Interim analysis of Acute Myeloid Leukaemia treated on the Red Cross Children's Hospital Rx 2071 (adapted from the MRC AML 15 protocol)

By

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DECLARATION

I, **Karla Mari Thomas** , hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 11 March 2017

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Abstract

Interim analysis of Acute Myeloid Leukaemia treated on the Red Cross Children's Hospital Rx 2071 (adapted from the MRC AML 15 protocol)

Background:

Due to the poor outcomes achieved in acute myeloid leukaemia (AML) treatment, the Red Cross War Memorial Children's Hospital (RCWMCH) Oncology service changed from a BFM-87 based protocol to one based on MRC-AML15 in 2007.

Rationale:

This study was designed to assess the outcomes and treatment – related toxicity among children treated with RCWMCH protocol Rx 2071.

Methods:

This was a retrospective review of AML patients treated with Rx2071 between 2007 and 2012 at RCWMCH. Patients with acute promyelocytic leukaemia (APL) and Down Syndrome were excluded. Risk was assigned by cytogenetics. Good risk patients were those with t(8;21), t(16,16) and inv(16). Poor and standard risk included all other cytogenetics according to MRC-AML15. Data pertaining to toxicity was obtained from patient folders.

Results:

Thirty five children were treated on Rx 2071 during the study period. Males comprised 51.4% (18/35) and females 48.6% (17/35). Age at diagnosis ranged from 0.33 to 12.51 years with the median being 5.68 years. Follow–up from remission in the patients who survived ranged from 1 year 10 months to 9 years 1 month with a median of 62.5 months. Fifteen patients had favourable cytogenetics. Event free survival (EFS) for the good risk group was 85.6%. Twenty patients presented with standard/poor risk cytogenetics. Five patients were deemed poor risk with one having major karyotype abnormalities and four not achieving remission. The remaining fifteen were deemed standard risk by cytogenetics. EFS in this group was 32.4%.

Two standard/poor risk patients were transplanted in first complete remission (CR1) and two patients were transplanted in second complete remission. (CR2)

Patients had a median of four neutropaenic fevers, and required a median of eight packed cell and eleven platelet transfusions. There were 39 positive blood cultures. Treatment related deaths were 8.57%.

Discussion:

The EFS for good risk patients is excellent but the EFS for standard/poor risk group is not on par with results being achieved in high income countries. The toxicity is not excessive on Rx2071. The results achieved on this protocol were superior to that of the previous BFM-based protocol.

Conclusion:

The results of this study support the continued use of Rx2071 at RCWMCH.

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List of Abbreviations

ADF	Alive disease free
ALL	Acute lymphoblastic leukaemia
Allo- HSCT	Allogeneic haematopoietic stem cell transplant
AML	Acute myeloid leukaemia
APL	Acute promyelocytic leukaemia
ATRA	All trans retinoic acid
AWD	Alive with disease
BFM	Berlin Frankfurt Munster
CCG	Children's Cancer Group
COG	Children's Oncology Group
CNS	Central nervous system
CR1	First complete remission
CR2	Second complete remission
CSF	Cerebral spinal fluid
DD	Died due to disease
DO	Died due to another cause
EFS	Event free survival
ELN	European Leukaemia Network
GSH	Groote Schuur Hospital
GvHD	Graft versus host disease
Hb	Haemoglobin
ITD	Internal tandem duplication

LAME	Leucémie Aiguë Myéloblastique Enfant
MAKA	Major karyotype aberrations
ml	Millilitre
MRI	Magnetic resonance imaging
MRC	Medical Research Council United Kingdom
MRD	Minimal residual disease
NHLS	National Health Laboratory Service
OS	Overall survival
PBC	Pediatric Blood and Cancer
POG	Paediatric Oncology Group
RCWMCH	Red Cross War Memorial Children's Hospital
SABMR	South African Bone Marrow Registry

Chapter 1:

Introduction:

1.1 Context:

Acute myeloid leukaemia (AML) constitutes 15 – 20% of all childhood and adolescent acute leukaemia. [1]

AML is a heterogonous family of neoplasms of the myeloid lineage. [1] The neoplastic cells in AML show an increased rate of self-renewal, aberrant differentiation and a decreased rate of auto destruction. [1]

The progress in treating AML has lagged behind that of acute lymphoblastic leukaemia (ALL). [2] Prior to 1970 the prognosis of children with AML was dire. [1, 3] A recent paper in the Journal of Clinical Oncology showed that five year overall survival (OS) rates in AML now range from 50% - 76%. [4] There has, thus been steady progress in the treatment of AML. [5,6]

The history of AML treatment in children is interesting. Prior to 1970 nearly all patients with AML died of their disease. [1] Anthracyclines and cytarabine were introduced alone and in combination in the 1970s and 1980s. [1] This lead to a minority of patients being cured. [1] The five year survival rate of children younger than fifteen with AML until the early 1990s was poor at 28%, [1] but there has been an improvement in the prognosis of AML over the last few decades. [5,7] National and international study groups were formed in the 1980s and 1990s. [4] Their goal was to improve the outcomes among children with cancer through cooperative research. [4] These study groups conducted various AML trials which intensified the doses of traditional chemotherapeutic agents. [1] The groups used similar approaches such as risk adapted treatment and the use of dose intense blocks with common drugs such as cytarabine, anthracyclines and etoposide. [7] There were, however, many differences among the study groups such as the number, designs and intensity of the treatment blocks, cumulative doses of certain drugs, the use of maintenance treatment, prophylaxis for central nervous system (CNS) relapse and the use of allogeneic haematopoietic stem cell transplantation (allo – HSCT). [7] A key factor in the improvement

of AML survival has been the advent of good supportive care which allows for dose intense treatment. [4,7]

The various AML studies have presented numerous questions which were highlighted by the late Robert Arceci in a review article. [1] These questions include: what is the optimal induction remission regimen?; [1] how many courses of chemotherapy are needed to cure AML?; [1] what is the optimal therapy to reduce CNS relapse?; [1] what is the role of allo - HSCT in first complete remission (CR1)? [1] and is there a role for maintenance therapy? [1] Various collaborative groups have different approaches to these questions. The Berlin Frankfurt Munster (BFM) group uses a one year maintenance block. [4,8] Despite the use of maintenance, the BFM results are similar to those groups that do not use maintenance therapy. [4] Two studies, LAME 91 and CCG 213 looked at the use of maintenance therapy in AML and found no benefit. [4,7,9] The new BFM AML trial, AML – BFM 2012 will randomly assign patients to maintenance and will assess the possibility that certain sub groups will benefit from maintenance. [4]

All studies have experienced success with intensifying doses but there is no consensus on how many courses of the chemotherapy are optimal. [1] Many of the study groups use five courses of chemotherapy in total with three courses being used for consolidation. [4] The United Kingdom's Medical Research Council (MRC) MRC AML 15 trial showed that four courses of chemotherapy were effective. [4,10] This was, however, shown primarily in adults. [4] There is no consensus on the optimal approach to central nervous system (CNS) prophylaxis in AML. [4]The BFM 87 trial showed that patients receiving cranial irradiation had a better relapse – free survival. [1,8] This was in contrast to many other studies such as the MRC, Paediatric Oncology Group (POG) and Children's Cancer Group (CCG) trials. These studies did not include cranial radiation and they had similar outcomes to the BFM 87 trial. [1,11,12] Most study groups are using intrathecal therapy as CNS prophylaxis and have moved away from cranial irradiation. [4]

The role of allo- HSCT in CR1 has led to debate among study groups. [4] Since the mid-1980s allo- HSCT has been recommended for patients with a matched sibling donor following induction chemotherapy. [11,13,14,15] There are no randomized studies comparing allo-HSCT to chemotherapy. [6] It is also not possible to do historical comparison of outcomes because of the differences in intensity of the chemotherapeutic agents used prior to

allo -HSCT. [4] Even though there is no consensus on the use of allo- HSCT, there is broad support among European and North American groups to use allo- HSCT in poor risk patients in CR1 [4] and there is general consensus among the various study groups that patients with acute promyelocytic leukaemia (APL), Down syndrome and those with t(8,21), inv(16) or t(16,16) do not need allo – HSCT in CR1. [13,16]

A recent review article in Journal of Clinical Oncology brought the issue of risk group stratification to light. [4] Cytogenetic patterns play a central role in risk stratification in AML. [4] There is general consensus among the study groups about the definition of high risk disease but groups differ in their definitions of low, standard and intermediate risk groups.[4] The following cytogenetic patterns are generally recognised by study groups across the world as markers of good risk disease: inversion 16; t(16;16) and t(8;21). [3,5,17] High risk cytogenetic patterns include the following: FLT3 mutations, monosomy 7, monosomy 5, abnormal 3q and complex karyotype abnormalities. [4,6,7] The European Leukaemia Network (ELN) calls for testing of FLT3 internal tandem duplication (ITD), CEPBA and NPM1 to further risk stratify.[18] The ELN deems CEPBA to be good risk whilst patients with FLT3ITD and wild type NPM1 mutations are classified as intermediate risk.[18] A bone marrow with >15% blasts after the first induction course is also deemed as poor risk on the MRC AML trials. [11] An international expert panel published recommendations on the diagnosis and management of AML.[19] They state that minimal residual disease (MRD) can be monitored and that though some study groups use MRD as part of risk stratification, its clinical benefit is still being investigated. [19]

Another factor contributing to the improved overall survival is better salvage after relapse. [20] The BFM group looked at a cohort of 379 patients with relapsed AML and found that a third can be salvaged. They recommend HSCT in CR2. [20]

Robert Arceci suggests that the ideal remission – reduction regimen is one that effectively results in cytoreduction without significant toxicity to the patient and one which has a positive impact on post remission treatment. [1] AML Studies in the past have shown relatively high mortality related to the leukaemia and treatment complications. [4,21] Patients with AML are at risk of invasive fungal infections, and viridans streptococcal and gram negative bacteraemia. [4,22] AML patients with hyperleukocytosis (white blood cell count > 100 x 10^9/L) are at increased risk of pulmonary leukostasis, CNS ischaemia and haemorrhage and early death as a result. [4]

The Red Cross War Memorial Hospital Children's Hospital (RCWMCH) AML treatment protocol, Rx 2941, from 1994 – 2007 was adapted from the BFM- 87 protocol. [23] The outcome for the standard/poor risk patients was poor with EFS of 27.3%. [23]The EFS for good risk patients was 74.4%. [23] The toxicity related death rate with Rx 2941 was 9%. [23] This resulted in a change in the RCWMCH AML treatment protocol and in 2007 an MRC – AML 15 based protocol, Rx 2071 was adopted.

The RCWMCH AML protocol Rx2071 has been used since 2007. This study was designed to assess the outcomes and treatment – related toxicity among children treated with RCWMCH protocol Rx 2071. This study will serve as a base to decide whether or not to continue to treat children with AML on Rx 2071. It will also allow us to identify any shortcomings of this current protocol.

1.2 Ethical Considerations:

This is a retrospective study therefore informed consent from each patient is thought to be unnecessary. The names of the patients will not be published or used in any presentations. This study implemented the ethical principle of beneficence and non-maleficence. The results of this study will benefit future patients with AML because it will determine whether or not the current treatment protocol is an improvement on the old regime based on event free survival. There were no adverse effects on any subjects due to this study.

This study will serve as the mini dissertation for an MPhil project and will be put forward for publication in a peer review journal.

Ethics approval was obtained from the University of Cape Town's Faculty of Health Science's Human Research Ethics Committee. The ethics reference number is HREC REF: 235/2012. (Appendix 2)

1.3 Journal for Publication

Pediatric Blood and Cancer (PBC) is the journal selected for publication. PBC has an impact factor of 2.634. PBC has a wide readership and publishes relevant research articles pertaining to childhood oncological and haematological topics. PBC is listed on the South African

Department of Higher Education's list of accredited journals. The publication – ready manuscript has been formatted according to the PBC author guidelines. (Appendix 4)

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Chapter 2: Publication ready manuscript

Acute Myeloid Leukaemia (AML) treated on the Red Cross War Memorial Children's Hospital (RCWMCH) Treatment protocol Rx 2071 (adapted from MRC-AML15)

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Abbreviations	
ADF	Alive disease free
ALL	Acute lymphoblastic leukaemia
Allo- HSCT	Allogeneic haematopoietic stem cell transplant
AML	Acute myeloid leukaemia
APL	Acute promyelocytic leukaemia
ATRA	All trans retinoic acid
AWD	Alive with disease
BFM	Berlin Frankfurt Munster
CCG	Children's Cancer Group
COG	Children's Oncology Group
CNS	Central nervous system
CR1	First complete remission
CR2	Second complete remission
CSF	Cerebral spinal fluid
DD	Died due to disease
DO	Died due to another cause

EFS	Event free survival
GSH	Groote Schuur Hospital
GvHD	Graft versus host disease
Hb	Haemoglobin
LAME	Leucémie Aiguë Myéloblastique Enfant
MAKA	Major karyotype aberrations
ml	Millilitre
MRI	Magnetic resonance imaging
MRC	Medical Research Council United Kingdom
NHLS	National Health Laboratory Service
OS	Overall survival
POG	Paediatric Oncology Group
RCWMCH	Red Cross War Memorial Children's Hospital
SABMR	South African Bone Marrow Registry

Abstract

Acute myeloid leukaemia (AML) treated on the Red Cross War Memorial Children's Hospital (RCWMCH) Treatment protocol Rx 2071 (adapted from MRC-AML15)

Background:

The Red Cross War Memorial Children's Hospital (RCWMCH) Oncology service changed from a BFM-87 based protocol to one based on MRC-AML15 in 2007 due to the poor outcomes achieved for patients with standard and poor risk acute myeloid leukaemia (AML).

This study was designed to assess the interim outcomes and treatment – related toxicity among children treated with RCWMCH protocol Rx 2071.

Procedure:

This was a retrospective review of AML patients treated with Rx2071 between 2007 and 2012 at RCWMCH. Patients with acute promyelocytic leukaemia (APL) and Down syndrome were excluded. Risk was assigned by cytogenetics. Good risk patients were those with t(8;21) and inv(16). Poor/standard risk included all other cytogenetics according to MRC-AML15.

Results:

Thirty five children were treated on Rx 2071 during the study period. Males comprised 51.4% (18/35) and females 48.6% (17/35). Age at diagnosis ranged from 0.33 to 12.51 years with the median being 5.68 years. Follow-up from remission in the alive patients ranged from 1 year 10 months to 9 years 1 month with a median of 62.5 months. Fifteen patients had favourable cytogenetics. EFS for the good risk group was 85.6%.Twenty patients presented with standard/poor risk cytogenetics. EFS in this group was 32.4%. Patients had a

median of four neutropaenic fevers, and required a median of eight packed cell and 11 platelet transfusions. There were 39 positive blood cultures.

Treatment related deaths were 8.57%.

Conclusion:

Results are superior to those achieved with the previous regime and the toxicity is not excessive.

Introduction

Acute myeloid leukaemia (AML) accounts for approximately 15 – 20% of acute leukaemia in children and adolescents. [1] The progress in treating AML has lagged behind that of acute lymphoblastic leukaemia (ALL). [2] Prior to 1970 nearly all patients with AML succumbed to their disease. [1,3]

The Red Cross War Memorial Children's Hospital (RCWMCH) Oncology Service had poor results in treating AML and this prompted a change in protocol in 1994. A Berlin Frankfurt Munster (BFM-87) based protocol, Rx 2941 was introduced. The outcome for the standard/ poor risk patients was an event free survival (EFS) of 27.3%. [4] The EFS for good risk patients was 74.4%. This protocol also resulted in 9% of the patients succumbing to toxicity related deaths. [4] As a result in 2007, United Kingdom's Medical Research Council (MRC) AML 15 based protocol, Rx 2071, was adopted.

Methods

This was a retrospective analysis of all patients with AML treated on Rx 2071 at RCWMCH between 2007 and 2012. Patients with acute promyelocytic leukaemia (APL) and Down syndrome were excluded from the analysis because they were treated on different protocols. Patients with APL received Rx2091 which includes all trans retinoic acid (ATRA) in induction and 6 – mercaptopurine and methotrexate in maintenance.

RCWMCH is an academic referral hospital attached to the University of Cape Town. The oncology unit treats children from the Eastern and Western Cape provinces. The hospital is a state funded institution and about 75% of the patients attending the oncology unit do not have medical insurance. The oncology unit sees 130 new patients each year. There are three full time oncologists, a reliable supply of chemotherapeutic agents, blood products and broad-

spectrum antibiotics as well as access to comprehensive support services including radiology with magnetic resonance imaging (MRI) capability, intensive care, cardiology, nephrology and dietetics. Patients who need allogeneic haematopoietic stem cell transplant (allo-HSCT) are sent to the transplant unit at Groote Schuur Hospital (3.8km away), which services adults and children.

Risk was assigned by cytogenetics. Good risk patients were those with t(8; 21), inv(16) and t(16;16). The following cytogenetic patterns were deemed to be poor risk: monosomy 5 or 7 del(5q), abn(3q),t(9;22) and major karyotype aberrations (>5 abnormalities). Standard risk cytogenetics consisted of cytogenetic patterns which were neither good nor poor risk. Patients who had more than 15% blasts on their day 28 bone marrow biopsies were also deemed poor risk. The risk assignment was as per the MRC – AML 15. [5, 6]

Rx2071 comprises of two ADE induction blocks of cytarabine (100mg/m^2 per dose), daunorubicin (50mg/m^2 x 3) and etoposide (100mg/m^2 x 5). ADE 1 has 20 doses of cytarabine while ADE 2 has 16 doses. A bone marrow aspirate and trephine is performed on day 28 of ADE 1. A patient is deemed poor risk if there are >15% blasts on the day 28 marrow irrespective of cytogenetics. The two consolidation blocks consist of high dose cytarabine. (3g/m^2 x 6) Patients who are in the standard/poor risk group are tissue typed and proceed to allo – HSCT after ADE 2 if a donor is available. If a sibling donor is not available, a donor is sought for on the local registry (or the international registry for insured patients). Mitoxantrone and intermediate dose cytarabine (1g/m^2) are reserved for poor risk patients with no donor option. (Figure 1) The cumulative anthracycline dose for the standard arm is 300mg/m^2. Triple intrathecal therapy (methotrexate, cytarabine and hydrocortisone) is given on day one of both ADE blocks. In the event of blasts in the diagnostic cerebral spinal fluid (CSF) triple intrathecal therapy is given twice weekly until the CSF is clear. An additional

two are given after the CSF has cleared and intrathecal therapy is administered monthly until the end of chemotherapy.

Neutropaenic fever in our unit was defined as two axillary temperatures of >/= 37.5 degrees Celsius on two occasions or one axillary temp of >/= 38 degrees Celsius in a patient with an absolute neutrophil count of less than 1000 per microlitre. This differs from international guidelines. [7] This policy has been consistent during the use of Rx2071 and Rx2941. We have instituted this policy to ensure that the vulnerable children in our setting are picked up early and started on appropriate anti microbials. An episode of prolonged neutropaenic fever was defined as one lasting for more than seven days.[8] Blood cultures were obtained in an aseptic manner in patients with neutropeanic fever. The blood cultures were sent to the National Health Laboratory Service (NHLS). Here they were processed and analysed using the BACTEC^{TM} 9240 automated blood culture system. Patients with febrile neutropaenia were started on empiric antibiotics (pipericillin/tazobactam and amikacin.)

Patients with AML all received co- trimaxazole as pneumocystis prohylaxis. Patients with prolonged neutropaenia were started on prophylactic oral fluconazole. This has been the standard of care in our unit during the use of both Rx2941 and 2071.

Blood products for transfusion were obtained from the Western Province Blood Transfusion Service. Packed red cell transfusions were given to patients with symptomatic anaemia or those with a haemoglobin (Hb) of seven grams per decilitre or less. Leucodepleted red cells were reserved for patients who were due to undergo stem cell transplantation. Platelet transfusions were given to patients who were actively bleeding or to those with platelets of 50 000/mm^3 in the face of severe mucositis or 20 000 /mm^3 and falling after chemotherapy. Apheresis single donor units of platelets were given to patients who reacted to pooled

platelets and those who were to undergo stem cell transplantation. The volumes of these units are approximately 275 millilitres (ml). Those patients not due for stem cell transplant received pooled units of platelets. One pooled unit of platelets is approximately 200ml.

Current outcomes were defined as follows: alive disease free (ADF), alive with disease (AWD), died due to disease (DD) and died due to another cause (DO).

Post remission follow up was defined as the time elapsed from first complete remission (CR1) to the latest follow up date or date of death.

Complete remission was defined as a bone marrow with <5 % blasts morphologically.

Data was obtained from patient folders and entered into a *Microsoft Access*™ database. Descriptive analyses were performed using *Excel*™. EFS was calculated with *Statistica*™. Kaplan – Meier curves were used to determine the EFS. EFS was calculated from the point of diagnosis to the date of an event (relapse or death) or the last follow up date. The standard/poor risk and good risk groups were compared using the log – rank test. A p value of 0.05 was regarded as statistically significant.

Ethics approval was obtained from the Human Research and Ethics committee at the University of Cape Town. The ethics reference number is HREC REF: 235/2012.

Results

Thirty five children were treated on Rx 2071 from 2007 to 2012. Males comprised 51.4% (18/35) and females 48.6% (17/35). Ages ranged from 0.33 to 12.51 years with the median age being 5.68 years.

Fifteen patients were in the good risk cytogenetics group with 14 having t(8;21) and one with inv(16). Twenty patients were in the standard/poor risk group. Nineteen patients had standard

risk cytogenetics. One patient with major karyotype aberrations (MAKA) was poor risk. Four patients did not achieve remission, one of whom presented with intracranial haemorrhage and died early in induction. Thirty one patients achieved CR1 (good risk: n= 15; standard/poor risk: n= 16). Two of the standard/poor risk patients were transplanted in CR1: one demised due to graft-versus-host-disease (GvHD). Ten patients relapsed (good risk: n= 2; standard/poor risk: n=8). Two patients received matched sibling allo – HSCT in CR2 (good risk: n= 1; standard/poor risk: n=1): one demised due to delayed onset acute GvHD. Twenty one patients are currently alive and disease free. (Figure 2)

Overall EFS was 54 %. (Figure 3) EFS for the good risk group was 85.6%. EFS for the standard/poor risk group was 32.4 %. (Figure 4) This difference was statistically significant (p value 0.003). (Figure 4)

Follow–up from remission in the patients who survived ranged from 1 year 10 months to 9 years 1 month with a median of 62.5 months.

Standard and poor risk patients are tissue typed and proceed to allo – HSCT after ADE2 if a donor is available. The donor pool for our patients was small. We had access to matched siblings and a limited number of matched unrelated donors on the South African Bone Marrow Registry (SABMR), which currently has 73000 registered donors but does not adequately represent the ethnic diversity of our patient population. [9] Only those patients with health insurance had access to international donors and thus many patients who qualify for allo – HSCT cannot access a transplant. [9]

Two of the twenty standard/poor risk patients eligible for allo – SCT were transplanted in CR1. One had a matched sibling allo – HSCT, relapsed and received a second allo – HSCT from the same donor in CR2. This patient is currently alive and disease free. The other patient received an unrelated cord allo – HSCT and died due to GvHD.

Two standard/poor risk patients had matched sibling donors but were not transplanted in CR1. One of these patients had two matched sibling donor options but the family refused the allo – HSCT and this patient is well and disease free. The other patient had one matched sibling donor but never achieved CR1. The remaining 16 standard/poor risk patients had no sibling donor options; three of them failed to achieve CR1, six remain in CR1 and seven relapsed.

Two patients were transplanted in CR2. One was a good risk patient who received a matched sibling allo – HSCT. This patient demised due to late onset acute GvHD. The other was the standard/poor risk patient who had received a matched sibling allo – HSCT in CR1. Eight patients who relapsed did not receive an allo – HSCT. Two had potential donors on accessible registries but never achieved CR2. The remaining six patients had no donor options and were palliated.

There were 135 episodes of neutropaenic fever with a median of four episodes per patient and 67.4% (91/135) met the case definition for prolonged neutropaenic fever while 32.6% (44/135) lasted fewer than seven days. There was a minimum of two episodes of neutropaenic fever per patient, excluding one patient who died early in induction. There were 32 positive bacterial blood cultures with a range of 0 to 4 positive cultures per patient. Gram positive organisms were most frequently cultured with *Staphylococcal* species accounting for ten cultures, *Streptococcal* species accounting for eight and *Enterococci* for one. Only two of

the streptococcal infections occurred after high dose cytarabine. Thirteen cultures grew gram negative bacilli, with one patient requiring inotropes. There were seven positive fungal blood cultures which were successfully treated with systemic anti fungals. One patient had mucor of the appendix and survived after complete resection of the affected area and systemic amphotericin B. It is standard of care in our unit for patients with AML to have central venous access devices but this study did not look at whether the positive blood cultures were from peripheral or central samples. Other recorded infections included two urinary tract infections, one liver abscess, one giardia gastroenteritis, one scalp abscess and one thrombophlebitis.

There was a mean of 7.8 and a median of eight packed cell transfusions per patient, with a range of 1 to 15. There was a mean of 11.3 and a median of 11 platelet transfusions per patient, with a range of 3 to 19.

There were no cases of cardiomyopathy.

There were 3 treatment related deaths. One patient presented with intracranial haemorrhage as a complication of disease prior to starting chemotherapy and died early in induction. Two patients succomed to GvHD post allo –HSCT. The treatment related toxicity was 8.57%.

Discussion

The progress in treating AML has not kept pace with developments in ALL. [2] A recent report demonstrated that five year overall survival (OS) rates in AML range from 50% - 76%. [10] There has, however, been steady progress leading to improved survival. [3,11,12,13] Some of the key factors contributing to the improved survival are the addition of cytarabine

and anthracyclines to chemotherapy regimens, [1,3] the formation of collaborative study groups, [10] improvements in supportive care [3,10,13] and the use of stem cell transplantation. [3,14,15]

Prior to 2007 patients with AML at RCWMCH were treated on a modified BFM-87 protocol, Rx 2941. This protocol consisted of four blocks of intense chemotherapy (induction, consolidation and two intensification blocks) as well as one year of maintenance therapy with thioguanine and cytarabine. The induction block of ADE was followed by a consolidation block consisting of six weeks using six drugs. The two intensification blocks consisted of high dose cytarabine and etoposide. CNS prophylaxis was given in the form of methotrexate intrathecal therapy during induction and cytarabine intrathecal therapy during consolidation. (Figure 5) Rx2071 differs in that it only has four blocks of chemotherapy. The consolidation blocks do not include etoposide but use high doses of cytarabine. Rx 2071 uses anthracyclines and cytarabine as its backbone and these drugs have been attributed to the improved survival in AML. [1,3] The total treatment time on Rx2071 is considerably shorter and this impacts positively on patient experience and workload without detracting from survival. Rx2071 also uses triple intrathecal therapy which may confer better CNS coverage.

The EFS for good risk patients was 85.6% on Rx2071 as compared to an EFS of 74.4% on Rx2941 (p- value 0.25). The EFS of the standard/poor risk group was 32.4% compared to 27.3% on Rx2941 (p-value 0.4) (Table 1) Rx2071 did not have excessive toxicity.(Table 1) The EFS for good risk patients is excellent but the EFS for the standard/poor risk group are not on par with results being achieved in high income countries. [10]

As mentioned before, international experience suggests that stem cell transplant has positively impacted on survival. [14,15] The role of allo- HSCT in CR1 has led to debate among various study groups. [9,16] Since the mid-eighties allo- HSCT has been

recommended for patients with matched sibling donor following induction chemotherapy, [16,17,18,19,20] but there are no randomized clinical trials comparing allo– HSCT to chemotherapy. [12,16] There is general consensus among the various study groups that patients with APL, Down syndrome and those with t(8,21), inv(16) or t(16,16) do not need allo- HSCT in CR1 [15,16] and that poor risk patients do need allo– HSCT in first remission. [9,21]

Our limited experience with transplantation in this group of patients prevents us from drawing any absolute conclusions about the benefits of this salvage strategy but we would still pursue transplantation in end-induction unremitted patients or those with standard or poor risk cytogenetics who have a suitable donor, as this likely offers the only chance of survival for these patients. The relative paucity of adequate local donor genotypes on the SABMR [9] and the fact that only those patients with health insurance have access to donors on international registries has resulted in very few of our patients being transplanted. A similar phenomenon emerged when looking at a historical cohort of patients who were treated on the previous BFM based protocol at RCWMCH. Of the 45 patients considered for allo – HSCT on Rx2941, only three were transplanted. [4]

The toxicity related deaths in Rx2071 were 8.57% . Only one of these deaths were in induction and this patient had presented with an intra cranial haemorrhage. Two patients died due to GvHD post transplant and these were thus not attributed directly to the chemotherapy on Rx2071. Toxicity related deaths accounted for 9% of the 78 patients treated on the previous BFM based protocol at RWMCH between 1994 and 2005. [4] These deaths were due to gram negative sepsis (n=3),fatal haemorrhages (n=2), treatment related myelodysplasia (n=1) and cardiomyopathy (n=1). AML studies in the past have shown relatively high mortality related to the leukaemia and treatment complications. [10,22,23,24] The Dutch Childhood Oncology Group found that they had higher treatment related mortality

with an increased intensity of their chemotherapeutic regime on their AML 92/94 protocol. [23] Patients with AML are at risk of invasive fungal infections, viridans streptococcal [25] and gram negative bacteraemias. [10,26] AML patients with hyperleukocytosis (white blood cell count > 100 x 109/L) are at increased risk of pulmonary leucocytosis, CNS ischaemia and haemorrhage resulting in early death. [10] Our study found that gram positive organisms were most commonly cultured . This is in keeping with other studies. [25,27,28,29] None of our patients succumbed to infection. Treatment related toxicity on Rx2071 was deemed acceptable given our supportive care architecture.

Good supportive care is essential in treating patients with intense chemotherapy regimens. [30,31] Supportive care at RCWMCH has improved over the years with more nursing staff being oncology trained. There is also comprehensive multi –disciplinary supportive services. We have fungal and antimicrobial prophylaxis policies and strict protocols for neutropaenic fever. This has contributed in decreasing toxic deaths.

The late Robert Arceci suggested that the ideal remission – reduction regimen is one that effectively results in cytoreduction without significant toxicity to the patient and has a positive impact on post remission treatment. [1] This treatment protocol goes some way to achieving this objective although room for improvement still exists in standard and poor risk patients.

Conflicts of interest:

There are no conflicts of interest to declare.

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TABLE 1 Event free survival (EFS) and toxicity differences between BFM 87 – based Rx 2941 and MRC AML-15 based Rx 2071

EFS/Toxicity	**BFM 87- based protocol**	**RCWMCH Rx2071**
EFS: good risk cytogenetics	74.4% (n= 29/37)	85.6% (n= 13/15)
EFS: standard/poor risk cytogenetics	27.3% (n= 13/41)	32.4% (n= 7/20)
Neutropeanic fevers	Mean = 3.3 Median = 3	Mean = 3.9 Median = 4
Packed cell transfusions	Mean = 8.2 Median = 7	Mean = 7.8 Median = 8
Platelet transfusions	Mean = 8.9 Median = 8	Mean = 11.3 Median = 11
Toxicity related deaths	9% (n= 7/78)	8.57% (n=3/35)

BFM = Berlin Frankfurt Munster; EFS = event free survival; MRC =Medical Research Council United Kingdom; RCWMCH = Red Cross War Memorial Children's Hospital

RX 2071 RED CROSS CHILDREN'S HOSPITAL ONCOLOGY SERVICE

ACUTE MYELOID LEUKAEMIA (2007)

(excluding Acute Promyelocytic Leukaemia)

Protocol based on MRC AML15

Good Risk: t(8;21) inv(16) t(16;16)

Standard Risk: all patients that are neither good nor poor risk with BM < 15% blasts after ADE 1

Poor Risk: monosomy 5 or 7 del(5q) abn(3q) t(9;22) MAKA(>5abn) **or** BM > 15% blasts after ADE 1

All but good risk cytogenetics should be considered for MRD BMT in 1st remission

IT Therapy: Methotrexate 1yr 5mg / 1-2yr 7.5mg / 2-3yr 10mg / >3yr 12.5mg
Cytarabine 1yr 15mg / 1-2yr 20mg / 2-3yr 25mg / >3yr 30mg
Hydrocortisone 1yr 5mg / 1-2yr 7.5mg / 2-3yr 10mg / >3yr 12.5mg

Cotrimoxazole prophylaxis 80 mg TMP/m^2 po BD three days weekly [0-6m: 2.5ml / 6m-6y: 5ml / >6y: 10ml]

1 INDUCTION

ADE 1

Day 1-10	Ara C 100 mg/m^2 over 30 minutes 12 hourly [20]
Day 1,3,5	Daunorubicin 50 mg/m^2 IV over 1 hour [3]
Day 1-5	Etoposide 100 mg/m^2 IV over 2 hours daily [5]
Day 1	Triple IT (doses above)

Bone Marrow at day 28 or when ANC > 0.5; Tissue typing after remission if full siblings

ADE 2 Requires ANC > 1 and Platelets > 100 at Day 1

Day 1-8	Ara C 100 mg/m^2 over 30 minutes 12 hourly [16]
Day 1,3,5	Daunorubicin 50 mg/m^2 IV over 1 hour [3]
Day 1-5	Etoposide 100 mg/m^2 IV over 2 hours daily [5]
Day 1	Triple IT (doses above)

Bone Marrow after ADE 2 if > 15% blasts after ADE 1

2 **CONSOLIDATION**

HDAC Requires ANC > 1 and Platelets > 100 at Day 1

Day 1,3,5 AraC 3 g/m^2 IV over 3 hours 12 hourly [6] (start pm)

prednisone eye drops 3 hourly for 6 days and then 6 hourly for a further 4 days

HDAC 2 Requires ANC > 1 and Platelets > 100 at Day 1

Day 1,3,5 AraC 3 g/m^2 IV over 3 hours 12 hourly [6] (start pm)

prednisone eye drops 3 hourly for 6 days and then 6 hourly for a further 4

For **Central nervous system disease** (>5 cells per hpf with blasts on cytospin):
2x weekly IT therapy until CSF clear plus two (minimum of 6) then monthly IT until the end of chemotherapy
18 Gy of cranial radiotherapy

For **poor risk AML with no matched donor** substitute HDAC 2 with MidAc

MidAc Requires ANC > 1 and Platelets > 100 at Day 1; Requires normal cardiac function on echocardiogram

Day 1-5 Mitoxantrone 10mg/m^2 IV over 1 hour daily [5]

Day 1-3 AraC 1g/m^2 IV over 2 hours 12hourly [6]

prednisone eyedrops 3 hourly for 5 days and then 6 hourly for further 5 days

FIGURE 1 MRC AML 15- based Rx2071 treatment protocol

ADE= cytarabine, daunorubicin,etoposide;ANC= absolute neutrophil count; CSF =cerebral spinal fluid; HDAC= high dose cytarabine; IT= Intrathecal; MidAc= mitoxantrone and cytarabine; MRC= Medical Research Council United Kingdom

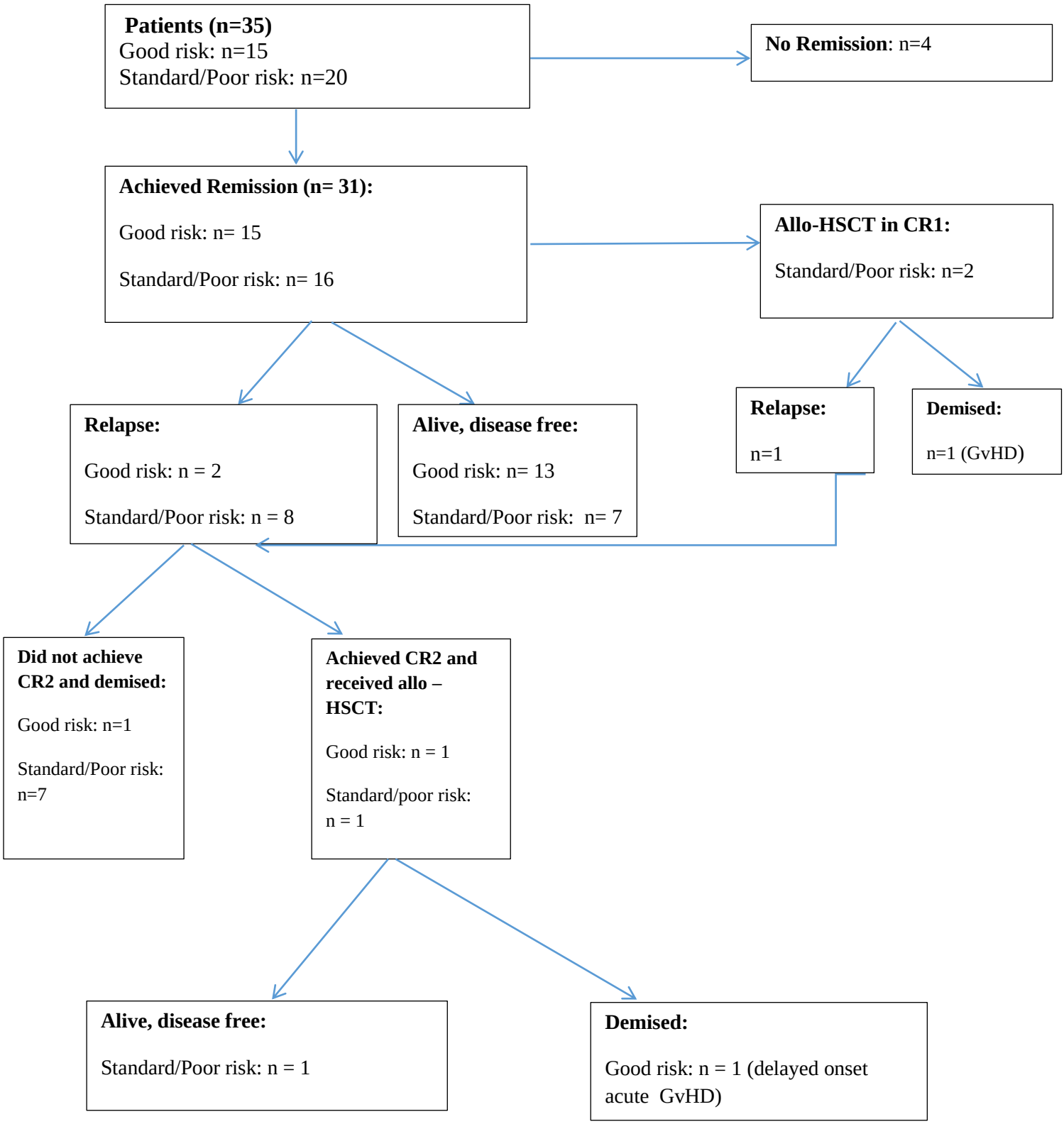


FIGURE 2 Consort diagram of Acute myeloid leukaemia (AML) patients treated on Rx2071 between 2007 and 2012
CR1= first complete remission; CR2 = second complete remission;GvHD = graft-versus-host-disease; n= number of patients

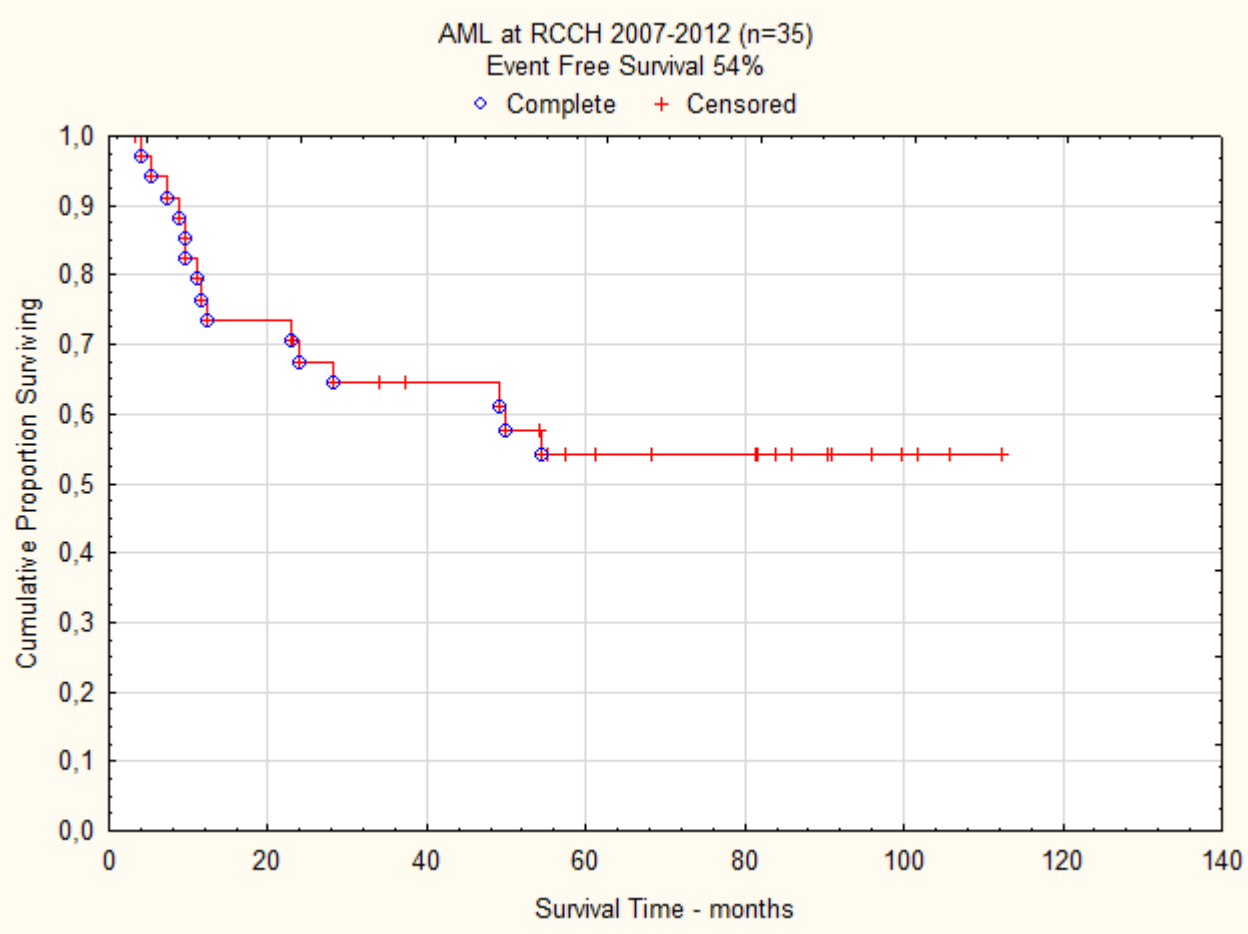


FIGURE 3 Event free survival of all patients treated on Rx2071 between 2007 and 2012
AML= acute myeloid leukaemia; EFS= event free survival; n= number of patients; RCCH= Red Cross Children's Hospital

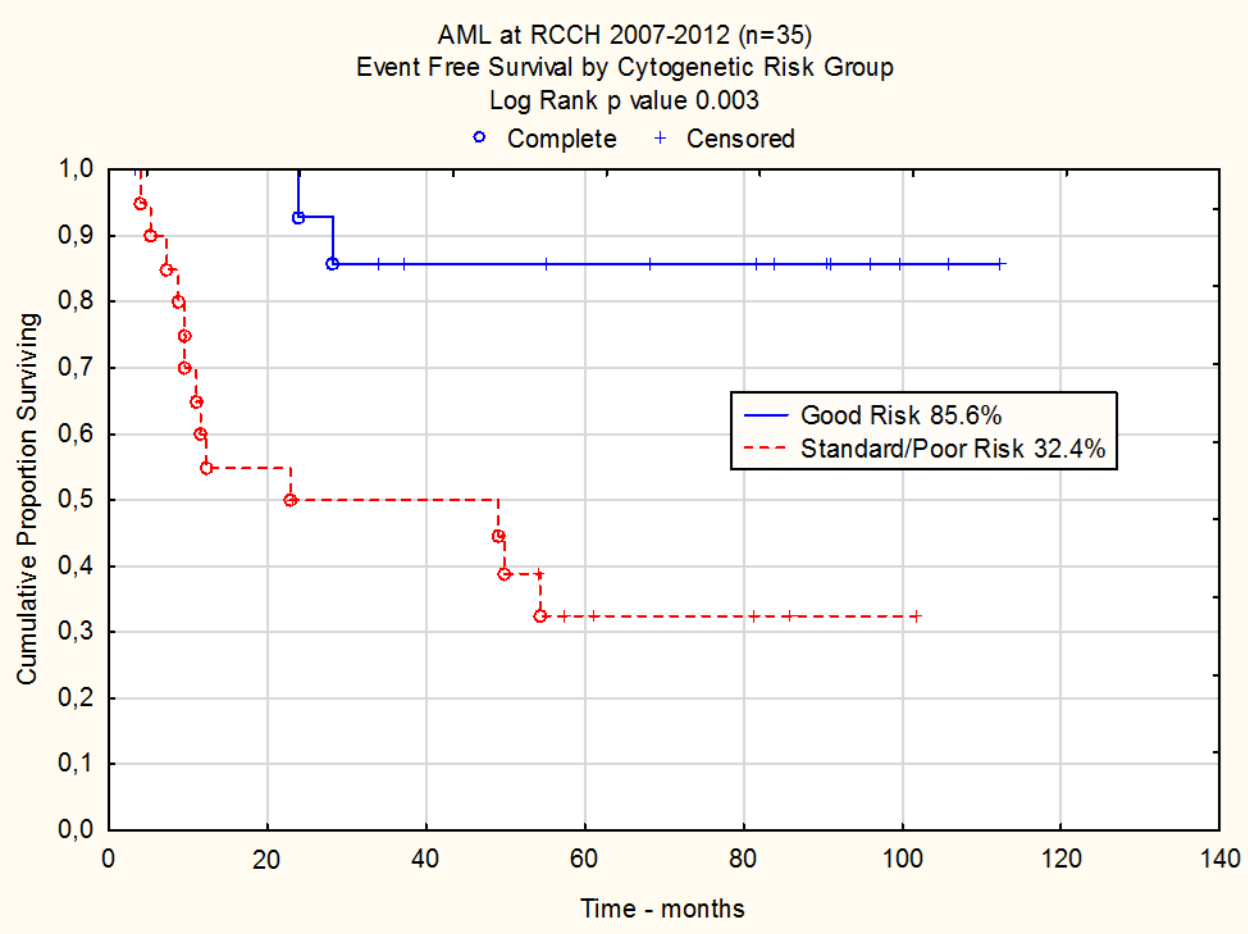


FIGURE 4 Event free survival based on cytogenetic risk groups
AML= acute myeloid leukaemia; EFS= event free survival; n= number of patients; RCCH= Red Cross Children's Hospital

RED CROSS CHILDREN'S HOSPITAL ONCOLOGY: ACUTE MYELOID LEUKAEMIA RX2941 (1994)

Protocol based on BFM 87. All but good risk cytogenetics should be considered for BMT in 1st remission

Bactrim prophylaxis 80 mg TMP/m^2 po BD three days weekly [0-6m: 2.5ml / 6m-6y: 5ml / >6y:10ml]

1.**INDUCTION**

Day 1,2	Ara C 100 mg/m^2 IV over 24 hours by continuous infusion
Day 3-8	Ara C 100 mg/m^2 IV over 30 minutes 12 hourly [12]
Day 3-5	Daunorubicin 30 mg/m^2 IV over 1 hour 12 hourly [6]
Day 6-8	Etoposide 150 mg/m^2 IV over 1 hour daily [3]
Day 1	Methotrexate IT …
	Age <1yr 5mg / 1-2yr 7.5mg / 2-3yr 10mg / >3yr 12.

Bone Marrow at day 28 or when ANC > 0.5 ; Tissue typing after remission if full siblings

2.**CONSOLIDATION** Requires ANC > 1 and Platelets > 100 at Day 1

Weeks 1-4	
Day 1, 8, 15, 22	Vincristine 1.5 mg/m^2 IV (maximum 2 mg)
	Adriamycin 30 mg/m^2 IV over 30 mins
Day 2-5, 9-12, 16-19, 23-26	Ara C 75 mg/m^2 IV or sc 4 days weekly
Day 1-28	Prednisone 40 mg/m^2 po daily (wean over 5 days)
Day 1-28	Thioguanine 60 mg/m^2 po daily
Day 1,15	Ara C IT …
	Age <1yr 10mg / 1-2yr 15mg / 2-3yr 20mg / >3yr30mg

Push through as quickly as possible; Delay each weekly block if plts < 50 or granulocytes < 0.5
TG dose reduction:50% if plts 50-100 or ANC 0.5-1; STOP if plts < 50 or ANC < 0.5

Weeks 5 and 6	Requires ANC > 1 and Platelets > 100 at Day 29 and 36
Day 29, 43	Cyclophosphamide 500 mg/m^2 IV with post-hydration
Day 30-33, 37-40	Ara C 75 mg/m^2 IV or sc 4 days weekly
Day 29-43	Thioguanine 60 mg/m^2 po daily
Day 29, 43	Ara C IT …
	Age <1yr 10mg / 1-2yr 15mg / 2-3yr 20mg / >3yr 30mg

TG dose reduction:50% if plts 50-100 or ANC 0.5-1; STOP if plts < 50 or ANC < 0.5

3 INTENSIFICATION I and II Requires ANC > 1 and Platelets > 100 at Day 1

Day 1-3	Ara C 3 g/m^2 IV over 3 hours 12 hourly [6]
Day 2-5	Etoposide 125 mg/m^2 IV over 1 hour daily [4]
Day 1-5	Steroid eye drops 3 hourly
Day 6-10	Steroid eye drops 6 hourly

4 CONTINUATION Requires ANC > 1 and Platelets > 100

Day 1-28	Thioguanine 40-60 mg/m^2 po daily
Day 1-4	Ara C 50 mg/m^2 IV or sc

TG dose reduction:50% if plts 50-100 or ANC 0.5-1
STOP if plts < 50 or ANC < 0.5
Repeat continuously for 12 months

Figure 5 BFM 87 based Rx2941 treatment protocol

ANC= absolute neutrophil count; BMT =bone marrow transplant; CSF =cerebral spinal fluid; IT =intrathecal; plts =platelets; TG= thioguanine

Appendices:

Appendix 1: Study Protocol

Interim analysis of Acute Myeloid Leukaemia treated on the Red Cross Children's Hospital Rx 2071 (adapted from the MRC AML 15 protocol)

Background:

Acute myeloid leukaemia (AML) accounts for approximately 15 – 20% of acute leukaemia in children and adolescents.[1]

AML is a heterogenous family of neoplasms involving the precursor cells of myeloid lineage.[1 ,2]It is characterised by proliferation of the myeloid precursors with an inability of these cells to differentiate into mature elements.[2]

The cure rates for AML vary between 40 and 50%, [1, 3] lagging behind the progress that has been made in the treatment of acute lymphoblastic leukaemia (ALL). [3]

The history of AML treatment is very interesting. Before 1970 nearly all those with AML died of their disease. [1] Anthracyclines and cytarabine were introduced in the 1970s and 1980s and their use resulted in a minority of patients being cured. [1] The 5 year survival rate of children younger than 15 with AML until the early 1990s was poor at 28%. [1] There has, however, been an improvement in the prognosis of AML over the last few decades. [4]Various clinical trials have looked at different treatment regimens for AML. The trials in the 1990s intensified the doses of traditional chemotherapeutic agents. [1] These trials all had similar stages of therapy namely: remission induction, consolidation and intensification with or without maintenance. The journal, Leukemia, invited groups from all over the world in 2005 to describe their experiences on treating AML.[4] These group utilized a similar

approach: risk – adapted therapy, consisting of dose – intense blocks using common drugs (cytarabine, anthracycline and etoposide).[4] There were, however, many differences such as, the design, intensity and number of blocks given, the use of maintenance treatment, the cumulative doses of the most important drugs given, the use of intrathecal therapy and cranial irradiation for prophylaxis of central nervous system (CNS) relapse and the indications for stem cell transplantation. [4] What proved vital to their success, regardless of the specific approach was an improvement in their capacity for supportive care, which decreased deaths from toxicity. All these groups reported that they had an improved outcome with a decline in early deaths, an increase in complete remission and a decrease in relapse rates; the net result being an increase in event free survival (EFS).[4]

The various treatment strategies have presented numerous questions which were highlighted in a recent review article by RJ Arceci. [1] Some of these questions are: what is the optimal remission – induction regimen?; how many courses of chemotherapy are needed to cure AML; is there a role for maintenance therapy? What is the optimal therapy for reducing CNS relapse? [1] Some studies have showed that there is no benefit of maintenance therapy.[4] despite the fact that the Berlin – Frankfurt – Munster (BFM) group use a maintenance block in their treatment.[5]The Red Cross Children's Hospital AML treatment protocol from 1994 – 2007 was adapted from the BFM 87 protocol. All studies have experienced success with intensifying doses but there is no consensus on how many courses of the chemotherapy are ideally needed.[1] The BFM 87 trial showed that patients receiving cranial irradiation had a better relapse – free survival.[1,5] This was in contrast to many other studies such as the MRC, POG and CCG trials. These studies did not include cranial radiation and they had similar outcomes to the BFM 87 trial.[1,6,7]Robert Arceci in his review article suggests that the ideal remission – reduction regimen is one that effectively results in cytoreduction

without significant toxicity to the patient and one which has a positive impact on post remission treatment. [1] Unfortunately, no such perfect regimen exists presently. [1]

The Red Cross Children's Hospital Oncology Service had poor results in treating AML and this prompted a change in protocol in 1994. [8] A BFM 87 based protocol, Rx 2941 was introduced. However, the outcome for the poor risk patients was poor with an EFS of 27.3%.[9]The EFS for good risk patients was 74.4%. This resulted in yet another change in AML treatment protocol and in 2007 a MRC – AML 15 based protocol, Rx 2071 was adopted. In the United Kingdom the Medical Research Council (MRC) has conducted trials using various treatment regimens. The MRC AML 10 trial used four blocks of chemotherapy and assessed the role of allogenic and autologous bone marrow transplant. This trial found that bone marrow transplant reduced relapse risk but did not contribute to an increase in overall survival.[6] The MRC AML 12 trial tested whether or not an extra course of chemotherapy would be beneficial and it found that there was no advantage in an extra course.[6] In this trial allogenic bone marrow transplant was offered to the standard and poor risk patients.[6] The MRC AML 15 trial compared an anthracycline based consolidation with a Cytosine – Arabinoside (cytarabine) based one.[10] It, thus will ask a particularly important question : how will the potentially less cardiotoxic Ara – C based consolidation compare to the standard but potentially cardiotoxic anthracycline based one? [10] As stated before Red Cross adopted an MRC based protocol in 2007 (see addendum 1). This protocol uses the cytarabine based consolidation arm (see addendum 2) .

Risk stratification is important. As suggested before, trials have offered certain treatment strategies based on risk. The BFM group looked at risk and found that certain cytogenetic patterns were associated with a favourable outcome. [11]The cytogenetic patterns deemed

good risk were: t(8;21), t(15;17) and inv16.[11]The prognostic accuracy of good risk cytogenetics has been widely reported and was demonstrated at Red Cross Children's Hospital with Rx 2941.[9]

As stated before it is important to that one has the most effective treatment regime without significant toxicity. At Red Cross Children's War Memorial Hospital (RCCWMH) the toxicity related deaths with Rx 2941 was 9%. [9]

The RCCWMH AML protocol Rx2071 has now been used for four years. Although we can't yet ascertain the true 5-year EFS, it is necessary to perform an interim analysis to ensure that early results are at least as good as those we observed with RCCH Rx2941.

Rationale:

To assess the interim outcomes of patients with AML, treated on the new AML treatment regime, Rx 2071 adopted by Red Cross Children's Hospital from 2007.

Objective:

The objectives of this analysis are:

- To determine the number ofstandard/ poor risk and good risk AML patients as defined by cytogenetics
- To assess the event free survival (EFS) of both poor/standard risk and good risk patients (as defined by cytogenetics) on the new AML treatment regimen
- To determine the toxicity associated with the new AML regimen

Methods

Study Design:

This will be a retrospective folder review.

Sample:

All patients with AML treated at Red Cross Children's hospital on the AML regime Rx 2071 from 2007 to 2012. Patients with Down Syndrome will be excluded, and patients with Acute Promyelocytic Leukaemia(who have been treated with a different protocol since 2009) will be excluded.

Measurement:

Instruments:

Database:

A database will be created in Microsoft access onto which various variables will be captured. The variables will be listed under columns. Each row will represent a patient. The variables will be as follows:

A: Patient folder number

B: Patient surname

C: Patient name

D: Date of diagnosis

E: Date of birth

F: Sex

G: FAB

H: FAB code

I: Immunophenotype

J: Cytogentics

K: Cytogenetics risk group

L: Karyotype

M: FISH

N: Remission achieved

O: Date of remission achieved

P: Treatment protocol used

Q: Neutropaenic fever

R: Positive blood cultures

S: Myelotoxicity

T: Follow up date

U: Currently

The data will be collected from the patients' folders.

Analysis:

Descriptive analyses will be performed using Microsoft Access and Excel. Event free survival will be calculated using Statistica. Kaplan – Meier curves will be used to determine the EFS. EFS will be calculated from the point of diagnosis to the date of an event (relapse or death) or the last follow up date. The normal/poor risk and good risk groups will be compared using the log – rank test. A p – value of 0.05 will be regarded as statistically significant.

Ethics and Communication:

This is a retrospective study therefore informed consent from each patient is thought to be unnecessary. The names of the patients will not be published or used in any presentations. This study does implement the ethical principle of beneficience. This study will benefit those patients with AML because it will determine whether or not the current treatment protocol is an improvement on the old regime based on event free survival.

This study will serve as the mini dissertation for an MPhil project. The results of this study will presented at the UCT School of adolescent and child Health research day.

References:

1. Arceci, RJ, Progress and controversies in the treatment of pediatric acute myelogenous leukemia. Current Opinion in Hematolog, 2002;**9**:353-360

2. Schiffer C, Anastasi J, Clinincal manifestations, pathologic features, and diagnosis of acute myeloid leukemia. Up to Date 20119 (http://www.uptodate.com/contents/acute-myeloid-leukemia-in-children-and-adolescents)

3. Ravindranath Y, Recent advances in pediatric acute lymphoblastic and myeloid leukemia. Current Opinion in Oncology 2003;**15**:23 - 35

4. Kaspers GJL, Creutzig U, Editorial: Pediatric acute myeloid leukaemia: international progress and future directions. Leukemia 2005;**19**:2025 - 2029

5. Creutzig U, Zimmerman M, Ritter J, et a,. Treatment strategies and long - term results in paediatric patients treated in four consecutive AML – BFM trials. Leukemia, 2005;**19**:2030-2042

6. Gibson BES, Wheatley K, Hann I, et al, Treatment strategy and long – term results in paediatric patients treated in consecutive UK AML trials. Leukemia 2005;**19**:2130-2138

7. Smith FO, Alonzo TA, Gerbing RB, et al, Long –term results of children with acute myeloid leukemia: a report of three consecutive Phase III trials by the Children's Cancer Group: CCG 251, CCG213 and CCG 2891. Leukemia 2005;**19**:2054-2062

8. Davidson A, Desai F, Hendricks M, Shuttleworth M, Hartley P. AML Therapy at RCCH, SIOP Africa presentation, 2008

9. Davidson A, Hartley P, Desai F, Hendricks M, Mathiassen W, Abstract: Acute Myeloid Leukaemia treated with a BFM – based protocol at a South African centre. SIOP Africa, 2008

10. AML 15 , Paediatric guidance variations for children, 2006

11. Creutzig U, Zimmerman M, Ritter J, et al, Definition of a standard – risk group in children with AML. British Journal of Haematology 1999;104:630 -639

Protocol Addendum 1:

RED CROSS CHILDREN'S HOSPITAL ONCOLOGY SERVICE

ACUTE MYELOID LEUKAEMIA (2007)
(excluding Acute Promyelocytic Leukaemia)

Protocol based on MRC AML15

Good Risk: t(8;21) inv(16) t(16;16)
Standard Risk: all patients that are neither good nor poor risk with BM < 15% blasts after ADE 1
Poor Risk: monosomy 5 or 7 del(5q) abn(3q) t(9;22) MAKA(>5abn) **or** BM > 15% blasts after ADE 1
All but good risk cytogenetics should be considered for MRD BMT in 1st remission

IT Therapy: Methotrexate 1yr 5mg / 1-2yr 7.5mg / 2-3yr 10mg / >3yr 12.5mg
Cytarabine 1yr 15mg / 1-2yr 20mg / 2-3yr 25mg / >3yr 30mg
Hydrocortisone 1yr 5mg / 1-2yr 7.5mg / 2-3yr 10mg / >3yr 12.5mg

Cotrimoxazole prophylaxis 80 mg TMP/m^2 po BD three days weekly [0-6m: 2.5ml / 6m-6y: 5ml / >6y: 10ml]

2 INDUCTION

ADE 1

Day 1-10	Ara C 100 mg/m^2 over 30 minutes 12 hourly [20]
Day 1,3,5	Daunorubicin 50 mg/m^2 IV over 1 hour [3]
Day 1-5	Etoposide 100 mg/m^2 IV over 2 hours daily [5]
Day 1	Triple IT (doses above)
	Bone Marrow at day 28 or when ANC > 0.5
	Tissue typing after remission if full siblings

ADE 2 Requires ANC > 1 and Platelets > 100 at Day 1

Day 1-8	Ara C 100 mg/m^2 over 30 minutes 12 hourly [16]
Day 1,3,5	Daunorubicin 50 mg/m^2 IV over 1 hour [3]
Day 1-5	Etoposide 100 mg/m^2 IV over 2 hours daily [5]
Day 1	Triple IT (doses above)
	Bone Marrow after ADE 2 if > 15% blasts after ADE 1

2 CONSOLIDATION

HDAC 1 Requires ANC > 1 and Platelets > 100 at Day 1
Day 1,3,5 AraC 3 g/m^2 IV over 3 hours 12 hourly [6] (start pm)
prednisone eyedrops 3 hourly for 6 days and then 6 hourly for a further 4 days

HDAC 2 Requires ANC > 1 and Platelets > 100 at Day 1
Day 1,3,5 AraC 3 g/m^2 IV over 3 hours 12 hourly [6] (start pm)
prednisone eyedrops 3 hourly for 6 days and then 6 hourly for a further 4 days

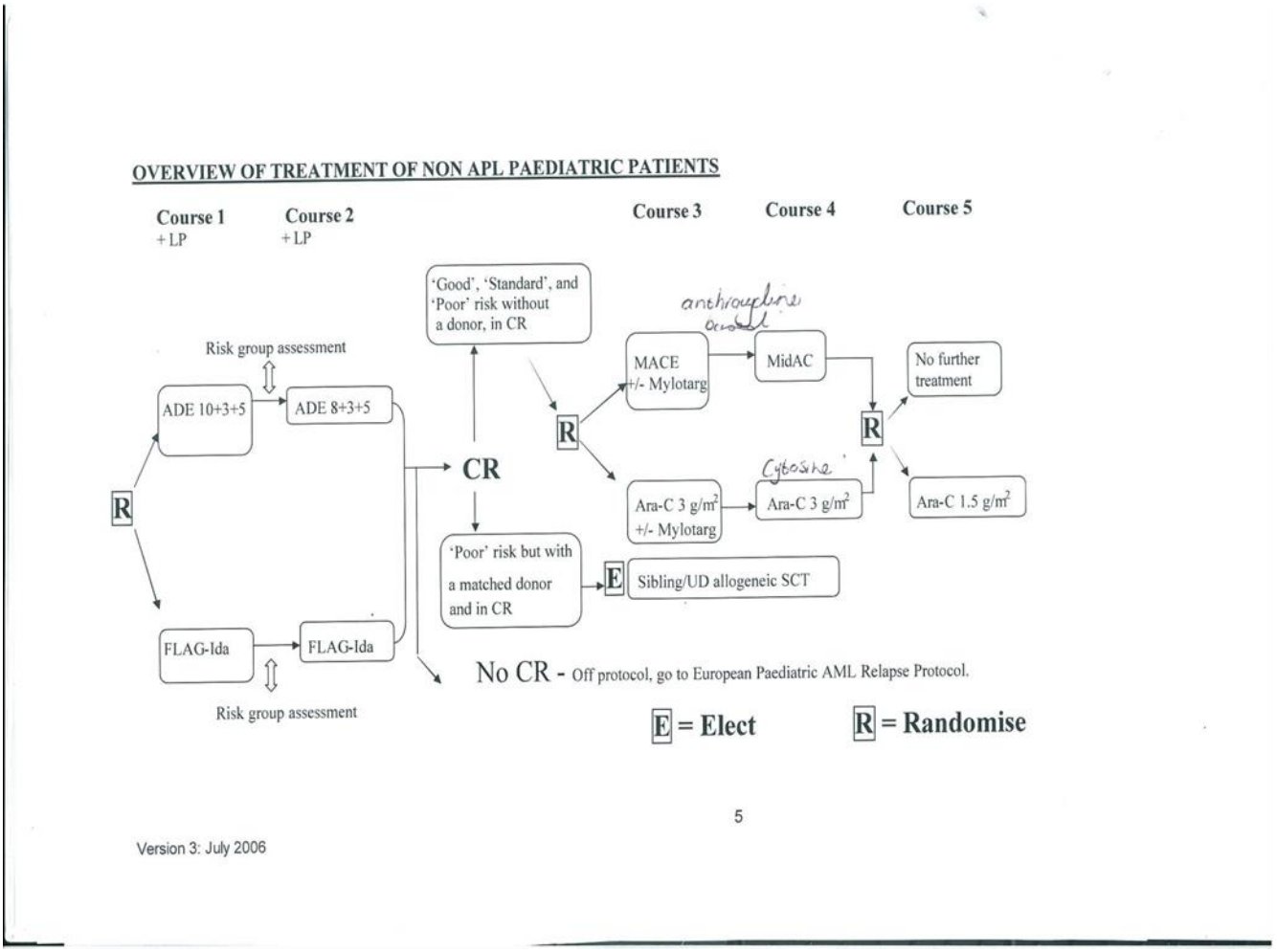
For **CNS disease** (>5 cells per hpf with blasts on cytospin):
2x weekly IT therapy until CSF clear plus two (minimum of 6) then monthly IT until the end of chemotherapy
18 Gy of cranial radiotherapy

For **poor risk AML with no matched donor** substitute HDAC 2 with MidAc

MidAc Requires ANC > 1 and Platelets > 100 at Day 1
Requires normal cardiac function on echocardiogram

Day 1-5	Mitoxantrone 10 mg/m^2 IV over 1 hour daily [5]
Day 1-3	AraC 1 g/m^2 IV over 2 hours 12 hourly [6]
	prednisone eyedrops 3 hourly for 5 days and then 6 hourly for a further 5 days

Protocol Addendum 2:



Appendix 2: Ethics Approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

19 February 2014

HREC REF: 235/2012

A/Prof A Davidson
Paediatrics
Red Cross War Memorial Children's Hospital

Dear A/Prof Davidson

PROJECT TITLE: ***INTERIM ANALYSIS OF ACUTE MYELOID LEUKAEMIA TREATED ON THE RED CROSS CHILDREN'S HOSPITAL RX 2071 (ADAPTED FROM THE MRC AML 15 PROTOCOL)***

Thank you for submitting your amendment to the Faculty of Health Sciences Human Research Ethics Committee.

The HREC has **noted and approved** the amendment on the above-mentioned study.

We acknowledge that Dr Karla Thomas is also involved in this study and using it for her MPhil degree.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely,

PROFESSOR MARC BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS

Appendix 3: Ethics Renewal

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

ETHICS COMMITTEE
- 4 MAR 2016
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)		
This serves as notification of annual approval, including any documentation described below.		
☑ Approved	Annual progress report	Approved until/next renewal date 30.3.2017
☐ Not approved	See attached comments	
Signature Chairperson of the HREC		Date Signed 4/3/2016

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	3/3/2016		
HREC REF Number	235/2012	Current Ethics Approval was granted until	30/3/2016
Protocol title	Interim analysis of Acute Myeloid Leukaemia treated on the Red Cross Children's Hospital Rx 2071 (adapted from the MRC AML 15 Protocol)		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ☐x No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof Alan Davidson		
Department / Office Internal Mail Address	Ward G1 Red Cross Children's Hospital alan.davidson@uct.ac.za		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☐x No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	☐ No N/A
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	☐ No N/A

Appendix 4: PBC instructions to authors

Author Guidelines

Wiley's Journal Styles

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Pediatric Blood & Cancer welcomes submitted manuscripts online at: http://mc.manuscriptcentral.com/pbc. Authors are encouraged to check for an existing account. If you are submitting for the first time, and you do not have an existing account, then create a new account. Once you have logged in, you will be presented with the Main Menu and a link to your Author Center. Enter your Author Center to submit your manuscript. At the end of a successful submission, a confirmation screen with manuscript number will appear and you will receive an e-mail confirming that the manuscript has been received by the journal. If this does not happen, please check your submission and/or contact the Editorial Office.

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Authorship

All authors should have contributed in a significant manner and be in agreement with all content in a manuscript. The corresponding author will take responsibility for this requirement being met.

Individuals should only be listed as authors if they have participated in both the conception and design of the study and in the data analysis or editing. All authors must explicitly approve the final draft of the paper before it is submitted. Purely technical contributions are not sufficient for authorship, but instead should be included in the acknowledgements.

Statements of equal authorship contribution may be included. At least one person's name must precede a group-attributed authorship (e.g., "Tom Jones for the Meningitis Study Group").

Contributions by individuals who made direct contributions to the work but do not meet all criteria should be noted in the Acknowledgements section of the manuscript. Medical writers and industry employees can be contributors. Their roles, affiliations, and potential conflicts of interest should be included in the author list or noted in the Acknowledgments and/or Contributors section concurrent with their contribution to the work submitted. Signed statements from any medical writers or editors declaring that they have been given permission to be named as an author or as a contributor, or in the Acknowledgments section is also required. Failure to acknowledge these contributors can be considered inappropriate, which conflicts with the journal's editorial policy. For questions about authorship criteria, please consult the ICMJE guidelines and/or contact the Editorial Office prior to submitting.

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COVER LETTER. The online submission program requires an author cover letter. Please note that the cover letter should:

--Be addressed to the journal's Editor-in-Chief;

--State what significant, new information the submission provides from that previously published in medical literature;

--Contain the manuscript title and all author names;

--Provide a brief summary of the findings and why they are important and appropriate for PBC;

--State what manuscript classification it fits, i.e., Research Article, Brief Report, etc;

--State that the manuscript has not been submitted elsewhere nor previously published;

--State any conflicts of interest;

--List names of 3 potential reviewers who: a) are experts in the field, b) are not from the authors' institutions, and c) have no other conflicts of interest;

--State who and why any colleagues should not be asked to review, if applicable;

--State that all authors have contributed to the manuscript in significant ways, have reviewed and agreed upon the manuscript content.

FILE ORDER. When uploading the files into the ScholarOne Manuscript Central system, please order the files as follows:

1. Text file -Word file (.doc) should be 8.5 x 11 inches with at least 1 inch margins on all sides

2. Table files - Each table should be uploaded in a separate Word file in numerical order

3. Figure files - Each figure should be uploaded as a separate TIF, EPS, PNG, or PDF file following in numerical order. Do not upload individual panels as separate files (e.g., "Figure 1a.tif" and "Figure 1b.tif.").

4. Supplemental files - Please note that Supplemental files will be published in the exact format provided at submission. These files must be clearly identified as "Supplemental" at the top of the first page.

5. For Revised and Resubmitted Manuscripts, Responses to Review Comments should be included in the “View and Respond to Decision Letter” field of the online submission system.

TITLE PAGE

--The complete title of the manuscript;

--The names of all authors (NOTE: While the number of authors should usually not exceed six, exceptions will be granted with adequate justification that can be included in the cover letter.)

--The complete affiliations of all authors;

--The name, address, phone, fax and email contact for the corresponding author;

--Word Count for:

a) Abstract (if applicable) and

b) Main Text (excludes title page, abstract, Conflicts of Interest, Acknowledgments, References, Tables, Figures, and Legends);

--The number of Tables, Figures, and Supplemental files;

--A short running title (not to exceed 50 characters);

--Three to six keywords to index the content.

--An abbreviations key in a table. This should just be a two-column list, with the abbreviation on the left, and the full term or phrase on the right. ALL abbreviations used in the manuscript should appear in this table. (Though abbreviations that are only used in a Table can simply be defined with footnotes.)

ABSTRACTS

--Abstracts should be included in the online submission form and in the manuscript file.

--Please do not include material in the Abstract that is not described in the main manuscript.

--See Article Types listing for specific formatting guidelines.

MAIN TEXT

--Double spaced with consecutive line numbering

--Font should 12pt in size, Times New Roman or Arial

--Order of elements: Title Page, Abstract, Introduction, Methods, Results, Discussion, Conflict of Interest statement, Acknowledgements, References, Legends

--ETHICS STATEMENT: If photographs include human subjects, the author must include an Ethics Statement in the main manuscript text, where appropriate. This statement should affirm that informed consent has been properly documented. When possible, any identifiers in the image (such as facial features or patient ID numbers) should be obscured prior to review.
--Use subheadings and paragraph titles whenever possible. Note, however, that the Discussion section should not have separate subsections. Subheadings should not be underlined or be followed by punctuation.

--No numbered or bulleted lists are allowed in the text file.

--See Journal Style section for further PBC style preferences.

REFERENCES

AMA – American Medical Association

References: All references should be numbered consecutively in order of appearance and should be as complete as possible. In text citations should cite references in consecutive order using Arabic superscript numerals. Sample references follow:
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Book:
2. Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons; 1990. 1223 p.

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--Authors are responsible for the accuracy of references.

The EndNote style file for PBC is no longer updated or supported (http://authorservices.wiley.com/jendnotes/#p).
--All references must be cited whether in text, figures or tables.

--Include the complete title of the article and inclusive page numbers.

--Articles accepted for publication and published on-line should be referenced like a journal article, except that the DOI (digital object identifier) and the date of prepublication should supplant the year, volume number, and page numbers. The cited article must be accessible to readers.

--Published abstracts may be cited in the references.

--Unpublished data and personal communications should not be listed as references.

TABLES

--Number tables consecutively with Arabic numerals.

--Do not include multi-part Tables (e.g., Table 1a, 1b, and 1c).

--The table number and title should be placed **above** the table.

Correct Example: TABLE 1 Graph demonstrating results

Incorrect Example: Table 1: Graph Demonstrating Results

--Abbreviations should be in footnotes beneath the table. Abbreviations should be in paragraph form and footnotes should be a vertical list.

FIGURES

--There is no charge to authors for color figures. Please use color for emphasis and clarity, but avoid use of unnecessary and background colors or shading.

--Figures should be numbered using Arabic numerals, i.e., Figure 1, Figure 2, etc., and cited in the manuscript as (Fig. 1), (Figs. 3A and 3B), etc. Do not include a label ("Figure X") in the image itself.

--The figure number and legend should be included in BOTH:

a) The legend list at the **end** of the manuscript AND

b) The Description field of the online submission form for that file.

--Figures and text within a figure should not be surrounded by boxed lines. Crop extra white space from around images. Do not include legend text in the figure files.

--Label each panel with a **capital letter in the upper left corner**

--Panels should be labeled as A and B (not A. or A- or A)).

--Presentation of growth charts may be facilitated by utilizing tools such as those listed below:

a) http://www.seattlechildrens.org/about/community-benefit/obesity-program/excel-based-clinical-tools-assist-growth-charts/

b) http://www.who.int/childgrowth/software/en/

c) https://itunes.apple.com/us/app/pediatric-growth-charts-by/id617601789?mt=8

--Upload figures as individual TIF, EPS, or PNG files. High resolution PDF files may also be uploaded.

--Upload composite figures (with multiple panels) as one file. Do not upload separate panel files for a single figure.

--The journal requires a minimum resolution of 300dpi for all figures.

- 1200 DPI/PPI for black and white images, such as line drawings or graphs
- 300 DPI/PPI for picture-only photographs
- 600 DPI/PPI for photographs containing pictures and line elements, i.e., text labels, thin lines, arrows

--Figures must be legible at 100% zoom in the file itself. We recommend that all text in figures be at least 6pt.

--Arrows should be included in radiographs or histology figures to point out areas of interest described in the figure legends below the figures.

--Please ensure that all axes are labeled clearly and in accordance with the journal's requirements for numbering (all numbers over 999 must contain commas, zeroes before decimals).

--Any axis in a given figure must have a centered label. Note that numbers on the y-axis should be oriented to read left to right. For example:

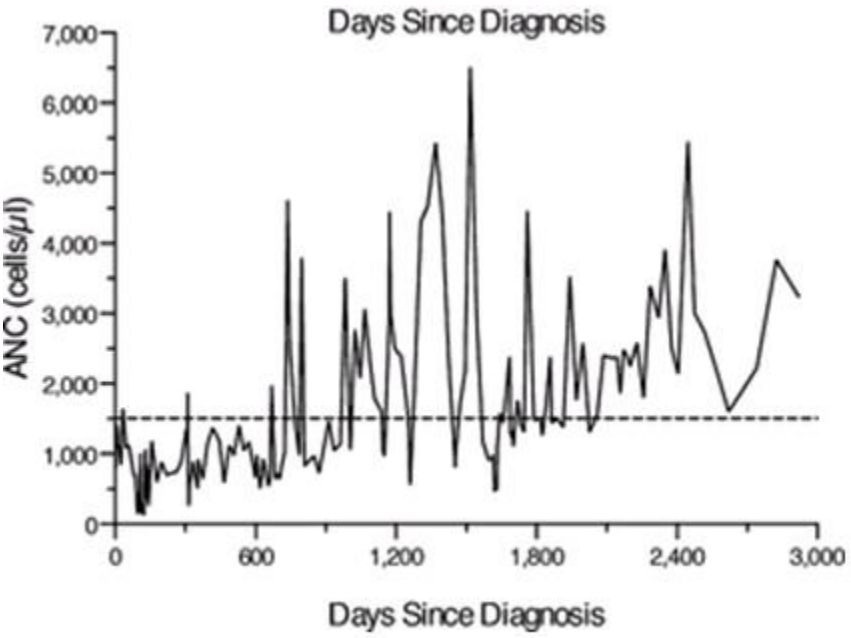

FIGURE 1 Platelet count and absolute neutrophil count (ANC) versus time since diagnosis. Treatment courses are indicated at top with doses as described in Table 1: Vbl, vinblastine; Pred, prednisone; Ritux, rituximab; MP, mercaptopurine. IVIg = short course intravenous immunoglobulin 1 g/kg/day for 2 days, given for immune thrombocytopenia.

Image from: Cooper, S. L., Arceci, R. J., Gamper, C. J., Teachey, D. T. and Schafer, E. S. (2015), Successful Treatment of Recurrent Autoimmune Cytopenias in the Context of Sinus Histiocytosis With Massive Lymphadenopathy Using Sirolimus. Pediatr. Blood Cancer. doi: 10.1002/pbc.25770

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--Supplementary material will be published as submitted and will not be corrected or checked for scientific content, typographical errors or functionality. The responsibility for scientific accuracy and file functionality remains entirely with the authors. A disclaimer will be displayed to this effect with any supporting information published.

--Supplementary material should always be provided in its final format, as it will not be copyedited or changed from its original format. It will not be available for review prior to publication.

--Upload these files as either “Supplementary Material for Review” or “Supplementary Material NOT for Review,” as appropriate.

--For each individually uploaded supplementary file, a corresponding legend must be included in the manuscript file.

--Supplementary tables or figures may be cited within the text (as Supplementary Table S1 and Supplementary Figure S1) and will be made available to readers online.

--Tables should be limited to 1-2 pages in a Word file, preferably in portrait orientation. Overly long tables should likely be reformatted as Supplementary material. In this case, uploading Excel files is suitable.

SPECIFIC JOURNAL STYLE GUIDELINES

--Either American or British style is acceptable. *American:* use Merriam-Webster's; *British:* Oxford Shorter Dictionary.
--The statement ‘data not shown’ is not allowed within the manuscript text as readers cannot evaluate if the data are not shown. Such information should either be included in the manuscript or provided as a Supplementary Material for Review file.

--Please do not use slang expressions, such as “On the other hand”.

--Avoid statements such as “This is the first study…” and “To our knowledge…” and “this is the largest.” These expressions are not meaningful.

>>Name Formatting

--Disease names should be written without apostrophes, as follows: Wilms tumor, Burkitt lymphoma, Hodgkin disease, Ewing sarcoma.

--Abbreviations should be defined on first usage, then using of abbreviation alone is acceptable: e.g., Wilms Tumor (WT), then referred to as WT in subsequent mention, without quotation marks.

--Abbreviations should follow the guidelines in the CBE Style Manual, 5th Edition (available from the Council of Biology Editors, Inc., One Illinois Center, Suite 200, 111 East Wacker Drive, Chicago, IL60601-4298).

--Gene and protein designations should be written in the international style approved by the HUGO Gene Nomenclature Committee at http://www.genenames.org/guidelines.html
--Use uncapitalized generic names (e.g., cyclophosphamide) for all drugs and pharmaceutical preparations.

--Trade names (capitalized) for appliances, etc., may be used in the Methods section, and the manufacturers identified by name.

>>Human Subjects

--Please do not refer to patients by their diseases, e.g. “Wilms tumor patients” or “ALL patients.” Instead, identify them as “patients with Wilms tumor” and “patients with ALL.”

--Patients should be referred to only by subject numbers and not with names, initials, or other potentially identifying characters.

--Manuscripts reporting the results of experimental investigations on human subjects must include a statement to the effect that procedures had received official institutional approval.

--There should be no dates in the text or on radiographs as these are potential patient identifiers.

>>Numbers

--All measurements must be in metric units.

--Decimal numbers should have a zero preceding the decimal point (e.g., 0.95g).

--Decimal points should be periods and not commas.

--Do not begin sentences with a number. For example, it should be “Three patients…” instead of “3 patients…”

--Numbers over 999 must include a comma, e.g., 2,000.

--P values should not be zero but refer to a number (e.g., p<0.0001 not p=0.0000).

II. ARTICLE TYPES

Submission Type	Description	Abstract	Word count**	References	Maximum # of Primary Figures and Tables (Combined) ***
RESEARCH ARTICLES	Articles should represent original and in-depth studies involving any aspect of clinical or laboratory investigation.	250 words Structured*	3,500 words	as needed	6
PRIORITY REPORTS	Upon approval by the journal’s editors, occasional timely and high-impact research articles may be fast-tracked for online publication within 4 weeks after final acceptance and print publication within 2 months of final acceptance. Cover letters for articles submitted as Priority Reports should include an explanation as to the reason for this designation.	150 words Structured	2,000 words	50	4
BRIEF REPORTS	Brief reports may include descriptions of single or several patients that demonstrate novel findings or add in a significant way to current knowledge. Brief reports may also include novel laboratory observations relating to clinical questions or advances in laboratory methodologies. Brief reports should include the following sections: Introduction, Results (including Methods or Case Descriptions), and Discussion. If brief, the Methods may be merged into	100 words Unstructured	1,200 words	20	2

	Results.				
CRITICAL REVIEWS	Reviews of important and timely subjects can be invited through the Editorial Board or submitted after a brief introduction to the Editor-in-Chief. Authors should consult the editor prior to submission by e-mailing PBCeditorialoffice@wiley.com. The inquiry should include the authors' names and affiliations, subject matter of the review, and rationale for publication in PB&C. Reviews should focus on the critical aspects of a subject, linking what is known to what areas remain controversial or unanswered. Historical accounts of important events relating to pediatric hematology/oncology are also acceptable. Requests for permission to submit manuscripts of greater length should be emailed to the Editor-in-Chief prior to submission.	100 words Unstructured	3,500 words	100	6 Illustrations and tables should be used only to provide summaries or a synthesis of ideas and/or data not included in the text.
REVIEWS OF BOOKS AND OTHER MEDIA FORMATS	Reviews of books, films or other media formats relevant to the scientific or clinical practice of medicine with particular importance to pediatric hematology/oncology can be invited or submitted independently. In the latter case, consultation with the Editor-in-Chief should be made prior to submission.	N/A	1,000 words	10	Include only if they highlight or clarify points made in the text.
HIGHLIGHTS	Highlights are submitted only at the invitation of the Editor-in-Chief. These will summarize findings from one or more recently accepted papers and put them into perspective in terms of past work and future challenges. Controversial areas should be included.	N/A	1,000 words	10	A single original figure or table that summarizes the content or text of the paper(s) is encouraged but not required.
COMMENTARY	Commentaries are usually invited but may be submitted independently after consultation with the Editor-in-Chief. Commentaries should focus on a controversial subject or a timely topic of relevance to the journal's readership. Commentaries will be reviewed and may require changes or be rejected.	N/A	1,200 words	10	Include only if they highlight or clarify points made in the text.
CORRESPONDENCE AND LETTERS	Letters to the Editor should usually be in reference to previously published manuscripts in Pediatric Blood and Cancer. Correspondence relating to important and timely publications or topics from other sources and brief descriptions of interesting laboratory or clinical observations may also be appropriate.	N/A	500 words	10	1 Illustrations or tables should only be included when absolutely

					necessary.
HISTORICAL PERSPECTIVES	A Historical Perspective should be submitted only after consultation with the journal's Editor-in-Chief. This occasionally-appearing series focuses on the history of pediatric hematology/oncology.	100 words Unstructured	1,500 words	20	2
ON CHILDREN, BLOOD, AND CANCER	These narratives, topical essays, historical vignettes, poems, and photographic essays will provide personal, artistic interpretations of the experiences of children with cancer or blood disease, or of caring for these children. Submissions may describe difficult, challenging, informative, or uplifting patient encounters or clinical experiences, or may explore other aspects of professional life in pediatric hematology/oncology. Like all work submitted to PBC, submissions should be original, and not previously published or under consideration elsewhere. Poems should be fewer than 50 lines and 400 words.	N/A	1,200 words	10	Include only if they highlight or clarify points made in the text. Photo essays may include up to 6 images.
SPECIAL REPORT	To be submitted only after consultation with the journal's Editor-in-Chief, the report should focus on a subject of current interest to readers and/or importance to the sponsoring societies.	100 words Unstructured	2,500 words	20	2
CLINICAL PRACTICE GUIDELINES	CPGs should concern important and timely subjects. In 2011, the Institute of Medicine updated its definition of a CPG to reflect essential components of evidence-based guidelines: "CPGs are statements that include recommendations intended to optimize patient care that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options." CPG submissions must receive pre-submission approval from the Editor-in-Chief. CPGs must meet the Institute of Medicine standards for trustworthiness as operationalized by the criteria for inclusion in the National Guideline Clearinghouse (http://www.guideline.gov/about/inclusion-criteria.aspx). Criteria include, but are not limited to, recommendations being based upon a systematic review (the search strategy should be included as an Appendix) and the creation of evidence tables. Use of an approach for recommendation development such as Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) is encouraged. An excellent resource for preparing a CPG can be found at:http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3928232/. Guideline developers are encouraged to evaluate their own CPG using the Appraisal of Guidelines for Research & Evaluation II tool (http://www.agreetrust.org/agree-ii/).	100 words Unstructured	4,500 words	150	Illustrations, tables, and tools for implementation such as clinical care algorithms are encouraged to augment the text.
MEETING REPORTS AND SUPPLEMENTS	Concise summaries of meetings that have important information to convey to the readers of Pediatric Blood & Cancer are welcomed but consultation with the Editor-in-Chief should occur before submission. Summaries should emphasize the issues discussed at the meeting along with why they are important or	100 words Unstructured	1,500 words	as needed	Include only if they highlight or clarify points made in the text.

	controversial. More extensive meeting reports with manuscripts from the speakers at the meeting are also welcomed but early consultation with the Editor-in-Chief must take place in order to determine the type and number of manuscripts, expected pages to be published, and the review process, as well as procurement of additional funding if required.

***** Structured headings: (Background, Procedure, Results, and Conclusions)

****** Word count excludes title page, Abstract, References, Tables, Figures, and Legends

******* Tables and figures should not simply repeat information in the text. Additional figures and tables can be included in Supplementary Materials

MEETING ABSTRACTS

*Abstract text should be 300 words or less. That word count excludes the abstract title, author names and affiliations.

*There should be 4 sections to the abstract: Background/Objectives, Design/Methods, Results, Conclusion. All text should be included in a single paragraph and contain no lists.

*Abstract text should be written in complete sentences and in correct English.

*There should be a period at the end of all sentences.

*Tables and figures (if allowed in the submitting society's instructions), only when critical to the content, may be included and must comply with PBC Author Guideline format.

*Abstract titles should be in all capital letters, e.g., INDUCTION OUTCOMES IN CHILDREN WITH ALL

*Author names should be listed below the abstract title. The first and last names of authors should be written in upper and lower case letters and should be boldface. No degrees of authors should be included.

*Author affiliations should be written in upper and lower case letters, e.g., Tata Memorial Centre

*Geographic location should be indicated by city and country, with state, province, or other subdivision added if necessary for disambiguation, e.g. London, UK; but Portland, OR, USA. Names of cities and countries with 2 words should be written in upper and lower case letters, e.g., Czech Republic, South Africa, Los Angeles, St. Louis. Common abbreviations may be used, e.g. UK, USA.

*Periods should be used in numbers for decimal points, not commas, e.g., P=0.015, and numbers beginning with a decimal point should be preceded by a zero.

*Disease names should be written without apostrophes, e.g., Hodgkin lymphoma, Non-Hodgkin lymphoma, Burkitt lymphoma, Ewing sarcoma

*Numbers containing more than 3 digits should have a comma, e.g., 3,000.

*Abbreviations should be defined on first usage, then using of abbreviation alone is acceptable: e.g., Wilms Tumor (WT), then referred to as WT in subsequent mention, no quotation marks, however.

*Abstracts need to be proofread for all spelling and grammatical errors.

*Abstracts that do not satisfy publication instructions will not be published.

CLINICAL TRIALS

- NOTE: Clinical trials can be submitted as *either* a Research Article or a Brief Report. There is not a separate article type for clinical trials.
- Reporting prospectively conducted trials is strongly encouraged and such trials will be prioritized

- Retrospective reporting of clinical data is potentially acceptable for publication. All such manuscripts need to comply with documentation of approval by an institutional ethical review board or equivalent. Retrospective therapeutic studies should be avoided, as they may circumvent necessary informed consent, safety, and monitoring standards.

- All manuscripts reporting clinical trials need to document that the trial or study was approved Institutional Review Board or equivalent.

- All manuscripts reporting clinical trials need to be registered with 'ClinicalTrials.Gov' and/or an equivalent site.

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