T-ALL treated under regimen B, and a sputum sample from a female with B-ALL treated under regimen A).

Table 3.4-8 Distribution of respiratory samples and positives during consolidation by source

	Respiratory samples N (%)	Positive respiratory samples N (%)
Total	**109**	**59**
Source		
Nose/throat swab	79 (72.5)	47 (79.7)
Sputum	16 (14.7)	6 (10.2)
NPA	6 (5.5)	2 (3.4)
BAL	4 (3.7)	2 (3.4)
ET secretions	3 (2.8)	1 (1.7)
Unknown	1 (0.9)	1 (1.7)

Table 3.4-9 Microorganism type in positive respiratory samples during consolidation by source

Positive respiratory samples				
	Total N (%)	Bacterial N (%)	Viral N (%)	Fungal N (%)
Total	**66**	**7**	**43**	**16**
Source				
Nose/throat swab	53 (80.3)	2 (28.6)	39 (90.7)	12 (75.0)
NPA	2 (3.0)	0 (0.0)	2 (4.6)	0 (0.0)
ET secretions	1 (1.5)	0 (0.0)	0 (0.0)	1 (6.3)
BAL	2 (3.0)	1 (14.3)	1 (2.3)	0 (0.0)
Sputum	7 (10.7)	4 (57.1)	0 (0.0)	3 (18.7)
Unknown	1 (1.5)	0 (0.0)	1 (2.3)	0 (0.0)

Table 3.4-10 Pathogens isolated during consolidation by microorganism type

Bacteria		**Viruses**	
Total	**7**	**Total**	**43**
Enterococcus species	3	Rhinovirus	27
Coliform species	1	Parainfluenzas	6
Moraxella catarrhalis	1	RSV	5
P. aeruginosa	1	Influenzas	3
Strep. pneumoniae	1	Metapneumovirus	1
		Adenovirus	1

3.4.4 Fungal pathogens

There were 74 samples from 32 patients (four treated under regimen A, 15 under regimen B and 13 under regimen C) sent for fungal testing during consolidation, 12 of which were positive from six patients. Sixty-nine (93.2%) of the samples sent for fungal testing were blood tests; 67 tested for Aspergillus antigen, one for β-d-glucan and one for Candida antigen.

Nine of the 12 positive samples were blood samples; seven were positive for Aspergillus antigen, one for β-d-glucan and one for Candida antigen. The remaining three positive samples were tested for microscopy and culture (MCS) from an arm skin biopsy, a lung tissue biopsy and cerebrospinal fluid (CSF). Seven positive samples were taken at a time of neutropenia; five were blood samples, four of which tested positive for Aspergillus antigen and one for mycology serology, the remaining two were the arm biopsy and lung tissue biopsy mentioned above.

The 12 positive fungal samples came from six patients:

- A female patient had a lung tissue biopsy positive for *Candida parapsilosis* (aged≥10, treated under regimen C).

- A female had two blood samples positive for Aspergillus antigen (within four days) and a CSF sample positive for *Aspergillus fumigatus* on MCS three days later (age 5-9, treated under regimen A).
- A male had two samples positive for Aspergillus antigen two days apart (aged 1-4 and treated under regimen B). This was the only male patient with positive fungal samples during consolidation.
- A female had a blood sample positive for Aspergillus antigen (age≥10 and treated under regimen C).
- A female had two samples positive for Aspergillus antigen three days apart (age 1-4 and treated under regimen C)
- Another female had two blood samples positive for β-d-glucan and Candida antigen each on the same day followed by an arm biopsy positive for *Candida tropicalis* ten days later (age≥10 and treated under regimen B).

All patients but one were diagnosed with B-ALL, and all but one moved to the next treatment phase after consolidation. The mean duration of consolidation in weeks for these patients was longer than patients without positive fungal samples (9.5 versus 6.3 weeks).

The only death in a patient with positive fungal samples was a male patient with B-ALL was treated under regimen B. He had two blood samples positive for Aspergillus antigen taken two days apart. The death, however, was caused by disseminated varicella zoster infection.

3.4.5 Summary of findings during consolidation

We saw that patients treated under regimen B had a higher rate of all samples and positive samples compared to those treated under regimen A and regimen C. High-risk patients have been reported to have a higher incidence of infectious complications during consolidation compared to standard-risk patients (7,11). More febrile episodes and more bloodstream infections in high-risk patients have been associated with the use of cytarabine (8,78).

At first glance, the regimen differences between B and C are, perhaps, somewhat surprising as it would be expected that patients treated under regimen C would be more likely to have more samples and positive samples. As shown in Figure 3.4-1, however, although shorter, the treatment in regimen B was more concentrated with myelosuppressive agents. By contrast, in regimen C, weeks three and four of consolidation provided a gap between the myelosuppressive agents of 6-mercaptopurine and cytarabine. This break from myelosuppressive intensity may have contributed to the lower sample rates of regimen C compared to regimen B. Another possible contributor, was the fact that some of the patients treated under regimen C would have been treated under regimen A in induction and therefore would have had “lighter” induction treatment compared to those in regimen B, depending on the day of treatment that they moved to regimen C. The cumulative effect of intensive chemotherapy in the patients treated under regimen B through both phases, may have contributed to the susceptibility to infection.

Almost 70% of the microbiological samples taken during consolidation were blood cultures, and around 10% of these were positive. Among patients who had at least one positive blood culture, all but one were treated under regimen B or C. The bacteria isolated were 70% Gram +ve and 30% Gram –ve; similar to the proportions found in induction. The most common Gram +ve bacteria were CONS and viridans group streptococci; *Pseudomonas aeruginosa* the most common Gram -ve. Respiratory samples accounted for nearly 20% of microbiological samples, and over half were positive, mostly with viruses. The most commonly isolated viruses were rhinovirus, parainfluenza viruses and RSV. A quarter of patients had one or more respiratory samples taken, and over two thirds of the patients tested had at least one respiratory sample that was positive. One in eight patients had at least one sample sent for fungal testing, with less than a fifth of these patients having at least one positive.

There were two deaths during consolidation: One patient (with two blood samples positive for Aspergillus antigen) died due to disseminated varicella

zoster septicaemia. Another patient died due to peritonitis without any positive microbiological samples from this patient (Section 3.1.3).

The next section presents the samples of the 247 patients who started interim maintenance phase treatment (two patients died, one moved to different treatment protocol, one started conditioning for haematopoietic stem cell transplant (HSCT), and two patients skipped consolidation and had interim maintenance after induction). All samples refer to interim maintenance I, i.e. the first interim maintenance phase for patients treated under UKALL 2003 randomised to receive two courses of this phase and the only interim maintenance course in all other patients.

3.5 Interim maintenance

Figure 3.5-1 shows the treatment era of the 247 patients who started interim maintenance between 4th November 2004 and 5th December 2016: 131 (53.0%) were treated in the UKALL 2003 era, 102 (41.3%) in the UKALL 2011 era, and 14 (5.7%) under interim guidelines (IG) between the two.

The length of interim maintenance was eight weeks except under regimens A and B of UKALL 2011 where this phase lasted nine weeks. In total, 108 (43.7%) patients were treated on regimen A, 61 (24.7%) on regimen B, and 78 (31.6%) regimen C.

During interim maintenance patients on regimens A and B received dexamethasone, vincristine, weekly oral methotrexate and daily 6-mercaptopurine; patients on the UKALL 2011 protocols also had intrathecal methotrexate. Patients treated under regimen C had intravenous and intrathecal methotrexate, vincristine and peg-asparaginase. In the UKALL 2011 trial there was a randomisation between standard and high-dose methotrexate. From the 62 patients treated under the UKALL 2011 trial

protocol, only 13 received high-dose methotrexate; four on regimen A, two on B and seven on C (some patients declined the second randomisation). High-dose methotrexate carries more risk of complication with mucositis and potential bacterial translocation from the gut into the circulation (116).

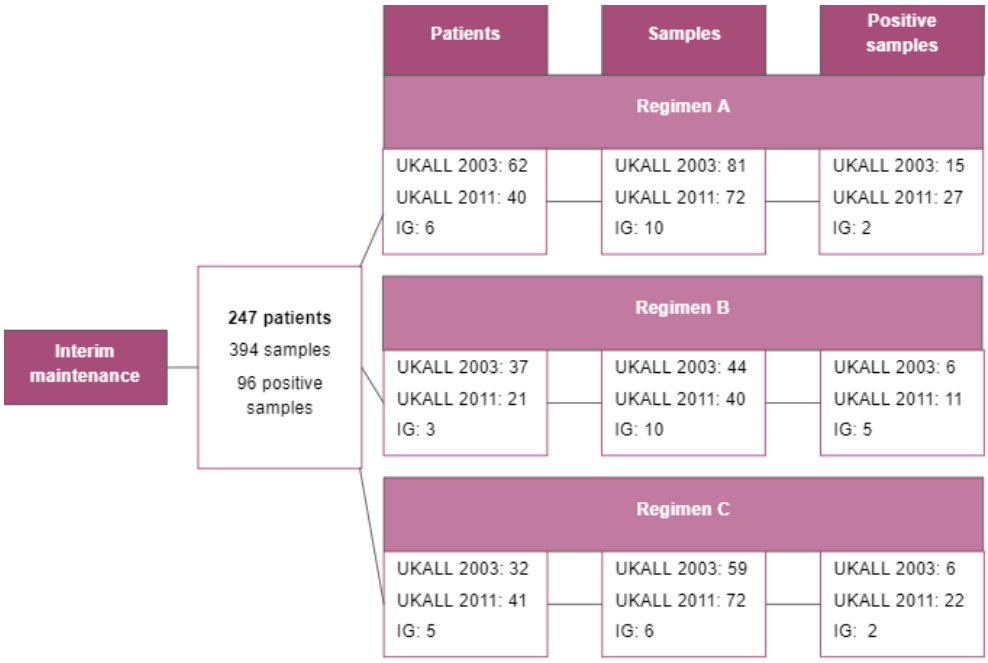

Figure 3.5-1 Patients and their samples by treatment protocol and regimen

3.5.1 Microbiological samples during interim maintenance

During interim maintenance, 124/247 (50.2%) patients had at least one sample taken yielding 394 microbiological samples in total (Table 3.5-1). Just under half (n=59) of the 124 patients had samples that were positive for at least one pathogen, but only 96/394 (24.4%) of the total samples were positive.

As seen in other phases, the number of samples per patient varied widely: 123 (49.6%) had none, 41 (16.6%) had one, 20 (8.1%) had two, 23 (9.3%) had three, 14 (5.7%) had four, and 26 (10.7%) had five or more (the maximum was 12). Likewise, the number of positive samples per patient also varied: 188 (76.1%) had none, 39 (15.8%) had one, ten (4.1%) had two, five (2.0%) had three, and five (2.0%) had four or more (the maximum was five).

Among the four patients with Down syndrome, two had at least one sample taken during interim maintenance, but none had any positive samples (Table 3.5-1). Only 33/124 patients with samples were diagnosed at the age of ten or over; only nine of whom were female (data not presented). Those treated under regimen C were more likely to have at least one sample compared to patients treated under regimens A and B (Fisher's exact p=0.04). The characteristics of patients who had at least one positive sample taken during interim maintenance did not differ greatly from those who did not.

The differences between patient characteristics and regimen of treatment by microbiological sample category are explored in Sections 3.5.2 to 3.5.4. Of the total 394 microbiological samples, 262 (66.5%) were blood cultures, 106 (26.9%) respiratory samples and 26 (6.6%) samples sent for fungal testing. Only 96/394 (24.4%) samples were positive; 41 (42.7%) blood cultures and 55 (57.3%) respiratory samples.

Table 3.5-1 Characteristics of patients and their samples during interim maintenance

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**247**	**123**	**124**	**59**	**394**	**96**
Diagnosis						
B-ALL	205 (83.0)	108 (87.8)	97 (78.2)	48 (81.4)	316 (80.2)	82 (85.4)
- hyperdiploidy	81 (32.8)	49 (39.8)	32 (25.8)	17 (28.8)	124 (31.5)	32 (33.3)
- NOS	78 (31.6)	44 (35.8)	34 (27.4)	14 (23.7)	97 (24.6)	21 (21.9)
- t(12;21)	42 (17.0)	14 (11.4)	28 (22.6)	14 (23.7)	82 (20.8)	23 (24.0)
- hypodiploidy	2 (0.8)	1 (0.8)	1 (0.8)	1 (1.7)	6 (1.5)	4 (4.2)
- MLL rearrangement	2 (0.8)	0 (0.0)	2 (1.6)	2 (3.4)	7 (1.8)	2 (2.1)
T-ALL	42 (17.0)	15 (12.2)	27 (21.8)	11 (18.6)	78 (19.8)	14 (14.6)
Predisposing conditions	4 (1.6)	2 (1.6)	2 (1.6)	0 (0.0)	5 (1.3)	0 (0.0)
Sex						
Female	110 (44.5)	63 (51.2)	47 (37.9)	26 (44.1)	155 (39.3)	45 (46.9)
Male	137 (55.5)	60 (49.8)	77 (62.1)	33 (55.9)	239 (60.7)	51 (53.1)
Age at diagnosis (years)						
1-9	185 (74.9)	92 (74.8)	93 (75.0)	49 (83.0)	298 (75.6)	76 (79.1)
10-17	62 (25.1)	31 (25.2)	31 (25.0)	10 (17.0)	96 (24.4)	20 (20.9)
Regimen						
A	108 (43.7)	62 (50.4)	46 (37.1)	25 (42.4)	163 (41.4)	44 (45.8)
B	61 (24.7)	30 (24.4)	31 (25.0)	17 (28.8)	94 (23.9)	22 (22.9)
C	78 (31.6)	31 (25.2)	47 (37.9)	17 (28.8)	137 (34.8)	30 (31.3)
Deprivation						
More affluent	131 (53.0)	71 (57.7)	60 (48.4)	26 (44.1)	174 (44.2)	43 (44.8)
More deprived	116 (47.0)	52 (42.3)	64 (51.6)	33 (55.9)	220 (55.8)	53 (55.2)

3.5.2 Blood culture samples

During interim maintenance, 108 (43.7%) patients had at least one blood culture sample taken, yielding 262 samples in total (Table 3.5-2). A quarter (n=27, 25.0%) of the 108 patients had blood cultures that were positive for at least one bacterial microorganism, but only 41/262 (15.6%) of the total blood cultures were positive.

Following the pattern seen for total samples in Section 3.5.1, the number of blood cultures per patient varied: 139 (56.3%) had none, 42 (17.0%) had one, 26 (10.5%) had two, 17 (6.9%) had three, and nine (3.6%) had four and 14 (5.7%) had five or more (the maximum was 12). Similarly, the number of positive samples per patient also varied: 220 (89.0%) had none that were positive, 18 (7.3%) had one, five (2.0%) had two, and four (1.7%) had three or more (the maximum was ten).

The characteristics of patients who had at least one sample, or at least one positive sample, during interim maintenance did not differ greatly from those who did not (Table 3.5-2). There were only 27 patients with positive blood cultures; 12 were treated under regimen C and 21 were diagnosed under the age of ten years.

Overall there were only 41 positive blood cultures with 20 from patients treated under regimen C. Seven of those came from patients who had high-dose methotrexate. None of the positive blood cultures came from patients who had high-dose methotrexate in regimen A or B (data not presented).

Table 3.5-2 Characteristics of patients and their blood culture samples during interim maintenance

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**247**	**139**	**108**	**27**	**262**	**41**
Diagnosis						
B-ALL	205 (83.0)	116 (83.5)	89 (82.4)	20 (74.0)	211 (80.5)	33 (80.5)
- hyperdiploidy	81 (32.8)	52 (37.4)	29 (26.8)	7 (25.9)	81 (30.9)	13 (31.7)
- NOS	78 (31.6)	46 (33.0)	32 (29.6)	5 (18.5)	65 (24.8)	5 (12.1)
- t(12;21)	42 (17.0)	17 (12.2)	25 (23.1)	5 (18.5)	53 (20.2)	9 (21.9)
- hypodiploidy	2 (0.8)	1 (0.7)	1 (0.9)	1 (3.7)	6 (2.3)	4 (9.7)
- MLL rearrangement	2 (0.8)	0 (0.0)	2 (1.8)	2 (7.4)	6 (2.3)	2 (4.8)
T-ALL	42 (17.0)	23 (16.5)	19 (17.6)	7 (26.0)	51 (19.5)	8 (19.5)
Sex						
Female	110 (44.5)	68 (48.9)	42 (38.9)	12 (44.4)	106 (40.4)	21 (51.2)
Male	137 (55.5)	71 (51.1)	66 (61.1)	15 (55.6)	156 (59.6)	20 (48.8)
Age at diagnosis (years)						
1-9	185 (74.9)	105 (75.5)	80 (74.0)	21 (77.8)	196 (74.8)	31 (75.6)
10-17	62 (25.1)	34 (24.5)	28 (26.0)	6 (22.2)	66 (25.2)	10 (24.4)
Regimen						
A	108 (43.7)	67 (48.2)	41 (38.0)	9 (33.3)	100 (38.2)	14 (34.1)
B	61 (24.7)	34 (24.5)	27 (25.0)	6 (22.2)	59 (22.5)	7 (17.0)
C	78 (31.6)	38 (27.3)	40 (37.0)	12 (45.5)	103 (39.3)	20 (48.9)
Deprivation						
More affluent	131 (53.0)	78 (56.1)	53 (49.0)	11 (40.7)	116 (44.3)	18 (43.9)
More deprived	116 (47.0)	61 (43.9)	55 (51.0)	16 (59.3)	146 (55.7)	23 (56.1)

Looking at the source of blood cultures, over half (148/262,56.5%), were taken from central lines, with 31/148 being positive with at least one microorganism (Table 3.5-3). Forty-seven blood cultures were paired samples (see Section

2.4.1) with only two positives. No source was documented for 46/262 (17.6%) blood cultures.

A total of 48 bacteria were isolated from 41 positive blood cultures; one sample had three and one had four bacteria. The majority (32/48, 66.6%) were Gram +ve and most of these (27/32, 84.3%) were isolated from central line cultures (Table 3.5-4). The most commonly isolated Gram +ve microorganism was coagulase-negative *Staphylococcus (*CONS) (16/32) followed by viridans group streptococci (3/32) and *Propionibacterium* species (3/32). There were only 16 Gram -ve bacteria isolated; 11 were isolated from central line cultures. The most commonly isolated Gram -ve bacteria were *Klebsiella* species (4/16), *Pseudomonas aeruginosa* (3/16) and *E. coli* (3/16). There were two positive paired cultures from two patients; with *E. coli* and CONS.

One hundred and six blood cultures were taken at time of neutropenia, only 16 of which were positive (Table 3.5-5). Regardless of neutropenia status, the most commonly isolated microorganism was CONS. Interestingly only one third of the bacteria (16/48, 33.3%) were isolated at time of neutropenia.

Twenty-six microorganisms were isolated in blood cultures of patients treated under regimen C (Table 3.5-6). CONS was the most common pathogen across all regimens. Gram -ve bacteria were more commonly isolated in regimen C (11/16).

Looking at the bacteria from the positive blood cultures from patients who received high-dose methotrexate, five Gram +ve and two Gram -ve were found. The following microorganisms were isolated: CONS in two samples two days apart from one patient; CONS, *Diphtheroid* species and *Clostridium perfringens* in three different samples and more than a week apart each from another patient; and finally *E. coli* in two samples from a third patient over a week apart (data not presented).

Table 3.5-3 Distribution of blood culture samples and positives during interim maintenance by source

	Blood cultures N (%)	Positive blood cultures N (%)
Total	**262**	**41**
Source		
Central line	148 (56.5)	31 (75.6)
Paired	47 (17.9)	2 (4.9)
Peripheral	21 (8.0)	3 (7.3)
Unknown	46 (17.6)	5 (12.2)

Table 3.5-4 Distribution of Gram category of bacteria isolated during interim maintenance by source and neutropenia status

	Microorganisms	
	Gram -ve N (%)	Gram +ve N (%)
Total	**16**	**32**
Source		
Central line	11 (68.7)	27 (84.4)
Paired	1 (6.3)	1 (3.1)
Peripheral	2 (12.5)	1 (3.1)
Unknown	2 (12.5)	3 (9.4)
Neutropenia		
Yes	5 (31.2)	11 (34.3)
No	10 (62.5)	21 (65.7)
Missing	1 (6.3)	0 (0.0)

Table 3.5-5 Isolated bacteria during interim maintenance by Gram category and neutropenia status

Bacterial microorganisms			
	Total	**Neutropenia**	**Non-neutropenia**
Total	**48**	**16**	**32**
Gram +ve bacteria			
Total	**32**	**11**	**21**
CONS	16	6	10
Viridans group streptococci	3	1	2
Propionibacterium species	3	2	1
Bacillus species	2	0	2
Micrococcus species	2	1	1
S. aureus	2	0	2
Diphtheroid species	1	0	1
Streptococcus pneumoniae	1	0	1
Streptococcus species	1	1	0
Clostridium perfringens	1	0	1
Gram -ve bacteria			
Total	**16**	**5**	**11**
Pseudomonas aeruginosa	3	0	3
E. coli	3	1	2
Klebsiella oxytoca	2	0	2
Klebsiella pneumoniae	2	2	0
Stenotrophomonas maltophilia	2	0	2
Aeromonas caviae	2	0	2
Moraxella osloensis	1	1	0
Environmental Gram -ve bacillus	1	1	0

Table 3.5-6 Isolated bacteria during interim maintenance by regimen

Bacteria in blood cultures					
Regimen A		**Regimen B**		**Regimen C**	
Total	**14**	**Total**	**8**	**Total**	**26**
CONS	6	CONS	2	CONS	8
Propionibacterium spp.	2	*S. aureus*	2	*P. aeruginosa*	2
Strep. pneumoniae	1	*P. aeruginosa*	1	*Klebsiella oxytoca*	2
Klebsiella pneumoniae	1	*Klebsiella pneumoniae*	1	*E. coli*	2
Viridans group *Streptococcus*	1	Viridans group *Streptococcus*	1	*Micrococcus* spp.	2
Moraxella osloensis	1	*E. coli*	1	*Aeromonas caviae*	2
Bacillus spp.	1			*Stenotrophomonas maltophilia*	2
Streptococcus spp.	1			Viridans group *Streptococcus*	1
				Clostridium perfingens	1
				Propionibacterium spp.	1
				Bacillus licheniform	1
				Diptheroid species	1
				Environmental Gram -ve bacillus	1

3.5.3 Respiratory samples

During interim maintenance, 63/247 (25.5%) patients had at least one respiratory sample with around two thirds (40/63, 63.5%) having samples that were positive for at least one pathogen. Of the total 106 respiratory samples, just over half (55/106, 51.8%), were positive (Table 3.5-7).

Following the pattern seen for the total samples (Section 3.5.1), the number of respiratory samples taken per patient varied. One hundred and eighty-four (74.5%) had none, 39 (15.8%) had one, 15 (6.0%) had two, six (2.4%) had three, and 3 (1.2%) had four or more (the maximum was seven). Similarly, the number of positive samples per patient also varied: 207 (83.8%) had none

positive, 31 (12.6%) had one, five (2.0%) had two, and four (1.6%) had three or more (the maximum was four).

The diagnostic characteristics of patients who had at least one respiratory sample and at least one positive respiratory sample taken during interim maintenance were broadly similar to those who did not (Table 3.5-7).

Table 3.5-7 Characteristics of patients and their respiratory samples during interim maintenance

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**247**	**184**	**63**	**40**	**106**	**55**
Diagnosis						
B-ALL	205 (83.0)	153 (83.1)	52 (82.5)	34 (85.0)	90 (84.9)	49 (89.1)
- hyperdiploidy	81 (32.8)	59 (32.0)	22 (34.9)	14 (35.0)	39 (36.8)	19 (34.5)
- NOS	78 (31.6)	65 (35.3)	13 (20.6)	10 (25.0)	25 (23.6)	16 (29.1)
- t(12;21)	42 (17.0)	26 (14.1)	16 (25.4)	10 (25.0)	25 (23.6)	14 (25.5)
- hypodiploidy	2 (0.8)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- MLL rearrangement	2 (0.8)	1 (0.5)	1 (1.6)	0 (0.0)	1 (0.9)	0 (0.0)
T-ALL	42 (17.0)	31 (16.9)	11 (17.5)	6 (15.0)	16 (15.1)	6 (10.9)
Sex						
Female	110 (44.5)	83 (45.1)	27 (42.8)	18 (45.0)	40 (37.7)	24 (43.6)
Male	137 (55.5)	101 (54.9)	36 (57.2)	22 (55.0)	66 (62.3)	31 (56.4)
Age at diagnosis (years)						
1-9	185 (74.9)	131 (71.2)	54 (85.7)	34 (85.0)	89 (83.9)	45 (81.8)
10-17	62 (25.1)	53 (28.8)	9 (14.3)	6 (15.0)	17 (16.1)	10 (8.2)
Regimen						
A	108 (43.7)	77 (41.8)	31 (49.2)	20 (50.0)	57 (53.8)	30 (54.5)
B	61 (24.7)	44 (23.9)	17 (27.0)	11 (27.5)	25 (23.6)	15 (27.3)
C	78 (31.6)	63 (34.3)	15 (23.8)	9 (22.5)	24 (22.6)	10 (18.2)
Deprivation						
1-3 (affluent)	131 (53.0)	103 (55.9)	28 (44.4)	17 (42.5)	48 (45.3)	25 (45.4)
4-5 (deprived)	116 (47.0)	81 (44.1)	35 (55.6)	23 (57.5)	58 (54.7)	30 (54.6)

Table 3.5-8 shows respiratory samples distributed by source. Most respiratory samples (77/106, 72.6%) and positive samples (45/55, 81.8%) were

nose/throat swabs. Of the seven bronchioalveolar lavage (BAL) samples two were positive (two different patients).

A total of 59 pathogens were isolated from 55 positive respiratory samples; three respiratory samples were positive for two pathogens. Most of the pathogens (47/59, 79.6%) isolated were respiratory viruses and most of those were from nose/throat swabs (39/47, 82.9%) (Table 3.5-9). There were only three samples with bacterial pathogens isolated, *Haemophilus* species and CONS, from sputum and bronchoalveolar lavage (BAL) samples (Table 3.5-10). Of the two positive BAL samples, one was positive for RSV and another positive for *Haemophilus influenza*e, adenovirus and *C. albicans*

As was the case in the previous treatment phases, the most commonly isolated virus was rhinovirus; found in 27/47 respiratory viral samples. Respiratory syncytial virus (RSV) was found in 9/47 positive samples and parainfluenza viruses in 5/47 (Table 3.5-10). Only seven of the isolated viruses came from samples of patients diagnosed at age ten or more (data not presented).

All nine samples that were positive for fungal pathogens isolated *Candida albicans*; seven of which from nose/throat swabs.

Table 3.5-8 Distribution of respiratory samples and positives during interim maintenance by source

	Respiratory samples N (%)	Positive respiratory samples N (%)
Total	**106**	**55**
Source of sample		
Nose/throat swab	77 (72.6)	45 (81.8)
NPA	13 (12.3)	4 (7.4)
Sputum	7 (6.6)	2 (3.6)
BAL	7 (6.6)	2 (3.6)
Unknown	2 (1.9)	2 (3.6)

Table 3.5-9 Microorganisms in positive respiratory samples during interim maintenance by source

	Positive respiratory samples			
	Total N (%)	Bacterial N (%)	Viral N (%)	Fungal N (%)
Total	**59**	**3**	**47**	**9**
Source of sample				
Nose/throat swab	46 (77.9)	0 (0.0)	39 (82.9)	7 (77.8)
NPA	4 (6.7)	0 (0.0)	4 (8.5)	0 (0.0)
Sputum	3 (5.0)	2 (66.7)	0 (0.0)	1 (11.1)
BAL	4 (6.7)	1 (33.3)	2 (8.6)	1 (11.1)
Unknown	2 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)

Table 3.5-10 Pathogens isolated during interim maintenance by microorganism type

Bacteria		**Viruses**	
Total	**3**	**Total**	**47**
Haemophilus species	2	Rhinovirus	27
CONS	1	RSV	9
		Parainfluenzas	5
		Influenzas	4
		Metapneumovirus	1
		Adenovirus	1

3.5.4 Fungal pathogens

There were 26 blood samples sent for Aspergillus antigen testing during interim maintenance from 23 patients; all of which were negative.

3.5.5 Microbiological samples during interim maintenance II

As outlined in Section 3.1.4, 93 of the patients treated under the UKALL 2003 trial had a second course of interim maintenance. This sections outlines their samples.

There were 129 samples from 43 patients; 97 blood cultures, 24 respiratory samples and 8 samples sent for fungal testing. Fourteen patients had at least one positive sample yielding 23 samples that were positive for at least one microorganism; 15 blood cultures and 8 respiratory samples.

A total of 18 bacteria were isolated from the 15 positive blood cultures (Table 3.5-11). CONS was the most commonly isolated (9/18) (isolated at the time of unknown neutrophil count on one occasion). Only two Gram -ve bacteria were isolated.

Sixteen patients had at least one respiratory sample during interim maintenance II and six of those had at least one positive. A total of 10 pathogens were isolated from the eight positive respiratory samples (Table 3.5-11). There were four viral pathogens (influenza A and rhinovirus on two occasions respectively), four bacterial (*H. influenzae*, *Strep. Pneumoniae*, *P. aeruginosa* and *Moraxella catarrhalis*) and two fungal pathogens (*C. albicans* and yeasts).

Table 3.5-11 Isolated bacteria during interim maintenance II by Gram category and neutropenia status

Bacterial microorganisms			
	Total	**Neutropenia**	**Non-neutropenia**
Total	**18**	**8**	**9**
Gram +ve bacteria			
Total	**16**	**7**	**8**
Microorganism			
CONS	9*	5	3
Micrococcus species	3	2	1
Enterococcus species	2	0	2
Strep. pneumoniae	1	0	1
Bacillus species	1	0	1
Gram -ve bacteria			
Total	**2**	**1**	**1**
E. coli	1	0	1
Alcaligenes faecalis	1	1	0

3.5.6 Summary of findings during interim maintenance

Patients treated under regimen C were more likely to have samples taken during interim maintenance; however they were no more likely to have samples that were positive than other groups of patients.

Two of every three microbiological samples during interim maintenance were blood cultures, 16% of them were positive, and two thirds of the bacteria were Gram +ve and a third Gram -ve. Blood cultures were taken from half of the patients in this phase, and a quarter of patients sampled were found to have at least one positive result. Among patients who received high-dose methotrexate, four out of the five bacteria isolated from their blood cultures were bacteria commonly found in the gastrointestinal tract and mucus membranes. High-dose methotrexate regimens are associated with higher risk of bacterial translocation from the gut, so although the number of positive blood cultures from these patients is low, this remains an interesting observation (117).

Over a quarter of samples were respiratory samples, half of which were positive. Two thirds of the pathogens found were respiratory viruses, with rhinovirus the most common followed by RSV and parainfluenza viruses. Respiratory samples were taken from a quarter of patients, and two thirds of sampled patients had at least one positive sample. A sample number of samples were sent for fungal testing and all were found to be negative.

There was one death during interim maintenance due to gastrointestinal bleeding.

The next section will present the samples of the 242 patients who started delayed intensification; one patient died, one started conditioning for haematopoietic stem cell transplant (HSCT), and three skipped delayed intensification and had maintenance after interim maintenance (see Section 3.1.4). All samples refer to delayed intensification I, i.e. the first delayed

intensification phase for patients treated under UKALL 2003 randomised to receive two courses of this phase, and the only delayed intensification in all other patients.

3.6 Delayed intensification

The goal of delayed intensification is to eliminate any residual drug-resistant cells. It has been shown to improve event-free survival in all risk groups (69,192).

Figure 3.6-1 shows the treatment era of the 242 patients who started delayed intensification between 29th December 2004 and 8th February 2017: 128 (52.6%) were treated in the UKALL 2003 era, 100 (41.3%) in the UKALL 2011 era, and 14 under the interim guidelines (IG) between the two. In total,108 (44.6%) patients were treated using regimen A, 57 (23.5%) regimen B, and 77 (31.9%) regimen C.

As described in Section 3.1.4, patients in the UKALL 2003 era had two courses of delayed intensification (separated by a second course on interim maintenance), unless randomised to receive one course. The additional chemotherapy phases were not found to be beneficial and in the UKALL 2011 era patients only received one course of delayed intensification. This Section presents the microbiological samples from the first course of delayed intensification (delayed intensification I) in the UKALL 2003 patients and the only course of delayed intensification in those treated in the UKALL 2011 era and interim guidelines (IG). In Section 3.6.5, there is a brief outline of the microbiological samples from patients treated under UKALL 2003 who received a second course (delayed intensification II).

The total length of delayed intensification was seven weeks, except under regimen C in the UKALL 2003 era where this phase lasted for eight weeks. In

the first part of delayed intensification patients received dexamethasone, vincristine, doxorubicin, peg-asparaginase and intrathecal methotrexate; and in the second part, cyclophosphamide, cytarabine, 6-mercaptopurine and intrathecal methotrexate. Patients treated under regimen C also had vincristine and peg-asparaginase in the second part of delayed intensification. Delayed intensification is the most intensive phase of treatment for patients treated under regimen A, as it is the first time they receive an anthracycline (doxorubicin), in combination with cyclophosphamide, cytarabine and peg-asparaginase.

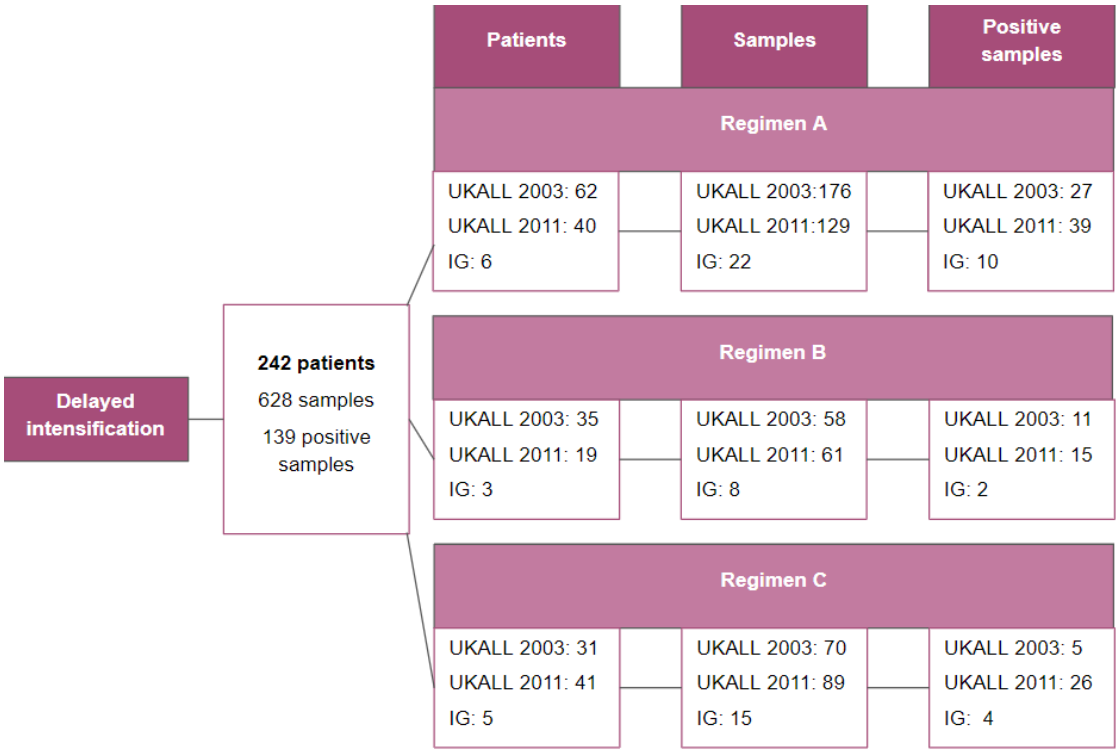

Figure 3.6-1 Patients and their samples distributed by treatment protocol and regimen

3.6.1 Microbiological samples during delayed intensification

During delayed intensification, 161 (66.5%) patients had at least one sample taken, with 628 microbiological samples in total (Table 3.6-1). Just under half (n=79) of the 161 patients had samples that were positive for at least one pathogen, but only 139/628 (22.1%) of the total samples taken were positive.

As seen in other phases, the number of samples per patient varied widely: 81 (33.5%) had none, 35 (14.5%) had one, 37 (15.3%) had two, 21 (8.7%) had three, 15 (6.2%) had four, 17 (7.0%) had five and 36 (14.9%) had six or more (the maximum was 23). Likewise, the number of positive samples per patient also varied: 163 (67.4%) had none positive, 50 (20.7%) had one, 14 (5.8%) had two, seven (2.9%) had three, and eight (2.3%) had four or more (the maximum was nine).

No notable differences were seen between the characteristics of patients with at least one sample versus those who had none (Table 3.6-1). However, significant differences were seen in the distribution of positive samples, which varied with lineage and age at diagnosis. Patients diagnosed with B-ALL were more likely to have at least one positive sample during delayed intensification (72/135, 53.3%), compared to those diagnosed with T-ALL (7/26, 26.9%, Fisher's exact p=0.018). So were patients diagnosed under the age of ten years (68/127, 53.5%), compared to those diagnosed at the age of ten or more (11/34, 32.3%, Fisher's exact p=0.034). There were only 17 positive samples from 11 patients diagnosed at the age of ten or more and only 11 positive samples from seven patients diagnosed with T-ALL. There were four patients with Down syndrome with three having at least one sample during delayed intensification and only one with a positive sample.

Table 3.6-1 Characteristics of patients and their samples during delayed intensification

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**242**	**81**	**161**	**79**	**628**	**139**
Diagnosis						
B-ALL	201 (83.0)	66 (81.5)	135 (83.8)	72 (91.1)	544 (86.6)	128 (92.0)
- hyperdiploidy	80 (33.0)	23 (28.4)	57 (35.4)	31 (39.2)	214 (34.1)	62 (44.6)
- NOS	75 (30.9)	31 (38.3)	44 (27.3)	21 (26.6)	175 (27.8)	29 (20.8)
- t(12;21)	42 (17.3)	12 (14.8)	30 (18.6)	18 (22.8)	139 (22.1)	34 (24.5)
- hypodiploidy	2 (0.8)	0 (0.0)	2 (1.2)	1 (1.3)	12 (1.9)	2 (1.4)
- MLL rearrangement	2 (0.8)	0 (0.0)	2 (1.2)	1 (1.3)	4 (0.6)	1 (0.7)
T-ALL	41 (17.0)	15 (18.5)	26 (16.2)	7 (8.9)	84 (13.4)	11 (8.0)
Predisposing conditions	5 (2.0)	2 (2.4)	3 (1.8)	1 (1.3)	22 (3.5)	1 (0.7)
Sex						
Female	109 (45.0)	37 (45.6)	72 (44.7)	35 (44.3)	281 (44.7)	55 (39.6)
Male	133 (55.0)	44 (54.4)	89 (55.3)	44 (55.7)	347 (55.3)	84 (60.4)
Age at diagnosis (years)						
1-9	182 (75.2)	55 (67.9)	127 (78.9)	68 (86.1)	520 (82.8)	122 (87.7)
10-17	60 (24.8)	26 (32.1)	34 (21.1)	11 (13.9)	108 (17.2)	17 (12.3)
Regimen						
A	108 (44.6)	33 (40.7)	75 (46.6)	40 (50.6)	327 (52.0)	76 (54.6)
B	57 (23.5)	23 (28.4)	34 (21.1)	15 (18.9)	127 (20.2)	28 (20.1)
C	77 (31.9)	25 (30.9)	52 (32.3)	24 (30.5)	174 (27.8)	35 (25.3)
Deprivation						
More affluent	128 (52.9)	46 (56.7)	82 (50.9)	40 (50.6)	300 (47.8)	59 (42.4)
More deprived	114 (47.1)	35 (43.3)	79 (49.1)	39 (49.4)	328 (52.2)	80 (57.6)

The differences between patient characteristics and regimen of treatment by microbiological sample category are presented in Sections 3.6.2 to 3.6.4. Of

the total 628 microbiological samples, 432 (68.8%) were blood cultures, 143 (22.8%) respiratory samples, and 53 (8.4%) samples were sent for fungal testing. Only 139/628 (22.1%) samples were positive; 47 (33.8%) blood cultures, 88 (63.3%) respiratory samples and four fungal samples (2.9%).

3.6.2 Blood culture samples

During delayed intensification, 158 (65.3%) patients had at least one blood culture sample, yielding 432 blood culture samples in total (Table 3.6-2). Around one quarter (n=36, 22.7%) had blood cultures positive for at least one bacterial microorganism, but only 47/432 (10.9%) of the total samples were positive.

Following the pattern seen for total samples in Section 3.6.1, the number of blood cultures per patient varied: 84 (34.7%) had none, 58 (24.0%) had one, 31 (12.8%) had two, 22 (9.0%) had three, 18 (7.4%) had four, and 29 (11.9%) had five or more (the maximum was ten). Similarly, the number of positive samples per patient also varied: 206 (85.1%) had none positive, 26 (10.7%) had one, seven (2.9%) had two, and three (1.4%) had three or more (the maximum was four).

The characteristics of patients with at least one blood culture, or at least one that was positive, were broadly similar to those of patients without (Table 3.6-2). Only 36 patients had at least one positive blood culture, 21 of whom were treated under regimen A, three under B, and 12 under C. There were only five patients diagnosed at age ten or older with positive blood cultures.

Table 3.6-2 Characteristics of patients and their blood culture samples during delayed intensification

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**242**	**84**	**158**	**36**	**432**	**47**
Diagnosis						
B-ALL	201 (83.0)	69 (82.1)	132 (83.5)	34 (94.4)	374 (86.6)	45 (95.7)
- hyperdiploidy	80 (33.0)	23 (27.4)	57 (36.1)	20 (55.5)	152 (35.2)	28 (59.6)
- NOS	75 (30.9)	32 (38.1)	43 (27.2)	8 (22.2)	124 (28.7)	9 (19.1)
- t(12;21)	42 (17.3)	14 (16.6)	28 (17.7)	6 (16.6)	88 (20.4)	8 (17.0)
- hypodiploidy	2 (0.8)	0 (0.0)	2 (1.3)	0 (0.0)	7 (1.6)	0 (0.0)
- MLL rearrangement	2 (0.8)	0 (0.0)	2 (1.3)	0 (0.0)	3 (0.7)	0 (0.0)
T-ALL	41 (17.0)	15 (17.9)	26 (16.5)	2 (5.6)	58 (13.4)	2 (4.3)
Sex						
Female	109 (45.0)	39 (46.4)	70 (44.3)	13 (36.1)	190 (43.9)	17 (36.2)
Male	133 (55.0)	45 (53.6)	88 (55.7)	23 (63.9)	242 (56.1)	30 (63.8)
Age at diagnosis (years)						
1-9	182 (75.2)	57 (67.8)	125 (79.1)	31 (86.1)	362 (83.8)	42 (89.4)
10-17	60 (24.8)	27 (32.2)	33 (20.9)	5 (13.9)	70 (16.2)	5 (10.6)
Regimen						
A	108 (44.6)	33 (39.3)	75 (47.5)	21 (58.3)	232 (53.7)	29 (61.7)
B	57 (23.5)	24 (28.6)	33 (20.9)	3 (8.3)	72 (16.6)	3 (6.4)
C	77 (31.9)	27 (32).1	50 (31.6)	12 (33.4)	128 (29.7)	15 (31.9)
Deprivation						
More affluent	128 (52.9)	49 (58.3)	79 (50.0)	15 (41.6)	209 (48.4)	18 (38.3)
More deprived	114 (47.1)	35 (41.7)	79 (50.0)	21 (58.4)	223 (51.6)	29 (61.7)

Overall, there were 47 positive blood cultures from 36 patients (Table 3.6-3). Looking at their source, over half (240/432, 55.5%) were taken from central lines, and 30/240 (12.5%) of these were positive for at least one

microorganism. Sixty-eight blood cultures were paired samples (see 2.4.1), with only five positives. No source was documented for 97/432 (22.5%) blood cultures.

A total of 50 bacteria were isolated from 47 positive blood cultures; three samples had two bacteria. The majority (36/50, 72.0%) were Gram +ve and 24/36 of these were isolated from central line cultures (Table 3.6-4). There were only 14 Gram -ve bacteria; nine were isolated from central line cultures and two in paired cultures; two samples positive for *Pseudomonas aeruginosa* from the same patient three days apart. There were three more positive paired blood culture samples (from three patients) all of which had a growth of coagulase-negative *Staphylococcus*.

Two hundred and ninety-six blood cultures were taken at the time of neutropenia. Only 32 of these were positive, with two thirds (23) of the bacteria isolated at time of neutropenia (Table 3.6-4). Overall, the most commonly isolated microorganism (32/50) was CONS (Table 3.6-5). The most commonly isolated Gram -ve bacteria were *Pseudomonas aeruginosa* (6/14) and *E. coli*. (4/14). Nine of the 14 Gram -ve bacteria were isolated at time of neutropenia. No full blood count results were available for the time that CONS was isolated on one occasion and *Micrococcus* species on another occasion.

Table 3.6-6 shows the microorganisms isolated by regimen. The majority of microorganisms (31/50) were isolated from 21 patients treated under regimen A, including half of the Gram -ve (7/14) bacteria that were isolated. There were only three microorganisms isolated from three patients treated under regimen B (all CONS), and 16 isolated from 12 patients on regimen C.

Table 3.6-3 Blood culture samples and positives during delayed intensification by source

	Blood cultures N (%)	Positive blood cultures N (%)
Total	**432**	**47**
Source		
Central line	240 (55.5)	30 (63.8)
Paired	68 (15.7)	5 (10.6)
Peripheral	27 (6.3)	3 (6.4)
Unknown	97 (22.5)	9 (19.2)

Table 3.6-4 Isolated bacteria during delayed intensification by Gram category and neutropenia status

	Microorganisms	
	Gram -ve	**Gram +ve**
Total	**14**	**36**
Source		
Central	9	24
Paired	2	3
Peripheral	1	2
Unknown	2	7
Neutropenia		
Yes	9	23
No	5	10
Missing	0	3

Table 3.6-5 Isolated bacteria during delayed intensification by Gram category and neutrophil count

Bacterial microorganisms			
	Total	**Neutropenia**	**Non-neutropenia**
Total	**50***	**32**	**15**
Gram +ve bacteria			
Total	**36**	**23**	**10**
Microorganism			
CONS	32*	20	10
Micrococcus species	3*	2	0
Gram-positive bacillus	1	1	0
Gram -ve bacteria			
Total	**14**	**9**	**5**
Microorganism			
Pseudomonas aeruginosa	6	5	1
E. coli	4	2	2
Enterobacter species	2	0	2
Rhizobacterium radiobacter	1	1	0
Sphingomonas paucimobilis	1	1	0

*Unknown neutrophil count at the time of isolation of CONS x 1 and Micrococcus species x 1

Table 3.6-6 Isolated bacteria during delayed intensification by regimen

Regimen A		**Regimen C**	
Total	**31**	**Total**	**16**
Microorganism		**Microorganism**	
CONS	21	CONS	8
P. aeruginosa	4	*E. coli*	3
Enterobacter species	2	*P. aeruginosa*	2
Micrococcus species	2	*Micrococcus* species	1
E. coli	1	*Rhizobacterium radiobacter*	1
Gram-positive bacillus	1	*Sphingomonas paucimobilis*	1

Three microorganisms, all CONS, were isolated from patients treated under regimen B

3.6.3 Respiratory samples

During delayed intensification, 79/242 (32.6%) patients had at least one respiratory sample, of which 56/79 (70.9%) had samples that were positive for at least one pathogen. Of the total 143 respiratory samples, over half (88/143, 61.5%) were positive (Table 3.6-7).

Following the pattern seen for total samples (Section 3.6.1), the number of respiratory samples per patient varied: 163 (67.4%) had none, 47 (19.4%) had one, 15 (6.2%) had two, seven (2.9%) had three, and 10 (3.1%) had four or more (the maximum was six). Similarly, the number of positive samples per patient also varied: 186 (76.9%) had none positive, 38 (15.7%) had one, eight (3.3%) had two, and 10 (4.0%) had three or more (the maximum was five).

With the exception of age, the diagnostic characteristics of patients who had at least one respiratory sample during delayed intensification were broadly similar to those who did not (Table 3.6-7). Patients diagnosed under the age of ten years were more likely to have at least one positive respiratory sample (50/66, 75.7%), compared to those diagnosed at ten years or more (6/13,

46.2%, Fisher's p=0.046). There were only 11 positive respiratory samples from six patients diagnosed at ten years or more.

Table 3.6-7 Characteristics of patients and their respiratory samples during delayed intensification

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**242**	**163**	**79**	**56**	**143**	**88**
Diagnosis						
B-ALL	201 (83.0)	132 (81.0)	69 (87.3)	51 (91.0)	124 (86.7)	79 (89.8)
- hyperdiploidy	80 (33.0)	54 (33.1)	26 (32.9)	19 (33.9)	47 (32.8)	34 (38.6)
- NOS	75 (30.9)	55 (33.7)	20 (25.3)	14 (25.0)	35 (24.5)	19 (21.6)
- t(12;21)	42 (17.3)	22 (13.5)	20 (25.3)	16 (28.6)	36 (25.1)	23 (26.1)
- hypodiploidy	2 (0.8)	0 (0.0)	2 (2.5)	1 (1.8)	5 (3.5)	2 (2.3)
- MLL rearrangement	2 (0.8)	1 (0.6)	1 (1.3)	1 (1.8)	1 (0.7)	1 (1.1)
T-ALL	41 (17.0)	31 (19.0)	10 (12.7)	5 (9.0)	19 (13.3)	9 (10.2)
Sex						
Female	109 (45.0)	73 (44.8)	36 (45.6)	26 (46.4)	64 (44.7)	37 (42.0)
Male	133 (55.0)	90 (55.2)	43 (54.4)	30 (53.6)	79 (55.3)	51 (58.0)
Age at diagnosis (years)						
1-9	182 (75.2)	116 (71.2)	66 (83.5)	50 (89.3)	115 (80.4)	77 (87.5)
10-17	60 (24.8)	47 (28.8)	13 (16.5)	6 (10.7)	28 (19.6)	11 (22.5)
Regimen						
A	108 (44.6)	71 (43.5)	37 (46.8)	28 (50.0)	64 (44.7)	44 (50.0)
B	57 (23.5)	38 (23.3)	19 (24.0)	13 (23.2)	42 (29.4)	24 (27.3)
C	77 (31.9)	54 (33.2)	23 (29.2)	15 (26.8)	37 (25.9)	20 (22.7)
Deprivation						
More affluent	128 (52.9)	87 (53.4)	41 (51.9)	29 (51.8)	69 (48.2)	40 (45.4)
More deprived	114 (47.1)	76 (46.6)	38 (48.1)	27 (48.2)	74 (51.8)	48 (54.6)

Table 3.6-8 distributes respiratory samples by source. Most samples (100/143, 69.9%) and positives (64/88, 72.7%) were nose/throat swabs. There were five bronchoalveolar lavage (BAL) samples with three positive (from three patients). None of the four samples from endotracheal tube (ET) secretions were positive.

A total of 100 pathogens were isolated from 88 positive respiratory samples; 11 samples were positive for two pathogens and one for three. Most of the pathogens (78/100) were respiratory viruses, mostly from nose/throat swabs (55/78) (Table 3.6-9 and Table 3.6-10). The most commonly isolated virus was rhinovirus (51/78) followed by parainfluenza viruses (13/78). All but seven of the 78 isolated viral pathogens came from patients diagnosed under the age of ten years (data not presented). There were only five bacterial pathogens: four isolated from nose/throat swabs and one from a BAL. Three pathogens were isolated in the three positive BAL samples: *Pneumocystis jirovecii* (PJP), CONS, and parainfluenza 1. Of the 16 samples that were positive for fungal pathogens, 14 were nose/throat swabs, which isolated *Candida albicans* on 12 occasions, and *Candida parapsilosis* in two.

Table 3.6-8 Distribution of respiratory samples and positives during delayed intensification by source

	Respiratory samples N (%)	Positive respiratory samples N (%)
Total	**143**	**88**
Source		
Nose/throat swab	100 (69.9)	64 (72.6)
NPA	28 (19.6)	17 (19.3)
Sputum	5 (3.5)	3 (3.5)
BAL	5 (3.5)	3 (3.5)
ET secretions	4 (2.8)	0 (0.0)
Unknown	1 (0.7)	1 (1.1)

Table 3.6-9 Types of microorganism in positive respiratory samples during delayed intensification by source

Positive respiratory samples					
	Total	**Bacterial**	**Viral**	**Fungal**	**PJP**
Total	**100**	**5**	**78**	**16**	**1**
Source of sample					
Nose/throat swab	73	4	55	14	0
NPA	19	0	19	0	0
Sputum	4	0	2	2	0
BAL	3	1	1	0	1
Unknown	1	0	1	0	0

Table 3.6-10 Pathogens isolated during delayed intensification by microorganism type

Bacteria		**Viruses**	
Total	**5**	**Total**	**78**
Coliform	2	Rhinovirus	51
CONS	1	Parainfluenzas	14
Streptococcus species	1	RSV	5
S. aureus	1	Influenzas	5
		Metapneumovirus	2
		Adenovirus	1

3.6.4 Fungal pathogens

There were 53 samples from 28 patients sent for fungal testing: 51 blood samples, one cerebrospinal fluid (CSF) and one lung tissue biopsy sample. Of the 51 blood samples, 49 were tested for Aspergillus antigen, one for Candida antigen and one for β-d-glucan. The other two samples were sent for microscopy and culture.

Only four samples from two patients were positive. A male patient had two blood samples positive for Aspergillus antigen within three days of each other and then a CSF sample positive for mycelium (unspecified which fungus) on microscopy and culture (MCS) ten days later (diagnosed at the age of two and treated under regimen A). A female patient with Down syndrome had a lung tissue biopsy positive for mycelium on MCS (diagnosed at the age of 13 years and treated under regimen C). Both patients moved to the next phase of maintenance.

3.6.5 Microbiological samples during delayed intensification II

As outlined in Section 3.1.4, 91 patients treated under the UKALL 2003 trial had a second course of delayed intensification. A brief description of the microbiological samples from these patients is outlined below.

There were 283 samples from 63 patients: 208 blood cultures, 51 respiratory samples and 24 samples sent for fungal testing. Thirty-two patients had at least one positive sample, yielding 56 samples that were positive for at least one microorganism: 31 blood cultures, 23 respiratory samples and two samples sent for fungal testing.

A total of 37 bacteria were isolated in the 31 positive blood cultures. The majority of bacteria were Gram +ve (31/37); most commonly isolated was CONS (18/37) (Table 3.6-11). Five of the six Gram -ve bacteria were isolated at time of neutropenia.

Table 3.6-11 Isolated bacteria during delayed intensification II by Gram category and neutrophil count

Bacterial microorganisms			
	Total	**Neutropenia**	**Non-neutropenia**
Total	**37**	**20**	**17**
Gram +ve bacteria			
Total	**31**	**15**	**16**
Microorganism			
CONS	18	9	9
Enterococcus species	4	2	2
Streptococcus pneumoniae	2	0	2
Viridans group streptococci	2	1	1
Vancomycin resistant Enterococcus	1	1	0
S. aureus	1	0	1
Diptheroid species	1	1	0
Propionibacterium species	1	0	1
Bacillus species	1	1	0
Gram -ve bacteria			
Total	**6**	**5**	**1**
E. coli	2	2	0
Pseudomonas aeruginosa	2	1	1
Enterobacter gallinarum	2	2	0

Thirty patients had at least one respiratory sample during delayed intensification II and 13 of those had at least one positive. A total of 24 pathogens were isolated from the 23 positive respiratory samples. There were 12 viruses, five bacterial and seven fungal pathogens (*C. albicans* isolated on six occasions and *C. glabrata* on one) (Table 3.6-12).

Table 3.6-12 Bacterial and viral pathogens isolated in positive respiratory samples during delayed intensification II

Bacteria		**Viruses**	
Total	**5**		**12**
Microorganism			
P. aeruginosa	2	Rhinovirus	4
Moraxella catarrhalis	1	RSV	4
H. influenzae	1	Parainfluenza 3	2
Strep. pneumoniae	1	Influenza A	1
		Metapneumovirus	1

As presented in Section 3.1.4, three patients died during delayed intensification II. Two of the three deaths were of infective cause; Gram -ve septicaemia with *E. coli* and liver failure with viral infection (one positive Aspergillus antigen test near the time of death). The non-infective death had a documented cause of extrapontine myelinolysis; there was growth of *Candida glabrata* in the blood near the time of death.

3.6.6 Summary of findings during delayed intensification I

As outlined above, delayed intensification is an intensive block of treatment comprising of high-intensity combination chemotherapy. It is the first part of treatment where patients treated under regimen A are exposed to anthracyclines in combination with cytarabine and peg-asparaginase, which as a combination causes severe myelosuppression. This is the most intensive treatment phase for patients in regimen A and as expected, comparatively high levels of sampling and numbers of positive samples was observed; over half of the samples came from patients in regimen A, who accounted for less than half of those sampled. More importantly, over half of the positive samples came from regimen A patients, who comprised half the patients with positive samples.

Although nearly 70% of samples during delayed intensification were blood cultures, just over 10% were positive. A fifth of samples were respiratory, and 60% of these were positive; with patients under the age of ten years being more likely to have at least one positive respiratory sample compared to those diagnosed over the age of ten years. Given that most of the patients diagnosed under ten years were treated under regimen A (107/182), the pattern seen in the total samples by regimen should be viewed in the context of age.

The interlinked relationship between age at diagnosis and regimen was also reflected in the finding that patients diagnosed under the age of ten years were more likely to have positive samples during delayed intensification; as most patients diagnosed under the age of ten were treated under regimen A. Similarly, patients with B-ALL were more likely to have positive samples, which was not surprising as T-ALL is more frequent in older patients.

With respect to microbiology, 50 bacteria were isolated from 47 positive blood cultures and 100 pathogens were isolated from 88 positive respiratory samples (78 viruses, 5 bacteria and 17 fungal pathogens). Finally, there were two patients with MCS positive for mycelium from CSF and lung tissue biopsy samples respectively.

The next section examines the samples of the 235 patients who started maintenance (in those treated in UKALL 2003 era: three patients died during delayed intensification II, and five patients had not started maintenance by the end of data collection).

3.7 Maintenance

Maintenance, which is initiated if the leukaemia remains in remission, aims to suppress the growth of leukaemia cells.

Figure 3.7-1 shows the treatment era and samples of the 235 patients who started maintenance between 11th May 2004 and 15th February 2017 (five of the included patients started maintenance after the end of data collection so they are not included in this section): 124 (52.8%) were treated in the UKALL 2003 era, 97 (41.3%) in the UKALL 2011 era, and 14 (5.9%) under interim guidelines (IG) between the two.

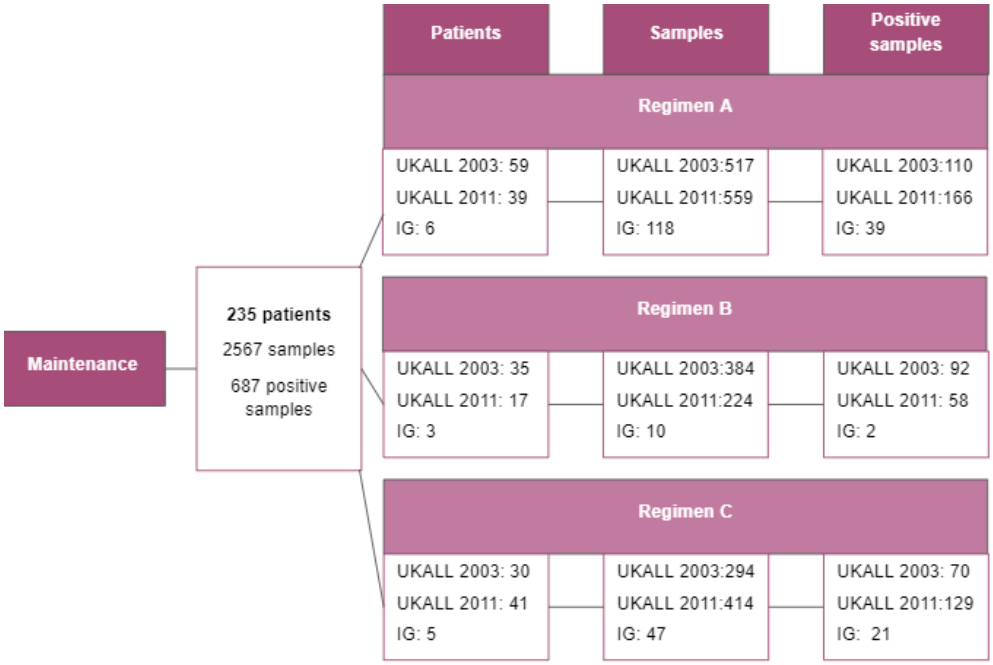

Figure 3.7-1 Patients and their samples by treatment protocol and regimen

In the UK, during the period studied, males had an extra year of treatment. The duration of treatment for female patients was two years from the start of interim maintenance, but for male patients it was three years. The extra year of treatment was given due to evidence that boys need longer maintenance treatment to maintain remission (53). The length of maintenance also varied slightly depending on regimen and treatment era (Table 3.7-1).

In all regimens, maintenance involved daily oral 6-mercaptorurine and weekly oral methotrexate with intrathecal methotrexate on day 15 of each cycle and pulses of vincristine (on days 1, 19 and 57 of each cycle) and oral dexamethasone (on days 1-5, 29-33 and 57-61 of each cycle). In the UKALL 2011 trial, patients who had high-dose methotrexate in interim maintenance did not have intrathecal methotrexate during maintenance (except those diagnosed with T-ALL or with a white cell count over 100 x 10^9/L at diagnosis). There was also a randomisation between maintenance with pulses of vincristine and dexamethasone or without. Only 15/45 patients treated under the UKALL 2011 trial were randomised to no pulses of vincristine and dexamethasone during maintenance.

Table 3.7-1 Duration of maintenance in weeks by regimen and sex

Regimen		Maintenance time in weeks; as specified in the protocol	
		Females	**Males**
UKALL 2003	A	74	126
	B	73	125
	C	71	123
UKALL 2011	A	90	142
	B	88	140
	C	83	135

3.7.1 Microbiological samples during maintenance

During maintenance, the majority of patients (208/235, 88.5%), had at least one sample, yielding 2,567 microbiological samples in total. Most (n=160/208, 76.9%) patients had positive samples; and 687/2567 (26.7%) of the total samples were positive

Sample rates and 95% confidence intervals per 100 person-weeks are shown in Table 3.7-2. The rate of patients with at least one sample was 3.7 per 100

persons-weeks (95%CI 3.2-4.3), and the rate of patients with at least one positive sample was 1.5 (95%CI 1.3-1.8). No differences by age or sex were observed in rates of patients sampled, but there were significant differences in the rates of patients with at least one sample in regimen B compared to A, and in regimen C compared to A. With a rate of 4.5 (95% CI 3.5-5.9) regimen B had a higher rate of sampled patients compared to regimen A at 3.1 (95% CI 2.5-3.8; χ^2=5.2, p=0.022). Patients on regimen C also had a higher rate of being sampled (4.3, 95% CI 3.3-5.4) compared to those on A (3.1, 95% CI 2.5-3.8; χ^2=4.1, p=0.04). No difference was observed between the rates of sampled patients in regimen B compared to C.

Examining the rate of all samples, significant differences were seen by sex, age and deprivation. At 11.6 (95% CI 11.1-12.2) per 100 person-weeks, the rate of all samples for males was significantly higher than that of females (10.2, 95% CI 9.5-10.9; χ^2=9.5, p=0.002). However, no significant sex differences were seen in the positive sample rates. Significant differences were also seen between the rates of all samples among patients diagnosed under the age of ten years compared to those diagnosed at ten years or more. The rate of all samples per 100 person-weeks among patients diagnosed under the age of ten was 12.6 (95% CI 12.1-13.2), which was significantly higher than that among those diagnosed at ten years or more (7.0, 95% CI 6.3-7.7; χ^2=126.5, p<0.001). More importantly, patients diagnosed under the age of ten years had a significantly higher rate of positive samples (3.4, 95% CI 3.1-3.7) compared to those diagnosed at ten years or more (1.8, 95% CI 1.5-2.2; χ^2=38.7, p<0.001).

Significant differences were also seen between the rates of all samples among patients categorized as living in more affluent areas (10.3 per 100 person weeks, 95% CI 9.7-10.9) compared to those living in more deprived areas (12.1, 95% CI 11.4-12.7) (χ^2=16.2, p=0.0001). A similar difference was seen for positive samples: the positive sample rate for patients living in more

affluent areas (2.7, 95% CI 2.4-3.0) being lower than that for those living in more deprived areas (3.3, 95% CI 3.0-3.7, χ^2=8.0, p=0.005).

Finally, significant differences were also evident between the rates of all samples by regimen. In regimen A, the rate of all samples per 100 person-weeks of 11.9 (95% CI 11.2-12.6) was significantly higher than that of all samples for regimen B (10.8; 95% CI 10.0-11.7) and C (10.4; 95% CI 9.7-11.2): χ^2=4.0, p=0.045 and χ^2=7.8, p=0.005, respectively. The opposite was seen in the rates of patients sampled by regimen, with patients treated under regimens B (4.5, 95% CI 3.5-5.9) and C (4.3, 95% CI 3.3-5.4) having a higher rate than those in regimen A (3.1, 95% CI 2.5-3.8) (χ^2=5.2, p=0.022 and χ^2=4.1, p=0.04, respectively). So although the rate of patients with samples was higher in those treated under regimens B and C compared to A, the rates of all samples was higher for regimen A. This observation may be confounded by age, as only patients diagnosed under the age of ten would be treated under regimen A and they were found to be more likely to have samples taken. No significant differences, however, were seen in the rates of all positive samples per 100 person-weeks among different treatment regimens.

The differences between patient characteristics and regimen of treatment by microbiological sample category are explored in Sections 3.7.2 to 3.7.4. Of the total 2,567 microbiological samples, 1,627 (63.4%) were blood cultures, 843 (32.8%) respiratory samples and 97 (3.8) samples sent for fungal testing. Only 687 samples were positive: 184 (26.8%) blood cultures, 498 (72.5%) respiratory samples and three (0.7%) samples sent for fungal testing.

Table 3.7-2 Person weeks and sampling rates per 100 person-weeks during maintenance, distributed by sex, age, deprivation and regimen

	Patients						Samples					
	At least one sample			At least one positive sample			All samples			All positive samples		
	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate (95%CI)	N	Person-weeks	Rate(95%CI)
Total	208	5593.6	3.7(3.2-4.3)	160	10510.7	1.5(1.3-1.8)	2567	23002.3	11.2(10.7-11.6)	687	23002.3	3.0(2.8-3.2)
Sex												
Male	120	3060.3	3.9(3.3-4.7)	97	5991.4	1.6(1.3-2.0)	1786	15343.3	11.6(11.1-12.2)	471	15343.3	3.1(2.8-3.4)
Female	88	2533.3	3.5(2.8-4.3)	63	4519.3	1.4(1.1-1.8)	781	7659.0	10.2(9.5-10.9)	216	7659.0	2.8(2.5-3.2)
Age												
1-9	154	4282.4	3.6(3.1-4.2)	121	7471.3	1.6(1.4-1.9)	2150	17026.7	12.6(12.1-13.2)	580	17026.7	3.4(3.1-3.7)
10-17	54	1311.2	4.1(3.2-5.4)	39	3039.3	1.3(0.9-1.8)	417	5975.6	7.0(6.3-7.7)	107	5975.6	1.8(1.5-2.2)
Deprivation												
More affluent	109	3157.5	3.5(2.9-4.2)	79	5921.6	1.3(1.1-1.7)	1232	11951.3	10.3(9.7-10.9)	320	11951.3	2.7(2.4-3.0)
More deprived	99	2436.1	4.1(3.3-4.9)	81	4589.5	1.8(1.4-2.2)	1335	11051.0	12.1(11.4-12.7)	367	11051.0	3.3(3.0-3.7)
Regimen												
A	87	2836.3	3.1(2.5-3.8)	71	4530.5	1.6(1.2-2.0)	1194	10039.0	11.9(11.2-12.6)	315	10039.0	3.1(2.8-3.5)
B	55	1211.2	4.5(3.5-5.9)	38	2957.9	1.3(0.9-1.8)	618	5738.0	10.8(10.0-11.7)	152	5738.0	2.6(2.3-3.1)
C	66	1546.2	4.3(3.3-5.4)	51	3022.3	1.7(1.3-2.2)	755	7225.3	10.4(9.7-11.2)	220	7225.3	3.0(2.7-3.5)

Rates and 95% confidence intervals (CI) are shown per 100 person-weeks. Person-weeks were calculated from the start of maintenance until the first sample, or the first positive sample, to examine the rate of the first sample or the first positive sample; for all samples and all positive samples, follow-up continued from the start of maintenance until the end of treatment, the end of data collection or death. Differences between rates were tested using the Mantel-Haenszel method; statistically significant differences were seen for the rates of all samples among boys compared to girls (χ^2=9.5, p=0.002), the rates of all samples among aged 1-9 years compared to 10 years or more (χ^2=126.5, p<0.001), the rate of all positive samples among ages 1-9 years compared to 10 years or more (χ^2=38.7, p<0.001), the rate of all samples among the more deprived compared to being more affluent (χ^2=16.2, p=0.0001), the rate of all positive samples among the more deprived compared to being more affluent (χ^2=8.0, p=0.005),the rate of at least one sample for regimen B compared to regimen A (χ^2=5.2, p=0.022), the rate of at least one sample for regimen C compared to regimen A (χ^2=4.1, p=0.04), the rate of all samples for regimen B compared to the rate of all samples for regimen A (χ^2=4.0, p=0.045), and the rate of all samples for regimen C compared to the rate of all samples for regimen A (χ^2=7.8, p=0.005).

3.7.2 Blood culture samples

During maintenance, most patients (201/235, 85.5%) had at least one blood culture over a third (83/201) of whom had at least one that was positive (Table 3.7-3). Of the 1,627 blood culture samples, 186 (11.4%) were positive for at least one bacterial microorganism.

Blood culture sample rates and 95% confidence intervals per 100 person-weeks are shown in Table 3.7-3. The rate of patients with at least one blood culture was 3.1 per 100 persons-weeks (95%CI 2.7-3.6), and the rate of patients with at least one positive blood culture was 0.5 (95% CI 0.4-0.6). No differences were observed in rates by sex, or regimen.

There were, however, significant age and deprivation differences in the rates of patients with at least one positive blood culture sample. Patients diagnosed under the age of ten had a rate of at least one positive blood culture of 0.6 (95% CI 0.4-0.7), which was significantly higher than the 0.3 in patients diagnosed at ten years or more (95% CI 0.2-0.5; χ^2=4.6, p=0.032). Similarly, a higher rate of having at least one positive blood culture sample was observed in patients living in more deprived areas (0.7, 95% CI 0.5-0.9) than in more affluent areas (0.4, 95% CI 0.3-0.5; χ^2=7.2, p=0.007).

Examining the rates of all samples, significant differences were seen by sex, age at diagnosis, deprivation and regimen. As in Table 3.7-3, at 7.4 (95% CI 7.0-7.8) per 100 person-weeks, the rate of all samples for males was significantly higher than that of females (6.4, 95% CI 5.9-7.0; χ^2=6.8, p=0.009). There was no difference though, in the rate of all positive samples in males compared to females**.** Significant differences, were, however, evident in the rates of all samples and all positive samples by age at diagnosis. Again, as shown in Table 3.7-3, at 7.9 (95% CI 7.4-8.4) per 100 person-weeks, the rate of all samples for those diagnosed under ten years was significantly higher than that of those diagnosed at age ten or more (4.1, 95% CI 3.6-4.6; χ^2=100.9, p<0.0001). Similarly, the rate of all positive samples was

significantly higher in patients diagnosed under ten (1.0, 95% CI 0.8-1.1) compared to those diagnosed at ten years or more (0.3, 95% CI 0.2-0.5; χ^2=22.4, p<0.0001). Significant differences were also seen between the rates of all samples and all positive samples among the more affluent compared to the more deprived patients (Table 3.7-3). The rate of all samples among the more deprived group 7.9 (95% CI 7.4-8.4), was significantly higher than the rate among the more affluent (6.3; 95% CI 5.9-6.8; χ^2=19.6, p<0.0001). Likewise, the rate of all positive samples in the more deprived (1.0, 95% CI 0.9-1.2) was higher than that in the more affluent group (0.6, 95% 0.5-0.8; χ^2=12.0, p=0.0005).

The only significant difference when comparing rates among regimens was between the rates of all samples in regimens A and C; at 7.7 (95% CI 7.1-8.2) the rate of all samples among patients treated under regimen A was significantly higher than those of regimen C (6.4, 95% CI 5.9-7.0; χ^2=9.2, p=0.002). No difference, was seen in the rate of total positive samples by regimen.

Table 3.7-3 Person weeks and sampling rates per 100 person-weeks for blood cultures distributed by sex, age, deprivation and regimen

	Patients						Samples					
	At least one sample			At least one positive sample			All samples			All positive samples		
	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate (95%CI)	N	Person-weeks	Rate(95%CI)
Total	201	6421.5	3.1(2.7-3.6)	83	16750.6	0.5(0.4-0.6)	1627	23002.3	7.1(6.7-7.4)	186	23002.3	0.8(0.7-0.9)
Sex												
Male	118	3528.6	3.3(2.8-4.0)	56	10371.0	0.5(0.4-0.7)	1135	15343.3	7.4(7.0-7.8)	131	15343.3	0.9(0.7-1.0)
Female	83	2892.9	2.9(2.3-3.6)	27	6379.6	0.4(0.3-0.6)	492	7659.0	6.4(5.9-7.0)	55	7659.0	0.7(0.6-0.9)
Age												
1-9	151	4632.1	3.3(2.8-3.8)	68	11941.3	0.6(0.4-0.7)	1382	17026.7	8.1(7.7-8.6)	166	17026.7	1.0(0.8-1.1)
10-17	50	1789.4	2.8(2.1-3.7)	15	4809.3	0.3(0.2-0.5)	245	5975.6	4.1(3.6-4.6)	20	5975.6	0.3(0.2-0.5)
Deprivation												
More affluent	107	3490.6	3.1(2.5-3.7)	34	9309.2	0.4(0.3-0.5)	756	11951.3	6.3(5.9-6.8)	73	11951.3	0.6(0.5-0.8)
More deprived	94	2931.0	3.2(2.6-3.9)	49	7441.6	0.7(0.5-0.9)	871	11051.0	7.9(7.4-8.4)	113	11051.0	1.0(0.9-1.2)
Regimen												
A	87	3014.6	2.9(2.3-3.6)	34	7351.5	0.5(0.3-0.6)	769	10039.0	7.7(7.1-8.2)	87	10039.0	0.9(0.7-1.1)
B	52	1360.7	3.8(2.9-5.0)	18	4263.8	0.4(0.3-0.7)	395	5738.0	6.9(6.2-7.6)	41	5738.0	0.7(0.5-1.0)
C	62	2046.2	3.0(2.4-3.9)	31	5135.3	0.6(0.4-0.9)	463	7225.3	6.4(5.9-7.0)	58	7225.3	0.8(0.6-1.0)

Differences between rates per 100 person-weeks were tested using the Mantel-Haenszel method; statistically significant differences were seen for the rate of all samples among boys compared to girls (χ^2=6.8, p=0.009), the rate of at least one positive sample among ages 1-9 years compared to 10 years or more (χ^2=4.6, p=0.032), the rate of all samples among ages 1-9 years compared to 10 years or more (χ^2=100.9, p<0.0001), the rate of all positive samples among ages 1-9 compared to 10 years of more (χ^2=22.4, p<0.0001), the rate of at least one positive sample among the less affluent compared to being more affluent (χ^2=7.2, p=0.007), the rate of all samples among the less affluent compared to being more affluent (χ^2=19.6, p<0.0001), the rate of all positive samples among the less affluent compared to being more affluent (χ^2=12.0, p=0.0005), the rate of all samples for regimen C compared to regimen A (χ^2=9.2, p=0.002).

Looking at the source of blood cultures, over half (869/1627) were taken from central lines, of which 130/869 (14.9%) were positive with at least one microorganism (Table 3.7-4, Table 3.7-5). Of the 332 blood cultures that were paired samples (see 2.4.1), only 17 were positive and no source was documented for 285/1627 (17.5%).

A total of 209 bacteria were isolated from 186 blood cultures; four samples had three bacteria and 15 had two. The majority (173/209, 82.8%) were Gram +ve, with 123/173 (71.1%) isolated from central lines (Table 3.7-5). There were only 36 Gram -ve bacteria; 25 from central line cultures and four from paired cultures. Of the 173 Gram +ve bacteria, 88 were coagulase-negative *Staphylococcus* (CONS), 28 *Streptococcus pneumoniae*, 14 *Corynebacterium* species and 11 viridans group streptococci (Table 3.7-6). The most commonly isolated Gram -ve microorganism was *E. coli* (8/36).

Most bacteria, 167/209 (80.0%), were isolated from samples taken during a time of non-neutropenia and were mostly positive for Gram +ve bacteria (138/167 , 82.6%) (Table 3.7-5). The neutrophil count was not known at the time of 12 positive samples (Table 3.7-6). Regardless of neutrophil count, the most commonly isolated bacteria were Gram +ve. The majority of isolated Gram -ve bacteria (29/36, 80.5%, however, were detected at times of non-neutropenia.

Table 3.7-4 Blood culture samples and positives during maintenance by source

Source	Total N (%)	Positive N (%)
Total	**1627**	**186**
Central line	869 (53.4)	130 (69.9)
Paired	332 (20.4)	17 (9.1)
Peripheral	141 (8.7)	14 (7.5)
Unknown	285 (17.5)	25 (13.5)

Table 3.7-5 Distribution of Gram category of bacteria isolated during maintenance by source and neutropenia status

	Microorganisms	
	Gram-ve N (%)	Gram +ve N (%)
Total	**36**	**173**
Source		
Central line	25 (69.4)	123 (71.1)
Paired	4 (11.1)	14 (8.1)
Peripheral	2 (5.6)	24 (13.9)
Unknown	5 (13.9)	12 (6.9)
Neutropenia		
Yes	7 (19.4)	23 (13.3)
No	29 (80.6)	138 (79.8)
Missing	0 (0.0)	12 (6.9)

Table 3.7-6 Isolated bacteria during maintenance by Gram category and neutropenia status

	Total	Neutropenia	Non-neutropenia	Unknown neutrophil count
Total	**209**	**30**	**167**	**12**
Gram +ve				
Total	**173**	**23**	**139**	**12**
Microorganism				
CONS	88	13	71	4
Streptococcus pneumoniae	28	1	26	1
Corynebacterium species	14	1	10	3
Viridans group streptococci	11	3	7	1
Enterococcus species	6	1	5	0
Actinobacteria	6	1	5	0
Microbacterium species	4	0	3	1
Propionibacterium species	4	0	4	0
Bacillus species	4	0	3	1
S. aureus	2	0	2	0
Streptococcus pyogenes		0	1	0
Abiotrophia defectiva	1	1	0	0
Gordonia species	2	0	1	1
Granulitella adiacens	1	1	0	0
Gram +ve coccus	2	1	1	0
Gram -ve bacteria				
Total	**36**	**7**	**28**	**0**
E. coli	8	3	5	0
Pseudomonas species	3	1	2	0
Klebsiella pneumoniae	3	0	3	0
Enterobacter cloacae	3	0	3	0
Moraxella catarrhalis	3	1	2	0
Haemophilus influenzae	2	0	2	0
Neisseria species	3	0	3	0
Stenotrophomonas maltophilia	2	0	2	0
Acinetobacter lwoffii	2	1	1	0
Rhizobium radiobacter	1	0	1	0
Bacteroides fragilis	1	0	1	0
Campylobacter jejuni	1	0	1	0
Gordonia species	1	0	0	0
Sphingobacerium multivorum	1	0	1	0
Gram-ve bacillus	2	1	1	0

3.7.3 Respiratory samples

Most of the microbiological samples during maintenance were respiratory samples; with 167/235 (71.0%) patients having at least one respiratory sample and 143/167 (85.6%) of these having at least one that was positive. Of the total 843 respiratory samples, over half, 498 (59.1%) were positive.

Respiratory sample rates and 95% confidence intervals per 100 person-weeks are shown in Table 3.7-7. The rate of patients with a respiratory sample was 1.7 per 100 persons-weeks (95%CI 1.4-1.9) and the rate of patients with at least one positive respiratory sample was 1.1 (95%CI 1.0-1.4). No difference was observed in rates of patients sampled by age, sex, deprivation category, or regimen. Significant differences were only seen by age in the rate of patients with at least one positive sample; those diagnosed under the age of ten having a higher rate at 1.2 (95% CI 1.0-1.5) compared to 1.0 (95%CI 0.7-1.3; χ^2=4.6, p=0.032) in those aged ten or more.

Examining the rates of all samples, significant differences were seen by sex and age at diagnosis. The rate of all samples among males at 3.9 (95% CI 3.6-4.2) was significantly higher than that of females, 2.1 (95% CI 1.8-2.4) (χ^2=4.4, p=0.036), but no difference was seen in the rate of all positive samples. Also, the rate of all samples was significantly higher in patients aged under ten years at diagnosis (4.1, 95% CI 3.8-4.4) compared to those aged ten years or more (2.5, 95% CI 2.1-2.9; χ^2=32.0, p<0.0001). Younger patients also had a higher rate of positive samples; 2.4 (95% CI 2.2-2.7) in those under ten years compared to a rate of 1.4 (95% CI 1.2-1.8) in those 10 years or more (χ^2=19.6, p<0.0001).

Table 3.7-7 Person weeks and sampling rates per 100 person-weeks for respiratory samples distributed by sex, age, deprivation and regimen

	Patients						Samples					
	At least one sample			At least one positive sample			All samples			All positive samples		
	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate(95%CI)	N	Person-weeks	Rate (95%CI)	N	Person-weeks	Rate(95%CI)
Total	167	9995.2	1.7(1.4-1.9)	143	12435.3	1.1(1.0-1.4)	843	23002.3	3.7(3.4-3.9)	498	23002.3	2.2(2.0-2.4)
Sex												
Male	99	5802.9	1.7(1.4-2.1)	87	7468.5	1.2(0.9-1.4)	591	15343.3	3.9(3.6-4.2)	339	15343.3	2.2(2.0-2.5)
Female	68	4192.3	1.6(1.3-2.1)	56	4966.8	1.1(0.9-1.5)	252	7659.0	3.3(2.9-3.7)	159	7659.0	2.1(1.8-2.4)
Age												
1-9	128	7140.8	1.8(1.5-2.1)	109	8883.4	1.2(1.0-1.5)	696	17026.7	4.1(3.8-4.4)	412	17026.7	2.4(2.2-2.7)
10-17	39	2854.4	1.4(1.0-1.9)	34	3551.9	1.0(0.7-1.3)	147	5975.6	2.5(2.1-2.9)	86	5975.6	1.4(1.2-1.8)
Deprivation												
More affluent	84	5441.6	1.5(1.2-1.9)	72	6739.4	1.1(0.8-1.3)	433	11951.3	3.6(3.3-4.0)	247	11951.3	2.1(1.8-2.3)
More deprived	83	4553.8	1.8(1.5-2.3)	71	5696.5	1.2(1.0-1.6)	410	11051.0	3.7(3.4-4.1)	251	11051.0	2.3(2.0-2.6)
Regimen												
A	75	4417.3	1.7(1.4-2.1)	66	5320.6	1.2(1.0-1.6)	389	10039.0	3.9(3.5-4.3)	226	10039.0	2.3(2.0-2.6)
B	39	2868.4	1.4(1.0-1.9)	32	3511.0	0.9(0.6-1.3)	187	5738.0	3.3(2.8-3.8)	110	5738.0	1.9(1.6-2.3)
C	53	2709.5	2.0(1.5-2.6)	45	3603.7	1.2(0.9-1.7)	267	7225.3	3.7(3.3-4.2)	162	7225.3	2.2(1.9-2.6)

Rates and 95% confidence intervals (CI) are shown per 100 person-weeks. Person-weeks were calculated from the start of maintenance until the first respiratory sample, or the first positive respiratory sample, to examine the rate of the first respiratory sample or the first positive respiratory sample; for all respiratory samples and all positive respiratory samples, follow-up continued from the start of maintenance until the end of treatment, the end of data collection or death. Differences between rates were tested using the Mantel-Haenszel method; statistically significant differences were seen for the rate of all samples among boys compared to girls (χ^2=4.4, p=0.036), the rate of at least one positive sample among ages 1-9 years compared to 10 years or more (χ^2=4.6, p=0.032), the rate of all samples among ages 1-9 years compared to 10 years or more (χ^2=32.0, p<0.0001), the rate of all positive samples among ages 1-9 compared to 10 years of more (χ^2=19.6, p<0.0001).

There were 582 pathogens isolated from 498 positive respiratory samples; 120 respiratory samples were positive for two pathogens, six for three, and four for four. Information on the source of the sample and pathogens isolated is presented in Table 3.7-8- to Table 3.7-10.

Most respiratory samples (639/843, 75.8%) and most that were positive (387/498, 77.7%) were nose/throat swabs. There were 14 bronchoalveolar lavage (BAL) samples (from 13 patients), ten of which were positive for at least one pathogen (from nine patients).

Most of the pathogens (473/582) isolated were respiratory viruses. 212/473 respiratory viral samples were rhinovirus, 79 parainfluenza viruses and 72 respiratory syncytial virus (RSV). There were 40 positive for bacteria, with *Haemophilus influenzae* isolated in 13/40 (32.5%). Of the 64 fungal pathogens, 51 were *Candida albicans* (33 nose/throat, 17 sputum, one BAL), 13 were other yeast species (11 nose/throat, two sputum). The ten BAL samples identified 19 pathogens. Five of these BAL samples were positive for *Pneumocystis jirovecii* (PJP) from five different patients; 4/5 of whom were diagnosed with ALL over the age of ten years. One of those BAL samples was also positive for *Candida albicans*. Of the remaining 13 pathogens isolated in BAL samples; three were bacteria (*Haemophilus*, *Pseudomonas* species and *S. aureus*) and ten viruses (rhinovirus in five samples, parainfluenza viruses in three and one each of adenovirus and metapneumovirus). There was only one sample from an ET (endotracheal tube) secretion and it isolated *Bordetella bronchiseptica*.

Table 3.7-8 Respiratory samples and positives during maintenance by source

	Respiratory samples N (%)	Positive respiratory samples N (%)
Total	**843**	**498**
Source		
Nose/throat swab	639 (75.8)	387 (77.7)
NPA	90 (10.7)	55 (11.0)
Sputum	86 (10.2)	38 (7.6)
BAL	14 (1.7)	10 (2.0)
ET secretions	1 (0.1)	1 (0.2)
Unknown	13 (1.5)	7 (1.4)

Table 3.7-9 Types of microorganisms in positive respiratory samples during maintenance by source

Positive respiratory samples					
	Total	**Bacterial**	**Viral**	**Fungal**	**PJP**
Total	**582**	**40**	**473**	**64**	**5**
Source					
Nose/throat swab	448 (76.9)	21 (52.5)	383 (80.9)	44 (68.7)	0 (0.0)
NPA	66 (11.3)	1 (2.5)	65 (13.7)	0 (0.0)	0 (0.0)
Sputum	40 (6.8)	14 (35.0)	7 (1.5)	19 (29.6)	0 (0.0)
BAL	19 (3.3)	3 (7.5)	10 (2.1)	1 (1.7)	5 (100.0)
ET secretions	1 (0.2)	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	8 (1.4)	0 (0.0)	8 (1.5)	0 (0.0)	0 (0.0)

Table 3.7-10 Pathogens isolated during maintenance by microorganism type

Viral		Bacterial		Fungal	
Total	**473**	**Total**	**40**	**Total**	**64**
Rhinovirus	212	*H. influenzae*	13	*C. albicans*	51
RSV	72	*Strep. pneumoniae*	5	Yeasts	3
Metapneumovirus	36	*S. aureus*	4	*C. parapsilosis*	3
Parainfluenza 1	31	*Coliform* species	3	*C. kefyr*	3
Parainfluenza 3	28	Beta haemolytic *Strep.*	4	*C. lusitaniae*	2
Influenza B	20	*Pseudomonas aeruginosa*	3	*C. krusei*	1
Influenza A H1	19	*Enterococcus* species	2	*C. krusei*	1
Parainfluenza 4	18	*Moraxella catarhallis*	2		
Adenovirus	15	*Bordetella bronchiseptica*	1		
Influenza A H3	15	CONS	1		
Influenza A	5	*Mycoplasma*	1		
Parainfluenza 2	2	*Neisseria meningitidis*	1		

3.7.4 Fungal pathogens

Of the 235 patients, 62 (26.4%) had samples sent for fungal testing, yielding a total of 97 samples; of which 95 were from blood, one lung tissue biopsy, and one renal stone biopsy. All blood samples were tested for Aspergillus antigen apart from two; one for microscopy and culture (MCS) and one for mycology serology. The two biopsy samples were sent for MCS testing.

Only three samples were positive. These came from the following patients; a female who had a blood sample positive for *Candida lusitaniae* on MCS (age group 1-4 and treated under regimen A). Another female patient had a lung tissue biopsy sample positive for *Aspergillus fumigatus* (age≥10 and treated under regimen B). Finally, a male patient had a blood sample positive for *Candida albicans* (age group 1-4 and treated under regimen A). All three patients survived and reached the end of treatment.

3.7.5 Samples from males in the third year of maintenance

Of 134 males scheduled to start maintenance, 18 had not reached their third year of maintenance by the end of data collection, and six were taken off the treatment protocol in the first two years of maintenance; five of whom had relapsed and one developed haemophagocytic lymphohistiocytosis (HLH) earlier during maintenance.

This section presents information about the samples from the 110 male patients during the extra year of maintenance; 80/110 (72.7%) had at least one sample and 48/80 (60%) of these had at least one positive sample. In total there were 488 samples during this time; 313 blood cultures, 156 respiratory samples and 12 samples sent for fungal testing. Of the 111 positive samples, 27 were blood cultures and 84 were respiratory samples.

As shown in Table 3.7-11, the characteristics of male patients with at least one sample and at least one positive sample were broadly similar to those without.

Table 3.7-11 Characteristics of male patients and their samples during the third year of maintenance

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**110**	**30**	**80**	**48**	**488**	**111**
Diagnosis						
B-ALL	85 (77.3)	21 (70.0)	64 (80.0)	37 (77.0)	415 (85.0)	88 (79.2)
T-ALL	25 (22.7)	9 (30.0)	16 (20.0)	11 (23.0)	73 (15.0)	23 (20.8)
Age at diagnosis (years)						
1-9	79 (71.8)	20 (66.7)	59 (73.7)	34 (70.8)	404 (82.8)	86 (77.4)
10-17	31 (28.2)	10 (33.3)	21 (26.3)	14 (29.2)	84 (11.2)	25 (22.6)
Regimen						
A	48 (43.6)	12 (40.0)	36 (45.0)	21 (43.7)	248 (50.8)	50 (45.0)
B	27 (24.5)	9 (30.0)	18 (22.5)	12 (25.0)	91 (18.6)	25 (22.5)
C	35 (68.1)	9 (30.0)	26 (32.5)	15 (31.3)	149 (30.6)	36 (32.5)
Deprivation						
More affluent	57 (51.8)	11 (36.6)	46 (57.5)	26 (54.1)	256 (52.4)	57 (51.3)
More deprived	53 (48.2)	19 (63.4)	34 (42.5)	22 (45.9)	232 (47.6)	54 (48.7)

Of the 110 males, 75 (68.2%) had at least one blood culture during the third year of maintenance, and only 17/75 (22.6%) of these were positive. Overall, there was a total of 315 blood cultures with 29 positive. As shown in Table 3.7-12, the characteristics of male patients with at least one sample and at least one positive sample were broadly similar to those without.

Table 3.7-12 Characteristics of male patients and their blood culture samples during the third year of maintenance

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**110**	**35**	**75**	**17**	**315**	**29**
Diagnosis						
B-ALL	85 (77.3)	24 (68.6)	61 (81.3)	13 (76.4)	275 (87.3)	25 (86.2)
T-ALL	25 (22.7)	11 (31.4)	14 (18.7)	4 (23.6)	40 (22.7)	4 (13.8)
Age at diagnosis (years)						
1-9	79 (71.8)	24 (68.6)	55 (73.3)	15 (88.2)	268 (85.0)	27 (93.1)
10-17	31 (28.2)	11 (31.4)	20 (26.7)	2 (11.8)	47 (25.0)	2 (6.9)
Regimen						
A	48 (43.6)	13 (37.1)	35 (46.6)	7 (41.2)	161 (51.1)	14 (48.3)
B	27 (24.5)	10 (28.5)	17 (22.6)	3 (17.6)	56 (17.8)	5 (17.2)
C	35 (68.1)	12 (34.4)	23 (30.8)	7 (41.2)	98 (31.1)	10 (34.5)
Deprivation						
More affluent	57 (51.8)	13 (37.1)	44 (58.6)	7 (41.2)	165 (52.4)	14 (48.3)
More deprived	53 (48.2)	22 (62.9)	31 (41.4)	10 (58.8)	150 (47.6)	15 (51.7)

Just over half of the males (59/110, 53.6%) had respiratory samples taken during the extra year of maintenance and 43/59 (72.8%) of these had samples that were positive. Overall, there was a total of 156 respiratory samples, just over half of which (84/156, 53.8%) were positive.

No differences were seen between the characteristics of patients with and without respiratory samples and positive respiratory samples (Table 3.7-13).

Table 3.7-13 Characteristics of male patients and their respiratory samples during the third year of maintenance

	Patients				Samples	
	Total N (%)	**No samples N (%)**	**At least one sample N (%)**	**At least one positive N (%)**	**Total N (%)**	**Positives N (%)**
Total	**110**	**51**	**59**	**43**	**156**	**84**
Diagnosis						
B-ALL	85 (77.3)	38 (74.5)	47 (79.6)	33 (76.7)	126 (80.8)	65 (77.3)
T-ALL	25 (22.7)	13 (25.5)	12 (20.4)	10 (23.3)	30 (19.2)	19 (22.7)
Age at diagnosis (years)						
1-9	79 (71.8)	33 (64.7)	46 (77.9)	30 (69.7)	125 (80.2)	61 (72.6)
10-17	31 (28.2)	18 (35.3)	13 (22.1)	13 (30.3)	31 (19.8)	23 (27.4)
Regimen						
A	48 (43.6)	18 (35.3)	30 (50.8)	20 (46.4)	77 (49.4)	38 (45.2)
B	27 (24.5)	15 (29.4)	12 (20.3)	10 (23.3)	32 (20.5)	20 (23.8)
C	35 (68.1)	18 (35.3)	17 (28.9)	13 (30.3)	47 (30.1)	26 (31.0)
Deprivation						
More affluent	57 (51.8)	25 (49.0)	32 (54.2)	23 (53.6)	85 (54.5)	45 (53.5)
More deprived	53 (48.2)	26 (51.0)	27 (45.8)	20 (46.4)	71 (45.5)	39 (46.5)

3.7.6 Summary of findings during maintenance

During maintenance, patients return to a “near normal” routine, with most returning to school/childcare settings. Boys, of course, have an extra year compared to girls, with the chemotherapy mostly administered at home, with regular hospital outpatient check-ups. Therefore, as noted in the general paediatric population, younger children would be expected to have more infections, particularly viral respiratory illnesses (7,8,11,12). Finally, with regards to patients living in more or less affluent areas, given that most of maintenance is expected to be delivered in an outpatient basis, differences were not unexpected.

Our findings revealed significant differences in sampling, and in some cases also in positive sampling rates by age at diagnosis, sex, regimen and deprivation. These are summarized and briefly discussed below.

Under ten year olds were more likely to have a positive blood culture and respiratory sample and had higher rates of both sample types and higher rates of positive samples in both sample types than the patients aged ten years or more. This was expected and there are a few potential contributors to this finding. Younger patients have an immature immune system with the additional effects of immunosuppressive and myelosuppressive therapy rendering them more susceptible to infections (8,12,112,120,121). They are more likely to develop symptoms of respiratory viral illnesses with pyrexia, and therefore more likely to be brought to the hospital for medical assessment. The patients are more likely to have percutaneously sited central lines which are more susceptible to line infection as they are exposed (126). Younger children are less able to vocalise their symptoms, and hence their carers may be more likely to attend hospital with any concerning behaviour or observation at home.

Another patient group of particular interest were male patients as they had an extra year of treatment. Overall, males had blood cultures and respiratory samples taken more frequently than females, but the rates of positive samples were similar in males and females. But, as seen in our data, there were not many samples and positives from males in the extra year of maintenance.

Patients living in more deprived areas were more likely to have a positive blood culture and had higher rates of blood cultures and positive blood cultures than those living in more affluent areas. No differences were seen by deprivation in respiratory samples. This was an interesting finding that potentially has clinical implications as it is possible that it may be modifiable.

3.8 Pathogens from total microbiological samples

There were 3,150 blood culture samples, 1,326 respiratory samples and 352 samples sent for fungal testing of which 396 blood cultures, 770 respiratory samples and 28 samples sent for fungal testing were positive (Figure 3.8-1). We have seen that most microbiological samples throughout treatment were blood cultures (3150/4828, 65.2%), but only a small proportion of these were positive (396/3150, 12.6%). By comparison, over half of the respiratory samples were positive with at least one bacterial, viral or fungal pathogen (770/1326, 58.1%). Induction was the only phase where most of the positive samples were blood culture samples. In all other phases, most of the positive samples were respiratory samples (Figure 3.8-1). So in line with published literature, the most common site of documented infection in children being treated for ALL was the upper respiratory tract (7,8,11,12).

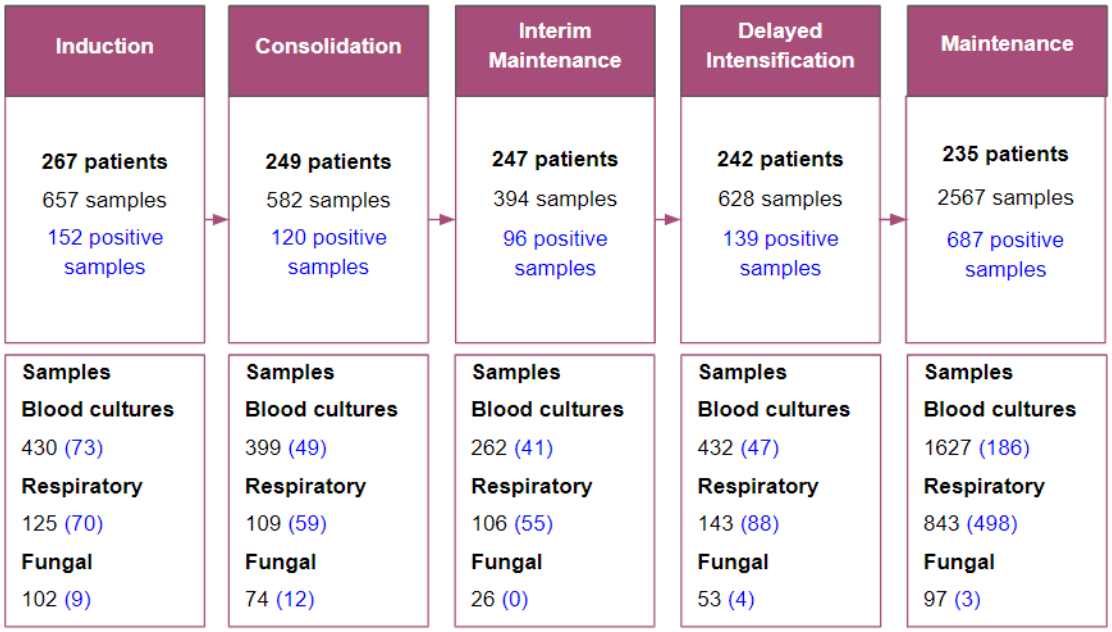

Figure 3.8-1 Patients and their samples by treatment phase

Although rare, samples positive for fungal testing were observed in all but the interim maintenance phase. Of the total 352 samples sent for fungal testing, only 28 were positive; nine in induction, twelve in consolidation, four in delayed intensification and three in maintenance. The majority of the positive fungal samples (21/28) were seen during induction (9/28) and consolidation (12/28).

Most microorganisms in blood cultures (338/445, 75.9%), were Gram +ve (Table 3.8-1). The most common pathogen in all treatment phases was coagulase-negative *Staphylococcus* (179/338). *Streptococcus pneumoniae* was the second most common overall, the majority (28/32) being isolated during maintenance. Viridans group streptococci and *S. aureus* were seen in most phases. *S. aureus*, however, was isolated mostly during induction (13/20). Viridans group streptococci were the third most common Gram +ve pathogen (30/338); mostly isolated during maintenance (11/30). Whilst other studies have reported that viridans group streptococci may account for up to 30% of bacteraemias (76,77,91), in our study they only represented 8.8% of the total isolated Gram +ve pathogens (30/338). This could be due to differences in testing procedures, local ecology, and may also have been affected by empirical antibiotic choices. *Enterococcus* species were only grown during induction and maintenance. There was only one sample positive for methicillin-resistant *Staphylococcus aureus* (MRSA) from a patient during induction.

Of the 107 Gram -ve bacteria, 27 were isolated in induction and 35 in maintenance (Table 3.8-2). Overall, *E. coli* (27/107) was the most common Gram -ve microorganism followed by *Pseudomonas aeruginosa* (18/107). This is in contrast to other studies were *P. aeruginosa* was the most frequently isolated Gram -ve microorganism. Overall, however, *E. coli*, *P. aeruginosa* and *Klebsiella* species were the most frequently isolated, which is in line with international reports of Gram -ve bacteria isolated in the blood cultures of children being treated for ALL (13,76).

Of the 427 microorganisms isolated at the time that a concurrent full blood count result was available, 321 were Gram +ve and 106 were Gram -ve. Of

the 171 microorganisms isolated at the time of neutropenia, most (125/171, 73.1%) were isolated during intensive phases of treatment: 63 in induction, 30 in consolidation, 32 in delayed intensification. Overall, 120/321 Gram +ve and 51/106 Gram -ve bacteria were grown during neutropenia (data not presented).

Table 3.8-1 Gram +ve bacteria in blood cultures by phase

	Total	Induction	Consolidation	Interim maintenance	Delayed intensification	Maintenance
	338	**59**	**37**	**32**	**36**	**174**
CONS	179	22	21	16	32	88
Streptococcus pneumoniae	32	3	0	1	0	28
Viridans group streptococci	30	8	8	3	0	11
Staphylococcus aureus	20	13	3	2	0	2
Corynebacterium species	18	0	3	1	0	14
Actinobacteria	15	2	1	2	3	8
Enterococcus species	13	7	0	0	0	6
Propionibacterium species	7	0	0	3	0	4
Other streptococci	6	1	1	1	0	3
Microbacterium species	4	0	0	0	0	4
Clostridium species	1	0	0	1	0	0
MRSA	1	1	0	0	0	0
Other	11	2	0	2	1	6

Table 3.8-2 Gram -ve bacteria in blood cultures by phase

	Total	Induction	Consolidation	Interim maintenance	Delayed intensification	Maintenance
Total	**107**	**27**	**15**	**16**	**14**	**35**
E. coli	27	10	2	3	4	8
Pseudomonas aeruginosa	18	3	5	3	6	1
Klebsiella species	10	2	1	4	0	3
Enterobacter species	10	5	0	0	2	3
Moraxella species	7	1	2	1	0	3
Stenotrophomonas maltophilia	5	0	1	2	0	2
Bacteroides fragilis	4	3	0	0	0	1
Neisseria species	3	0	0	0	0	3
Other *Pseudomonas* species	3	0	1	0	0	2
Acinetobacter species	2	0	0	0	0	2
Aeromonas caviae	2	0	0	2	0	0
Haemophilus influenzae	2	0	0	0	0	2
Rhizobacterium radiobacter	2	0	0	0	1	1
Sphingomonas paucimobilis	2	0	1	0	1	0
Capnocytophaga sputigena	1	0	1	0	0	0
Fusobacterium species	1	0	1	0	0	0
Campylobacter jejuni	1	0	0	0	0	1
Other	7	3	0	1	0	3

Looking at the source of blood cultures and the isolated bacteria it can be seen that around 65% (220/335) Gram +ve microorganisms were isolated from central line cultures, mostly implanted ones (135/220) (Table 3.8-3). There were 27 paired cultures with Gram +ve isolates, 16 of which had a growth of CONS. Although there were fewer Gram -ve isolates, the opposite trend was seen, with more isolated from percutaneously sited lines (40/107) compared to implanted ones (25/107) (Table 3.8-4). Gram -ve bacteria have been reported to be more frequently isolated from percutaneously sited CVLs compared to implanted ones (126); although the numbers are very small, our findings confirmed this. Only ten paired cultures had Gram -ve isolates. The predominance of Gram -ve bacteria from percutaneous line cultures may be due to exogenous infection from infected line tubing; for instance lines sitting in the nappy area of young children or near drains when bathed etc. There were only ten patients diagnosed above the age of ten with positive blood cultures from a percutaneously sited line; 24 microorganisms were isolated from their line cultures, 17 Gram +ve and seven Gram -ve (data not presented).

Table 3.8-3 Gram +ve bacteria in blood cultures by source

	Total	**Implanted CVL**	**Percutaneous CVL**	**Paired**	**Peripheral**	**Unknown**
Total	**338**	**135**	**85**	**27**	**37**	**54**
CONS	179	75	42	16	15	31
Streptococcus						
-pneumoniae	32	18	2	5	2	5
-viridans-group	30	9	7	2	7	5
-other	6	2	3	0	1	0
S. aureus	20	3	4	3	6	4
Corynebacterium species	18	8	7	1	`1	1
Actinobacteria	16	4	5	0	2	5
Enterococcus species	13	2	7	0	1	3
Propionibacterium species	7	6	1	0	0	0
Microbacterium species	4	0	4	0	0	0
MRSA	1	0	1	0	0	0
Other	12	8	2	0	2	0

Table 3.8-4 Gram -ve bacteria in blood cultures by source

	Total	Implanted CVL	Percutaneous CVL	Paired	Peripheral	Unknown
Total	**107**	**25**	**40**	**10**	**17**	**15**
E. coli	27	4	8	4	6	5
Pseudomonas species	21	6	7	3	1	4
Klebsiella species	10	2	7	0	0	1
Enterobacter species	10	3	3	2	1	1
Moraxella species	7	0	1	0	5	1
Stenotrophomonas maltophilia	5	0	5	0	0	0
Neisseria species	3	2	1	0	0	0
Acinetobacter species	2	0	0	0	2	0
Haemophilus influenzae	2	1	1	0	0	0
Rhizobacterium radiobacter	2	0	2	0	0	0
Other	18	7	5	1	2	3

Of the 682 positive respiratory samples, 473 (69.4%) were taken during maintenance (Table 3.8-5). This is not surprising as maintenance spans over years as opposed to the few weeks of other phases. Also, children have resumed some normality by the time they get to maintenance, with most being back at school/nursery and socialising with other children in non-hospital settings. Therefore, they are more likely to be exposed to respiratory pathogens. During this phase, the most frequently isolated virus was rhinovirus (341/682), which was expected as rhinovirus is common and also tends to linger in the respiratory tract of patients making it likely to be present in repeat samples. Respiratory syncytial virus (RSV) was the second most common and was encountered in all phases. RSV can cause severe infections involving the lower respiratory tract and is likely to have been a clinically significant pathogen. Similarly, metapneumovirus can cause complicated respiratory tract infections – found in 40/682 positive viral samples. Other respiratory viruses seen in all phases included parainfluenza 1, 3 and 4 and influenza B. Although the data were collected during the time of the influenza

H1 pandemic (2009-2010), it was only isolated in 21 samples, 19 of which in maintenance.

Haemophilus influenzae was the most frequently isolated bacterial pathogen from respiratory samples (15/71), though mostly seen in maintenance (Table 3.8-6). *Coliform* (9/71) and *Enterococcus* species (9/71) were seen in most phases. *Streptococcus pneumoniae* which is likely to have been isolated at the time of lower respiratory tract infection was isolated from 7/71 samples and *Mycoplasma* was only found in one sample.

Of the 133 fungal pathogens isolated from respiratory samples, 109 (81.9%) were *Candida albicans* (Table 3.8-7). The significance of these isolates, which mostly came from nose/throat swabs, is unknown. *Pneumocystis jirovecii* was isolated in six samples – once in delayed intensification and five times in maintenance

Table 3.8-5 Respiratory viruses in respiratory samples by phase

	Total	Induction	Consolidation	Interim maintenance	Delayed intensification	Maintenance
	682	**41**	**43**	**47**	**78**	**473**
Rhinovirus	341	24	27	27	51	212
RSV	99	8	5	9	5	72
Parainfluenza 3	44	3	2	2	9	28
Metapneumovirus	40	0	1	1	2	36
Parainfluenza 1	39	1	1	1	5	31
Influenza B	31	1	1	4	5	20
Parainfluenza 4	24	3	2	1	0	18
Influenza A H1	21	1	1	0	0	19
Adenovirus	18	0	1	1	1	15
Influenza A H3	16	0	1	0	0	15
Influenza A	5	0	0	0	0	5

Table 3.8-6 Bacterial microorganisms in respiratory samples

	Total	Induction	Consolidation	Interim maintenance	Delayed intensification	Maintenance
Total	**71**	**16**	**7**	**3**	**5**	**40**
Haemophilus species	15	0	0	2	0	13
Coliform species	9	3	1	0	2	3
Enterococcus species	9	4	3	0	0	2
Staphylococcus aureus	8	3	0	0	1	4
Streptococcus pneumoniae	7	1	1	0	0	5
CONS	6	3	0	1	1	1
Other streptococci	5	0	0	0	1	4
Pseudomonas aeruginosa	5	1	1	0	0	3
Moraxella catarhallis	4	1	1	0	0	2
Bordetella bronchiseptics	1	0	0	0	0	1
Mycoplasma	1	0	0	0	0	1
Neisseria meningitidis	1	0	0	0	0	1

Table 3.8-7 Fungal microorganisms isolated in respiratory samples

	Total	Induction	Consolidation	Interim maintenance	Delayed intensification	Maintenance
Total	**133**	**28**	**16**	**9**	**16**	**64**
Candida albicans	109	24	11	9	14	51
Aspergillus fumigatus	2	0	2	0	0	0
Yeasts	9	3	2	0	0	4
Candida parapsilosis	6	1	0	0	2	3
Candida kefyr	4	0	1	0	0	3
Candida lusitaniae	2	0	0	0	0	2
Candida krusei	1	0	0	0	0	1

As already mentioned in earlier sections, only 28 samples sent for fungal testing were positive; 22 blood samples, three lung tissue biopsy samples, one arm skin tissue biopsy and two cerebrospinal fluid (CSF) samples (Table 3.8-8). 15/28 positive samples were blood samples tested for fungal serology - mostly Aspergillus antigen testing (11/15). The seven remaining blood samples were mostly positive for yeasts; there was *C. tropicalis* growth from two line cultures (same patient*), C. albicans* from line culture in two occasions and paired cultures in another, and *C. lusitaniae* from a line culture. Finally, *Aspergillus fumigatus* was grown from a blood culture sample of unknown source. The remaining fungal pathogens were isolated from other biopsy sites and CSF sampling. Although, invasive fungal diseases are more common at times of profound prolonged neutropenia, the only MCS samples with a fungal isolate taken at time of neutropenia were a CSF and a lung biopsy sample with *Aspergillus fumigatus*, a skin biopsy with *C. tropicalis*, and a line blood culture with *C. lusitaniae*. Overall, only 13/28 positive samples for fungal testing were taken at time of neutropenia.

Table 3.8-8 Positive fungal tests and pathogens from blood/biopsies by phase

	Total	Induction	Consolidation	Delayed intensification	Maintenance
Total	**28**	**9**	**12**	**4**	**3**
Aspergillus antigen	11	2	7	2	0
Candida antigen	2	1	1	0	0
β-d glucan	2	1	1	0	0
Aspergillus fumigatus	3	1	1	0	1
Candida tropicalis	3	2	1	0	0
Candida albicans	3	2	0	0	1
Mycelium	2	0	0	2	0
Candida lusitaniae	1	0	0	0	1
Candida parapsilosis	1	0	1	0	0

No samples were positive for fungal pathogens during interim maintenance.

Invasive fungal infections are associated with high mortality rates, and so the outcomes of patients with positive fungal samples were reviewed. There were three deaths around the time that the samples that were positive for fungal pathogens were taken, and all affected patients were male. One patient who died during induction had *Candida albicans* septicaemia (also the main cause of death on the death certificate). Another patient who died of disseminated varicella zoster disease during consolidation, also had a blood sample that was positive for Aspergillus antigen a few days before death. Finally, another patient died of pneumonia during maintenance who tested positive for *Candida parapsilosis* in a nose/throat swab taken a few days prior to their death- the significance of this isolate is unknown.

3.8.1 Microbiological samples from patients with Down syndrome

Examining the microbiological samples from patients with Down syndrome, only one patient who moved to a different protocol post induction treatment did not have any samples or positive samples. The remaining four patients

had a range of 11-19 samples per patient during treatment; two patients had one positive blood culture each (Enterococcus species and coagulase-negative Staphylococcus), another had two nose/throat swabs positive for respiratory viruses and another one had a blood culture positive for CONS and a lung tissue biopsy positive for mycelium on microscopy and culture.

3.8.2 Patients with microbiological samples across all treatment phases

It was noted that of the 51 patients who had samples across all treatment phases, only four patients had positive samples in all phases (Table 3.8-9). No significant differences were seen between the characteristics of patients who had samples in all phases compared to those who did not. Proportionately more patients diagnosed with T-ALL had samples (13/51) and blood culture samples (10/39) in all phases compared to patients with B-ALL (where the figures were 38/51 and 29/39). Also, proportionately more patients treated under regimen C (24/51) had samples in all phases compared to regimen A (17/51) and B (10/51). The difference was more marked for blood culture samples with 20/39 patients with blood cultures in all treatment phases having been treated under regimen C. Most patients with samples in all phases (41/51) were diagnosed under the age of ten years.

Table 3.8-9 Characteristics of patients with samples in all phases

	Total N (%)	Samples in all phases N (%)	Blood cultures in all phases N (%)
Total	**267**	**51**	**39**
Diagnosis			
B-ALL	221 (82.8)	38 (74.5)	29 (74.3)
- hyperdiploidy	86 (32.2)	14 (27.5)	12 (30.7)
- NOS	80 (29.9)	12 (23.5)	9 (23.0)
- t(12;21)	44 (16.5)	9 (17.6)	5 (12.8)
- t(9;22)	6 (2.3)	0 (0.0)	0 (0.0)
- hypodiploidy	3 (1.1)	1 (1.9)	1 (2.5)
- MLL rearrangement	2 (0.8)	2 (3.9)	2 (5.1)
T-ALL	46 (17.2)	13 (25.5)	10 (25.7)
Regimen			
A	117 (43.8)	17 (33.3)	12 (30.7)
B	68 (25.5)	10 (19.6)	7 (17.9)
C	82 (30.7)	24 (47.1)	20 (51.4)
Sex			
Female	118 (44.2)	20 (39.2)	16 (41.0)
Male	149 (55.8)	31 (60.8)	23 (59.0)
Age at diagnosis (years)			
1-9	197 (73.8)	41 (80.4)	32 (82.0)
10-17	70 (26.2)	10 (19.4)	7 (18.0)

Chapter 4 Discussion

The central aim of this thesis was to explore infection frequencies and patterns in children with acute lymphoblastic leukaemia (ALL) and to identify patient groups who are particularly at risk. The prevalence of microbiologically documented bacterial, viral and fungal infections was reviewed during treatment in its entirety, and in the context of different treatment phases. Gaining more understanding through this retrospective review of the prevalence of infection in children with ALL, aimed to provide some insight into the mechanisms behind susceptibility and inform the way we manage infection.

To this end, data on the microbiological samples collected during the course of treatment in a cohort of 267 patients aged 1-17 years who were diagnosed September 2004 and February 2017 were analysed. Patients diagnosed in the population-based Haematological Malignancy Research Network (HMRN) were included, regardless of trial participation.

To date, this is the only UK cohort of patients with ALL treated in a paediatric setting whose microbiological history during treatment has been studied. This Chapter presents the key results and strength and weaknesses of my work, along with an evaluation of the findings and comparison with existing evidence.

4.1 Evaluation of study findings

4.1.1 Infection related mortality

With cure rates of over 90% in developed regions of the world, death in children with ALL is a relatively uncommon, but nonetheless a devastating event (1–4). In such settings, the leading cause of treatment related mortality (TRM) is infection, with the incidence of reported infection-related mortality (IRM) ranging between 1.7% and 2.3% (5–10). In the cohort studied in this thesis, 13 patients died of infection-related causes demonstrating a higher

overall IRM of 4.8% (13/267). Importantly, this real-world study included all patients, regardless of trial participation; which may have contributed to the higher IRM compared to trial reports. Higher IRM was seen in patients treated in the UKALL 2003 era (8/13 infection related deaths) with lower IRM in patients treated under the UKALL 2011 era (4/13) and one death due to infection in the patients treated under the interim guidelines; the figures of IRM were 5.6%, 3.7% and 6.25% respectively. Treatment intensity modification and evolution of supportive care are the most likely causes of the difference in IRM between the UKALL 2003 and UKALL 2011 eras. Patients treated under the UKALL 2003 trial protocol were randomised between one and two courses of delayed intensification but all patients treated under the UKALL 2011 protocols only had one course of delayed intensification. The provision of supportive care measures has evolved over time, with better microbiological diagnostic tools, more broad spectrum antimicrobial agents and more vigilant clinical reviews during neutropenia with early initiation of treatment. Causes of death were collected from death certificates and classified as infective or non-infective based on a verified classification system (185). As outlined in Section 3.1.3, six deaths occurred at the time of bacteraemia, with three Gram +ve (a case of *Abiotrophia defectiva* causing infective endocarditis and *S. aureus* bacteraemia on two occasions) and three Gram -ve microorganisms isolated (one case of *Klebsiella pneumoniae* and *E. coli* on two occasions).

The UKALL 2003 trial reported a 5-year cumulative incidence of IRM of 2.4%, with infection accounting for 30% of deaths and 64% of treatment related deaths (10,193). Around two-thirds (68%) of infection-related deaths were caused by bacterial infection, predominantly with Gram -ve pathogens (64%), 20% were caused by fungal infection and 12% were caused by viral pathogens. There are no published reports of the UKALL 2011 trial to date.

In UKALL 2003, patients with Down syndrome were found to have a sevenfold higher risk of TRM compared to non-Down syndrome children (10). There were no deaths in the five patients with Down syndrome in my cohort; but two patients with ataxia telangiectasia died during treatment, one with

candidaemia and the other during palliation post infection-related complications (Section 3.1.3).

Due to the known increased risk of treatment-related toxicity and particularly infection-related morbidity and mortality, patients with Down syndrome were treated less intensively and received ciprofloxacin prophylaxis during induction additional to the standard co-trimoxazole prophylaxis during the rest of treatment (194).

4.1.2 Microbiological samples and pathogens

In line with other published reports, the findings presented in this thesis demonstrated that infection affects most children with ALL, with 254/267 (95.1%) patients having at least one microbiological sample taken during treatment, and 230/254 (90.5%) of these having at least one positive sample (76). More specifically looking at blood culture samples, most of the included patients had at least one blood culture (252/267, 94.4%), and almost two-thirds of these had at least one positive blood culture (162/252, 64.3%).

Considerable variability in the burden of infection between patients was observed (Figure 3.2-2). Some patients had a lot of samples and samples yielding positive results across all treatment phases, whereas others seemed to go through treatment with very few instances of suspected or microbiologically confirmed infection. This suggests that there may be individual factors that result in increased rates of infection. The role of individuality has been discussed in other studies (7,8,78).

We have identified different patient groups who are likely to be at increased risk of infection during different parts of treatment. The main findings per microbiological sample type and per phase of treatment are outlined below.

Blood culture samples

Similar to other studies, Gram +ve bacteria were isolated more frequently 338/445 (75.9%) (7,12,13,77,78,80,84). The predominance of Gram +ve

bacteria in recent years has been linked with more intensive chemotherapy, possibly driven by a higher risk of invasive infection due to less virulent skin flora in the context of severe myelosuppression with prolonged and profound neutropenia (76,195). The most common pathogen in all treatment phases was coagulase-negative *Staphylococcus* (179/338), followed by *Streptococcus pneumoniae* (32/338), viridans group streptococci (30/338) and *S. aureus* (20/338). CONS is commonly associated with the presence of an indwelling central venous line (CVL); accounting for 30%-50% of the Gram +ve bloodstream infections in children with cancer (76,111). In our study, CONS represented just over half of isolated Gram +ve bacteria (179/338) (Table 3.8-2). The interpretation of its significance has been impacted upon through the lack of truly paired cultures; it can be assumed that on most occasions it was only isolated in the CVL culture with a contemporaneous peripheral sample that was negative.

Of the 107 Gram -ve bacteria, 27 were isolated in induction and 35 in maintenance. Overall, *E. coli* (27/107), *Pseudomonas aeruginosa* (18/107) and *Klebsiella* species (10/107) were the most frequently isolated, which is in line with international reports (13). However, the ordering is slightly different. As mentioned in Section 3.8.1, other studies have reported that *Pseudomonas aeruginosa* was the most frequently isolated Gram -ve microorganism representing up to 20% of Gram -ve infections (5,8,10,76), but in our study, *Pseudomonas aeruginosa* was isolated in 16.8% (18/107) of blood cultures with a Gram -ve isolate and *E. coli* was found in 25.2% (27/107). This difference may be due to variations in supportive care measures.

Respiratory samples

This study has included all respiratory samples tested for microscopy and culture (MCS), viral PCR, and *Pneumocystis jirovecii* (PJP). Although blood cultures were the most frequent sample type (3150/4828), most positive microbiological samples were respiratory samples (770/1194), confirming the finding that the respiratory tract is the most common site of infection in children treated for ALL (7,8,11,12). There were 892 pathogens isolated from 770

positive respiratory samples: 71 bacteria, 682 viral, 133 fungal pathogens and six cases of PJP. As outlined in Section 1.6, limited studies are available that explore the specific role of respiratory pathogens, and respiratory viruses in the infections of children treated for ALL. In the 682 respiratory samples positive for viral polymerase chain reaction (PCR), rhinovirus was by far the most common (341/682, 50%) followed by parainfluenza viruses (107/682, 16%), RSV (99/682, 14%) and influenza viruses (73/682, 11%). Rhinovirus may be over-represented as it can be repeatedly isolated in patients who have contracted it (152,154,155). The fact that rhinovirus represents half of the isolated respiratory pathogens needs to be taken into consideration when interpreting the results as it may have over-estimated the positivity of respiratory samples sent if it was repeatedly isolated from the same patients. Further analysis with the exclusion of this virus, or an approach to assessing repeat isolates from the same patients, would be required to untangle this.

Patients with mild upper respiratory tract symptoms may not have presented and therefore may be consistently underrepresented in this study. Furthermore, the PCR assay used did not include enterovirus which could have led to more positive samples if tested for. Overall, the quality of respiratory samples is likely to have varied with age, compliance of the patient and sampling method; this may have underestimated the number of positive respiratory samples. Also, although the study included the 2009 H1N1 influenza A pandemic, it was only isolated on ten occasions during that time, mostly from nose/throat swabs and with no associated mortality. Due to lack of clinical information, we cannot comment on the role of H1N1 influenza A in the development of lower respiratory tract infection which has been reported in other studies (144,196–198).

In many instances of suspected infection, respiratory pathogens (mostly viral) were the only isolates. There is existing evidence of the significant burden caused by respiratory viral infection in children with ALL (144,145). The understanding of the clinical gravity of respiratory viral infections in children treated for leukaemia is therefore of paramount importance in order to guide

the management of febrile episodes in these patients and potentially reduce their exposure to antibiotics.

Fungal testing of blood and biopsy samples

In order to assess the incidence of microbiologically documented fungal infections, all samples sent for serological fungal testing or isolated fungal pathogens via MCS were included. Reporting fungal infection was limited to microbiologically documented infection (which included serological testing), as insufficient clinical information was available to classify fungal infections according to the Mycoses Study Group/European Organisation for Research and Treatment of Cancer guidelines (157,199). Only 28 samples sent for fungal testing were positive: 22 blood samples, three lung tissue biopsy samples, one arm skin tissue biopsy and two cerebrospinal fluid (CSF) samples. However, 15/28 (54%) blood samples were positive for Aspergillus antigen testing, therefore not providing definitive microbiological evidence of invasive fungal disease. The presence of a positive Aspergillus antigen test can be suggestive of invasive fungal infection when used in clinical context and in conjunction with other physical, radiological, or laboratory findings. In some instances, as outlined in Chapter 3, the serological tests were paired with MCS samples from blood or other biopsies confirming invasive fungal disease. In the 13 remaining samples, *Aspergillus fumigatus* was isolated in three samples, Candida species in eight and mycelium in two. Neutropenia is known to be a major risk factor for invasive fungal infections (91,96,161,162,200). However, in our study only 13/28 positive samples for fungal testing were taken at time of neutropenia with four of those being samples positive on MCS from biopsy sites and one blood culture.

4.1.3 Infection per phase of treatment

Only two studies have reported on infections in children with ALL in the context of different phases of treatment (8,11). The present study of microbiological samples per phase adds to this knowledge, suggesting that different patient groups may be at increased risk during various parts of treatment.

Induction

That infection, suspected and confirmed, is common during induction is well known; with 30%-74% of children having at least one febrile episode (7,11,78,112,113). Indeed, the majority of serious infections happen during induction; with increased risk of infectious related complications and infection-related mortality being comparatively high during this phase (10,12,109). In the present study, 166/267 (62.2%) patients had at least one microbiological sample taken during induction, and half (n=83) had at least one sample that was positive. Patient groups potentially more likely to have positive microbiological samples included females, those diagnosed under the age of ten years, and those treated with more intensive chemotherapy regimens (four-drug induction). A high proportion of the infection-related deaths (8/13) occurred during induction; confirming that most serious infections happen at the beginning of treatment.

The finding that female patients are potentially more at risk could be due to differences reported in chemotherapy toxicity and immunologic responses between males and females (5,8,10). Other studies have reported longer and more profound neutropenic episodes in females compared to males, particularly after prolonged steroid use, which is seen during induction (5,8,10). Younger patients have been reported to be more at risk of infections throughout treatment for ALL (8,12,112,120,121). As outlined in Chapter 1, this could be due to differences in prior exposure to pathogens and differences in response to intensive chemotherapy and to steroids (5,8,12). Myelosuppression due to chemotherapy might induce a more profound immunocompromised status in younger patients who are more likely to have lower neutrophil counts but also impaired immunoglobulin production (8).

Finally, more intensive treatment was associated with higher risk of positive microbiological samples during induction. This is unlikely to have been affected by age as younger children would have mostly been allocated regimen A (least intensive treatment) at diagnosis. Their regimen may have changed after cytogenetic results and when response to treatment information

became available (later during induction or post-induction). Therefore, as a proportion, more patients diagnosed at the age of ten or more would have received a four-drug (intensive) induction from the start. The higher positivity in microbiological samples may have been driven by the higher number of samples taken from patients who received a four-drug induction; as patients treated under regimen C tend to remain in hospital during the whole of the induction phase. They were, therefore, there to be sampled with any fever or clinical concerning episode. This may have contributed to the higher number of microbiological samples and also positives from patients treated under regimen C.

Proportionately more Gram -ve bacteria were isolated in blood cultures during induction compared to any other treatment phase; with 27/86 of bacteria isolated during induction being Gram -ve (27/107, 25% total Gram -ve bacteria isolated).

Fungal pathogens have also been reported to be more common during induction (8,114,159). In this study, although over 100 samples were sent for fungal testing, there were only nine positive samples which were associated with morbidity, and in one case mortality. One patient died with candidaemia (*C. albicans*). Three more had fungal pathogens isolated in blood cultures (*Aspergillus fumigatus, C. tropicalis* and *C. albicans*) which led to treatment delays and/or modifications

Consolidation

During consolidation, patients treated under regimen B were likely to have more samples and more positive samples compared to regimens A and C. As outlined in Section 3.4, although shorter, the treatment in regimen B was intensive with myelosuppressive agents in close chronological proximity whereas, in regimen C there was a two-week interval between the myelosuppressive agents of 6-mercaptopurine and cytarabine. Other studies have reported a higher incidence of febrile episodes, with bacteraemias in particular, after high-dose cytarabine and asparaginase (7,11). This is most

likely driven by the prolonged neutropenia caused by this chemotherapy combination (8,78). In the UKALL 2003 published analysis of infectious deaths, a trend towards increased IRM with increasing intensity of treatment was noted, with significance limited to comparison between regimen B versus A, but not C versus regimen A. Our finding of higher risk of infection in regimen B, ties in with this (10).

Interim maintenance

During interim maintenance, patients who were treated under regimen C were more likely to have samples. However, no patient groups were identified as being more likely to have positive samples.

Delayed intensification

During delayed intensification, patients diagnosed with B-ALL under the age of ten years were more likely to have at least one positive sample. This was most likely driven by age as 175/221 (79.1%) of patients diagnosed with B-ALL were under the age of ten. There was, overall, a low number of positive blood cultures; 47 positive samples of the 432 blood cultures taken (10.8%). Delayed intensification entails intensive combination chemotherapy with significant myelosuppressive effects, so high frequency of blood culture sampling was expected, however there were very few positive samples. This may have been influenced by the pause between the two parts of delayed intensification for count recovery. Of note, patients in regimen A, for whom delayed intensification is the most intensive treatment phase, were found to have more samples and positives compared to those treated in regimens B or C (although this was not statistically significant). This finding, of course, goes hand in hand with younger patients (diagnosed under the age of ten years) being more likely to have a least one positive sample during delayed intensification. All patients treated under regimen A in delayed intensification were diagnosed under the age of ten years, but there were 23 patients diagnosed under the age of ten years treated under regimen B and 52 under regimen C. This phase of treatment may be the first time that patients on

regimen A become seriously unwell. Although statistical significance was not demonstrated, this is an important observation as we know clinically that patients treated under regimen A can encounter complicated infections during delayed intensification.

Maintenance

An overall heavy burden of infection was seen during this phase which is a very clinically important observation. More specifically, a lot of different bacteraemia were noted during maintenance, although the setting of the treatment has shifted more towards oral chemotherapy given at home by this point on their pathway. This poses the big question of timing of central line removal in these patients. Our local practice has been to mostly keep the CVLs in place until the end of treatment particularly for younger patients and guided by family wishes. The burden of bacterial infections should be taken into consideration when making these decisions and this study has posed the important clinical question of timing of line removal which may vary across different centres.

Some interesting findings were observed during maintenance. Younger patients had higher rates of positive blood cultures and positive blood samples than patients aged ten years or more. Patients living in more deprived areas had higher rates of blood cultures and positive blood cultures compared to those living in more affluent areas. No differences were seen by deprivation in respiratory samples.

The susceptibility of younger patients to infection has been outlined in the above section on induction. As maintenance is the part of treatment when patients are mostly at home, it was interesting to see that although the level of infection was lower in maintenance (as expected), this was the only phase during which differences were seen based on deprivation. Although area-based deprivation is a crude marker of individual circumstances, this observation is nonetheless important since it could reflect factors/circumstances that are potentially modifiable.

That children from less advantaged areas of the world often have poorer outcomes is well known, and the literature on global inequalities is growing (e.g. 201,202). Even within the UK, where all children are treated under the standardised care of the NHS, geographical/deprivation difference in survival have been detected in previous decades (203,204). Examining individual-based by treatment phase (as done in this thesis) has, however, not been previously undertaken.

During the two to three years of maintenance, the only planned hospital presentations are for intravenous vincristine and intrathecal methotrexate administration; all other chemotherapy administration relies on the parents/carers/patients. In this regard, maintenance is a unique treatment phase as the treating responsibility shifts from the health care professionals to the parents/carers, and there is some indication that this is the point where socio-economic/lifestyle effects may emerge (203). Indeed, issues with compliance/treatment adherence in children and young adults have been identified in other settings (205,206). Importantly, the findings in this thesis suggest that patients who lived in a deprived setting had higher positive blood culture frequencies than those living in more affluent areas. Clearly, further research investigating the consistency and potential causes of these findings is required, both within the broader context of HMRN region, and possibly beyond. Importantly, as noted above, should causes be identified, then targeted intervention strategies could, perhaps be developed.

Regarding males during the extra year of maintenance, we know clinically that during that time boys can have a lot of infection related complications. By that point, they have been immunocompromised for two years and are vulnerable to viral infections, particularly with varicella zoster virus and herpes simplex virus (11). Non-respiratory viruses were not included in this study, therefore we have not been able to demonstrate this.

4.1.4 What have we learnt?

There are many contributors to this finding which together pose the question; are we doing the right things for our patients? Only a small proportion of the blood cultures taken were positive (396/3150, 12.6%). In the era of stratification for management of febrile neutropenia, this is a highly relevant observation, as it tangibly demonstrates that for all the blood cultures taken, few are found to be positive

The incidence and types of bacterial pathogens and their susceptibility is continuously changing and shows great variability in different geographical areas and health care settings (131). In this UK cohort, we have seen a predominance of Gram +ve bacteria (338/445, 75.9%); most commonly seen were CONS (179/338), *Streptococcus pneumoniae* (32/338) and viridans group streptococci (30/338). Of the 107 Gram -ve bacteria, *E. coli* (27/107) was the most common followed by *Pseudomonas aeruginosa* (18/107) and *Klebsiella* species (10/107).

It is known that although fever is the most consistent and sometimes the only manifestation of infection in neutropenic patients, in only 20-25% of febrile episodes is a causative pathogen identified (101,102). In around half the patients there are no clinical or microbiological manifestations of infection and a quarter of patients have an identified site of infection, but no positive microbiological samples. The latter may have various causes including, lack of specimens (such as sputum or bronchoalveolar lavage samples), due to a suboptimal immune response resulting in fewer clinical signs of infection, or the use of antimicrobial prophylaxis/recurrent exposure to broad spectrum antibiotics rendering the cultures negative. So, for the samples taken during neutropenia, we know that only a few are likely to yield a positive result.

Another consideration would be if the “bar” is set too low; are we sampling more patients than we need to? The cut-offs for definition of febrile neutropenia and initiation of its management may be too low and therefore we may be seeing a low positivity rate because we are sampling inappropriately.

This is a consideration not just for febrile neutropenia but for our clinical approach to infection overall, regardless of full blood count results. Because infections in children with cancer can be lethal and these patients can deteriorate quickly, we may have developed a “knee jerk” reaction of taking blood cultures and starting antibiotics on more patients than needed. The optimal management of infection in immunocompromised children can be challenging, as it is difficult to identify which patients are more at risk of severe infection, and of those who will survive. There is a lot of ongoing research into the risk-stratified management of febrile neutropenia in children treated for ALL who are exposed to broad spectrum antibiotics for at least 48 hours with every presentation with fever (106,207). Antibiotic resistance is a very important consideration (208,209). The flora of each patient is bound to change over the course of treatment through exposure to broad-spectrum antibiotics. At diagnosis each patient presents with their own microbiome, but with exposure to antibiotics, resistance development is inevitable as broad spectrum antibiotics eradicate sensitive microorganisms and provide the environment for the emergence of antimicrobial resistant bacteria. The choice of empirical antibiotic is likely to affect this process.

Finally, another factor that may have influenced the low positivity rates in blood cultures may be that on many occasions only one blood culture sample could be drawn before initiating antibiotic therapy. These children can be very unwell at presentation, not allowing for series of blood cultures to be drawn. Repeat blood cultures may have been taken after the initiation of antibiotic treatment, but we know that bacteraemia is detected nearly twice as frequently in initial blood cultures compared with repeat blood cultures obtained when the initial blood culture is negative (210). It can therefore be expected that the study underestimates the number of bacteraemia.

Even if we are sampling the right patients at the right time, the quality of samples may be suboptimal. There is evidence to suggest that underfilled blood culture bottles may not yield positives due to lack of adequate sampling volume (211). This is a common phenomenon in paediatric patients whose peripheral blood culture samples may not be possible, and when taken may

be underfilled due to technical difficulties in obtaining the sample, or poor peripheral circulation in in the very unwell child. Samples from CVLs are more likely to be of the appropriate volume due to the ease of aspiration. This may be reflected in the number of positive cultures in each source category in this study.

The present study demonstrates, however, high positivity in the respiratory samples, with diverse viral pathogens. The PCR panel that was used during the time of this study includes rhinovirus which is known to “linger” in the upper respiratory tract of patients long after it is first isolated. As rhinovirus was by far the most common viral isolate, many of these samples may represent re-current isolates/“colonisation” of the upper respiratory tract rather than the cause of active infection. The clinical significance of bacterial pathogens isolated from nose/throat swabs and fungal pathogens isolated in the respiratory tract is also unclear; they likely represent flora/colonisation rather than be the cause of active infection.

Respiratory infections are a common cause of fever in children, both in the general population and in the immunocompromised host. In the majority of cases or investigations the only microbiological evidence of infection identified was a respiratory virus. In the context of febrile neutropenia, even with a viral illness, children would have been exposed to broad spectrum antibiotics despite the lack of evidence of systemic bacterial disease. It is, therefore, important to investigate the role of respiratory viral pathogens in children treated for cancer, as well as aid in the stratification of management of febrile neutropenia and in the stewardship of antibiotic use in order to avoid over-exposure to antibiotics that leads to antimicrobial resistance.

We have seen different patient groups to be at risk of positive microbiological samples during different phases of treatment. These findings may assist clinicians in their decision-making process when managing infection-related problems in children with ALL. Recommendations for the management of these patient groups are outlined in Section 4.4.

4.2 Strengths and limitations

This is the largest single UK study on infection in patients treated for ALL in a paediatric setting whose microbiological samples have been studied in depth. It is also the only study of infection in a UK paediatric cohort of patients with ALL that has included all patients regardless of trial participation. The cohort is population-based relating to ‘real world’ data. All patients diagnosed and treated in the catchment area received the same NHS care regardless of trial participation or socio-economic status. This is of particular importance as there is no selection bias that may be driven by trial participation. The inclusion of all patients, trial and non-trial, provides a true insight into the infections seen in all children treated for ALL in the HMRN region. The duration covered is also a major strength as this study does not just offer a snapshot but a true representation of infectious complications over time.

The analysis was done reflecting actual treatment time and not the expected duration of each phase, i.e. exact durations of each treatment phase were known and they include the delays within the phases. Within each treatment phase, some trial participants would have received less intensive treatment as the randomisations were mostly focused on reducing treatment burden; therefore including all patients regardless of trial participation has captured all infection episodes within the different treatment regimes.

Microbiological samples other than blood cultures were also included so as to describe all documented microbiological infections. This included respiratory samples and all samples sent for fungal testing (including serology) and not just blood culture samples which has been the case in most published data to date.

This study provides a comprehensive description of the microbiological samples from paediatric patients during the entire treatment for ALL analysed in the context of different treatment phases. The subset analysis by microbiological sample types provides new insights into the epidemiology of bacterial, viral and fungal pathogens in patients treated for ALL in the UK.

The robust data available from HMRN, was enhanced with data on microbiological samples, haematological results, and chemotherapy information on each individual patient. It is, therefore, the most comprehensive collection of data on microbiological samples among ALL patients in the UK. Of particular importance are the data on chemotherapy. Treatment information was available through the HMRN database, but in order to describe the infective episodes to the level of detail required for this thesis I had to extract information of the exact dates of start and end of each treatment phase along with all the treatment delays and modifications. This enabled microbiological samples to be mapped to the exact treatment phase of each individual patient.

Microbiological samples have been used in this thesis as a surrogate for suspicion of infection. Inherent in this approach is the bias of presentation with suspected infections, as only patients who presented or were already in hospital with suspected infection were sampled.

The incidence of infection varies among patients and between institutions due to the variation in local environmental and/or prophylactic and empirical treatment antimicrobial use (76). To that effect this work informs clinical practice about the epidemiology of bacterial, viral and fungal pathogens affecting patients in the UK. Although, variability of pathogens depending on pattern of infection, local ecology and antibiotic resistance may differ in other UK centres, this is a good first step towards the identification of pathogens affecting children with ALL treated in the NHS setting. In this context, it is also worth noting that the socio-demographic characteristics of the large catchment population (~4 million) is similar to the UK as a whole.

The biggest limitation of this study is the lack of clinical information on the infection episodes. The reason for presentation/admission, symptomatology, physical examination and parameters along with imaging and other biochemical tests are not available. Due to this, it was not possible to assess the gravity of each isolated pathogen. The effect of each microbiologically documented infection has not been identified nor its role on morbidity; we do

not know if the patients were unwell, if they required CVL removal, if they required oxygen or higher level of care such as intensive care, invasive or non-invasive ventilation, inotropic support etc. The infections have not been described in episodes, i.e., we have described the microbiological samples and positives, but not in the context of their succession in each patient during an admission/presentation. There is also lack of clinical context about the treatment delays, and the extent to which infections contributed to them. Finally, there is no information on the management of these infections, antibiotic escalation or addition of different antimicrobial agents, but this was not within the scope of this study.

The lack of clinical information has other implications which should be taken into consideration when interpreting the findings. We do not know if the samples taken were from a patient who was already an inpatient and developed fever or from a patient presenting with infective symptoms from home, i.e., if they are likely to be community or hospital acquired. A minority of microbiological samples would have been repeat samples to monitor clearance of previously isolated microorganisms. Others may be referring to the same episode of prolonged unexplained fever reflecting the level of concern in the clinical team resulting in the repeat sampling of the same patient; additional work could consider time adjacency of samples and the presence of positive samples within the identified sampling sequences to determine infection episodes. Some samples may have been taken in the context of an infected CVL insertion site to exclude infection of the line hub. Overall, whether or not each isolate was a contaminant or a cause of acute infection could not be determined. This is, of course, the nature of all research related to microbiology, as even with known clinical context the role of isolated pathogens in the causation of infection cannot be entirely deciphered, especially in immunocompromised patients. With a myriad of concurrent events and risk factors present in any given time it is nearly impossible to “blame” it all on a causative microorganism, even in patients with positive microbiological samples.

Microbiological samples of different types have not been analysed in a way to assess contemporaneous isolates from samples taken on the same day. Many patients would have had blood culture samples and respiratory samples taken on the same day, which is often the case when presenting with fever and respiratory tract symptoms. These samples have not been looked at in this context. We have looked at samples taken at time of neutropenia or not, but we cannot comment on whether the samples relate to episodes of febrile neutropenia, fever of unknown source, or clinically documented infection. It was also not known if the microbiological samples referred to a new presentation to the hospital or fever/suspicion of infection arising during an inpatient stay. With particular emphasis on neutropenia, we have not presented the samples in the context of duration of neutropenia. It is known that profound neutropenia that lasts over seven days is a major risk factor for invasive infection, but the data presented here have not been interrogated in that way (8,78,80,88–97). The sensitivities of bacteria grown in positive blood cultures have not been included in the present analysis since antimicrobial management was not within the scope of the study. Finally, polymicrobial infections have not been discussed; as described in Chapter3, some microbiological samples had two or more isolates. These samples have not been analysed separately, These samples have not been analysed separately, instead they are each included in the total analysis.

Another limitation is the historical nature of the data that was collected. All the described events were in the past, and the description of them is only as good as the data recorded at the time. The additional data collection involved results available in the Leeds Teaching Hospitals patient record (single centre). As the data extracted affected results reported from the laboratories, the effects of the retrospective collection on the quality of data are likely to be minimal. The totality of microbiological samples available for data collection may have been slightly impacted on by presentation in other areas, or in the community. There may have been presentations with infective symptoms to GP practices; although the included microbiological samples would have not been collected in primary care (with the exception of maybe the occasional respiratory sample testing). The few patients who lived geographically closer to paediatric

oncology shared care units (POSCU) may have presented to these with episodes of suspected infection, and any samples taken have not been included. As outlined in the results chapter, these patients are few in number and the lack of some of their samples is unlikely to have impacted on the patterns identified.

Variations in supportive care and management of infection over the years may have also affected the results. Over the 13-year period of the study, microbiological sampling indications and methods of sampling are likely to have changed. As outlined in methodology, the sensitivity of blood culture analysis improved as newer and automated methods became available. Also, the serological tests for suspected fungal infection were introduced in the later years of the study, and it is likely that there will have been other unrecognised factors influencing the results of a study running over a 13-year period. Finally, sampling techniques are likely to have affected the yield of results as the quality of obtained samples, particularly respiratory ones, is likely to have varied based on operator doing the sampling and the compliance of the patient being sampled.

As described in Chapter 2, the way the sample information was recorded during data collection may also have affected the results. The recording of microbiological samples has been used as a surrogate marker for suspicion of infection and as such certain decisions were taken in the way the data were recorded. More specifically, the blood culture samples were collected as “paired” only if all lumen samples of the CVL and the peripheral sample taken at the time of fever, were all negative or all had the same microorganism(s) isolated. If not, they were collected as central and peripheral samples separately which may have underestimated the number of paired samples. The analysis has not flagged samples taken on the same day; so, if a patient had a positive blood culture from a CVL on the same day as a negative peripheral blood culture, this will not have been described. Differential times of positivity in paired blood cultures were not included either. Therefore, we cannot comment on potential CVL infections versus true bacteraemias. The lack of documentation on source of sample in some blood cultures would have

had a similar effect. In respiratory samples, all samples taken on the same day and sent for all types of testing were also collected as one sample, and of course in reality they were not. I did this to avoid overestimating the suspicion of infection, which is implied by sampling, as we only test symptomatic patients. Another factor is that respiratory samples sent for fungal testing that yielded fungal pathogens through MCS are described with the rest of the respiratory samples, whereas samples taken from blood or other normally sterile sites that were tested for fungal pathogens that yielded fungal pathogens through MCS were described in a separate section. It was decided to include samples taken from all respiratory sites that were positive for fungal pathogens such as yeasts and *Candida* species, even though the majority were likely to represent colonisation and not the cause of active infection. Overall, we were not able to distinguish common contaminants from invasive infection and this may have affected the description of microbiologically documented infections.

Although the findings in this thesis are based on a sizeable cohort of children with ALL, the numbers are still small. Some of the findings could be due to chance given the cohort size. The data have been primarily described in a dichotomous manner, trying to identify differences within patient groups and patient characteristics. There are also some limitations in the statistical methods that were used. Additional work could include applying rate based analysis to all phases, or regression analyses could aid in the understanding of relative importance of factors such as lineage, age and regimen in the patterns of infection in children with ALL. Recurrent event analyses approaches could also be applied to help with the understanding of repeat infections in the same patient. Such analyses will be applied to the data if required in future publications.

4.3 Recommendations and future work

4.3.1 Recommendations

Although this thesis has not presented enough information to change practice, it has demonstrated that certain patient groups could be more at risk of having

positive microbiological samples; and in so doing highlighted promising areas for future research. This work has focused on describing the infections seen in a large cohort of patients treated for ALL in a paediatric setting so as to provide the basis of future research. Understanding the timing and types of infections seen in these patients is an important first step to guiding future work in this area. Based on the results, potential clinical interventions could be considered which include close clinical monitoring, the use of prophylactic antimicrobial agents and administration of immunoglobulins.

During particularly myelosuppressive parts of treatment, certain patient groups could be considered for closer clinical monitoring. For instance, frequent monitoring during count recovery could be considered. This could happen in an inpatient or outpatient setting and would enable medical teams to closely monitor for fever and signs of infection and act promptly with the initiation of empirical antimicrobial therapy. To an extent, of course, this is already happening as parents monitor their children closely at home throughout treatment. This could be supplemented by input of outreach staff with the combination of day unit assessment at set intervals. Admission for count recovery monitoring, although more extreme, could be considered on an individual case basis on the balance of risks depending on prior treatment and infection related morbidity.

The use of antimicrobial prophylaxis is another potential consideration, especially in patient groups at risk. The results have shown that younger patients, in particular, tend to be more at risk of both bacterial and respiratory viral infections. They could, therefore, be considered for prophylaxis as a separate group, as is already the case for patients with Down syndrome. Future work is needed to pave the way towards more individualised antimicrobial prophylaxis approaches for these patients. For instance, single arm feasibility studies could be an important first step; patients could be randomised to receive antibiotic prophylaxis versus the current practice of only receiving PJP prophylaxis. This could initially involve only the younger (under the age of ten years at diagnosis) patients based on the results of this study which support previously published data (8,12,112,120,121). Although

antibiotic prophylaxis could prove to be of benefit, this needs to be considered taking into account the pros and cons of this approach. The potential benefits of the use of prophylactic antibiotics include reduction in fever, bacteraemia, sepsis and mortality. There are also potential negative consequences though, which include drug toxicities, *Clostridium difficile* infections, invasive fungal disease and antibiotic resistance (212,213).

Routine prophylaxis for PJP with co-trimoxazole is currently universally recommended and is highly effective in the prevention of this life-threatening opportunistic infection (193,214). The use of fluoroquinolone prophylaxis may reduce the risk of bacterial infections and it is recommended in some adult ALL guidelines (without an increase in the incidence of fungal infections) (102,215,216). Its use in children, however, is controversial as its potential benefit needs to be weighed against the risk of developing resistant organisms and *Clostridium difficile* infections (193,217). The use of levofloxacin prophylaxis in children treated for acute myeloid leukaemia (AML) or relapsed ALL was shown to significantly reduce the rates of fever, neutropenia and bacteraemia, compared to no prophylaxis (218). Antibiotic prophylaxis could also be considered in those patients found to struggle with recurrent infections; the findings presented here showing high between patient variability in sampling rates. Individual predisposition to infection has been documented in other studies and the identification of patient-specific factors rendering them susceptible to infection is likely to be the key in the management of infection in children with ALL in the future. For instance, ciprofloxacin prophylaxis is given to children with Down syndrome during induction as they have been shown to be at risk of TRM and IRM in particular (194).

However, further assessment of the efficacy and potential harms of antibiotic prophylaxis should be evaluated. A recent guideline for antibacterial prophylaxis devised by an international panel of experts, does not recommend the consideration of using systemic antibacterial prophylaxis in children being treated for ALL; not even during the induction phase (212). The evidence-base underpinning this recommendation was weak; again highlighting the need for

more research focusing on the infections of children with ALL. The panel recommended that prophylactic antibiotic use should be considered in patients with relapsed ALL receiving intensive chemotherapy expected to result in severe neutropenia (<0.5 x 10^9/L) for at least seven days. The panel, also, recognised the need for further data so as to identify subgroups of paediatric patients with ALL who might particularly benefit from prophylaxis and to describe the effectiveness of prophylaxis in this group. The role of prophylaxis in blocks of intensive chemotherapy associated with prolonged severe neutropenia also needs to be evaluated (212). If antibacterial prophylaxis is considered it is recommended that it should only be given during the period of severe neutropenia. This approach could be applied to patient groups seen to be at risk of more positive samples in this study.

Antifungal prophylaxis with voriconazole or micafungin has been found to reduce the rates of bloodstream fungal infection in children with acute leukaemia during intensive chemotherapy; its use, though, can be complicated by the potential interaction with vincristine (216). The use of antifungal prophylaxis could also lead to resistant fungal pathogens. Another potential consideration, would be the intravenous replacement of immunoglobulin for patients with hypogammaglobulinaemia or frequent infections (8). Currently, however, there is no consensus recommendation on replacement immunoglobulin infusions in children receiving chemotherapy for ALL (193).

The use of prophylactic haematopoietic colony-stimulating factors in order to reduce the duration and complications of febrile neutropenia in children with cancer has also been investigated (219). Colony-stimulating factors were found to reduce the duration of febrile neutropenia by 20% and also reduced the length of hospital admissions, but did not impact on infection related mortality. Its efficacy in children with ALL is uncertain, but it can still be a consideration in patients with life-threatening infections, particularly in the intensive care unit setting.

The analysis presented here found that patients living in more deprived settings are at higher risk of having positive blood cultures during maintenance. As outlined in Section 3.7.4, many factors could be contributing to this, including differences in CVL care, threshold of presentation to the hospital in cases of suspected infection, awareness among carers/parents, behavioural differences in the children, family circumstances, and potential language barriers. The discrepancy of risk of bacteraemia depending on deprivation is an important observation that could potentially be addressed and modified. Indeed, a few simple steps could, for example, be put in place in order for the children living in more deprived areas to be safeguarded against potentially complicated infections. As a multi-disciplinary team, we may be complacent with our recommendations and guidelines to the parents/carers and thought needs to be put into explaining aspects of care in different family groups and settings. Educating and supporting the whole family – depending on the age and cognition of each individual patient, could be key.

Looking at ways to prevent respiratory viral infections, prevention of viral exposure and immunisation (of patients and family members) against viral organisms are the mainstay of decreasing viral morbidity and mortality. In the inpatient setting, isolating patients who develop symptoms of coryza and/or cough promptly is important. During the current COVID-19 pandemic with, with its increased emphasis on personal and environmental hygiene, isolation and less mixing we have seen a big reduction in transmissible infections (220,221). The power of these interventions, although perhaps not to this extreme, could be applied to protect children with ALL from respiratory viral pathogens during the winter months. Emphasis on hygiene, strategies of less mixing in outpatient clinics, potential use of face coverings could certainly be considered. Particulate filtration mask use, for instance, has been advised in children treated for ALL at St Jude Hospital during induction, delayed intensification and periods of neutropenia (8).

Influenza, in particular, can cause significant morbidity and mortality in immunocompromised children, and vaccination is the basis for primary

prevention (151). There is only one published study investigating the use of oseltamivir for eight weeks during the influenza season as prevention of influenza infection in immunocompromised children. Oseltamivir can prevent the progression to pneumonia and can be used from the age of one and zanamivir can be used in children over the age of seven years. The risk of developing resistance to antiviral agents is unknown (222).

Respiratory syncytial virus (RSV) can, also, cause complicated lower respiratory tract infections in immunocompromised children (148–150). Palivizumab, a humanised monoclonal immunoglobulin is used for prophylaxis in premature neonates with chronic lung disease, but there is no evidence available about its use in children with cancer. In patients who require treatment for RSV, there is no demonstrated benefit in the use of intravenous immunoglobulin in addition to supportive care and the role of ribavirin is also unclear.

On a non-clinical note, this project has demonstrated the impact of suboptimal data linkage on research, and potentially clinical practice. The time invested in collecting and linking the additional data could have been utilised in more meaningful ways. In the context of such a robust national health system, data sharing should have been in place, locally and nationally. Addressing this issue would mean that studies like the one presented in this thesis could happen alongside the relevant treatment protocols, as is the case in other countries. This could lead to real-time integration of big data and clinical practice and would give a new meaning to the “bench to bedside” process.

4.3.2 Future work

There are exciting future prospects following this research. First, the findings of this study need to be submitted for publication in order to formally contribute to existing knowledge on infections in children with ALL. The first step would be to publish the results as a whole in the context of each phase of treatment. Further publications focusing on respiratory viruses, fungal pathogens and the issues identified during maintenance could follow. There are a lot of additional

data points available, regarding respiratory viral infections and paediatric intensive care admissions which are worth sharing.

Data linkage to Hospital Episode Statistics (HES) is also in place for the HMRN cohort that could add crucial information to the existing results. The infection samples could be studied in the context of admissions and infection episodes which may inform us further on patient groups at risk and the effects of microbiological positivity on morbidity. This could help unravel the role of certain microorganisms as we could assess the number and length of admissions complicated by infection and their concurrent microbiological pathogens. It may not be possible to establish causative relationships, but nonetheless it would be an important next step in informing the way we manage infection, suspected and confirmed. An example of this approach would be the assessment of hospitalisation with respiratory viral illnesses and the impact on treatment delays. This is an identified burden on children treated for ALL, and is particularly important in the context of risk stratified management of febrile neutropenia in these patients.

Data on concurrently collected microbiological samples and pathogens isolated from different sample types at the same time, or negative samples at the time of other positive modalities, are also available. Information on recurrent sampling, when it occurred and exact duration of treatment delays is also available. This is an area that would benefit from exploring, as we know clinically that a lot of treatment delays are secondary to suspected or proven infection. Furthermore, delays not only impact on the total duration of treatment, but also increase the risk of relapse (223).

The finding of inequality in the risk of positive blood cultures by deprivation during maintenance is interesting and should, in theory, not have been seen in the setting of the NHS which has equal access of care. Although the role of patient and caretaker characteristics in the adherence to home-based maintenance therapy have been explored, the socio-economic inequalities have not fully been investigated in the UK (224). It is important to highlight that conclusions cannot be drawn from this work regarding the socio-economic

status and infection risk as the marker used (IMD) is an area-based tool and not individual to each patient.

The analysis demonstrate that certain individuals have a heavier microbiological record than others. More research is needed to shed light into patient specific inherent factors leading to more infection in certain individuals. A lot of current research is focusing on individuality and the identification of patients more at risk of complicated infections. Tools are being developed for the stratification of management of febrile neutropenia based on patient specific parameters (103,104). New biomarkers can aid in the identification of the point in every febrile neutropenic episode at which intravenous antibiotics could be stopped and reduce the length of hospital stay. Procalcitonin, in particular, is a biomarker more specific to bacterial infection which peaks at six hours and decreases rapidly in response to antibiotic treatment (225). It has the benefits of being easy to analyse, rapid and widely available. Procalcitonin has been studied in several patient groups in order to diagnose sepsis and monitor response to treatment. Trials have demonstrated its value in guided decision making for antibiotic cessation in adult intensive care and neonatal unit settings (226,227). Systematic reviews support further investigation in its utility in children with cancer presenting with febrile neutropenia (228).

There is a near complete lack in national guidance for the use of empirical antibiotics in children with haematological malignancies. However to my knowledge, there is a new initiative to form a multi-disciplinary steering group in order to develop antimicrobial guidelines and promote antimicrobial stewardship in this population. There is also ongoing work nationally on the antifungal stewardship in children, including those with haematological malignancies, which I have been involved with (submitted for publication) (229).

Another potential consideration is the use of alternative measures for the prevention or reduction of morbidity of infection. A great example is the use of probiotics to prevent or reduce mucositis and infection in children with cancer

(230). The era of focusing on the microbiome and its role in pathophysiology of infection may provide us with more tools in the fight against infection.

Epidemiological studies, such as this, focusing on the pathogens seen in children with ALL and identifying patient groups at risk could inform future research in the use of biomarkers for the diagnosis of and monitoring the response to treatment for infection. It is important to balance the management of infection between over-treating the infection and under-treating the patient which can have catastrophic implications.

4.4 Conclusions

Infections in children with ALL, suspected or microbiologically documented, are frequent and potentially life-threatening events. The research presented in this thesis has shown that the majority of children with ALL are often investigated for infection and some have a heavy microbiological footprint with high incidence of microbiological samples during their entire treatment. In the positive samples, a lot of respiratory viral pathogens were identified across all phases of treatment. In the few positive blood culture samples, there was a predominance of Gram +ve bacteria with CONS as the most common bacterial microorganism across all treatment phases.

There is great variability in the number of samples and positive samples in different patients. Some patients have few microbiological samples taken, whilst others have repeated clusters of samples, many of which are positive throughout treatment. Also, certain patient groups have been identified as having more positive microbiological samples during different phases of treatment including females during induction, those diagnosed under the age of ten years during induction and delayed intensification, those treated under regimen B during consolidation, those treated under regimen A during delayed intensification, and those living in more deprived areas during maintenance.

In summary, understanding the determinants of bacterial, viral and fungal pathogens during different phases of treatment is clearly an important topic;

and it is hoped that the findings presented in this thesis, coupled with the additional research that may occur as a result, will help shape and inform future research and clinical practice to the benefit of patients.

Appendix A Previous clinical trials for childhood ALL in the UK

Over the last 40 years a UK wide network of clinicians supported by the Medical Research Council (MRC) have been planning and delivering childhood ALL trials. The network has achieved the standardisation of therapy and uniformity of outcomes across the UK, and are also overseeing clinical trials (231,232). Previous studies assessed treatment efficacy and toxicity.

The two trials that tested the benefit of post-remission intensification therapy were UKALL X (1985-1992) comparing the outcomes after none versus one versus two intensification courses, and UKALL XI (1992-1997) comparing two versus three intensification courses. The cumulative effect of three post-remission intensification courses was a 20% improvement in five year EFS (233,234). A worldwide meta-analysis confirmed the benefits of intensification therapy (235). UKALL XI, also, showed that intensive intrathecal chemotherapy is as effective as cranial radiotherapy in the prevention of CNS relapses in the majority of patients (236).

These findings were confirmed by a meta-analysis which included more than 13,000 patients (237). UKALL XI, also, reduced anthracycline exposure and the associated risk of cardiac toxicity. The MRC UK childhood ALL study 97/99/01 recruited more than 90% of all new ALL cases. The trial tested the efficacy and toxicity of dexamethasone and thioguanine compared to prednisolone and mercaptopurine, respectively. This trial was closed to accrual as a result of an interim analysis showing significantly fewer CNS and systemic relapses in the group receiving dexamethasone and significantly higher risk of veno-occlusive disease of the liver in the patients receiving thioguanine.

A.1 UKALL 2003 (2003-2011)

Building on the findings of the previous UK wide trials, UKALL 2003 trial aimed to assess whether molecular MRD techniques could be used for risk-stratification in order to guide effective treatment with minimal toxicity (69). It

was the first UK trial that focused on reducing treatment without compromising efficacy in low-risk patients. At the same time, the trial aimed to evaluate if further post-remission intensification treatment would improve the outcomes of high-risk patients.

The trial recruited patients aged 1-24 years diagnosed with ALL between October 2003 and June 2011. The upper age limit was increased twice while the trial was open, due to better outcomes in young adults treated under a paediatric protocol.

At the time, 85% cure rates were reached with standard treatment. However, in previous protocols (UKALL VIII) 50% cure rates were reached with less intensive treatment and, hence less adverse effects and toxicities. The treatment intensity received by patients with ALL was based on known risk factors for relapse (1,238,239). Even with 85% cure rates, 15% of children would experience relapse and require further treatment, and most relapses were seen in the patients classed as low- and moderate-risk at diagnosis (1,238,239). As the assessment tools prior to MRD failed to predict accurately the patients who would relapse, defining low- and high-risk groups with more accuracy was more important than ever. Assessment of MRD after induction for remission had been shown to be the most sensitive and reliable marker of risk of relapse (66,240); hence the trial used the molecular techniques of measuring MRD in the bone marrow once remission had been achieved by standard morphological criteria. Treatment was, therefore, stratified based on MRD findings. Overall, UKALL 2003 focused on the identification of prognostic subgroups that would allow intensity of treatment to be modified based on risk of relapse (69).

A.1.1 Risk stratification and randomisation

Initially, patients were stratified in three risk groups based on the following criteria:

a) Standard Risk Group: patients over one and under ten years of age at diagnosis with a pre-treatment white cell count of less than 50 x 10^9/L and who

did not have adverse cytogenetic findings; i.e. cytogenetic abnormalities involving the rearrangement of the MLL gene, hypodiploidy, BCR-ABL.

b) Intermediate Risk Group: patients over ten years of age at diagnosis, or any patient with a pre-treatment white cell count of more than 50 x 10^9/L, who did not have the aforementioned adverse cytogenetic findings.

c) High Risk Group: all children with adverse cytogenetic findings (plus those with amplification of RUNX1), and children with slow early response (SER) to treatment irrespective of initial risk group. SER was defined as having more than 25% blast cells in their bone marrow on day 8 (regimen B) or 15 (regimen A). These patients were not eligible for MRD randomisation and were moved to regimen C on day 15 for the regimen A patients and day 8 for the regimen B patients. Exception were patients aged 16 and over who were treated as clinical intermediate risk (regimen B), irrespective of the day 8 or 15 bone marrow response and were eligible for MRD stratification and randomisation.

Based on these risk groups, patients were treated as follows:

a) Standard risk: regimen A - three-drug induction.

b) Intermediate risk: regimen B - four-drug induction.

c) High risk: not eligible for MRD randomisation, so allocated regimen C which also comprised of four-drug induction.

The low- and intermediate- risk patients who had morphological complete remission at day 29 of induction were eligible for MRD randomisation. This randomisation was as follows:

a) MRD Low Risk Group (MRD negative or positive <5 x 10^{-5} at day 29): continued on previously assigned regimens (A or B) and received one or two delayed intensifications. This randomisation closed in August 2009, while the trial was still recruiting, because the patients receiving two delayed

itensification courses did not have better outcomes and suffered more treatment related morbidity and mortality (69).

b) MRD High Risk Group (MRD positive >1 x 10^{-4} at day 29): were randomised between previously assigned regimen (A or B) and regimen C. However, patients with high-risk MRD who were randomised to receiving intensive regimen C treatment had significantly better five year EFS, compared to the high-risk patients randomised to receive standard treatment (regimen A or B) with half the relapse rate (OR 0.57, 95% CI 0.34 – 0.95, p=0.03) (2).

c) MRD Indeterminate Risk Group (No MRD results or MRD between 5 x 10^{-5} and 1 x 10^{-4} at day 29): continued on previously assigned regimen (A or B) and received two delayed intensifications.

A.1.2 UKALL 2003 treatment phases

- **Induction**

Regimen A (our weeks):Three-drug induction with:

- oral dexamethasone 6mg/m^2 /day for 28 days (maximum single dose 10mg/day)
- weekly vincristine (1.5mg/m^2 intravenously weekly, maximum single dose 2mg) on days 2, 9,16,23
- 2 doses of pegylated asparaginase (1000iu/m2 intramuscular) on day 4 and day 18
- 2 doses of intrathecal methotrexate (aged dependent dose). Days 1,8

During the last week of induction, patients would start daily oral mercaptopurine and would carry on with that until the end of consolidation (week 8).

Regimen B

As for regimen A plus four doses of daunorubicin (25mg/m^2 intravenously on days 2, 9, 16, 23) and one extra week of mercaptopurine (until the end of the fourth week of consolidation).

Regimen B (five weeks): additional to regimen A, four doses of daunorubicin n 25 mg/m^2 IV on days 2, 9, 16, and 23 and one extra week of mercaptopurine (as consolidation lasted for five weeks in regimen B).

Regimen C (five weeks): patients who moved to regimen C from regimen A or regimen B had three weeks of induction under regimen C. Daily dexamethasone, three doses of vincristine, two doses of daunorubicin, one dose of pegylated asparaginase. On the last week of induction, patients would start daily oral mercaptopurine and would carry on with that until the end of the second week of augmented BFM consolidation.

- **Consolidation**

Regimen A (three weeks): daily oral mercaptopurine 75 mg/m^2 daily (days 2–29) (weeks 5–8) and weekly intrathecal methotrexate (days 1, 8, 15,22).

Regimen B- standard BFM consolidation (five weeks): daily oral mercaptopurine 60mg/m^2 (days 1-28), weekly intrathecal methotrexate (days 1, 8, 15,22). Two doses of cyclophosphamide 1g/m^2 IV (days 1,15) and 16 cytarabine 75 mg/m^2 (days 2-5, 9-12, 16- 19, 23-26)

Regimen C (nine weeks)- augmented BFM consolidation: daily oral mercaptopurine 60 mg/m^2 (days 1–14), weekly intrathecal methotrexate (days 1, 8, 15 and 22). Two doses of cyclophosphamide 1 g/m^2 IV (days 1,29) and cytarabine in four pulses 75 mg/m^2 (days 2–5, 9–12, 30–33 and 37–40). Four doses of vincristine 1.5 mg/m^2 IV (days 16, 23, 44,51) and two doses of pegylated asparaginase 1000 iu/m^2 (days 16,44)

Patients with refractory CNS disease at diagnosis received cranial irradiation during consolidation.

- **Interim Maintenance I**

Regimen A and B (eight weeks): daily oral mercaptopurine 75 mg/m^2 (until the penultimate week), weekly oral methotrexate 20 mg/m^2 (days 1, 8, 15, 22, 36, 43 and 50), one dose of intrathecal methotrexate, two pulses of vincristine 1.5 mg/m^2 IV (days 1, 29) and steroid as randomized for days 1-5 and 29-33

Regimen C (eight weeks)- Capizzi I: five escalating doses of intravenous methotrexate starting on day 2 at 100 mg/m^2 IV. and increasing by 50 mg/m2 every 10 days as permitted, two doses of pegylated asparaginase 1000 iu/m^2 (days 3, 23), five doses of vincristine 1.5 mg/m^2 IV (days 2, 12, 22, 32, 42) and two doses of intrathecal methotrexate (days 1, 31).

- **Delayed Intensification I**

Regimen A and B (seven weeks): The delayed intensification courses consisted of two parts. The first half entailed one dose of pegylated asparaginase 1000units/m^2 on day 4, one dose of intrathecal methotrexate, three doses of vincristine 1.5 mg/m^2 IV (days 2, 9, 16) and doxorubicin 25 mg/m^2 IV (days 2, 9, 16) and two dexamethasone pulses 10mg/m^2 orally on days 2-8 and 16-22 (alternate weeks). The second half lasted for four weeks and consisted of oral mercaptopurine for two weeks 75 mg/m^2 (days 29–42), one dose of cyclophosphamide 1g/m^2 IV on day 29, two doses of intrathecal methotrexate (days 1, 29, 36) and eight doses of cytarabine in two pulses; 75 mg/m^2 IV or SC (days 30–33 and 37–40).

Regimen C (eight weeks): two doses of vincristine additional to the above regimen.

- **Interim Maintenance II**

This phase was for patients receiving two delayed intensifications.

Patients in regimens A and B were randomised to receive one or two delayed intensification courses. All patients allocated regimen C received two delayed intensifications (standard of treatment at the time).

Regimen A (eight weeks): same as interim maintenance I, but without any intrathecal therapy.

Regimen B (eight weeks): same as interim maintenance I.

Regimen C (eight weeks): same as interim maintenance I.

- **Delayed Intensification II**

Patients assigned to standard treatment received two delayed intensification courses separated by a second interim maintenance course (above), and those assigned to reduced treatment received only one delayed intensification course followed by maintenance.

The delayed intensification II courses for each regimen were identical to the delayed intensification I for that regimen.

- **Maintenance**

All patients received oral mercaptopurine and methotrexate, monthly vincristine and steroid pulses, and intrathecal methotrexate every three months.

Male patients received treatment for three years and female patients for two years from the start of interim maintenance I.

A.1.3 UKALL 2003 Regimens

- **Regimen A**

Patients received three-drug induction for five weeks (weeks 1-5) followed by standard BFM consolidation for three weeks (weeks 6-8). Interim maintenance I for eight weeks (weeks 9-16) followed by delayed intensification I for seven weeks (weeks 17-23). For patients allocated two delayed intensifications, interim maintenance II was delivered during weeks 24-31 followed by delayed intensification II during weeks 32-38. Finally, maintenance chemotherapy was delivered from week 39 until week 112 for girls and week 164 for boys.

- **Regimen B**

Patients received four-drug induction for five weeks (weeks 1-5) followed by standard BFM consolidation for five weeks (weeks 6-10). Interim Maintenance I for eight weeks (weeks 11-18) followed by delayed intensification I for seven weeks (weeks 19-25). For patients allocated two delayed intensifications, interim maintenance II was delivered during weeks 26-33 followed by delayed intensification II during weeks 34-40. Finally, maintenance chemotherapy was delivered from week 41 until week 114 for girls and week 166 for boys.

- **Regimen C**

Patients received four-drug induction for five weeks (weeks 1-5) followed by augmented BFM consolidation for nine weeks (weeks 6-14). Interim Capizzi maintenance I with pegylated asparaginase and escalating doses of intrevenous methotrexate for eight weeks (weeks 15-22) followed by delayed intensification I for eight weeks (weeks 23-30). Subsequently, interim Capizzi maintenance II for eight weeks (weeks 31-38) and then delayed intensification II during weeks 39-46. Finally, maintenance chemotherapy was delivered from week 47 until week 118 for girls and week 170 for boys.

The different regimens and randomisations of the UKALL2003 trial are presented in Figure A.1-1.

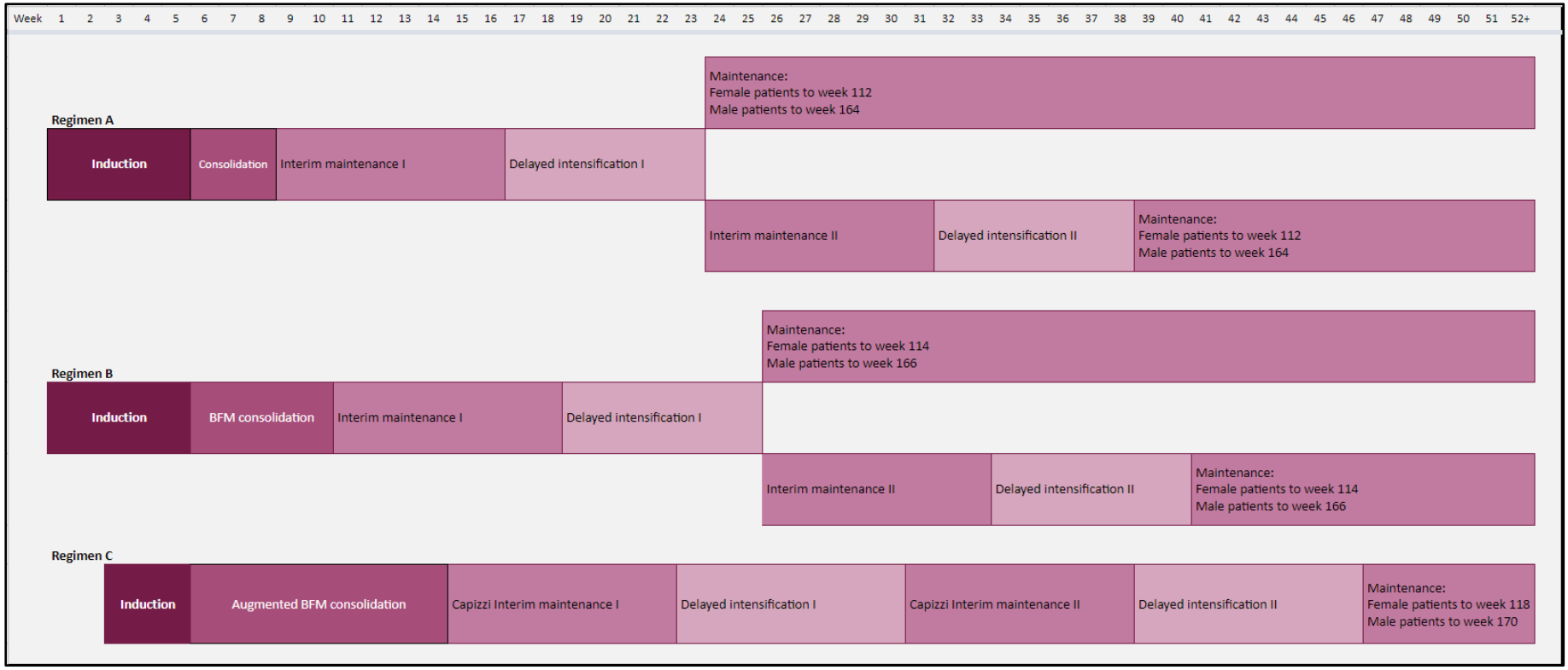

Figure A.1-1: UKALL 2003 phases per regimen and randomisation

A.1.4 Count recovery and planned chemotherapy administration

An absolute neutrophil count of 0.75 x 10^9/L and over and a platelet count of 75 x 10^9/L and over were required for the start of every phase. This was termed as “count recovery”.

Patients treated under all regimens were required to have achieved count recovery at the start of the second half of each delayed intensification cycle to start.

For patients in regimen A, delayed intensification I was given between weeks 17-23. Once started, therapy during weeks 17-20 was not interrupted for myelosuppression alone. Therapy due on day 1 of week 21 was delayed until count recovery was achieved and then, again, once started was not interrupted for myelosuppression alone. Any serious infection, and neutropenia with fever, with proven or suspected infection, warranted chemotherapy interruption at any point during delayed intensification.

The same applied for the patients who were allocated delayed intensification II during weeks 32-38. Once started, therapy during weeks 32-35 was not interrupted for myelosuppression alone. Therapy due on day 1 of week 36 was delayed until count recovery was achieved and then, again, once begun was not interrupted for myelosuppression alone.

For patients in regimen B, delayed intensification I was given between weeks 19-25. Once started, therapy during weeks 19-22 was not interrupted for myelosuppression alone. Therapy due on day 1 of week 23 was delayed until count recovery was achieved and then, again, once started was not interrupted for myelosuppression alone. Any serious infection and neutropenia with fever, with proven or suspected infection, warranted chemotherapy interruption at any point during delayed intensification.

The same applied for the patients who were allocated delayed intensification II during weeks 34-40. Once started, therapy during weeks 34-37 was not interrupted for myelosuppression alone. Therapy due on day 1 of week 38 was delayed until count recovery was achieved and then, again, once started was not interrupted for myelosuppression alone.

Finally, for patients in regimen C, delayed intensification I was given between weeks 23-30. Once started, therapy during weeks 23-28 was not interrupted for myelosuppression alone. Reconsolidation, scheduled to begin on day 1 of week 29 was delayed until count recovery was achieved and then, again, once started was not interrupted for myelosuppression alone. Any serious infection and neutropenia with fever with proven infection warranted chemotherapy interruption.

For delayed intensification II during weeks 39-46, once started, therapy during weeks 39-42 was not interrupted for myelosuppression alone. Therapy due on day 1 of week 43 was delayed until count recovery was achieved and then again once started was not interrupted for myelosuppression alone.

For patients in regimen C, there were additional potential delays of chemotherapy. Augmented BFM consolidation was interrupted for myelosuppression on day 29, but once the cyclophosphamide was given at day 1 or 29, therapy continued except in patients who were febrile with a proven infection. Escalating Capizzi maintenance phase I and II were interrupted for serious infection.

A.1.5 Antimicrobial prophylaxis

Patients received co-trimoxazole (trimethoprim and sulphamethoxazole) as PJP prophylaxis for two consecutive days throughout treatment.

A.1.6 UKALL 2003 results

UKALL 2003 reported improved outcomes and showed that MRD can be used to identify individual patients who may benefit from de-escalation of treatment.

The results of UKALL 2003 represented an improvement in EFS of 6% over the previous trial (ALL 97/99) and were amongst the best reported internationally. The trial showed that treatment reduction is feasible for children who are predicted to have low risk of relapse based on low MRD by the end of induction (69). In the low-risk patients, no significant difference in EFS was seen between the group that was given one delayed intensification and that given two, but additional treatment toxicity burdened the patients who received two delayed intensification courses. Overall, in the context of such high EFS, the treatment toxicity was extremelly high with a TRM in remission of 3.2% and 25% of patients suffering at least one non-haematological severe adverse event.

In the MRD high-risk patients, 5-year EFS was better in the augmented treatment group than in the standard group (62). However, more adverse events were seen in the augmented treatment group, particularly with the asparaginase and intravenous methotrexate.

There were poor outcomes in patients with CNS relapse with a 5-year EFS of only 30%. Very early bone marrow relapse, although rare (2.7% of patients), was associated with a dismal prognosis with less than 20% survival rate, even after SCT in second remission.

A.2 UKALL 2011: 2012-2018

Building on the findings of UKALL 2003, the subsequent trial UKALL 2011 aimed to refine the treatment stratification based on MRD. The aim of improved survival with less treatment burden remained; the trial entailed less toxicity and treatment burden for all patients through the administration of a single course of delayed intensification to all patients. There was also a reduction in augmented treatment for non-MRD low-risk patients.

The trial aimed to further improve survival by addressing the issues from UKALL 2003 findings mentioned in Section A.1.6. In order to attempt to reduce treatment related toxicity in balance with risk of relapse, UKALL 2011

randomisations modified therapy in the following: dexamethasone at induction, the duration of delayed intensification and the approach to CNS directed and maintenance therapy.

The trial recruited patients aged one (1st birthday) to 24 years diagnosed with ALL between April 2012 and December 2018. The trial also recruited patients with diffuse large B-cell lymphoma.

A.2.1 Changes from UKALL 2003

The major changes from UKALL2003 protocol were as follows.

All patients with T-ALL received a four-drug induction (regimen B).

There were five adverse cytogenetic abnormalities: iAMP21, t(17;19)(q22;p13), MLL rearrangement, near haploidy, and low hypodiploidy.

All patients receive a single delayed intensification.

The addition of an extra week in the interim maintenance phase in regimens A and B and an extra week in the consolidation phase in regimen C.

A.2.2 Risk stratification

Patients were divided in standard and high-risk treatment groups and were allocated to three treatment regimens (A, B or C) of increasing intensity.

Standard risk: patients over one and under ten years of age at diagnosis with a pre-treatment white cell count of less than 50 x 10^9/L.

High risk: patients over ten years of age at diagnosis, or any age with a pre-treatment white cell count of more than 50 x 10^9/L. Also, all patients with T-ALL and LBL.

Regimen C, the highest intensity of treatment, was not initiated at the start of treatment. Children being treated on regimens A or B, were moved to regimen

C if they had cytogenetic abnormalities associated with more aggressive disease, or if their early response to treatment based on MRD was suboptimal.

Post-induction treatment was stratified based on Day 29 MRD result (or tumour volume assessment in patients with LBL).

MRD Low Risk Group (MRD <0.005%) received consolidation as per previously assigned regimen at induction.

MRD Risk Group (MRD ≥0.005%) were allocated to regimen C at that point and received augmented BFM consolidation. They had a repeat MRD level at the end of consolidation and if it was more than 0.5%, they were at high risk of relapse and were taken off the UKALL 2011 protocol.

A.2.3 Randomisation

Both randomisations of the UKALL 2011 are aimed at reducing toxicity, and the second randomisation, also assesses the effect of high dose methotrexate in improving CNS prophylaxis.

Randomisation 1 (R1) – dexamethasone randomisation

In induction, a higher dose and shorter dexamethasone schedule (10mg/m^2/day for a total of 14 days) versus the standard UK schedule (6mg/m^2/day for 28 days).

The first randomisation aimed to reduce toxicity. It was, however, discontinued after a futility analysis in April 2017 showed that there was less than 10% power of showing a significant improvement with short dexamethasone (52).

Randomisation 2 (R2) – methotrexate and pulses randomisation

The second randomisation related to providing more effective CNS prophylaxis and to reducing the burden of treatment.

In interim maintenance therapy, patients were randomised to receiving either high dose methotrexate without prolonged intrathecal therapy or the standard UK CNS-directed therapy during interim maintenance with prolonged intrathecal therapy.

In maintenance therapy, the effect in patients on bone marrow relapse risk and quality of life of receiving monthly pulses of vincristine and dexamethasone.

Patients with T-ALL or white cell count of more than 100 x 10^9 at diagnosis, received intrathecal methotrexate during maintenance regardless of randomisation (for the first 6 cycles of maintenance).

A.2.4 UKALL 2011 treatment phases

- **Induction**

The first randomisation happened upon recruitment to trial. Patients were randomised to standard (standard dose for 28 days) or short dexamethasone (higher dose of 14 days) course.

<u>Regimen A (five weeks):</u>

Three-drug induction with:

- oral dexamethasone 6mg/m^2 /day for 28 days (maximum single dose 10mg/day)
- weekly vincristine (1.5mg/m^2 intravenously weekly, maximum single dose 2mg) on days 9,16,23,20
- 2 doses of pegylated asparaginase (1000iu/m2 intramuscular) on day 4 and day 18
- 3 doses of intrathecal methotrexate (aged dependent dose). Days 1,8,29

During the last week of induction, patients would start daily oral mercaptopurine (75mg/m^2 /day) and would carry on with that for four weeks (until the end of the third week of consolidation).

Regimen B

As for regimen A plus four doses of daunorubicin (25mg/m^2 intravenously on days 2, 9, 16, 23) and one extra week of mercaptopurine (until the end of the fourth week of consolidation).

Regimen C started on D15 (same as regimen B from day 15 onwards).

- **Consolidation**

Regimen A (three weeks):

- daily oral mercaptopurine (75mg/m^2 /day)
- weekly intrathecal methotrexate (days 1, 8, 15).

Regimen B (five weeks):

- daily oral mercaptopurine 60mg/m^2 /day once per day to day 28 of standard BFM consolidation (5 weeks in total from the start in week 5 of induction).weekly intrathecal methotrexate for three weeks (days 1, 8, 15)
- 2 doses of cyclophosphamide 1000mg/m^2 intravenously on days 1 and 15
- Cytarabine 75mg/m^2/day intravenously or subcutaneously 4 consecutive days in weeks 6,7,8,9 (standard BFM consolidation).

Regimen C (ten weeks):

- daily oral mercaptopurine e 60mg/m2 /day once a day for 21 days, beginning week 5 of induction to end of week 2 of consolidation, and again for 14 days on days 29-42 of consolidation
- 2 doses of cyclophosphamide (1000mg/m^2 intravenous) on days 1 and 29
- Cytarabine 75mg/m^2/day intravenously or subcutaneously 4 consecutive days in weeks 6,7,10, 11
- 4 doses of vincristine 1.5mg/m^2 intravenous (maximum single dose 2mg) on day 16, 23, 44 and 51
- 2 doses of pegylated asparaginase 1000 units/m^2 intramuscular on days 16 and 44 (augmented BFM consolidation).

- **Interim Maintenance**

This was the point of the second randomisation. If patients were randomised to high dose intravenous methotrexate therapy (Protocol M), they would have no subsequent intrathecal methotrexate during maintenance, but could be randomised to either pulses or no pulses of vincristine and dexamethasone during maintenance. If they were randomised to either standard (regimens A and B) or Capizzi interim maintenance (regimen C) they would be randomised to maintenance therapy with or without pulses, and all patients would receive intrathecal methotrexate during maintenance.

Standard interim Maintenance

Regimens A and B (nine weeks):

daily oral mercaptopurine 75mg/m^2 /day (apart from week 17 in regimen A and week 19 in regimen B as intrathecal methotrexate given that week)

weekly oral methotrexate 20mg/m^2 (days 1, 8, 22,29,36,50,57)

2 doses of intrathecal methotrexate (days 15,43)

2 doses of vincristine 1.5mg/m^2 IV (days 1 and 29) and

10 days of dexamethasone 6mg/m^2/day (days 1-5 and 29-33).

Capizzi interim maintenance (eight weeks): This regimen was standard treatment for patients in regimen C.

5 doses of vincristine 1.5mg/m^2 (days 2, 12, 22, 32, 42)

4 doses of intravenous methotrexate 100mg/m^2 IV (day 2. Escalating subsequent doses as tolerated on days 12, 22, 32, 42)

2 doses of pegylated asparaginase 1000 units/m^2 IM (days 3, 23)

2 doses of intrathecal methotrexate (days 1, 31).

High dose methotrexate Interim Maintenance

Regimens A and B:

daily oral mercaptopurine 25mg/m^2/day (days 1-56) (apart from week 17 in regimen A and week 19 in regimen B), four doses of)

4 doses of IV methotrexate 5g/m^2 (days 8, 22, 36,50) with folinic acid 15mg/m^2 IV (42,48 and 54 hours after start of methotrexate infusion),

4 doses of intrathecal methotrexate (days 8, 22, 36,50) (Protocol M).

Regimen C (eight weeks): (Protocol M-A).

daily oral mercaptopurine 25mg/m^2/day (days 1-49)

4 doses each of IV methotrexate 5g/m^2 (days 1, 15, 29, 43) with folinic acid 15mg/m^2 IV (42,48 and 54 hours after start of methotrexate infusion),

4 dose of intrathecal methotrexate (days 1, 15, 29, 43)

2 doses of pegylated asparaginase 1000 units/m^2 IM (days 2, 23)

- **Delayed Intensification**

Regimen A and B (seven weeks):

- dexamethasone 10mg/m^2/day orally for 7 days week 20 and 22
- vincristine 1.5mg/m2 intravenously days 2,9,16 (maximum single dose 2mg)
- doxorubicin 25mg/m2 intravenously days 2,9,16
- pegaspargase 1000iu/m2 IM day 4
- methotrexate 12mg intrathecal day 1
- cyclophosphamide 1000mg/m2 intravenously day 29
- mercaptopurine 60mg/m2/day orally day 29-42
- cytarabine 75mg/m2/day intravenously or subcutaneously 4 consecutive days weeks 24,25

If delayed intensification is part of regimen C the dexamethasone is given days 2-5 and 16-22, cytarabine is given in weeks 28 and 29, and vincristine given on days 2, 9, 16, 43 and 50. Intrathecal methotrexate is also given on days 29 and 36, and pegaspargase is also given on day 43.

- **Maintenance**

Continuing therapy depended on the randomisation at the point of interim maintenance as described above. Male patients received treatment for three years and female patients for two years from the start of interim maintenance (two years from the start of interim maintenance for all the patients with LBL).

Patients randomised to receive standard interim maintenance would be allocated to maintenance with or without pulses of vincristine and

dexamethasone, but would have intrathecal methotrexate during maintenance.

Patients randomised to receive high dose methotrexate (protocol M) interim maintenance would be allocated to maintenance with or without pulses of vincristine and dexamethasone, but would not have intrathecal methotrexate during maintenance.

A.2.5 UKALL 2011 Regimens

- **Regimen A**

Patients treated under regimen A received a three-drug induction for five weeks (weeks 1-5) followed by consolidation for three weeks (weeks 6-8). Interim maintenance for nine weeks (weeks 9-17) followed by delayed intensification for seven weeks (weeks 18-24). Finally, maintenance chemotherapy was delivered until week 114 for girls and week 166 for boys.

- **Regimen B**

Patients treated under regimen B receive a four-drug induction for five weeks (weeks 1-5) followed by standard BFM consolidation for five weeks (weeks 6-10). Interim maintenance for nine weeks (weeks 11-19) followed by delayed intensification for seven weeks (weeks 20-26). Finally, maintenance chemotherapy was delivered until week 114 for girls and week 166 for boys.

- **Regimen C**

Regimen C commences post induction. Patients receive augmented BFM consolidation for 10 weeks (weeks 6-15). Capizzi interim maintenance or high-dose methotrexate and asparaginase (Protocol M-A), depending on randomisation (weeks 16-23) followed by delayed intensification for eight

weeks (weeks 24-31). Finally, maintenance chemotherapy delivered until week 114 for girls and week 166 for boys.

The different regimens and randomisations of the UKALL 2011 trial are presented in Figure A.2-1.

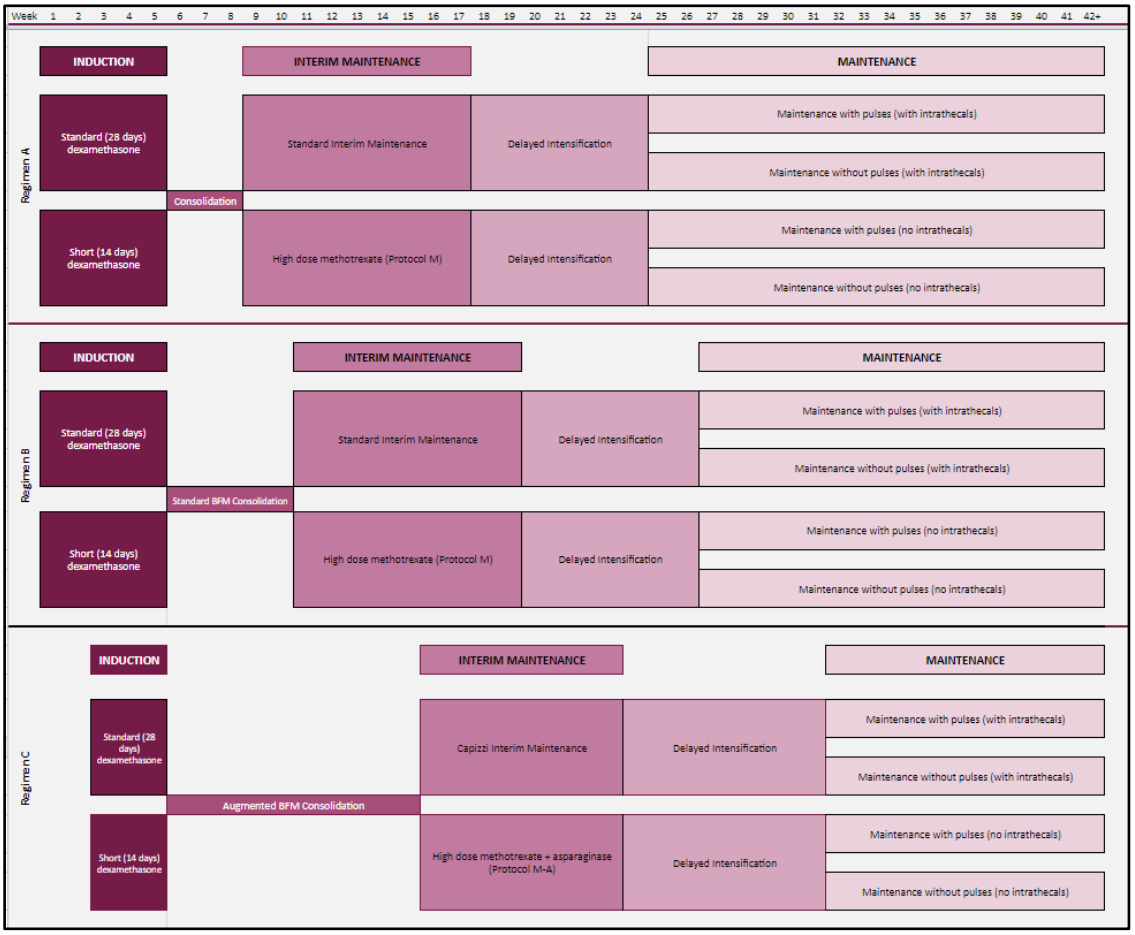

Figure A.2-1: UKALL 2011 phases over time. Each regimen is demonstrated with all possible randomisation combinations.

A.2.6 Count recovery and planned chemotherapy administration

A neutrophil count of more than 0.75 x 10^9/L and platelet count of more than 75 x 10^9/L was required for the start of every phase.

During interim maintenance, if randomised to protocol M, a neutrophil count of more than 0.5 x 10^9/L and platelet count of more than 50 x 10^9/L was required for the start of each subsequent pulse of high dose methotrexate.

Patients treated under all regimens were required to have count recovered at the start of the second half of each delayed intensification cycle to start.

For patients in regimen A, delayed intensification was given between weeks 18-24. Once started, therapy during weeks 18-21 was not interrupted for myelosuppression alone. Therapy due on day 1 of week 22 was delayed until count recovery was achieved and then, again, once started was not interrupted for myelosuppression alone. Any serious infection, suspected or proven, or neutropenia with fever warranted chemotherapy interruption at any time during delayed intensification.

For patients in regimen B, delayed intensification was given between weeks 20-26. Once started, therapy during weeks 20-23 was not interrupted for myelosuppression alone. Therapy due on day 1 of week 24 was delayed until count recovery was achieved and then, again, once started was not interrupted for myelosuppression alone. Any serious infection, suspected or proven, or neutropenia with fever warranted chemotherapy interruption at any time during delayed intensification.

Finally, for patients in regimen C, delayed intensification was given between weeks 24-31. Once started, the treatment was not interrupted for pancytopenia alone. Treatment would be delayed for any serious infection, suspected or proven, or neutropenia with fever.

For patients in regimen C, there were additional potential delays of chemotherapy. Augmented BFM consolidation was interrupted for myelosuppression on day 29, but once the cyclophosphamide was given at day 1 or 29, therapy continued except in patients who were febrile with a proven infection. Capizzi interim maintenance was interrupted for serious infection.

A.2.7 Antimicrobial prophylaxis

Patients received co-trimoxazole (trimethoprim and sulphamethoxazole) as PCP prophylaxis for two consecutive days throughout treatment.

A.3 Non trial treatment protocols and interim guidelines

- **UKALL 2003 non-trial protocol**

The patients who were not recruited in the UKALL 2003 received the standard of treatment at the time which consisted of two courses of delayed intensification and allocation to different regimens based on early response to treatment on bone marrow testing.

- **Interim Guidelines and UKALL 2011 non-trial protocol**

Patients diagnosed between July 2011 and the end of April 2012 were treated under the interim guidelines. These patients were allocated to regimens based on MRD-risk and received standard dose dexamethasone during induction, had one course of delayed intensification and maintenance with pulses of vincristine which is the same treatment received by the patients who were not recruited in the UKALL 2011 and were treated while the trial was open.

Appendix B Authored abstracts

The following jointly authored abstracts have been written as a result of the work in this thesis:

‘Paediatric intensive care admissions in children with acute lymphoblastic leukaemia’. Poster at the European Haematology Association Congress, 2018. Presenting author. Contributing authors: Dr Eleanor Kane, Prof Eve Roman, Dr Srdan Rogosic, Prof Sally Kinsey
https://library.ehaweb.org/eha/2018/stockholm/215507/melpomeni.rompola.pediatric.intensive.care.admissions.in.children.with.acute.html?f=menu=6*ce_id=1346*ot_id=19060*media=3

'Respiratory viruses: a threat to children with acute lymphoblastic leukaemia'. Poster at the 27th European Congress of Clinical Microbiology and Infectious Diseases, 2017. Presenting author. Contributing authors: Dr Eleanor Kane, Prof Eve Roman, Dr Fathi Azzuni, Dr Antony Hale, Dr Beki James, Prof Sally Kinsey
https://www.escmid.org/escmid_publications/escmid_elibrary/?tx_solr%5Bsort%5D=relevance%20asc&tx_solr%5Bfilter%5D%5B0%5D=main_filter_eccmid%253Atrue&tx_solr%5Bfilter%5D%5B1%5D=pub_date%253A201701010000-201712312359&tx_solr%5Bfilter%5D%5B2%5D=entry_type%253APoster%2Bpresentation&tx_solr%5Bfilter%5D%5B3%5D=main_category%253AViral%2BInfection%2B%2526%2BDisease&tx_solr%5Bpage%5D=5

'Impact of respiratory viral infections in immunocompromised paediatric haematology patients'. E-poster presentation at the European Haematology Association Congress, 2016. Presenting author. Contributing authors: Dr Eleanor Kane, Prof Eve Roman, Dr Fathi Azzuni, Dr Antony Hale, Dr Beki James, Prof Sally Kinsey
https://library.ehaweb.org/eha/2016/21st/132730/melpomeni.rompola.impact.of.respiratory.viruses.in.immunocompromised.pediatric.html

Appendix C HMRN Data Collection sheet

HMRN Data Collection: B-lymphoblastic leukaemia NOS [9811/3]

Please enter data into boxes, and amend any incorrect or missing details:

Patient Name:		HILIS ID:	
Date of Birth:		NHS No:	
HMDS Number:		Report Date:	
Source:		Specimen:	

Demographics:

Gender:	M / F	Date of diagnosis:	
Address at diagnosis:			
GP address:			
1st appointment on:		Palliative date:	
Date of death:			

Antecedent / concurrent events:

Event:	
Therapies:	chemotherapy / radiotherapy / both

Treatment history:

Centre:					
Treatment:					
Trial:					
Start date:		End date:		Response:	

Presentation data:

CNS disease:	

Comments:

Additional Treatment Histories

Centre:					
Treatment:					
Trial:					
Start date:		End date:		Response:	

Centre:					
Treatment:					
Trial:					
Start date:		End date:		Response:	

Centre:					
Treatment:					
Trial:					
Start date:		End date:		Response:	

Centre:					
Treatment:					
Trial:					
Start date:		End date:		Response:	

Centre:					
Treatment:					
Trial:					
Start date:		End date:		Response:	

Centre:					
Treatment:					
Trial:					
Start date:		End date:		Response:	

Appendix D Standard Operating Procedure for central line access and sampling

The Leeds Teaching Hospitals NHS
NHS Trust

Central Venous catheter: Insertion site dressing and Needle free device change and weekly flush

Essential equipment

- Personal protective equipment (Gloves/Apron) COVID 19 precautions PPE (Please follow current LTHT guidelines)
- Sterile dressing pack (containing sterile towel, gauze and gloves)
- Alcohol-based skin-cleaning(**Chloraprep Applicator** , 2% Chlorhexidine in 70% alcohol solution)
- Semi-permeable transparent dressing (IV300/Tegaderm Film)- Check patient allergy status
- Needle free device for each lumen (SmartSite/ Bio-Connector)
- 10ml syringe(s)
- Drawing up needle
- **Sani-cloth wipe**(chlorhexidine 2% in 70% alcohol solution) (more if a double lumen line)
- 1 pair of sterile Gloves
- NB: For a PICC line you will require another 10ml syringe for the Heparin sodium flushing solution (50units/5mls) for each lumen, and heparin sodium (50units/5mls) ampoules

Optional equipment

- StatLock stabilization device
- Wound Swab to be sent for MC&S

Pre-procedure

Action

Introduce yourself to the patient, explain and discuss the procedure with them, and gain consent to proceed.

Procedure

This procedure should be performed using an aseptic non-touch technique (despite sterile gloves)

1. Wash hands with soap and water.
2. Take a blue tray/or trolley and wipe with a Clinell Detergent Wipe.
3. Place all equipment required for the procedure around the blue tray
4. Take the trolley to the patient's bedside.
5. Assist the patient into a sitting position, if possible.
6. Open the sterile dressing pack in the clean blue tray/or on the trolley.
7. Open the **Chloraprep Applicator, dressing**, **Sani-cloth sterile wipes(CHG 2%)** and **sterile gloves** onto the centre of the sterile field.
8. Open other supplementary packs, including the **10ml syringe(s), drawing up needle, needle free device(s),** and tip contents gently onto the centre of the sterile field.
9. Open the ampoule of 0.9% sodium chloride and **place outside the sterile field.**

10. Use the orange waste bag to move items into accessible positions on sterile field then attach waste bag to the side of the trolley.

11. Check the clamp on each lumen is closed.

12. Wash hands with soap and water and dry/or alcohol gel.

13. **Time to put on sterile gloves**

14. Place the sterile towel under the patient's PICC/Hickman line.

15. The previously opened 0.9% sodium chloride ampule should be drawn up using a sterile needle and syringe without contaminating your sterile gloves. (you can do this by holding the ampule with the corner of the sterile field, or sterile piece of gauze).

16. Prime the needle free device(s) and place back onto the sterile field.

17. Use a Sani-cloth (CHG 2%) wipe to hold the line near the needle free device. Using gauze from dressing pack, twist off the needle free device, and discard in the waste bag.

18. Use a Sani-cloth(CHG 2%) to clean the hub/end of the line for 15 seconds and let this dry. Attach the "ready primed" needle free device
NB: Do not rest the line down on the towel while waiting to dry, hold in the air.
(Repeat process from step 17 & 18 with second lumen if applicable)

19. Flush the lumen with the 10ml 0.9% sodium chloride syringe using a pulsating technique. Discard the empty syringe into the clinical waste bag.

    If second lumen is present use 10ml 0.9% Sodium Chloride Syringe to flush

    If flushing a PICC that is not in regular use (at least daily), please draw up the Heparin Sodium Flushing Solution (50units/5mls), and use to flush each lumen using ANTT principles.

20. At the end of the flushing procedure, use a new sanicloth (CHG 2%) to clean the needle free device, for 15 seconds and let this dry.

Insertion site dressing change

21. Wearing gloves (non- sterile), remove the old dressing and discard it into waste bag.

22. If the site is red or discharging, take a swab for MC&S

23. Remove and dispose of gloves, use alcohol gel and put on **sterile gloves**

24. Remove statlock if present - hold PICC line to stop it slipping out.

25. Clean the area with the Chloraprep applicator (2%CHG), starting from the insertion site and working your way out. Allow the area to dry

26. Reapply the StatLock if necessary (if after 3 weeks since Tunnelled line insertion these are not needed)

27. Apply the appropriate dressing,

    NB: If a securacath (orange) device is in place with PICC line it is important that the dressing is not applied too tightly, and that pressure is not put on the orange part of the securacath. The dressings should be applied so that it is comfortable for the patient.

    With PICCs especially double lumen PICCs it is very important to ensure that he line has not become twisted or flipped over during cleaning as this will mean that the inner lumens are narrowed and the line will not work anymore.

28. Document the date and time on the dressing or in patient notes

29. Remove gloves and dispose of waste bag in the appropriate bin.

NB The Aseptic Non -Touch Technique should always be used for taking bloods - these can be taken pre changing the needle free device or after.

Appendix E Regression analyses

Regression models were applied to counts of samples, or positive samples, during each treatment phase. Since, in each phase, there was a reasonable number of patients who did not have any samples- because there may not have been suspicion of infection-, hurdle models were applied (190). The hurdle model comprises two components: the first component estimates the risk of ever being sampled (or having at least one positive sample) compared to never being sampled; the second is restricted to those who were sampled, and compares whether the sampling rates are different between groups. Here the risk of ever being sampled compared to never was estimated using logistic regression and are described using odds ratios (OR) with 95% confidence intervals (95%CI), while sampling rates amongst those with at least one sample are compared using negative binomial regression and are described using rate ratios (RR) with 95% confidence intervals. Hurdle models were implemented in R using the **pscl** package (191).

Findings for the regression models including the variables sex (female versus male), age (10+ years versus 1-10 years), deprivation (more deprived quintiles 4-5 versus less deprived 1-3), and treatment regimens (Regimen B, Regimen C compared to Regimen A) are presented by treatment phase and sample type (all, blood, respiratory, and fungal) in Table E-1 to Table E-20. Results are not presented where there were less than 10 patients with samples; this was the case for positive fungal samples in all phases.

Overall, the findings of the adjusted regression models support those presented in the Chapter 3. For instance, during induction, patients aged 10 or older were less likely to have been sampled- and were less likely to have had a positive sample- than those under the age of 10; and patients on the four-drug regimen were more likely to have had a positive sample, and were sampled more often than those on the three-drug regimen (Table E-1). These patterns were generally seen for blood and respiratory samples (Table E-2 and Table E-3 respectively). During consolidation, patients were more likely to be sampled- and to have at least one positive sample- if they were on Regimen B or C compared to those on Regimen A, but only the

sampling rate, and rate of positives, was elevated for Regimen B relative to Regimen A in total and for all three sample types (Table E-5 to Table E-8).

Regression models for interim maintenance 1 (Table E-9 to Table E-12) showed that patients aged 10 or older were less likely to be sampled- or to have at least one positive sample- than younger patients; and were more likely to have been sampled if treated on Regimen C, compared to Regimen A. The former finding applied to respiratory samples while the latter was seen for blood samples. Findings for the delayed intensification 1 phase (Table E-13 to Table E-16) were similar with respect to respiratory samples and age to those seen in interim maintenance 1. In maintenance, the sampling rate, and rate of positives, was lower among patients aged 10 or more compared to those aged under 10, and females were less likely than males to have at least one positive sample (Table E-17). These findings in maintenance were seen for both blood and respiratory samples (Table E-18 and Table E-19 respectively); in addition, for blood samples, patients from more deprived areas had a higher sampling rate and were more likely to have had at least one positive blood sample than those from more affluent areas (Table E-20).

Table E-1: Risk of being sampled, and rate ratios if sampled during induction.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate (95%CI)	RR(95%CI)	p-value
All Samples									
Total	267	166(62.2)			657	1418.8	46.3(42.8-50.0)		
Sex, M	149	92(61.7)	1(ref)		328	791.7	41.4(37.1-46.2)	1(ref)	
Sex, F	118	74(62.7)	1.02(0.61-1.69)	0.94	329	627.1	52.5(46.9-58.5)	1.45(0.92-2.26)	0.09
Age, 1-<10	197	129(65.5)	1(ref)		504	1051.6	47.9(43.8-52.3)	1(ref)	
Age, 10+	70	37(52.9)	0.48(0.25-0.91)	0.03	153	367.1	41.7(35.3-48.8)	1.00(0.58-1.72)	0.99
IMD, More affluent	139	81(58.3)	1(ref)		319	743.9	42.9(38.3-47.9)	1(ref)	
IMD, Less affluent	128	85(66.4)	1.37(0.83-2.27)	0.22	338	674.9	50.1(44.9-55.7)	1.12(0.72-1.73)	0.62
Regimen A	117	71(60.7)	1(ref)		190	580.9	32.7(28.2-37.7)	1(ref)	
Regimen B or C	150	95(63.3)	1.54(0.86-2.75)	0.15	467	837.9	55.7(50.8-61.0)	1.87(1.18-2.96)	0.01
Positive Samples									
Total	267	83(31.1)			152	1418.8	10.7(9.1-12.6)		
Sex, M	149	40(26.8)	1(ref)		68	791.7	8.6(6.7-10.9)	1(ref)	
Sex, F	118	43(36.4)	1.61(0.94-2.75)	0.08	84	627.1	13.4(10.7-16.6)	1.32(0.64-2.73)	0.45
Age, 1-<10	197	66(33.5)	1(ref)		118	1051.6	11.2(9.3-13.4)	1(ref)	
Age, 10+	70	17(24.3)	0.43(0.22-0.87)	0.02	34	367.1	9.3(6.4-12.9)	1.36(0.57-3.22)	0.49
IMD, More affluent	139	44(31.7)	1(ref)		68	743.9	9.1(7.1-11.6)	1(ref)	
IMD, Less affluent	128	39(30.5)	0.89(0.52-1.52)	0.67	84	674.9	12.4(9.9-15.4)	2.54(1.18-5.44)	0.02
Regimen A	117	30(25.6)	1(ref)		51	580.9	8.8(6.5-11.5)	1(ref)	
Regimen B or C	150	53(35.3)	2.29(1.26-4.16)	0.01	101	837.9	12.1(9.8-14.6)	1.08(0.50-2.32)	0.85

Table E-2: Risk of having a blood sample, and rate ratios if at least one blood sample during induction.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate (95%CI)	RR(95%CI)	p-value
All Samples									
Total	267	152(56.9)			430	1418.8	30.3(27.5-33.3)		
Sex, M	149	81(54.4)	1(ref)		214	791.7	27.0(23.5-30.9)	1(ref)	
Sex, F	118	71(60.2)	1.26(0.76-2.07)	0.37	216	627.1	34.4(30.0-39.4)	1.29(0.88-1.91)	0.20
Age, 1-<10	197	119(60.4)	1(ref)		333	1051.6	31.7(28.4-35.3)	1(ref)	
Age, 10+	70	33(47.1)	0.44(0.23-0.83)	0.01	97	367.1	26.4(21.4-32.2)	0.94(0.58-1.52)	0.81
IMD, More affluent	139	76(54.7)	1(ref)		218	743.9	29.3(25.5-33.5)	1(ref)	
IMD, Less affluent	128	76(59.4)	1.17(0.71-1.91)	0.54	212	674.9	31.4(27.3-35.9)	1.06(0.72-1.56)	0.79
Regimen A	117	63(53.8)	1(ref)		136	580.9	23.4(19.6-27.7)	1(ref)	
Regimen B or C	150	89(59.3)	1.81(1.02-3.21)	0.04	294	837.9	35.1(31.2-39.3)	1.59(1.04-2.44)	0.03
Positive Samples									
Total	267	52(19.5)			73	1418.8	5.1(4.0-6.5)		
Sex, M	149	24(16.1)	1(ref)		33	791.7	4.2(2.9-5.9)	1(ref)	
Sex, F	118	28(23.7)	1.67(0.90-3.10)	0.10	40	627.1	6.4(4.6-8.7)	1.08(0.47-2.47)	0.86
Age, 1-<10	197	41(20.8)	1(ref)		57	1051.6	5.4(4.1-7.0)	1(ref)	
Age, 10+	70	11(15.7)	0.51(0.23-1.13)	0.10	16	367.1	4.4(2.5-7.1)	1.22(0.43-3.43)	0.71
IMD, More affluent	139	27(19.4)	1(ref)		35	743.9	4.7(3.3-6.5)	1(ref)	
IMD, Less affluent	128	25(19.5)	0.96(0.51-1.77)	0.88	38	674.9	5.6(4.0-7.7)	1.68(0.71-3.95)	0.23
Regimen A	117	18(15.4)	1(ref)		25	580.9	4.3(2.8-6.4)	1(ref)	
Regimen B or C	150	34(22.7)	2.18(1.09-4.35)	0.03	48	837.9	5.7(4.2-7.6)	0.90(0.35-2.32)	0.82

Table E-3: Risk of having a respiratory sample, and rate ratios if at least one respiratory sample during induction.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate (95%CI)	RR(95%CI)	p-value
All Samples									
Total	267	61(22.8)			125	1418.8	8.8(7.3-10.5)		
Sex, M	149	32(21.5)	1(ref)		54	791.7	6.8(5.1-8.9)	1(ref)	
Sex, F	118	29(24.6)	1.23(0.68-2.22)	0.49	71	627.1	11.3(8.8-14.3)	1.85(0.91-3.77)	0.09
Age, 1-<10	197	48(24.4)	1(ref)		100	1051.6	9.5(7.7-11.6)	1(ref)	
Age, 10+	70	13(18.6)	0.46(0.22-0.97)	0.04	25	367.1	6.8(4.4-10.1)	1.03(0.43-2.46)	0.95
IMD, More affluent	139	28(20.1)	1(ref)		51	743.9	6.9(5.1-9.0)	1(ref)	
IMD, Less affluent	128	33(25.8)	1.32(0.74-2.38)	0.35	74	674.9	11(8.6-13.8)	1.47(0.73-2.95)	0.28
Regimen A	117	19(16.2)	1(ref)		29	580.9	5.0(3.3-7.2)	1(ref)	
Regimen B or C	150	42(28.0)	2.73(1.41-5.30)	<0.01	96	837.9	11.5(9.3-14.0)	1.69(0.73-3.87)	0.22
Positive Samples									
Total	267	42(15.7)			70	1418.8	4.9(3.8-6.2)		
Sex, M	149	22(14.8)	1(ref)		30	791.7	3.8(2.6-5.4)	1(ref)	
Sex, F	118	20(16.9)	1.19(0.61-2.32)	0.62	40	627.1	6.4(4.6-8.7)	3.00(0.76-11.8)	0.12
Age, 1-<10	197	33(16.8)	1(ref)		55	1051.6	5.2(3.9-6.8)	1(ref)	
Age, 10+	70	9(12.9)	0.57(0.24-1.35)	0.20	15	367.1	4.1(2.3-6.7)	1.47(0.26-8.39)	0.67
IMD, More affluent	139	18(12.9)	1(ref)		28	743.9	3.8(2.5-5.4)	1(ref)	
IMD, Less affluent	128	24(18.8)	1.50(0.77-2.94)	0.23	42	674.9	6.2(4.5-8.4)	1.53(0.40-5.90)	0.54
Regimen A	117	15(12.8)	1(ref)		21	580.9	3.6(2.2-5.5)	1(ref)	
Regimen B or C	150	27(18.0)	1.86(0.88-3.92)	0.10	49	837.9	5.8(4.3-7.7)	1.54(0.38-6.26)	0.55

Table E-4: Risk of having a fungal sample, and rate ratios if at least one fungal sample during induction.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate (95%CI)	RR(95%CI)	p-value
All Samples									
Total	267	49(18.4)			102	1418.8	7.2(5.9-8.7)		
Sex, M	149	29(19.5)	1(ref)		60	791.7	7.6(5.8-9.8)	1(ref)	
Sex, F	118	20(16.9)	0.89(0.47-1.68)	0.72	42	627.1	6.7(4.8-9.1)	0.94(0.40-2.22)	0.89
Age, 1-<10	197	33(16.8)	1(ref)		71	1051.6	6.8(5.3-8.5)	1(ref)	
Age, 10+	70	16(22.9)	1.09(0.52-2.29)	0.83	31	367.1	8.4(5.7-12)	0.79(0.32-1.99)	0.62
IMD, More affluent	139	24(17.3)	1(ref)		50	743.9	6.7(5.0-8.9)	1(ref)	
IMD, Less affluent	128	25(19.5)	1.17(0.63-2.19)	0.62	52	674.9	7.7(5.8-10.1)	1.13(0.51-2.52)	0.76
Regimen A	117	15(12.8)	1(ref)		25	580.9	4.3(2.8-6.4)	1(ref)	
Regimen B or C	150	34(22.7)	1.91(0.92-3.97)	0.08	97	837.9	9.2(7.3-11.5)	1.25(0.50-3.12)	0.63

Results are not presented for positive fungal samples since only five patients had nine positive fungal samples during induction.

Table E-5: Risk of having a sample, and rate ratios if at least one sample during consolidation.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	248	151(60.9)			582	1578.3	36.9(33.9-40.0)		
Sex, M	138	86(62.3)	1(ref)		330	869.9	37.9(34.0-42.3)	1(ref)	
Sex, F	110	65(59.1)	0.95(0.54-1.65)	0.84	252	708.4	35.6(31.3-40.2)	1.01(0.68-1.51)	0.95
Age, 1-<10	187	111(59.4)	1(ref)		406	1091.9	37.2(33.7-41.0)	1(ref)	
Age, 10+	61	40(65.6)	0.48(0.21-1.07)	0.07	176	486.4	36.2(31.0-41.9)	0.85(0.54-1.36)	0.51
IMD, More affluent	133	83(62.4)	1(ref)		323	835.9	38.6(34.5-43.1)	1(ref)	
IMD, Less affluent	115	68(59.1)	0.79(0.46-1.38)	0.41	259	742.4	34.9(30.8-39.4)	1.07(0.72-1.59)	0.74
Regimen A	109	46(42.2)	1(ref)		107	332.6	32.2(26.4-38.9)	1(ref)	
Regimen B	59	39(66.1)	4.11(1.78-9.50)	<0.01	184	343.1	53.6(46.2-62.0)	1.76(0.97-3.17)	0.06
Regimen C	80	66(82.5)	8.56(3.94-18.6)	<0.01	291	902.6	32.2(28.6-36.2)	0.76(0.46-1.25)	0.28
Positive Samples									
Total	248	68(27.4)			120	1578.3	7.6(6.3-9.1)		
Sex, M	138	37(26.8)	1(ref)		60	869.9	6.9(5.3-8.9)	1(ref)	
Sex, F	110	31(28.2)	1.19(0.66-2.17)	0.56	60	708.4	8.5(6.5-10.9)	2.01(0.64-6.30)	0.23
Age, 1-<10	187	50(26.7)	1(ref)		89	1091.9	8.2(6.5-10.0)	1(ref)	
Age, 10+	61	18(29.5)	0.50(0.24-1.05)	0.07	31	486.4	6.4(4.3-9.0)	0.49(0.15-1.58)	0.23
IMD, More affluent	133	41(30.8)	1(ref)		77	835.9	9.2(7.3-11.5)	1(ref)	
IMD, Less affluent	115	27(23.5)	0.63(0.34-1.15)	0.13	43	742.4	5.8(4.2-7.8)	0.84(0.29-2.44)	0.75
Regimen A	109	14(12.8)	1(ref)		17	332.6	5.1(3.0-8.2)	1(ref)	
Regimen B	59	21(35.6)	5.52(2.30-13.2)	<0.01	41	343.1	11.9(8.6-16.2)	4.92(0.64-38.1)	0.13
Regimen C	80	33(41.2)	6.17(2.89-13.2)	<0.01	62	902.6	6.9(5.3-8.8)	1.50(0.29-7.79)	0.63

Table E-6: Risk of having a blood sample, and rate ratios if at least one blood sample during consolidation.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	248	143(57.7)			399	1578.3	25.3(22.9-27.9)		
Sex, M	138	80(58)	1(ref)		237	869.9	27.2(23.9-30.9)	1(ref)	
Sex, F	110	63(57.3)	1.08(0.62-1.88)	0.79	162	708.4	22.9(19.5-26.7)	0.78(0.54-1.14)	0.20
Age, 1-<10	187	105(56.1)	1(ref)		281	1091.9	25.7(22.8-28.9)	1(ref)	
Age, 10+	61	38(62.3)	0.46(0.21-1.01)	0.05	118	486.4	24.3(20.1-29.1)	0.91(0.59-1.40)	0.67
IMD, More affluent	133	79(59.4)	1(ref)		218	835.9	26.1(22.7-29.8)	1(ref)	
IMD, Less affluent	115	64(55.7)	0.77(0.44-1.35)	0.37	181	742.4	24.4(21.0-28.2)	1.13(0.78-1.65)	0.51
Regimen A	109	41(37.6)	1(ref)		74	332.6	22.3(17.5-27.9)	1(ref)	
Regimen B	59	37(62.7)	4.43(1.94-10.1)	<0.01	120	343.1	35.0(29.0-41.8)	1.48(0.83-2.65)	0.18
Regimen C	80	65(81.2)	9.81(4.55-21.2)	<0.01	205	902.6	22.7(19.7-26.0)	0.72(0.44-1.20)	0.21
Positive Samples									
Total	248	33(13.3)			49	1578.3	3.1(2.3-4.1)		
Sex, M	138	18(13)	1(ref)		26	869.9	3.0(2.0-4.4)	1(ref)	
Sex, F	110	15(13.6)	1.26(0.57-2.80)	0.57	23	708.4	3.2(2.1-4.9)	1.16(0.44-3.09)	0.76
Age, 1-<10	187	23(12.3)	1(ref)		36	1091.9	3.3(2.3-4.6)	1(ref)	
Age, 10+	61	10(16.4)	0.48(0.20-1.16)	0.10	13	486.4	2.7(1.4-4.6)	0.36(0.10-1.28)	0.11
IMD, More affluent	133	22(16.5)	1(ref)		35	835.9	4.2(2.9-5.8)	1(ref)	
IMD, Less affluent	115	11(9.6)	0.46(0.20-1.05)	0.07	14	742.4	1.9(1.0-3.2)	0.39(0.11-1.32)	0.13
Regimen A	109	1(0.9)	1(ref)		1	332.6	0.3(0-1.7)	1(ref)	
Regimen B	59	13(22)	45.7(5.52-378)	<0.01	19	343.1	5.5(3.3-8.6)	∞(0-∞)	-
Regimen C	80	19(23.8)	45.0(5.75-352)	<0.01	29	902.6	3.2(2.2-4.6)	∞(0-∞)	-

Table E-7: Risk of having a respiratory sample, and rate ratios if at least one respiratory sample during consolidation.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	248	66(26.6)			109	1578.3	6.9(5.7-8.3)		
Sex, M	138	39(28.3)	1(ref)		62	869.9	7.1(5.5-9.1)	1(ref)	
Sex, F	110	27(24.5)	0.86(0.48-1.54)	0.62	47	708.4	6.6(4.9-8.8)	1.35(0.57-3.20)	0.50
Age, 1-<10	187	49(26.2)	1(ref)		82	1091.9	7.5(6.0-9.3)	1(ref)	
Age, 10+	61	17(27.9)	0.68(0.32-1.43)	0.31	27	486.4	5.6(3.7-8.1)	0.51(0.2-1.32)	0.17
IMD, More affluent	133	37(27.8)	1(ref)		64	835.9	7.7(5.9-9.8)	1(ref)	
IMD, Less affluent	115	29(25.2)	0.84(0.47-1.51)	0.57	45	742.4	6.1(4.4-8.1)	0.78(0.33-1.80)	0.55
Regimen A	109	20(18.3)	1(ref)		23	332.6	6.9(4.4-10.4)	1(ref)	
Regimen B	59	18(30.5)	2.38(1.03-5.50)	0.04	34	343.1	9.9(6.9-13.8)	4.42(0.99-19.8)	0.05
Regimen C	80	28(35)	2.69(1.33-5.45)	0.01	52	902.6	5.8(4.3-7.6)	1.89(0.47-7.55)	0.37
Positive Samples									
Total	248	45(18.1)			59	1578.3	3.7(2.8-4.8)		
Sex, M	138	26(18.8)	1(ref)		32	869.9	3.7(2.5-5.2)	1(ref)	
Sex, F	110	19(17.3)	0.94(0.48-1.83)	0.85	27	708.4	3.8(2.5-5.5)	2.08(0.52-8.35)	0.30
Age, 1-<10	187	36(19.3)	1(ref)		46	1091.9	4.2(3.1-5.6)	1(ref)	
Age, 10+	61	9(14.8)	0.43(0.18-1.04)	0.06	13	486.4	2.7(1.4-4.6)	1.03(0.25-4.27)	0.97
IMD, More affluent	133	24(18)	1(ref)		33	835.9	3.9(2.7-5.5)	1(ref)	
IMD, Less affluent	115	21(18.3)	0.97(0.50-1.88)	0.93	26	742.4	3.5(2.3-5.1)	0.60(0.14-2.53)	0.49
Regimen A	109	13(11.9)	1(ref)		13	332.6	3.9(2.1-6.7)	1(ref)	
Regimen B	59	12(20.3)	2.87(1.11-7.45)	0.03	17	343.1	5.0(2.9-7.9)	∞(0-∞)	0.91
Regimen C	80	20(25)	3.08(1.38-6.87)	0.01	29	902.6	3.2(2.2-4.6)	∞(0-∞)	0.91

Table E-8: Risk of having a fungal sample, and rate ratios if at least one fungal sample during consolidation.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	248	32(12.9)			74	1578.3	4.7(3.7-5.9)		
Sex, M	138	16(11.6)	1(ref)		31	869.9	3.6(2.4-5.1)	1(ref)	
Sex, F	110	16(14.5)	1.51(0.70-3.29)	0.30	43	708.4	6.1(4.4-8.2)	1.55(0.76-3.17)	0.23
Age, 1-<10	187	18(9.6)	1(ref)		43	1091.9	3.9(2.9-5.3)	1(ref)	
Age, 10+	61	14(23)	1.25(0.53-2.97)	0.61	31	486.4	6.4(4.3-9.0)	0.77(0.37-1.60)	0.48
IMD, More affluent	133	17(12.8)	1(ref)		41	835.9	4.9(3.5-6.7)	1(ref)	
IMD, Less affluent	115	15(13)	1.08(0.49-2.35)	0.85	33	742.4	4.4(3.1-6.2)	0.94(0.47-1.87)	0.85
Regimen A	109	4(3.7)	1(ref)		10	332.6	3.0(1.4-5.5)	1(ref)	
Regimen B	59	15(25.4)	8.22(2.31-29.3)	<0.01	30	343.1	8.7(5.9-12.5)	0.51(0.17-1.60)	0.25
Regimen C	80	13(16.2)	4.89(1.47-16.3)	0.01	34	902.6	3.8(2.6-5.3)	0.39(0.13-1.15)	0.09

Results are not presented for positive fungal samples since only six patients had 12 positive fungal samples during consolidation.

Table E-9: Risk of having a sample, and rate ratios if at least one sample during interim maintenance 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	247	124(50.2)			394	2149.4	18.3(16.6-20.2)		
Sex, M	137	77(56.2)	1(ref)		239	1186.6	20.1(17.7-22.9)	1(ref)	
Sex, F	110	47(42.7)	0.59(0.35-0.99)	0.05	155	962.9	16.1(13.7-18.8)	1.02(0.69-1.52)	0.91
Age, 1-<10	185	93(50.3)	1(ref)		298	1609.6	18.5(16.5-20.7)	1(ref)	
Age, 10+	62	31(50)	0.70(0.35-1.41)	0.32	96	539.9	17.8(14.4-21.7)	1.15(0.70-1.90)	0.58
IMD, More affluent	131	60(45.8)	1(ref)		174	1126	15.5(13.2-17.9)	1(ref)	
IMD, Less affluent	116	64(55.2)	1.40(0.84-2.34)	0.20	220	1023.4	21.5(18.7-24.5)	1.19(0.81-1.75)	0.38
Regimen A	108	46(42.6)	1(ref)		163	943.4	17.3(14.7-20.1)	1(ref)	
Regimen B	61	31(50.8)	1.65(0.78-3.49)	0.19	94	536.9	17.5(14.1-21.4)	0.77(0.45-1.31)	0.33
Regimen C	78	47(60.3)	2.19(1.15-4.18)	0.02	137	669.1	20.5(17.2-24.2)	0.80(0.49-1.29)	0.35
Positive Samples									
Total	247	59(23.9)			96	2149.4	4.5(3.6-5.5)		
Sex, M	137	33(24.1)	1(ref)		51	1186.6	4.3(3.2-5.7)	1(ref)	
Sex, F	110	26(23.6)	0.96(0.53-1.75)	0.89	45	962.9	4.7(3.4-6.3)	1.50(0.67-3.34)	0.32
Age, 1-<10	185	49(26.5)	1(ref)		76	1609.6	4.7(3.7-5.9)	1(ref)	
Age, 10+	62	10(16.1)	0.38(0.16-0.92)	0.03	20	539.9	3.7(2.3-5.7)	3.02(0.93-9.77)	0.06
IMD, More affluent	131	26(19.8)	1(ref)		43	1126	3.8(2.8-5.1)	1(ref)	
IMD, Less affluent	116	33(28.4)	1.57(0.86-2.85)	0.14	53	1023.4	5.2(3.9-6.8)	0.75(0.33-1.69)	0.49
Regimen A	108	25(23.1)	1(ref)		44	943.4	4.7(3.4-6.3)	1(ref)	
Regimen B	61	17(27.9)	2.10(0.91-4.85)	0.08	22	536.9	4.1(2.6-6.2)	0.23(0.06-0.89)	0.03
Regimen C	78	17(21.8)	1.16(0.55-2.41)	0.70	30	669.1	4.5(3.0-6.4)	0.72(0.26-2.01)	0.53

Table E-10: Risk of having a blood sample, and rate ratios if at least one blood sample during interim maintenance 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person -Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	247	108(43.7)			262	2149.4	12.2(10.8-13.8)		
Sex, M	137	66(48.2)	1(ref)		156	1186.6	13.1(11.2-15.4)	1(ref)	
Sex, F	110	42(38.2)	0.68(0.41-1.14)	0.14	106	962.9	11.0(9.0-13.3)	1.12(0.74-1.69)	0.59
Age, 1-<10	185	80(43.2)	1(ref)		196	1609.6	12.2(10.5-14.0)	1(ref)	
Age, 10+	62	28(45.2)	0.87(0.44-1.73)	0.69	66	539.9	12.2(9.5-15.6)	1.03(0.62-1.73)	0.90
IMD, More affluent	131	53(40.5)	1(ref)		116	1126	10.3(8.5-12.4)	1(ref)	
IMD, Less affluent	116	55(47.4)	1.29(0.77-2.16)	0.33	146	1023.4	14.3(12.0-16.8)	1.26(0.84-1.88)	0.26
Regimen A	108	41(38)	1(ref)		100	943.4	10.6(8.6-12.9)	1(ref)	
Regimen B	61	27(44.3)	1.37(0.65-2.89)	0.41	59	536.9	11.0(8.4-14.2)	0.85(0.48-1.52)	0.58
Regimen C	78	40(51.3)	1.73(0.92-3.27)	0.09	103	669.1	15.4(12.6-18.7)	1.22(0.75-1.97)	0.43
Positive Samples									
Total	247	27(10.9)			41	2149.4	1.9(1.4-2.6)		
Sex, M	137	15(10.9)	1(ref)		20	1186.6	1.7(1.0-2.6)	1(ref)	
Sex, F	110	12(10.9)	1.02(0.45-2.30)	0.97	21	962.9	2.2(1.4-3.3)	3.00(0.75-12.1)	0.12
Age, 1-<10	185	21(11.4)	1(ref)		31	1609.6	1.9(1.3-2.7)	1(ref)	
Age, 10+	62	6(9.7)	0.68(0.23-1.99)	0.48	10	539.9	1.9(0.9-3.4)	1.93(0.40-9.41)	0.42
IMD, More affluent	131	11(8.4)	1(ref)		18	1126.0	1.6(0.9-2.5)	1(ref)	
IMD, Less affluent	116	16(13.8)	1.66(0.73-3.77)	0.22	23	1023.4	2.2(1.4-3.4)	0.58(0.16-2.13)	0.41
Regimen A	108	9(8.3)	1(ref)		14	943.4	1.5(0.8-2.5)	1(ref)	
Regimen B	61	6(9.8)	1.48(0.44-4.94)	0.52	7	536.9	1.3(0.5-2.7)	0.22(0.02-3.00)	0.26
Regimen C	78	12(15.4)	2.19(0.83-5.75)	0.11	20	669.1	3.0(1.8-4.6)	1.07(0.24-4.72)	0.93

Table E-11: Risk of having a respiratory sample, and rate ratios if at least one respiratory sample during interim maintenance 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	247	63(25.5)			106	2149.4	4.9(4.0-6.0)		
Sex, M	137	36(26.3)	1(ref)		66	1186.6	5.6(4.3-7.1)	1(ref)	
Sex, F	110	27(24.5)	0.86(0.48-1.56)	0.63	40	962.9	4.2(3.0-5.7)	0.52(0.21-1.30)	0.16
Age, 1-<10	185	54(29.2)	1(ref)		89	1609.6	5.5(4.4-6.8)	1(ref)	
Age, 10+	62	9(14.5)	0.34(0.14-0.83)	0.02	17	539.9	3.1(1.8-5.0)	2.12(0.56-8.04)	0.27
IMD, More affluent	131	28(21.4)	1(ref)		48	1126	4.3(3.1-5.7)	1(ref)	
IMD, Less affluent	116	35(30.2)	1.58(0.88-2.84)	0.13	58	1023.4	5.7(4.3-7.3)	0.82(0.33-1.99)	0.65
Regimen A	108	31(28.7)	1(ref)		57	943.4	6.0(4.6-7.8)	1(ref)	
Regimen B	61	17(27.9)	1.65(0.72-3.77)	0.23	25	536.9	4.7(3.0-6.9)	0.38(0.11-1.22)	0.10
Regimen C	78	15(19.2)	0.74(0.36-1.55)	0.43	24	669.1	3.6(2.3-5.3)	0.43(0.13-1.45)	0.17
Positive Samples									
Total	247	40(16.2)			55	2149.4	2.6(1.9-3.3)		
Sex, M	137	22(16.1)	1(ref)		31	1186.6	2.6(1.8-3.7)	1(ref)	
Sex, F	110	18(16.4)	0.98(0.49-1.96)	0.95	24	962.9	2.5(1.6-3.7)	0.73(0.21-2.54)	0.62
Age, 1-<10	185	34(18.4)	1(ref)		45	1609.6	2.8(2.0-3.7)	1(ref)	
Age, 10+	62	6(9.7)	0.42(0.14-1.21)	0.11	10	539.9	1.9(0.9-3.4)	9.94(0.86-114)	0.07
IMD, More affluent	131	17(13)	1(ref)		25	1126	2.2(1.4-3.3)	1(ref)	
IMD, Less affluent	116	23(19.8)	1.66(0.83-3.31)	0.15	30	1023.4	2.9(2.0-4.2)	0.56(0.14-2.34)	0.43
Regimen A	108	20(18.5)	1(ref)		30	943.4	3.2(2.1-4.5)	1(ref)	
Regimen B	61	11(18)	1.49(0.58-3.83)	0.41	15	536.9	2.8(1.6-4.6)	0.17(0.02-1.58)	0.12
Regimen C	78	9(11.5)	0.69(0.29-1.67)	0.41	10	669.1	1.5(0.7-2.7)	0.05(0-1.13)	0.06

Table E-12: Risk of having a fungal sample, and rate ratios if at least one fungal sample during interim maintenance 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	247	23(9.3)			26	2149.4	1.2(0.8-1.8)		
Sex, M	137	15(10.9)	1(ref)		17	1186.6	1.4(0.8-2.3)	1(ref)	
Sex, F	110	8(7.3)	0.72(0.29-1.81)	0.49	9	962.9	0.9(0.4-1.8)	0.74(0.02-22.82)	0.86
Age, 1-<10	185	11(5.9)	1(ref)		13	1609.6	0.8(0.4-1.4)	1(ref)	
Age, 10+	62	12(19.4)	2.92(1.01-8.46)	0.05	13	539.9	2.4(1.3-4.1)	1.40(0.08-23.5)	0.82
IMD, More affluent	131	10(7.6)	1(ref)		10	1126	0.9(0.4-1.6)	1(ref)	
IMD, Less affluent	116	13(11.2)	1.63(0.67-3.98)	0.28	16	1023.4	1.6(0.9-2.5)	∞(0-∞)	-
Regimen A	108	5(4.6)	1(ref)		6	943.4	0.6(0.2-1.4)	1(ref)	
Regimen B	61	9(14.8)	1.77(0.44-7.04)	0.42	10	536.9	1.9(0.9-3.4)	0.69(0.02-29.7)	0.85
Regimen C	78	9(11.5)	1.66(0.47-5.85)	0.43	10	669.1	1.5(0.7-2.7)	0.92(0.01-75.4)	0.97

Results are not presented for positive fungal samples since no patients had any positive fungal samples during interim maintenance 1.

Table E-13: Risk of having a sample, and rate ratios if at least one sample during delayed intensification 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	242	161(66.5)			628	2072.4	30.3(28.0-32.8)		
Sex, M	133	89(66.9)	1(ref)		347	1137.6	30.5(27.4-33.9)	1(ref)	
Sex, F	109	72(66.1)	0.94(0.55-1.62)	0.83	281	934.9	30.1(26.6-33.8)	0.97(0.72-1.31)	0.84
Age, 1-<10	182	127(69.8)	1(ref)		520	1542.9	33.7(30.9-36.7)	1(ref)	
Age, 10+	60	34(56.7)	0.60(0.29-1.23)	0.16	108	529.6	20.4(16.7-24.6)	0.77(0.50-1.20)	0.25
IMD, More affluent	128	82(64.1)	1(ref)		300	1094.4	27.4(24.4-30.7)	1(ref)	
IMD, Less affluent	114	79(69.3)	1.23(0.72-2.12)	0.45	328	978.0	33.5(30.0-37.4)	1.18(0.87-1.60)	0.28
Regimen A	108	75(69.4)	1(ref)		327	870.7	37.6(33.6-41.9)	1(ref)	
Regimen B	57	34(59.6)	0.89(0.39-1.99)	0.77	127	474.3	26.8(22.3-31.9)	0.91(0.59-1.42)	0.69
Regimen C	77	52(67.5)	1.07(0.54-2.12)	0.85	174	727.4	23.9(20.5-27.8)	0.66(0.45-0.95)	0.02
Positive Samples									
Total	242	79(32.6)			139	2072.4	6.7(5.6-7.9)		
Sex, M	133	44(33.1)	1(ref)		84	1137.6	7.4(5.9-9.1)	1(ref)	
Sex, F	109	35(32.1)	0.91(0.53-1.58)	0.75	55	934.9	5.9(4.4-7.7)	0.64(0.27-1.50)	0.31
Age, 1-<10	182	68(37.4)	1(ref)		122	1542.9	7.9(6.6-9.4)	1(ref)	
Age, 10+	60	11(18.3)	0.37(0.16-0.85)	0.02	17	529.6	3.2(1.9-5.1)	0.50(0.11-2.27)	0.37
IMD, More affluent	128	40(31.2)	1(ref)		59	1094.4	5.4(4.1-7.0)	1(ref)	
IMD, Less affluent	114	39(34.2)	1.11(0.64-1.92)	0.71	80	978.0	8.2(6.5-10.2)	2.63(1.08-6.40)	0.03
Regimen A	108	40(37.0)	1(ref)		76	870.7	8.7(6.9-10.9)	1(ref)	
Regimen B	57	15(26.3)	1.02(0.45-2.33)	0.96	28	474.3	5.9(3.9-8.5)	1.53(0.43-5.43)	0.51
Regimen C	77	24(31.2)	0.99(0.51-1.92)	0.99	35	727.4	4.8(3.4-6.7)	0.58(0.21-1.61)	0.29

Table E-14: Risk of having a blood sample, and rate ratios if at least one blood sample during delayed intensification 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	242	158(65.3)			432	2072.4	20.8(18.9-22.9)		
Sex, M	133	88(66.2)	1(ref)		242	1137.6	21.3(18.7-24.1)	1(ref)	
Sex, F	109	70(64.2)	0.89(0.52-1.53)	0.68	190	934.9	20.3(17.5-23.4)	0.95(0.69-1.30)	0.74
Age, 1-<10	182	125(68.7)	1(ref)		362	1542.9	23.5(21.1-26.0)	1(ref)	
Age, 10+	60	33(55.0)	0.63(0.30-1.29)	0.20	70	529.6	13.2(10.3-16.7)	0.71(0.44-1.16)	0.17
IMD, More affluent	128	79(61.7)	1(ref)		209	1094.4	19.1(16.6-21.9)	1(ref)	
IMD, Less affluent	114	79(69.3)	1.37(0.80-2.36)	0.25	223	978.0	22.8(19.9-26.0)	1.06(0.77-1.46)	0.73
Regimen A	108	75(69.4)	1(ref)		232	870.7	26.6(23.3-30.3)	1(ref)	
Regimen B	57	33(57.9)	0.80(0.36-1.77)	0.58	72	474.3	15.2(11.9-19.1)	0.66(0.40-1.06)	0.09
Regimen C	77	50(64.9)	0.92(0.47-1.81)	0.81	128	727.4	17.6(14.7-20.9)	0.72(0.49-1.06)	0.09
Positive Samples									
Total	242	36(14.9)			47	2072.4	2.3(1.7-3.0)		
Sex, M	133	23(17.3)	1(ref)		30	1137.6	2.6(1.8-3.8)	1(ref)	
Sex, F	109	13(11.9)	0.60(0.28-1.27)	0.18	17	934.9	1.8(1.1-2.9)	1.14(0.34-3.88)	0.83
Age, 1-<10	182	31(17.0)	1(ref)		42	1542.9	2.7(2.0-3.7)	1(ref)	
Age, 10+	60	5(8.3)	0.75(0.23-2.40)	0.63	5	529.6	0.9(0.3-2.2)	0(0-∞)	0.77
IMD, More affluent	128	15(11.7)	1(ref)		18	1094.4	1.6(1.0-2.6)	1(ref)	
IMD, Less affluent	114	21(18.4)	1.70(0.82-3.54)	0.15	29	978.0	3(2.0-4.3)	1.47(0.40-5.42)	0.56
Regimen A	108	21(19.4)	1(ref)		29	870.7	3.3(2.2-4.8)	1(ref)	
Regimen B	57	3(5.3)	0.26(0.06-1.04)	0.06	3	474.3	0.6(0.1-1.8)	0(0-∞)	0.88
Regimen C	77	12(15.6)	0.77(0.33-1.81)	0.55	15	727.4	2.1(1.2-3.4)	0.83(0.23-3.02)	0.77

Table E-15: Risk of having a respiratory sample, and rate ratios if at least one respiratory sample during delayed intensification 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	242	79(32.6)			143	2072.4	6.9(5.8-8.1)		
Sex, M	133	43(32.3)	1(ref)		79	1137.6	6.9(5.5-8.7)	1(ref)	
Sex, F	109	36(33.0)	1.01(0.58-1.74)	0.99	64	934.9	6.8(5.3-8.7)	0.98(0.48-2.02)	0.96
Age, 1-<10	182	66(36.3)	1(ref)		115	1542.9	7.5(6.2-8.9)	1(ref)	
Age, 10+	60	13(21.7)	0.39(0.17-0.87)	0.02	28	529.6	5.3(3.5-7.6)	1.23(0.44-3.48)	0.69
IMD, More affluent	128	41(32.0)	1(ref)		69	1094.4	6.3(4.9-8.0)	1(ref)	
IMD, Less affluent	114	38(33.3)	1.04(0.60-1.79)	0.89	74	978.0	7.6(5.9-9.5)	1.60(0.76-3.37)	0.22
Regimen A	108	37(34.3)	1(ref)		64	870.7	7.4(5.7-9.4)	1(ref)	
Regimen B	57	19(33.3)	1.62(0.72-3.64)	0.24	42	474.3	8.9(6.4-12.0)	1.67(0.60-4.64)	0.33
Regimen C	77	23(29.9)	1.06(0.54-2.05)	0.87	37	727.4	5.1(3.6-7.0)	0.72(0.30-1.74)	0.46
Positive Samples									
Total	242	56(23.1)			88	2072.4	4.2(3.4-5.2)		
Sex, M	133	30(22.6)	1(ref)		51	1137.6	4.5(3.3-5.9)	1(ref)	
Sex, F	109	26(23.9)	1.03(0.56-1.90)	0.92	37	934.9	4.0(2.8-5.5)	0.69(0.29-1.67)	0.41
Age, 1-<10	182	50(27.5)	1(ref)		77	1542.9	5.0(3.9-6.2)	1(ref)	
Age, 10+	60	6(10.0)	0.22(0.08-0.62)	<0.01	11	529.6	2.1(1.0-3.7)	1.17(0.29-4.76)	0.83
IMD, More affluent	128	29(22.7)	1(ref)		40	1094.4	3.7(2.6-5.0)	1(ref)	
IMD, Less affluent	114	27(23.7)	1.03(0.56-1.90)	0.93	48	978.0	4.9(3.6-6.5)	1.98(0.83-4.73)	0.13
Regimen A	108	28(25.9)	1(ref)		44	870.7	5.1(3.7-6.8)	1(ref)	
Regimen B	57	13(22.8)	1.77(0.73-4.27)	0.20	24	474.3	5.1(3.2-7.5)	1.35(0.44-4.16)	0.60
Regimen C	77	15(19.5)	0.97(0.46-2.02)	0.93	20	727.4	2.7(1.7-4.2)	0.57(0.18-1.82)	0.34

Table E-16: Risk of having a fungal sample, and rate ratios if at least one fungal sample during delayed intensification 1.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	242	28(11.6)			53	2072.4	2.6(1.9-3.3)		
Sex, M	133	14(10.5)	1(ref)		26	1137.6	2.3(1.5-3.3)	1(ref)	
Sex, F	109	14(12.8)	1.22(0.55-2.70)	0.62	27	934.9	2.9(1.9-4.2)	0.89(0.39-2.00)	0.78
Age, 1-<10	182	23(12.6)	1(ref)		43	1542.9	2.8(2.0-3.8)	1(ref)	
Age, 10+	60	5(8.3)	0.81(0.24-2.70)	0.73	10	529.6	1.9(0.9-3.5)	1.37(0.34-5.50)	0.66
IMD, More affluent	128	12(9.4)	1(ref)		22	1094.4	2.0(1.3-3.0)	1(ref)	
IMD, Less affluent	114	16(14.0)	1.59(0.71-3.53)	0.26	31	978.0	3.2(2.2-4.5)	1.39(0.60-3.23)	0.44
Regimen A	108	15(13.9)	1(ref)		31	870.7	3.6(2.4-5.1)	1(ref)	
Regimen B	57	5(8.8)	0.69(0.20-2.40)	0.56	13	474.3	2.7(1.5-4.7)	1.07(0.33-3.41)	0.92
Regimen C	77	8(10.4)	0.75(0.28-2.00)	0.57	9	727.4	1.2(0.6-2.3)	0.11(0.01-0.87)	0.04

Results are not presented for positive fungal samples since only two patients had four positive fungal samples during delayed intensification 1.

Table E-17: Risk of having a sample, and rate ratios if at least one sample during maintenance.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	235	208(88.5)			2567	22913.3	11.2(10.8-11.6)		
Sex, M	132	120(90.9)	1(ref)		1786	15325.1	11.7(11.1-12.2)	1(ref)	
Sex, F	103	88(85.4)	0.61(0.27-1.38)	0.24	781	7588.2	10.3(9.6-11.0)	0.88(0.68-1.13)	0.32
Age, 1-<10	177	154(87.0)	1(ref)		2150	16998.2	12.6(12.1-13.2)	1(ref)	
Age, 10+	58	54(93.1)	1.04(0.28-3.95)	0.95	417	5915.1	7.0(6.4-7.8)	0.48(0.34-0.67)	<0.01
IMD, More affluent	122	109(89.3)	1(ref)		1232	11877.9	10.4(9.8-11.0)	1(ref)	
IMD, Less affluent	113	99(87.6)	0.85(0.37-1.92)	0.69	1335	11035.4	12.1(11.5-12.8)	1.15(0.89-1.47)	0.28
Regimen A	104	87(83.7)	1(ref)		1194	10019.8	11.9(11.3-12.6)	1(ref)	
Regimen B	58	55(94.8)	3.41(0.79-14.7)	0.10	618	5677.4	10.9(10-11.8)	1.12(0.79-1.60)	0.52
Regimen C	73	66(90.4)	1.79(0.64-5.01)	0.27	755	7216.1	10.5(9.7-11.2)	1.03(0.75-1.40)	0.86
Positive Samples									
Total	235	160(68.1)			687	22913.3	3.0(2.8-3.2)		
Sex, M	132	97(73.5)	1(ref)		471	15325.1	3.1(2.8-3.4)	1(ref)	
Sex, F	103	63(61.2)	0.57(0.32-0.99)	0.05	216	7588.2	2.8(2.5-3.3)	0.95(0.68-1.33)	0.77
Age, 1-<10	177	121(68.4)	1(ref)		580	16998.2	3.4(3.1-3.7)	1(ref)	
Age, 10+	58	39(67.2)	1.00(0.47-2.15)	1.00	107	5915.1	1.8(1.5-2.2)	0.39(0.25-0.61)	<0.01
IMD, More affluent	122	79(64.8)	1(ref)		320	11877.9	2.7(2.4-3.0)	1(ref)	
IMD, Less affluent	113	81(71.7)	1.37(0.78-2.40)	0.27	367	11035.4	3.3(3.0-3.7)	1.16(0.85-1.59)	0.36
Regimen A	104	71(68.3)	1(ref)		315	10019.8	3.1(2.8-3.5)	1(ref)	
Regimen B	58	38(65.5)	0.86(0.38-1.93)	0.71	152	5677.4	2.7(2.3-3.1)	1.23(0.79-1.91)	0.37
Regimen C	73	51(69.9)	1.03(0.51-2.07)	0.94	220	7216.1	3.0(2.7-3.5)	1.26(0.86-1.85)	0.23

Table E-18: Risk of having a blood sample, and rate ratios if at least one blood sample during maintenance.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	235	201(85.5)			1627	22913.3	7.1(6.8-7.5)		
Sex, M	132	118(89.4)	1(ref)		1135	15325.1	7.4(7.0-7.8)	1(ref)	
Sex, F	103	83(80.6)	0.49(0.23-1.04)	0.06	492	7588.2	6.5(5.9-7.1)	0.86(0.66-1.12)	0.26
Age, 1-<10	177	151(85.3)	1(ref)		1382	16998.2	8.1(7.7-8.6)	1(ref)	
Age, 10+	58	50(86.2)	0.74(0.26-2.12)	0.58	245	5915.1	4.1(3.6-4.7)	0.44(0.31-0.62)	<0.01
IMD, More affluent	122	107(87.7)	1(ref)		756	11877.9	6.4(5.9-6.8)	1(ref)	
IMD, Less affluent	113	94(83.2)	0.68(0.32-1.44)	0.32	871	11035.4	7.9(7.4-8.4)	1.30(1.01-1.68)	0.04
Regimen A	104	87(83.7)	1(ref)		769	10019.8	7.7(7.1-8.2)	1(ref)	
Regimen B	58	52(89.7)	1.91(0.59-6.22)	0.28	395	5677.4	7.0(6.3-7.7)	1.17(0.82-1.66)	0.39
Regimen C	73	62(84.9)	1.19(0.47-2.99)	0.71	463	7216.1	6.4(5.8-7.0)	1.04(0.76-1.42)	0.79
Positive Samples									
Total	235	83(35.3)			186	22913.3	0.8(0.7-0.9)		
Sex, M	132	56(42.4)	1(ref)		131	15325.1	0.9(0.7-1.0)	1(ref)	
Sex, F	103	27(26.2)	0.46(0.26-0.82)	0.01	55	7588.2	0.7(0.5-0.9)	1.16(0.53-2.51)	0.71
Age, 1-<10	177	68(38.4)	1(ref)		166	16998.2	1.0(0.8-1.1)	1(ref)	
Age, 10+	58	15(25.9)	0.43(0.20-0.94)	0.03	20	5915.1	0.3(0.2-0.5)	0.23(0.07-0.74)	0.01
IMD, More affluent	122	34(27.9)	1(ref)		73	11877.9	0.6(0.5-0.8)	1(ref)	
IMD, Less affluent	113	49(43.4)	1.95(1.11-3.41)	0.02	113	11035.4	1.0(0.8-1.2)	1.38(0.68-2.82)	0.38
Regimen A	104	34(32.7)	1(ref)		87	10019.8	0.9(0.7-1.1)	1(ref)	
Regimen B	58	18(31.0)	1.38(0.61-3.10)	0.44	41	5677.4	0.7(0.5-1.0)	0.99(0.41-2.40)	0.98
Regimen C	73	31(42.5)	1.85(0.94-3.66)	0.08	58	7216.1	0.8(0.6-1.0)	0.70(0.31-1.57)	0.39

Table E-19: Risk of having a respiratory sample, and rate ratios if at least one respiratory sample during maintenance.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate (95%CI)	RR(95%CI)	p-value
All Samples									
Total	235	167(71.1)			843	22913.3	3.7(3.4-3.9)		
Sex, M	132	99(75.0)	1(ref)		591	15325.1	3.9(3.6-4.2)	1(ref)	
Sex, F	103	68(66.0)	0.64(0.36-1.13)	0.13	252	7588.2	3.3(2.9-3.8)	0.86(0.62-1.21)	0.39
Age, 1-<10	177	128(72.3)	1(ref)		696	16998.2	4.1(3.8-4.4)	1(ref)	
Age, 10+	58	39(67.2)	0.82(0.38-1.77)	0.61	147	5915.1	2.5(2.1-2.9)	0.53(0.34-0.84)	0.01
IMD, More affluent	122	84(68.9)	1(ref)		433	11877.9	3.6(3.3-4.0)	1(ref)	
IMD, Less affluent	113	83(73.5)	1.23(0.69-2.17)	0.49	410	11035.4	3.7(3.4-4.1)	0.92(0.66-1.27)	0.60
Regimen A	104	75(72.1)	1(ref)		389	10019.8	3.9(3.5-4.3)	1(ref)	
Regimen B	58	39(67.2)	0.87(0.38-1.99)	0.74	187	5677.4	3.3(2.8-3.8)	1.22(0.76-1.96)	0.41
Regimen C	73	53(72.6)	1.06(0.51-2.18)	0.88	267	7216.1	3.7(3.3-4.2)	1.17(0.79-1.74)	0.43
Positive Samples									
Total	235	143(60.9)			498	22913.3	2.2(2.0-2.4)		
Sex, M	132	87(65.9)	1(ref)		339	15325.1	2.2(2.0-2.5)	1(ref)	
Sex, F	103	56(54.4)	0.61(0.36-1.03)	0.07	159	7588.2	2.1(1.8-2.4)	0.98(0.68-1.42)	0.92
Age, 1-<10	177	109(61.6)	1(ref)		412	16998.2	2.4(2.2-2.7)	1(ref)	
Age, 10+	58	34(58.6)	1.05(0.51-2.16)	0.90	86	5915.1	1.5(1.2-1.8)	0.43(0.26-0.71)	<0.01
IMD, More affluent	122	72(59.0)	1(ref)		247	11877.9	2.1(1.8-2.4)	1(ref)	
IMD, Less affluent	113	71(62.8)	1.17(0.69-1.99)	0.57	251	11035.4	2.3(2.0-2.6)	1.01(0.71-1.43)	0.96
Regimen A	104	66(63.5)	1(ref)		226	10019.8	2.3(2.0-2.6)	1(ref)	
Regimen B	58	32(55.2)	0.67(0.31-1.45)	0.31	110	5677.4	1.9(1.6-2.3)	1.39(0.84-2.29)	0.20
Regimen C	73	45(61.6)	0.88(0.45-1.71)	0.70	162	7216.1	2.2(1.9-2.6)	1.35(0.88-2.06)	0.16

Table E-20: Risk of having a fungal sample, and rate ratios if at least one fungal sample during maintenance.

	Persons	Sampled(%)	OR(95%CI)	p-value	Samples	Person-Weeks	Sample Rate(95%CI)	RR(95%CI)	p-value
All Samples									
Total	235	62(26.4)			97	22913.3	0.4(0.3-0.5)		
Sex, M	132	39(29.5)	1(ref)		60	15325.1	0.4(0.3-0.5)	1(ref)	
Sex, F	103	23(22.3)	0.69(0.38-1.26)	0.22	37	7588.2	0.5(0.3-0.7)	1.45(0.76-2.76)	0.26
Age, 1-<10	177	48(27.1)	1(ref)		72	16998.2	0.4(0.3-0.5)	1(ref)	
Age, 10+	58	14(24.1)	0.61(0.28-1.37)	0.23	25	5915.1	0.4(0.3-0.6)	1.46(0.69-3.12)	0.32
IMD, More affluent	122	30(24.6)	1(ref)		43	11877.9	0.4(0.3-0.5)	1(ref)	
IMD, Less affluent	113	32(28.3)	1.19(0.66-2.14)	0.57	54	11035.4	0.5(0.4-0.6)	1.57(0.83-2.99)	0.17
Regimen A	104	24(23.1)	1(ref)		36	10019.8	0.4(0.3-0.5)	1(ref)	
Regimen B	58	18(31.0)	1.92(0.83-4.42)	0.13	36	5677.4	0.6(0.4-0.9)	1.54(0.71-3.35)	0.27
Regimen C	73	20(27.4)	1.41(0.68-2.92)	0.35	25	7216.1	0.3(0.2-0.5)	0.46(0.16-1.36)	0.16

Results are not presented for positive fungal samples since only three patients had three positive fungal samples during maintenance.

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