DISSERTATION REPORT

FACULTY OF HEALTH SCIENCES

INTERNAL MEDICINE

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A retrospective audit of the cytogenetic profile and management outcome in Acute Myeloid Leukemia patients treated at Charlotte Maxeke Johannesburg Academic Hospital (2017 - 2021).

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment for the requirements of the degree of Master of Medicine (Internal Medicine).

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I. Ethics Committee Approval (HREC)

This study was approved by the Human Research Ethics Committee (16/9/2021), of the University of the Witwatersrand. Clearance certificate number: **M210820**.

II. Declaration

I, Dr Mbongi Mpanza, declare that this research project is my own work, which is being submitted for the degree Master of Medicine (in the dissertation format) in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has never been submitted for any degree or examination at this or other University.

3..Day.. Of…October…2022

III. Dedication

I would like to thank my family for believing in me and for their continuous support, my partner, Dr Onela Golela for supporting me during this challenging time, and my mother for her words of encouragement.

IV. Abstract

Introduction

Acute Myeloid Leukemia (AML) is a highly heterogenous blood cancer that affects the non-lymphoid lineage. It is a most common acute leukemia in adults. The worldwide incidence is relatively low with inordinately high cancer mortality. The recent advances done by on-going research has elevated our understanding of cytogenetic and abnormalities associated with AML. This understanding further aids in stratifying AML patients into favourable, intermediate, and poor prognosis groups. However, despite these insights into disease, patient outcome often remains poor.

In this review, we discuss findings in AML at CMJAH, with particular focus into cytogenetic profile and molecular gene mutations. These recurrent genetic alterations provide novel insights into the pathogenesis, clinical characteristics, and outcome of these patients. These alterations play a major role in prognosticating the outcome in AML and are also important in developing novel therapies.

The most common type of AML, called *de novo* AML, occurs sporadically with no prior history of underlying myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN) or exposure to potentially leukaemogenic therapies or agents. The less common is secondary AML which has poorer prognosis and is define as any leukemic process which may arise from previous haematological disorder like MDS, NPM or can be a result of prior cytotoxic chemotherapy or radiation therapy (t-AML).T-AML is defined as AML that develops from prior cytotoxic drugs, radiation or immunosuppressive agents which was given for unrelated illness. T-AML accounts for 7%-8% of all AML and is known to have a dismal outcome with an adverse cytogenetic and molecular profile. To our current knowledge there is no local study that has previously analysed t-AML in detail hence this study may provide such critical data.

Aims and Objectives

The primary aim of the study is to review the specific cytogenetic and molecular abnormalities in AML patients treated at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between the years 2017 - 2021. The study will also review all cases of therapy-related AML treated at CMJAH in the past 10 years.

Study Objectives

The primary objective is to examine all results of AML patients treated at CMJAH from 2017 - 2021(Cohort A), paying attention to:

- Patient's demographics
- Clinical and Laboratory findings
- Cytogenetic and molecular features
- Treatment and outcomes (outcome refer to remission vs. progressive disease)

The secondary objective is to review all therapy-related AML patients treated at CMJAH between 2011 – 2020 (Cohort B), with special interest to:

- Cytogenetics and molecular profile
- Treatment plans and outcomes

Methods

Files of adults with AML were collected at Charlotte Maxeke Academic Johannesburg Hospital, Medical Oncology unit, for patients that were diagnosed and treated between 01/01/2017 to 31/07/2021 (Cohort A). For Cohort B, all files of patients with therapy related AML that were treated between period 01/01/2011 to 31/12/2020 were collected. A detailed analysis of patient's demographics, clinical presentation, laboratory findings, cytogenetic and molecular studies as well as treatment and outcome were done.

Results

A total of 90 AML patients were analysed .This included 82 *de novo* AML and 8 therapy-related AML. The median age was 40 years with an interquartile range (IQR) of 32-59 years. The majority were in the age group 19-40 years (52.22%, n=47). There were 48.89% (n=44) patients in the intermediate cytogenetic group, 32 (35.56%) in favourable group and 14 (15.56%) in the unfavourable group. Majority of patients were females (54.44%) and of black ethnicity (71.11%). In both cohorts, the most common presentation was bone marrow failure in a form of anaemia. About 63% of patients were HIV negative while 13% were positive with the rest being unknown.

The most common FAB subtype found in our study was AML M2 (20%, n=18).

Using the WHO AML classification most patients (51.11%) fell on the category of AML Not Otherwise Specified (AML NOS). Commonest mutational abnormalities found were PML-RARA and RUNX1-RUNX1T1, 13, 33% and 20% respectively. While t(8;21) was the most common cytogenetic abnormality detected (20%).

Approximately 80% of patients received induction chemotherapy and of those, 24.44**%** achieved complete remission (CR), while 52.22% had refractory disease (RD). The overall outcome of this study showed 88.88% mortality, 5.55% lost to follow up and 5.55% of patients were still alive.

Conclusion

This retrospective study of combined cohorts of de novo AML (2017 - 2021) and t-AML patients (2011-2021) had 90 patients, had predominantly female patients (54.44% vs. 45.46% males). Most patients were in the age group 19-40 years. Anaemia, bicytopenia or pancytopenia were the main clinical features. The most common cytogenetic profile found was normal profile while common mutational abnormality found was RANX1-RANX1T1.

In the t-AML group, the cytogenetic features were normal at only in 25.0%, with 75,0% of patients having normal molecular studies.

Standard treatment with the 7+3 protocol (Cytarabine + Anthracycline) was given, with a CR of 24.44**%** for the overall study and 37.5% in t-AML cohort. The overall mortality was very high with 88.88% death from both cohorts, and in both groups, older patients had a significantly higher mortality (p= 0.0091), with death occurring within the first year of the diagnosis.

V. Acknowledgements

I would like to send a big thank you to my supervisors; Prof P Ruff and Dr D Tshabalala for their guidance and continuous support. I would like to send a heartfelt gratitude to my academic mentor and role model; Prof T Mgwebi (Sis T) for her immense assistance during the protocol and dissertation write up.

I would like to thank Prof Ruff and his staff for allowing me to access the departmental files with the assistance of the filing clerk, Mr Azwindini Maudu. I would also like to thank CMJAH CEO for allowing me to do my research project at this institution.

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IX. List of abbreviations and acronyms

AML	Acute Myeloid/Myelogenous Leukemia
APL	Acute Promyelocytic Leukemia
ARF	ADP Ribosylation Factor
AR	Age Standardized Risk
ATRA	All-Trans Retinoic Acid
CBF	Core Binding Factor Protein
CEBPA	CCAAT Enhancer Binding Protein Alpha
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
FAB	French American British score
FLT 3	FMS-like Tyrosine Kinase 3
HiDAC	High Dose Cytarabine
HIV	Human Immunodeficiency Virus
NHLS	National Health Laboratory Services
NPM1	Nucleophosmin1
WHO	World Health Organization
KMT2A	Histone-lysine N-methyltransferase 2A
CR	Complete Remission
MRD	Minimal Residual Disease
OS	Overall Survival
RD	Refractory Disease
TRM	Treatment-Related Mortality

CHAPTER1: INTRODUCTION

1.1 General introduction

Leukemias are a highly heterogeneous group of haemopoietic cancers that comprise of several diverse and biologically distinct subgroups of blood disorders, one of which is acute myeloid leukemia (AML). This disorder involves abnormal proliferation and differentiation of a clonal population of a myeloid stem cell. In addition to chromosomal rearrangements, molecular changes have also been implicated in the formation of AML. In fact, genetic mutations are identified in more than 97% of all AML cases (1).

In humans, haematopoiesis is a continuous process initiated from a reservoir of multipotent stem cells which differentiate to form mature cellular components of the blood. Haematopoietic stem cells initially differentiate to either lymphoid or myeloid cells. The lymphoid cells will form mature T and B lymphocytes including plasma cells, whilst myeloid cells give rise to mature erythrocytes, granulocytes, monocytes, eosinophils, basophils, and platelets. This complicated process of cellular differentiation and maturation is both stimulated and regulated at a molecular level by varies colony stimulating growth factors. Any cytogenetic disturbances that occur at the level of the haematopoietic precursor cell can alter normal cellular proliferation and differentiation, resulting in an accumulation of immature lymphoid or myeloid precursors within the bone marrow and peripheral blood (2).

This clonal proliferation and bone marrow infiltration by these immature precursor cells cause displacement of normal haematopoietic elements occurs, leading to leukemia.

AML can arise secondary to underlying haematological malignancies, consequence of prior exposure to cytotoxic drugs or radiation, but the vast majority of cases occur as a *de novo* malignancy in previously healthy individuals (3).

The aetiology of most AML is unknown, however established risk factors include smoking, exposure to chemicals (benzene, formaldehyde), chronic myeloproliferative neoplasms, cytotoxic chemotherapy drugs, high dose radiation exposure, genetic syndromes (Fanconi anaemia, Bloom syndrome) and family history (4). The prevalence of AML is notably higher in male patients and older individuals.

Most clinical manifestations of AML reflect accumulation of poorly differentiated malignant myeloid cells within the bone marrow, peripheral blood, but rarely, in other organs. Typically, patients will present with features of bone marrow failure, such as anaemia, bleeding and/ or infection. The diagnosis of AML is established when a patient has 20% or more of blasts in the bone marrow or peripheral blood. The diagnosis is further supported when these blasts are confirmed to be myeloid of origin (5).

The treatment of AML is instituted based on the accurate prognostic assessment of the newly diagnosed patient. Clinical features like age and performance status are used to determine the risk of resistance and treatment-related mortality (TRM), whilst the cytogenetic profile and molecular derangements constitute the single strongest prognostic factors for complete remission (CR) and overall survival (OS)(6). Therefore, for treatment purposes in clinical practice, AML is classified into favourable, intermediate, and adverse risk groups based on the cytogenetic profile. Regarding treatment, eligible patients first undergo induction chemotherapy to achieve CR. Unfortunately, minimal residual disease (MRD) often persists, and relapse will occur if treatment is discontinued. Therefore, a favourable response to induction therapy must be followed by consolidation therapy to remove any residual disease in order to achieve a long-lasting remission (6) (7).

1.2 Epidemiology of Acute Myeloid Leukemia

In AML incidence, increases with age. Advanced age is one of the strongest contributing risk factors for the development and prognosis of AML, with around 74% of patients presenting at 55 years or older (US Surveillance, Epidemiology, and End Results data; https://seer.cancer.gov/) (8).

In its latest statistics (2020), Global Cancer Incidence Mortality and Prevalence (GLOBOCAN) estimated the age-standardized worldwide incidence of leukemia to be 2.5 per 100,000 compared to the 5.2 per 100,000 recorded in 2018 while mortality has been recorded at the age standardized rate of 3.1 per 100,000, which is almost equal to the 3.5 per 100,000 documented in 2018(9).

Leukemia thus represents a small proportion of malignant diseases with a disproportionately large contribution to malignant related mortality. It is however important to note that the GLOBOCAN data which is collated by the International Agency for Research on Cancer (IARC), does not differentiate between the different types of Leukemias, therefore, the statistics is not AML specific.

In its 2019 report, the South African National Cancer Registry (SANCR) revealed an age-standardized incidence of leukemia before the age 74 to be 0.72 per 100,000 and 0.63 per 100,000 for males and females, respectively. These incidences once again were not specific to AML but applied to all Leukemias whether acute or chronic.

1.3 Classification of AML

The World Health Organization (WHO) approach to disease classification attempts to define clinicopathologic entities based on a combination of clinical features, morphology, immunophenotype, cytogenetics and molecular genetics (10).

For AML, this approach, first used in the 3rd edition, WHO classification in 2001 (10), was a major departure from the primarily morphologic and cytochemical approach used by the original French American-British Cooperative Group in 1976 (FAB classification) in which AML was classified by morphologic features of the blast cells.

The 2016 WHO classification of AML utilizes all aspects of the diagnostic approach by having entities defined primarily by clinical history, cytogenetic results, molecular genetic results and morphologic and immunophenotype. All this information is now necessary to properly classify cases of AML. This requires a more coordinated effort from all the specialists that are involved in diagnosing and treating AML patients.

This approach eventually results in a diagnosis that more clearly defines disease groups, including prognostic features and potential new therapeutic targets (10). The attached tables show the traditional FAB classification (Table 1.1) and the recent updated 2016 WHO classification (Table 1.2). It is important to note that there are provisional entities that have been added onto the 2016 revised classification awaiting further review and final decision.

Table 1.1: AML FAB subtype Classification System

MO	Undifferentiated AML
M1	AML without maturation (poorly differentiated)->90% blasts
M2	AML with maturation (more differentiated)-<90% blasts
M3	Acute Promyelocytic Leukemia (APL)
M4	Acute Myelomonocytic Leukemia
M5a	Acute Monoblastic Leukemia->80% blasts
M5b	Acute Monocytic Leukemia-<80% blasts
M6	Acute Erythroblastic Leukemia
M7	Acute Megakaryoblastic Leukemia

Note. Adapted from "Acute Myelogenous Leukemia and Acute Promyelocytic Leukemia " by WHO. 2014. Retrieved from http://www.who.int/selection medicines/committees:/expert/20/applications/AML APL.pdf

Table1.2: 2016 WHO Classification of AML

AML with recurrent genetic abnormalities	AML with t(8;21); RUNX1-RNX1T1 AML with inv (16) or t(6;16);CBFB-MYH11 APL PML-RARA AML with t(9;11); KMT2A MLLT3 AML with t(6;9); DEK-NUP214 AML with inv (3) or t(3;3); GATA2, MECOM AML with t(1;22); RBM15-MKL1 AML with mutated NPM1 AML with biallelic mutations of CEBPA Provisional entities: AML with BCR-ABL1 AML with mutatedRUN1
AML with myelodysplasia-related changes	
Therapy-related myeloid neoplasms	
AML, not otherwise specified (NOS)	AML with minimal differentiation AML without maturation
	AML with maturation Acute myelomonocytic leukemia Acute monoblastic/monocytic leukemia Pure Erythroid leukemia Acute megakaryoblastic leukemia Acute basophilic leukemia Acute panmyelosis with myelofibrosis
Myeloid sarcoma	
Myeloid proliferations related to Down syndrome	Transient abnormal myelopoiesis (TAM) Myeloid leukemia associated with Down syndrome

Note. Adapted from "Acute Myelogenous Leukemia and Acute Promyelocytic Leukemia " by WHO. 2014. Retrieved from http://www.who.int/selection medicines/committees:/expert/20/applications/AML APL.pdf

CHAPTER 2: LITERATURE REVIEW

2.1 Cytogenetic and molecular studies

Cytogenetic profile constitutes a single strongest prognostic factor for CR and OS. Based on cytogenetic abnormalities patients are stratified into either good, intermediate, or poor prognosis for response to chemotherapy as well as overall disease outcome (6).

Cytogenetic abnormalities that display better prognosis to chemotherapy are those that have balanced fusion genes such as t(8;21), t(15;17) and inv(16). The intermediate group is largely dominated by normal karyotype AML (NK-AML) and to a lesser extent the group of patients that are not included in either favourable or adverse prognosis groups (Table 1.3 below). The adverse prognostic group is associated with resistance to chemotherapy, a high relapse rate and poor overall outcomes. It includes t(6;9),inv(3) and complex karyotypes(11). The understanding of molecular mutation and its heterogenicity has become apparent in the past 15 years. These molecular abnormalities add value in further sub classifying AML and most importantly in the development of new targeted therapies(12).

The Cancer Genome Atlas Research Network analysed the genomes of 200 patients with AML. The genes that were significantly mutated were organized into several functional categories. These categories included mutations in signalling genes (FLT3-ITD), myeloid transcription factors (RUNX1), nucleophosmin 1 gene (NPM1), spliceosome-complex gene such as SRSF2, cohesin complex gene (STAG2 and RAD21), epigenetic homeostasis genes (ASXL1), tumour suppressor genes (TP53) and DNA methylation genes (IDH 1/IDH 2). Using this information a few targeted therapies have been developed and some recently approved for use in AML (13).

A local cohort done in 2014 which was looking at the incidence of FLT3-ITD and NPM1 mutation in the South African context, showed a frequency of 7,5% and 12% of NPM1 and FLT3-ITD-ITD, respectively which is lower compared to international data(14).

Table1.3 European Leukemia Net (ELN) Molecular Genetic and Cytogenetic

Alterations in AML.

Risk Profile	**Subsets**
Favourable	t(8;21) (q22;q22); *RUNX1-RUNX1T1* ***; t(15 ;17)*** inv(16) (p13.1q22) or t(16;16) (p13.1;q22); CBFB-MYH11 Mutated *NPM1* without *FLT3-ITD-ITD* (normal karyotype) Biallelic mutated *CEBPA* (normal karyotype)
Intermediate -1	Mutated *NPM1* and *FLT3-ITD-ITD* (normal karyotype) Wild-type *NPM1* and *FLT3-ITD-ITD* (normal karyotype) Wild-type *NPM1* without *FLT3-ITD-ITD* (normal karyotype)
Intermediate-2	t(9;11) (p22;q23); *MLLT3-KMT2A* Cytogenetic abnormalities not classified as favourable or adverse
Adverse	Inv(3) (q21q26.2) or t(3;3) (q21;q26.2); GATA2-MECOM t(6;9) (p23;q34); DEK-NUP214 t(v;11) (v;q23); KMT2A rearranged. -5 or del(5q); -7; abn (17p); complex karyotype

Note. Adapted from "Molecular Genetic Markers in Acute Myeloid Leukemia " by S.Yohe. 2015. *J. Clin Med*, 4(3), 462

2.2 Therapy-related AML

Therapy-related AML (t-AML) is one of the most severe long-term sequelae of anticancer chemotherapy and radiotherapy. Over the past 15 years the number of t-AML have emerged four-fold in the United States alone. This devastating cancer is more aggressive and tends to be resistant to standard chemotherapy of AML. Thus, the median overall survival of patients with t-AML is 8-10 months, with a 5-year survival rate not exceeding 10% (15).

T-AML most often occurs after anticancer chemotherapy with alkylating agents and topoisomerase 2 inhibitors, as well as after radiotherapy. Typically, tumour cells resulting from chemotherapy with alkylating agents and radiotherapy are characterized by partial loss of chromosome 5 or 7, or loss of entire chromosome 7 with their latency period lasting an average of 5 years (16). In contrast, topoisomerase 2 inhibitors induced t-AML is characterized by 11q23 translocation involving the KMT2A gene, with a latency period of about 1-2 years and generally responds better to chemotherapy compared to the one induced by alkylating agents(16). However, t-AML only occurs in 1 out of 10 patients who have the same type of cured primary tumour, similar clinical course of the diagnosis and have been treated using the same protocol.

Numerous attempts to reveal the reason for predisposition to t-AML have yielded rather ambiguous results. In some cases, t-AML has been treated with less leukaemogenic cytostatic drugs. Although, this approach has worked in some cases where leukaemogenic chemotherapy was replaced by equally potent but less marrow toxic drugs, it is however, dangerous in that it may lead to failure in curing the primary tumour. In other cases, positive results have been obtained after replacing the MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) protocol, which was used for Hodgkin lymphoma during 1971-1984, replaced by more effective but less marrow toxic ABVD (Adriamycin, bleomycin, vincristine and dacarbazine) just to mention a few(16)

2.3 Treatment and Outcome

The general therapeutic strategy in patients with AML has not changed significantly in more than four decades in our institution.

The primary assessment is to determine if the patient is eligible for intensive induction chemotherapy or not. Eligible patients first undergo induction therapy to achieve CR. Unfortunately, MRD often persists, and relapse will occur if treatment is abruptly discontinued. Therefore, favourable response to induction therapy must be followed by consolidation therapy to remove any residual disease and provide long-lasting remission (7).

Standard induction therapy entails a seven-day continuous intravenous infusion with cytarabine(100-200mg/m^2) and three days of intravenous anthracycline with daunorubicin 60-90mg/m^2 (idarubicin10-12mg/m^2may replace daunorubicin in younger patients).This regimen is commonly known as the '7+3 regimen'(5). The same protocol for AML treatment is used at CMJAH with no change for more than 30 years. The commonest reason for not treating certain patient is when the risk of TRM outweighs the benefit of supportive therapy and that is generally based on clinical and functional status of the patient.

Numerous studies have looked at anthracycline dosing, comparing the current dose to either higher or lower doses but the results have not shown any additional benefits. One such a study was done in the United Kingdom, where 60mg/m^2 vs. 90mg/m^2 of daunorubicin showed no difference in the rate of CR or rate of OS (17).

Compared to anthracycline however, recent studies of cytarabine confirmed earlier studies which demonstrated increased toxicity without improvement in efficacy with higher doses of bolus cytarabine (2000-3000mg/m^2) in the induction phase. Although there was one randomized study that showed prolonged OS with cytarabine at 3000mg/m^2 compared to 100mg/m^2 continuous infusion in cycle1, it was only in the patients younger than 45 years of age. Furthermore, the bulk of the evidence suggests that cytarabine at doses above 1000mg/m^2 should not be included in the induction regimens (18).

With the current regimen, a complete response of 60-80% is achieved in adults 60 years and younger, whereas inferior results are seen in adults above 60 years (40-60%). It is however important to note that, age as an isolated entity should not be a reason to withhold intensive therapy. A holistic analysis of disease-related and patient-related factors must be considered. Locally, there is lack of conclusive data regarding treatment outcomes in AML.

Following induction therapy patients should receive consolidation treatment, either chemotherapy or stem-cell transplant. In adults who are 60 years and younger, an increasingly preferred regimen is 2-4 cycles of intermediate dose cytarabine. The most appropriate dose and number of cycles remain inconclusive; however, current data shows that doses of 2000-3000mg/m^2 are above the plateau of maximal therapeutic effect. This method is generally used in patients with a favourable cytogenetic profile with cure rate of around 60-70% (5).

Prospective randomized trials comparing single agent high dose of cytarabine with multiagent therapy in adult patients who are 60 years and younger have not shown a significant difference in survival. However, in patients with unfavourable cytogenetic profile where extended CR is unlikely, Allogeneic Haematopoietic Stem Cell Transplant (Allo-HSCT) is recommended. Allo-HSCT offers the chance for relapse-free survival and cure to many AML patients. It might be performed in first CR or delayed until after the first relapse due to relevant treatment-related morbidity and mortality. Unfortunately, in our setting its not available as a treatment

option.

There are newer recently approved medicines that are showing promising results as add-on to the current regimen. A recent meta-analysis of five randomized trials showed that adding *gemtuzumab ozogamicin* (GO) (a humanized anti-CD33 monoclonal antibody conjugated with the cytotoxic agent calicheamicin) to induction therapy did not increase response rates; it did however reduce the risk of relapse and improved survival among younger and older adults with a favourable-risk and intermediate-risk cytogenetic profile (19).

Recent studies have also confirmed these GO findings; furthermore, dosing that showed beneficial outcome was 3mg/m^2on days 1, 4, and 7 compared to a single dose of GO at 3mg/m^2. GO is currently only available in clinical trials and through a compassionate use program approved by the US Food and Drug Administration (FDA), but it is not available in South Africa.

A trial that evaluated the use of midostaurin (FLT3-ITD inhibitors) during both intensive induction and consolidation phase in patients with FLT3-ITD-mutated AML showed that midostaurin use increased CR rate thus patients with FLT3-ITD-mutated AML may be considered to receive intensive chemotherapy in combination with midostaurin (20).

CPX-351, an encapsulation in a nano-scale liposome of cytarabine and daunorubicin (Vyxeos) at a synergistic 5:1 molar ration, showed promising results in secondary and therapy-related AML. Moreover, when compared to '7+3' in adults above 60 years with high-risk AML defined as AML with myelodysplasia or therapy related AML, it produced higher response rates and longer OS (21).

2.4 Significance of the study

The latest SANCR report revealed that although AML is a rare cancer in South Africa, it has one of the highest cancer mortalities. Unfortunately, there are only a handful of studies done locally that assess the frequency of specific cytogenetic and molecular findings in AML patients in the South African context. AML predominantly affects the elderly with an average age of 67 years at diagnosis and many have concomitant comorbidities. This often makes it difficult to decide on the best treatment plan.

Introduction of newer investigational modalities such as the Next Generation Sequencing make for better understanding of this highly heterogenous disease. Furthermore, development of targeted therapies may improve treatment plans and outcomes. Therefore, the results of this study compared with international trends and statistics can be used to reflect on local trends for the betterment of treatment plans and outcomes in AML.

CHAPTER 3: AIMS AND OBJECTIVES

3.1 Aims

The primary aim of the study was to review the specific cytogenetic and molecular trends in AML patients treated at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between years 2017 - 2021. The study also reviewed all cases of therapy-related AML treated at CMJAH in the past 10 years.

3.2 Study Objectives

3.2.1 Primary Objectives

The primary objective was to examine all results of AML patients treated at CMJAH in 2017-2021(Cohort A), paying attention to:

3.2.1.1 Patient's demographics

3.2.1.2 Clinical and Laboratory findings

3.2.1.3 Cytogenetic and molecular features

3.2.1.4 Treatment and outcome (outcome refer to remission vs. progressive disease) Secondary Objectives

3.2.2 The secondary objective was to review all therapy-related AML patients treated at CMJAH between the years 2011 - 2020 (Cohort B), with special interest to:

3.2.2.1 Cytogenetics profile

3.2.2.2 Treatment plans and treatment outcomes

CHAPTER 4: METHODS

4.1 Study setting

The study was conducted at Division of Medical Oncology, Department of Internal Medicine , at Charlotte Maxeke Johannesburg Academic Hospital, situated in Johannesburg, South Africa.

4.2 Study Design

This was a retrospective descriptive, cross-sectional study that reviewed data collected from medical records of Division of Medical Oncology, Internal Medicine Department at CMJAH and sample results obtained from National Health Laboratory Services (NHLS) database.
Study population: 82 files of patients that were treated for *de novo* AML at CMJAH between 2017 – 2021, (Cohort A). 8 files of patients who were diagnosed and treated for therapy-related AML at CMJAH between 01/01/2011and 31/12/2020 (Cohort B). Hence 90 files were reviewed for this study.

4.3 **Inclusion criteria were**:

4.3.1 Patients 18 years and above of age

4.3.2 Confirmed diagnosis of AML at CMJAH between 01/01/2017 to 31/07/2021(Cohort A)

4.3.3 All patients 18 years or older with confirmed therapy-related AML treated at CMJAH between 01/01/2011 to 31/01/2020 (Cohort B)

4.4 Exclusion criteria were:

4.4.1 Patients who are less than18 years of age

4.4.2 Diagnosis of AML at CMJAH before the year 2011

4.5 Data collection

This study used data collection tool (Attached on Appendix) to obtain demographic information of qualifying subjects. Clinical and laboratory findings were retrieved from the files as well as treatment given and outcome thereof. But most importantly, cytogenetic, and molecular studies information was retrieved from the NHLS database.

4.6 Data analysis

The quantitative data was reported as means and standard deviation for normally distributed variables and median and interquartile ranges were used otherwise. Frequencies and percentages were used for categorical variables. Visual presentation of categorical variables was done using pie charts and bar charts. Bivariate analysis was done using the Chi-square test to assess the association between two categorical variables. Time to mortality was assessed using the Kaplan-Meier curves and the log-rank test was used to assess for a significant difference in the mortality rates between groups of a categorical variable. The proportional hazard Cox model was used to determine factors associated with mortality using univariate and multiple regression models. The proportional hazard test was performed as a post estimation to assess if the constant hazard assumption was violated or satisfied. The significance of the analysis was set at 5% and all the analysis was performed using Stata 16 (StataCorp. 2021. College Station, TX, USA).

4.7 Ethics and Approvals

Ethics approval was obtained from the University of Witwatersrand Human Research Ethics Committee (HREC). Permission was obtained Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and from the Head of Medical Oncology Department before the commencement of the study.

The confidentiality was maintained by not having patient's identifiers and by making sure that only the researcher had access to the study material/information. An ethical consideration related to data collection in this study encompassed principle of respect of human dignity, principle of anonymity and confidentiality, principle of beneficence and justice. All data were kept in a password protected computer where access was only restricted to the researcher. Informed consent was not necessary as the study involved retrospective reviewing files of the subjects. All the data collected were used for the purpose of the study only.

CHAPTER 5: RESULTS

Figure 1: Age distribution of participants

The study had a total of 90 participants. The median age was 40 years with an interquartile range (IQR) of 32-59 years. Figure 1 shows the age distribution of the study participants. Majority of the patients (52.22%, n=47) were in the age group 19-40years, followed by the 41-60 age group with 23 (25.56%) patients and the over 60 years had 20 (22.22%) of the patients.

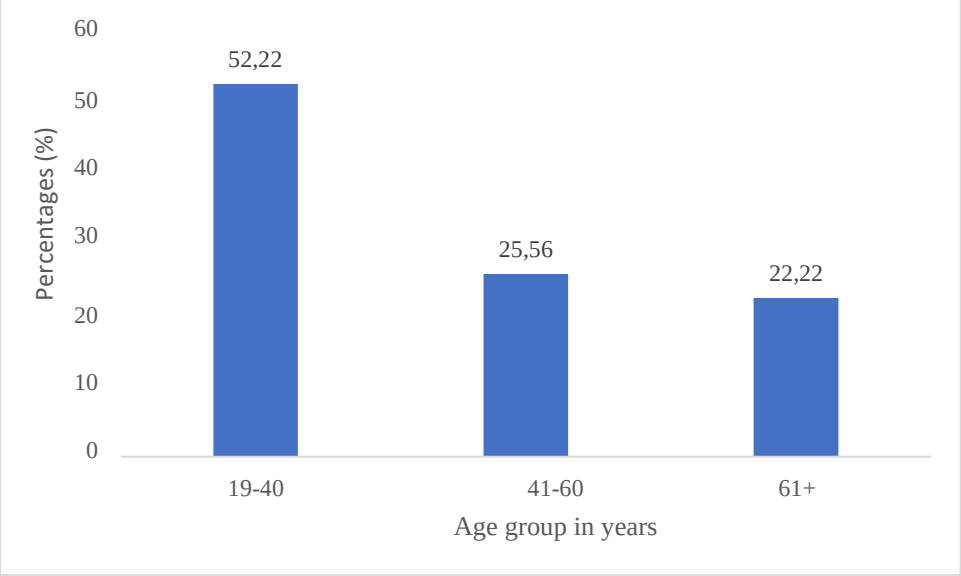


Table 1: Descriptive socio-demographic characteristics of the participants

The majority of the patients were females (54.44%, n=49) from a black ethnicity 71.11%(n=64).

Variable	Categories	Frequencies	Total
Age categories	19-40 41-60 60+	47 23 20	52.22 25.56 22.22
Gender	Female Male	49 41	54.44 45.56
Ethnicity	Black Asian White	64 1 25	71.11 1.11 27.78

Table 2: Descriptive socio-demographic characteristics of the participants in the t-AML cohort

There were 8 patients in the therapy-related AML. The median age in this cohort was 58.5

(IQR: 41-67) years. Majority of them were females from black ethnicity (62.5%, n=5)

Variable	Total n=8
Age Median(IQR)	 58.5(41-67)
Gender Female Male	 5(62.5) 3(37.5)
Ethnicity Black White	 5(62.5) 3(37.5)

Table 3: Clinical and prognostic characteristics of the participants stratified by cytogenetic classification.

Of the 90 patients, there were 48.89%(n=44) patients in the intermediate group, 14(15.56%) in the unfavourable group and 32(35.56%) in the favourable group. The majority of the participants had anaemia (97.78%, n=88), 76.67%(n=69) had no infection, 51.11%(n=46) had bicytopenia, 63.33%(n=57) were HIV negative and 73.33%(n=66) had no dysplasia.

Variable	**Intermediate n(%) 44(48.89)**	**Unfavourable n(%) 14(15.56)**	**Favourable n(%) 32(35.56)**	**Total**
Anaemia				
No	2(4.55)	0	0	2(2.22)
Yes	42(95.45)	14(100)	32(100)	88(97.78)
Infection				
No	34(77.27)	11(78.57)	24(75.0)	69(76.67)
Yes	10(22.73)	3(21.43)	8(25.0)	21(23.33)
Other presentations				
Bicytopenia	24(54.55)	7(50.0)	15(46.88)	46(51.11)
Bleeding	2(4.55)	0	8(25.0)	10(11.11)
Pancytopenia	7(15.91)	4(28.57)	5(15.63)	16(17.78)
Other	11(25.0)	3(21.43)	4(12.5)	18(20.0)
HIV				
Negative	28(63.64)	9(64.29)	20(62.5)	57(63.33)
Positive	5(11.36)	3(21.43)	4(12.5)	12(13.33)
Unknown	11(25.0)	2(14.29)	8(25.0)	21(23.33)
Dysplasia				
No	33(75.0)	4(28.57)	29(90.63)	66(73.33)
Yes	7(15.91)	10(71.43)	1(3.13)	18(20.0)
Missing	4(9.09)	0	2(6.25)	6(6.67)

Table 4: Clinical characteristics of the participants in the therapy-related AML cohort.

All the patients in this cohort had anaemia, 87.5% (n=7) had no infection, 62.5% (n=5) had bicytopenia, 62.5%(n=5) were HV negative, 75%%(n=6) had no dysplasia and 3(37.5%) had complete remission. Of these who had complete remission, 2 of them relapsed while 1 remains in remission. There were 5(62.5%) patients who had refractory disease.

Variable	**Total (n=8)**
Anaemia	
Yes	8(100%)
Infection	
No	7(87.5%)
Yes	1(12.5%)
Other presentations	
Bicytopenia	5(62.5%)
Pancytopenia	3(37.5%)
HIV	
Negative	5(62.5%)
Positive	1(12.5%)
Unknown	2(25.0%)
Outcome (multiple outcomes)	
CR	3(37.5%)
Relapse	2(25.0%)
Remission	1(12.5%)
RD	5(62.5%)

CR-Complete remission, RD-Refractory disease

Figure 2 : FAB classification subtype of all the patients.

Of the 34 participants with FAB information, 14.44%(n=13) were classified under M3 while 20% (n=18) were classified under M2. There was a huge number (48.88%) of patients with missing FAB classification.

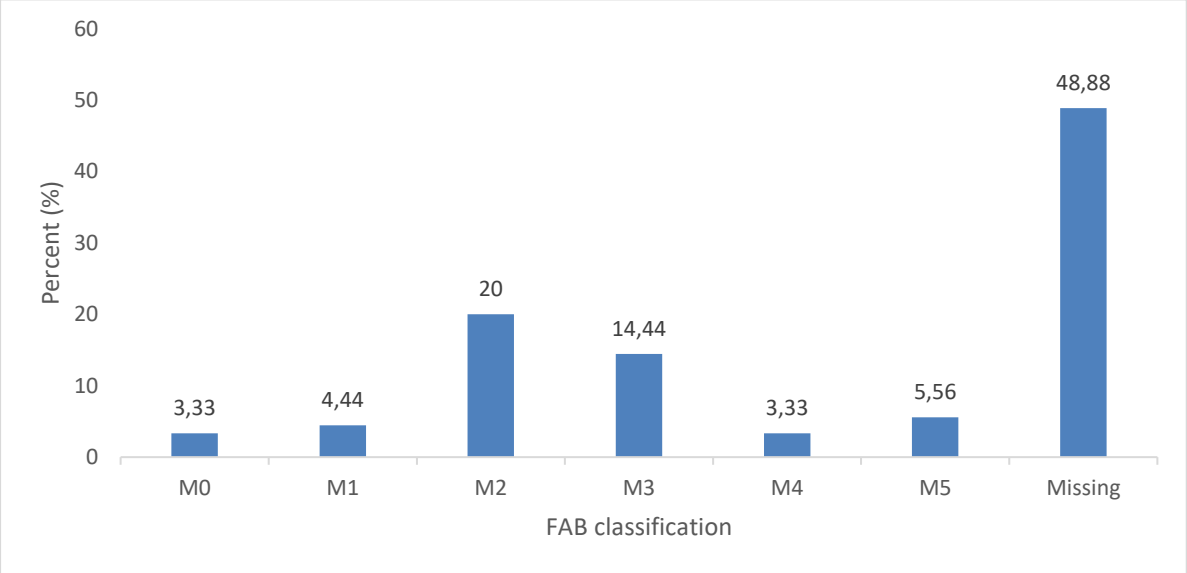


Table 5: Bone marrow morphological, cytogenetic, and molecular features of all participants

The majority of the patients had normal molecular analysis (n=49, 54,44%). Where abnormal results were present, 12 were PML-RARA and 18 were RUNX1-RUNX1T1. The t(8,21) was the most common cytogenetic abnormality detected (n=18, 20.0%).

Variables	Categories	Frequency (n)	Percentage (%)
FAB (n=90)	M0	3	3.33
	M1	4	4.44
	M2	18	20.0
	M3	13	14.44
	M4	3	3.33
	M5	5	5.56
	Missing	44	48.88
Molecular (n=90)	Normal	49	54.44
	DNMT3A/IDH2	1	1.11
	FLT3	2	2.22
	FLT3, NPM1	2	2.22
	KMT2A	1	1.11
	NPM1	4	4.44
	PML RARA	12	13.33
	RARA,FLT3	1	1.11
	RUNX1	18	20.0
Cytogenetics (n=90)	Deletion 5	2	2.22
	Deletion 7	4	4.44
	Deletion 5&7	4	4.44
	Monosomy 8	1	1.11
	Mon 3,5,14,15,16	1	1.11
	Normal	45	50.5
	t(11,17)	1	1.11
	t(15,17)	13	14.44
	t(6,9), Mono 7	1	1.11
	t(8,21)	18	20.0

Table 6: Bone marrow morphological, cytogenetic, and molecular features of all participants in the t-AML cohort.

Among AML cohort, there was one patient under M3 and one patient under M4 classifications. One patient had PML-RARA, and one patient had RUNX1-RUNX1T1. Deletion 5 & 7 was observed in 2 patients.

Variables	Categories	Frequency (n)	Percentage (%)
FAB	M3	1	12.5
	M4	1	12.5
	Missing	6	75.0
Molecular	RARA	1	12.5
	RUNX1	1	12.5
	Normal	6	75.0
Cytogenetics	Deletion 7	1	12.5
	Deletion 5 & 7	2	25.0
	Normal	2	25.0
	t(11,17)	1	12.5
	t(15,17)	1	12.5
	t(8,21)	1	12.5

Table 7: Treatment of AML and treatment outcomes

About 70(77.78%) participants received induction chemotherapy while 23.33% did not get any dose of chemotherapy. Of 70 participants that received induction chemotherapy 16(17.78%) proceeded to consolidation therapy, 3(3.33%) are currently on maintenance chemotherapy and 2 are just under surveillance. A total of 22(24.44%) participants had complete remission while 52.22% experienced refractory disease. Of the 22 patients that went to CR, 12 relapsed and later died, 5 were lost to follow-up and 5 are in remission and still alive. Of the 47 participants that had RD, 6 received salvage chemotherapy in a form of HiDAC (high dose Cytarabine).

Induction	**Frequency (n)**	**Percentage (%)**
Induction chemotherapy		
Yes	70	77.78
No	20	22.22
Salvage Chemotherapy		
Yes	6	6.67
No	17	18.89
Unknown	24	26.66
N/A	43	47.77
Consolidation		
Yes	16	17.78
No	74	82.22
Maintenance therapy		
No	87	96.67
Yes	3	3.33
Outcome (multiple outcomes)		
CR	22	24.44
Relapse	12	13.33
LTFU	5	5.56
Remission and Alive	5	5.56
No Treatment	21	23.33
RD	47	52.22

CR; Complete Remission, RD; Refractory Disease; LFTU; Lost to Follow Up.

*No treatment includes Refused Chemo, for Best supportive care and died awaiting chemotherapy

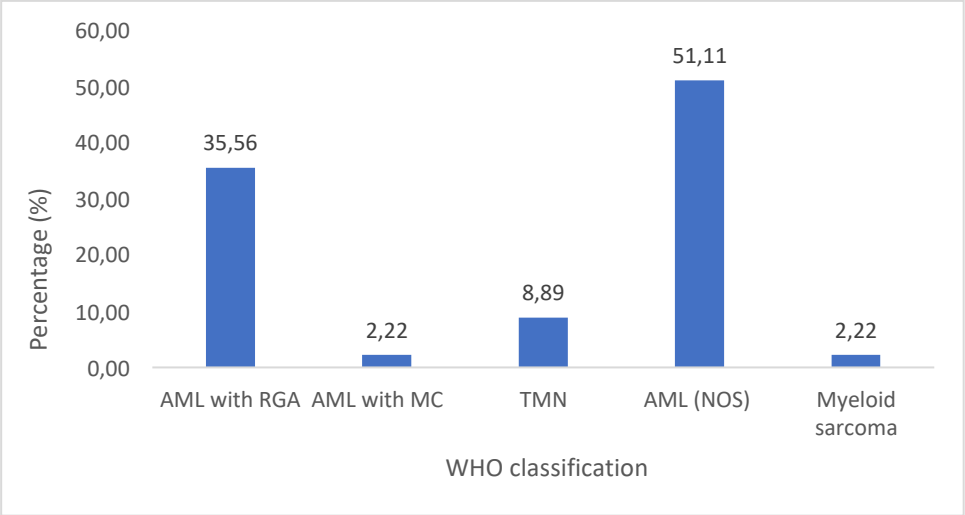
Table 8: Therapy-related AML treatment results.

A total of 7(87.5%) were on chemotherapy. Specifically, 7(87.5%) received induction chemotherapy, 3(37.5%) proceeded to consolidation chemotherapy and only one person was on maintenance chemotherapy. Three (37.5%) of those in the t-AML cohort were in complete remission, of those, 1 was lost to follow up, 1 relapsed and died and one alive and in remission. Overall mortality was as high as 75% in this cohort.

Induction	**Frequency (n)**	**Percentage (n)**
Induction chemotherapy		
Yes	7	87.5
No	1	12.5
Consolidation		
Yes	3	37.5
Missing	5	62.5
Maintenance therapy		
No	7	87.5
Yes	1	12.5
Outcome (multiple outcomes)		
CR	3	37.5
LTFU	1	12.5
Remission and Alive	1	12.5
Relapsed	1	12.5
RD	5	62.5
Died		
Yes	6	75.0
No	2	25.0

Figure 3: WHO classification.

There were 32 (35.56%) of AML patients with recurrent genetic abnormalities, 2(2.22%) had AML with myelodysplasia-related changes, 8 (8.89%) had therapy-related myeloid neoplasms, 46(51.11%) had not otherwise specified AML and 2(2.22%) had myeloid sarcoma.



RGA; recurrent genetic abnormalities, MC; Myelodysplastic changes, TMN; therapy-related myeloid neoplasm, NOS; Not otherwise specified, *Myeloid sarcoma-extramedullary tumor of immature granulocyte cells

Figure 4: Outcome of all AML patients.

There were 80(88.88%) of the AML patients who later died, 5(5.55%) became lost to follow-up and 5(5.55%) were alive at the end of the study.

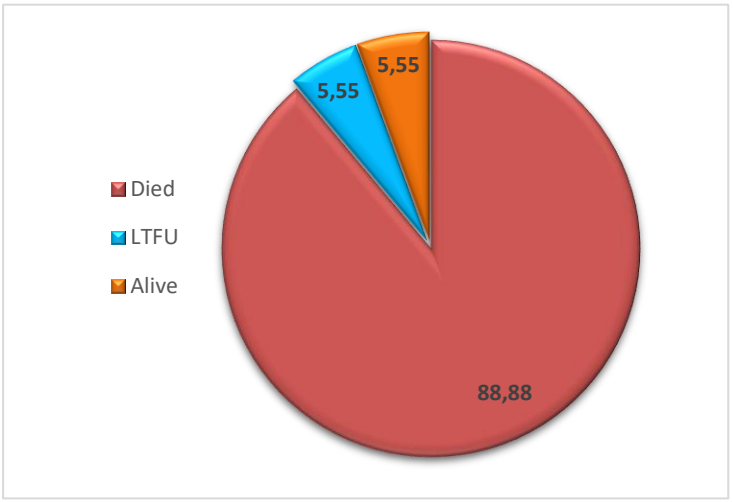


Figure 5: Survival probability of the AML patients over time.

The mortality rate was very high as 202.32 per 100 person-years (95%CI: 161.1-254.1) and this was very steep. Most deaths happened in the first year, the first half of the year to be more precise.

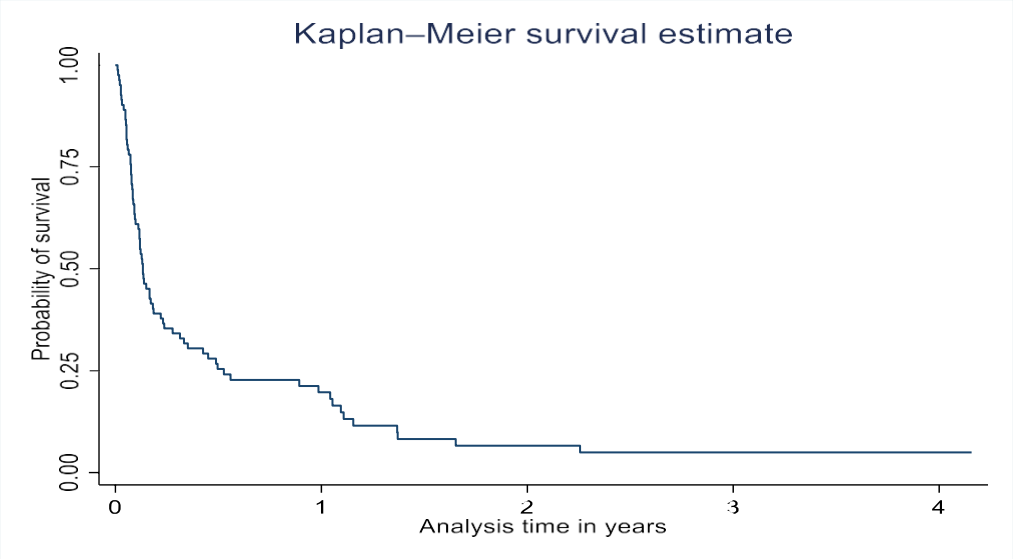


Figure 6: Survival probability stratified by HIV status, gender, age, and infection status

The bivariate association of mortality was assessed for HIV status, gender, age groups, having an infection. The Kaplan-Meier curves were used to describe the survival probabilities of AML patients (Figure 6). Those who were HIV positive were less likely to die compared to those who were HIV negative; however, this was not statistically significant (Log-rank p-value=0.5773). There was no significant difference in the survival of males and females (Log-rank p-value=0.7767). The older the patients, the more risk of death they had, and this was statistically significant (Log-rank p-value=0.0091). There was no significant difference in the survival by infection status (Log-rank p-value=0.5217).

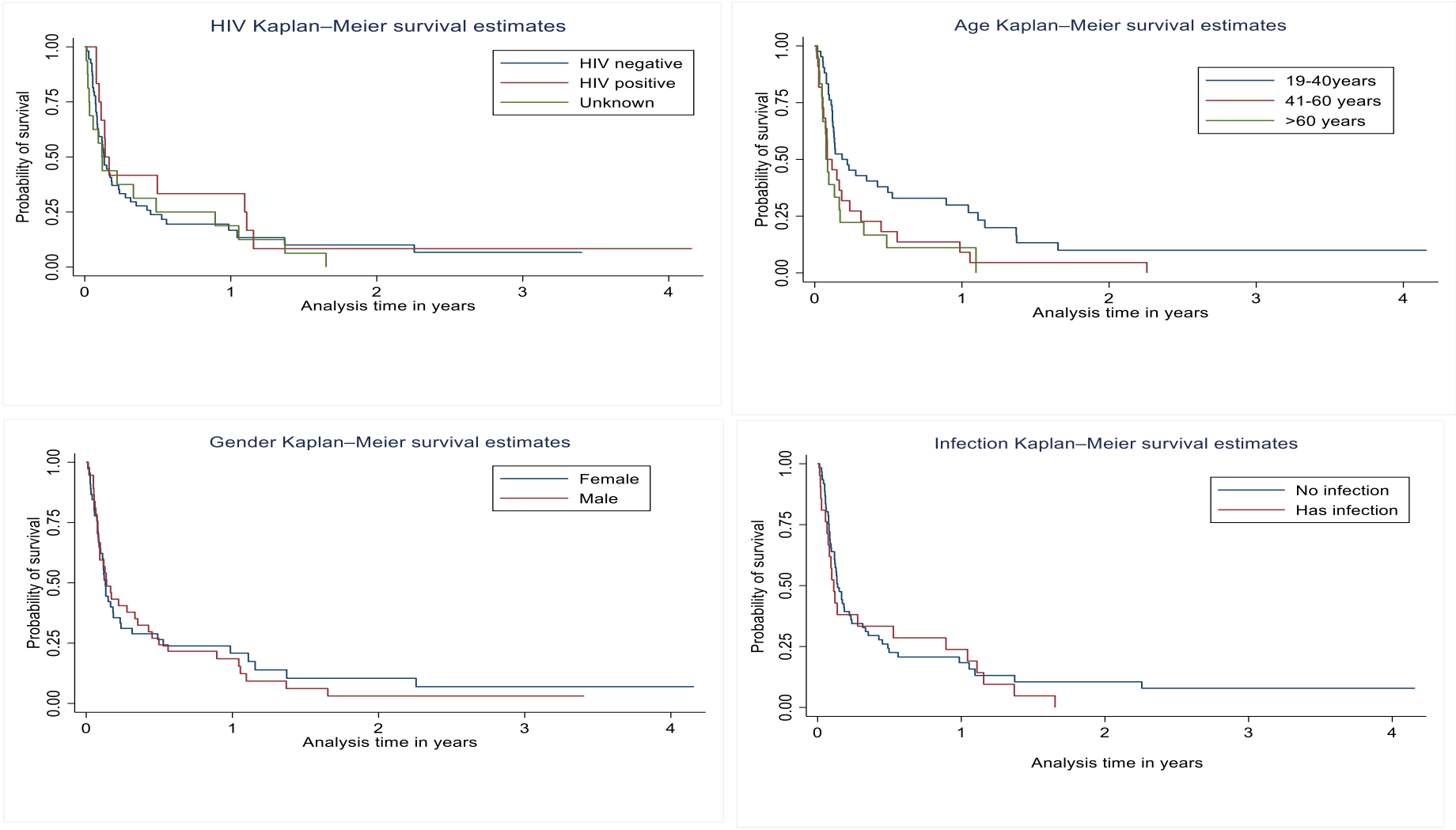
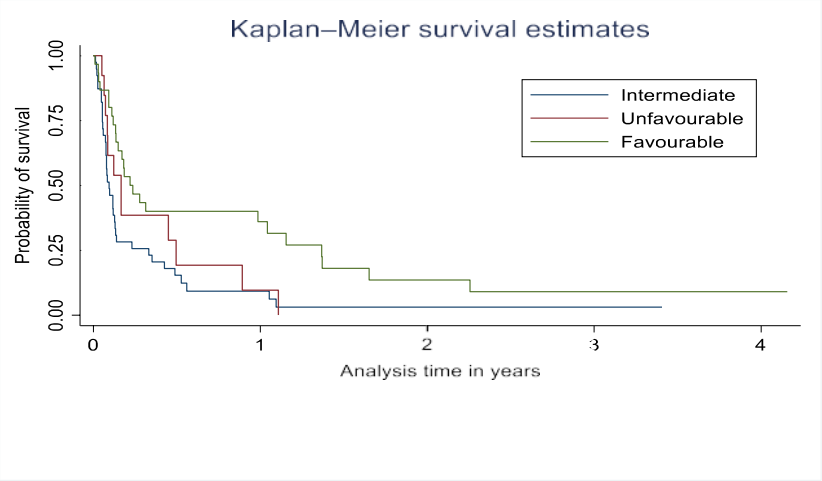


Figure 6: Survival probability stratified by HIV status, gender, age, and infection status

Figure 7: Survival probability stratified by cytogenetics

The Kaplan-Meier curves were used to describe the survival probabilities of AML patients by cytogenetics (Figure 7). Those who were intermediate were more likely to die followed by those who were unfavourable, and this was statistically significant (Log-rank p-value=0.019).



Univariate Cox regression analysis

Cox regression was used to determine the factors that were associated with mortality among the patients. Those who had unfavourable cytogenetics were 55% (Hazard ratio (HR)=0.45; 95%CI: 0.27-0.76) less likely to die compared to those who were intermediate cytogenetics. There was relationship with age, the older the patients get the more at risk they were to die. Those who were aged 41-60 years were 1.85 times (95%CI: 1.08 – 3.16) more likely to die while those who were above 60 years were 2.26 times (95%CI: 1.24 – 4.09) more likely to die compared to those who were aged below 40 years.

Table 9: Factors associated with mortality

Induction	**Univariate Cox regression**			**Adjusted Cox regression**		
	HR	**95%CI**	**P-value**	**AHR**	**95%CI**	**P-value**
Cytogenetics						
Intermediate	1(base)0			1(base)		
Unfavourable	.76	0.40-1.46	0.411	0.81	0.41–1.61	0.543
Favourable	0.45	0.27-0.76	**0.003**	0.47	0.26–0.85	**0.011**
Sex						
Female	1(base)			1(base)		
Male	1.07	0.68–1.69	0.777	0.92	0.57-1.48	0.723
Age categories						
19-40years	1(base)1			1(base)2		
41-60years	.85	1.08–3.16	**0.026**	.17	1.18–3.99	**0.013**
>60years	2.26	1.24–4.09	**0.007**	2.17	1.02–4.63	**0.045**
HIV						
Negative	1(base)			1(base)0		
Positive	0.76	0.39–1.47	0.41	0.73	0.35–1.51	0.388
Unknown	1.14	0.64–2.01	0.664	0.96	0.53–1.75	0.899
Infection						
No	1(base)			1(base)		
Yes	1.18	0.71–1.97	0.523	2.03	1.12–3.67	**0.02**
Race						
Black	1(base)			1(base)		
Other races	1.41	0.85–2.32	0.181	1.00	0.56–1.81	0.997
CR						
Complete remission	1(base)4			1(base)3		
No chemotherapy	.81	2.46–9.42	**<0.001**	.57	1.63–7.79	**0.001**
Residual disease	2.78	1.61–4.79	**<0.001**	2.73	1.46–5.11	**0.002**

Those who had the residual disease were 2.78 times (95%CI: 1.61-4.79) more likely to die while those who had no chemotherapy were 4.81times (95%CI: 2.46-9.42) more likely to die compared to those who had complete remission.

Multiple Cox regression analysis

Multiple Cox regression was fitted to estimate the adjusted risk of mortality. Adjusting for other factors, those who had unfavourable cytogenetics were 74% (adjusted hazard ratio (AHR)=0.26;95%CI:0.26–0.85) less likely to die compared to those who were intermediate. Adjusting for other factors, there was a relationship with age; the older the patients get the more at risk they were to die. Those who were aged 41-60 years were 2.17 times (95%CI: 1.18–3.99) more likely to die while those who were above 60 years were 2.17 times (95%CI: 1.02 – 4.63) more likely to die compared to those who were aged below 40 years. Adjusting for other factors, those who has an infection were 2.03 times (95%CI: 1.12 – 3.67) more likely to die than those who had no infection. Adjusting for other factors, those who had the residual disease were 3.57 times (95%CI: 1.63 – 7.79) more likely to die while those who did not receive chemotherapy were 2.73 times (95%CI: 1.46 – 5.11) more likely to die compared to those who obtained complete remission.

CHAPTER 6: DISCUSSION

The cohort of this study was composed of 82 *de novo* patients and 8 therapy-related AML patients. Young adults with a mean age of 40 years were majority females (54.44%) and of black descent (71.11%). However, this racial distribution may not be of significant, and it should not be taken into consideration because it reflects the general demographic of the country, with Blacks representing 86% of the entire population (22).

The reason for this unequal gender distribution with females more admitted than males is not known. The researcher could only assume that it depends on different demographics where the studies were conducted (23, 24). This present cohort had more females than males, 54.44% (49/90) and 45.56% (41/90). Even if the female proportion was higher than the male counterparts, the difference was not statistically significant. Likewise, some studies have dealt with male patients predominantly, without mention of female counterparts, for the same demographic gender distribution (24).

AML patients always need to be characterised in groups for a better prognosis assessment shown in table 3 and table 4. According to Hulegardh and colleagues (6), a characterisation in three groups fits this purpose, with a favourable, intermediate, and adverse group, each relying on the cytogenetic profile (6). Hence, following this international standard, the researcher has classified patients in this cohort into similar categories. In this audit most participants fell under intermediate group 44 (48.89%), followed by favourable group 32 (35,56%) while unfavourable had lowest number of 14 (5.55%).

Females were in the more favourable socio-demographic category than males (59.38% vs. 40.63%); however, males were in the unfavourable group than females (57.14% vs. 42.86%), while the outcome is discussed in that respect.

In cohort B with AML therapy-related patients, the median age was higher than the one in cohort A, representing respectively 58.5% (IQR:41-67) compared to cohort A with more females (62.5% vs. 37.5%) and predominantly black ethnicity (62.5%). Overall, these demographic data demonstrate that AML affects more females than males and in relatively young age group (19-40 years) in this specific cohort. Cannot make conclusion-due to small numbers.

Strickland and Vey (25) discussed more specific aspects in line with Cohort B of this study. They define the therapy-related AML as consecutive to "ionising radiotherapy, cytotoxic therapy, or immunosuppressive therapy for unrelated disease" (26, 27). So, in this condition, one should also retain therapeutic interventions for solid tumours (breast, prostate cancers) or other malignancies (e.g., Lymphomas-Hodgkin and non-Hodgkin) or autoimmune disease (e.g., rheumatoid arthritis). Among these prior pathologies, breast cancer and NHL are the most frequent, with 4.60 and 5.85 times higher, respectively (26, 28).

Such observation, however, was not recorded in the present cohort, but it can be part of a further multicentric study on this topic. As already stated earlier on, t-AML is a rare disease, estimated between 7% to 8% and developing at different timing according to the prior pathology (26, 29). Of note, one should expect the t-AML rate to increase considering the increased number of patients treated for any cancer (30), and this t-AML group carries short OS, relapse-free survival and worse outcome compared to *de novo* AML. However, the findings of this present study do align with Strickland and Vey's conclusion because the outcome in terms of CR was higher in t-AML than in de novo-AML patients. For Strickland and Vey (25), the sequence of evolution of t-AML is 2-3 years in patients under cytotoxic therapy with topoisomerase II inhibitor, 5-7 years in patients on chemotherapy with alkylating agents, for an undetermined period "following various immunosuppressive therapies" (25).

As for the factors related to poor outcomes, Kayser and colleagues (29) pointed to "old age, adverse cytogenetics or comorbidities, certain mutations and poor bone marrow reserve secondary to prior therapy". Of note, these factors also prevail in de novo-AML patients.
The absence of this specific comorbidity**,** namely the HIV status, has likely played a protective role in this group. In other words, HIV status do not play a role in worse prognosis.
The patients should be assessed on a prognostic basis through a genetic screening on admission, following a confirmed diagnosis of AML. Such a step precedes any treatment, except in the case of a formally established infection. Therefore, on the understanding that the myeloid progenitor loses the capacity to differentiate into mature cells (24, 31), cancerous cells then undergo genetic variations leading to AML. So, genetic screening serves on the one hand, to characterise patients in different groups and, on the other hand, to guide therapeutic interventions (32). As already referred to earlier, patients were in three groups according to the prognosis. Different genes mutations found in all patients were numerous (depicted in table 5), but the most common were 20% RUNX1-RUNX1T1 (n=18), NMP1(4.44%), PML-RARA (13.33%) and 54.44% (n=49) of participants had no abnormal gene mutations detected.
The cytogenetic features for all patients were normal in 50,5% (n:45), with t(8;21) 20.0% being the commonest cytogenetic profile. In t-AML on the other hand, common FAB classes were for M3 and M4 (12,5% each). Furthermore, the molecular types were PML-RARA (12.5%); the cytogenetic features were normal in 25% and 25% of deletion in chromosome 5 and 7. The rest of subtypes represented 12.5% [(t(11,7), t(15,17), t(8,21)] (table 5 and table 6).
The researcher would make the following implication: looking at Rehman and colleagues (2021) reporting on RUNX1 mutations and elevated FLT3-ITD gene expression. RUNX1 and FLT3-ITD, recognised as the primary genes contributing to normal haematopoiesis, are disrupted (i.e., at point mutations) in this cohort as well as in all cases of AML (24). Of note,

RUNX1, also called, among other names, AML1 protein, is composed of three domains: i) Runt homology domain (RHD), ii) transcriptor activation domain (TAD), and iii) suppression domain. Concerning FLT3-ITD, it is FM like tyrosine3 "that belongs to class iii tyrosine kinase". According to Murphy and colleagues (33), FLT3-ITD is "a type of tyrosine kinase that has an expression on early haematopoietic precursor cells and contributes to the survival and differentiation of stem cells" (33).

The main common finding between Rehman and colleagues' study (24) and the present report is that in both studies, the CR was lower in older patients, irrespective of their gender. Age remains a significant factor in the outcome of AML, and it may explain such discrepancy. The older patients (above 60 years) were by as far as 2.26 times more likely to die compared to the younger patients (41-60 years), who had 1.85 times the risk to experience death (p:0.007 vs. p:0.026). A similar result was also in the report by Hsin An Hou & Hwei-Fang Tien, 2020) (p:0.009) (34). Besides patients' age, the presence of comorbidities impacts negatively on patients' survival, as reported by Hsin An Hou and Hwei-Fang Tien (34) in their review on “genomic landscape in acute myeloid leukemia and its implications in risk classification and targeted therapies”. Hence, patients' age is more predictive of death than gender.

So, using sequencing technique and polymerase chain reaction (PCR), the study findings indicated mutations and abnormalities in FLT3-ITD, which is in line with other studies. Like in Rehman and colleagues' cohort, (24) RUNX1 mutations were prevalent in older male patients (>60 years), and all patients with RUNX1 and FLT3-ITD had a lower CR and event-free survival rate and also lower OS rate than patients with wild-type RUNX1 and FLT3-ITD gene. The prognosis was worst in case they had up-regulated FLT3-ITD gene expression.

The researcher could not discuss the incidence of cytogenetics features, like FLT3-ITD and NPM1, for comparison with another South African study and for the usefulness of extracting the disease trends in our context (14). However, cytogenetics and molecular studies play a

huge role on prognosis.

This is the reason why new drugs should aim at either blocking these RUNX1 mutations or reducing the levels of FLT3-ITD for efficient management of AML, on the condition that a higher proportion of the cytogenetic architecture is still normal.

So, for Nguyen and colleagues (35), all AML patients have abnormal cytogenetics (chromosomal translocations, deletions), while some other authors hold the opinion that it is only in 50% of patients (23). They all agree that patients who do not respond to chemotherapy evolve poorly, and the survival is rarely beyond two years (23,35, 36). However, some cytogenetics have a good prognosis and others not, in contrast to molecular features (mutations, over expressed signalling pathway) that often respond better to targeted therapies. Generally, treatment in AML is based on patients' cytogenetic profiles.

Authors stressed the importance of such determination and identified groups with TP53 mutations as the worse responding to the therapeutic agents (35). For Nguyen and colleagues (35), these TP53 mutations occur with E2F4, CD 34, CD 109, MN1, NMLB and CD 200 being the most expressed genes. As a result, these over expressed genes damage immune functions, cell proliferation and DNA.

However, in the current study, analyses did not reach that depth, but this does not alter the validity of this argument. Indeed, the information provided in Nguyen and colleagues' report (35) is valuable in designing new pharmacological agents. The assumption is that new drugs would be more efficient if they target the molecular characteristics, especially in AML patients unresponsive to existing standard chemotherapies (34).

Current treatment is based on intensive induction therapy with a combination chemotherapy regimen, known as 7+3, standing for continuous cytarabine infusion for 7 days and 3 days of anthracycline (e.g., Daunorubicin or idarubicin) (5) (Table 7). However, the physicians should hold this regimen if the patient is in poor general condition (i.e., comorbidities and

less fitness). In that case, a non-intensive therapy is advisable with hypomethylating agents alone (e.g., azacytidine, decitabine, venetoclax) or in combination with azacytidine or low dose cytarabine

In this cohort, nearly all patients received standard induction chemotherapy (77.78%), with 17.78% (n=16) went on to receive consolidation therapy (table 7). Even though maintenance chemotherapy was administered in only 3.33% (n=3), less than a quarter (n=22, 24.44%) achieved complete remission. Majority of patients in this cohort had refractory disease (52.22%). Of note, among those with RD, only 6 received salvage chemotherapy. Salvage chemotherapy in our setting includes the following protocols: HiDAC (Cytarabine 2-3g/m^2 12 hourly over 6 days) or FLAG-IDA (Fludarabine, Cytarabine, Idarubicin and Filgrastim) or MEC (Mitoxantrone, Etoposide and Cytarabine). Unfortunately, we do not have any of the novel therapies hence none were included in either induction or consolidation phase of therapy. Of note, in addition none of the participants in our study received Stem-cell transplantation. The lack of Allo-HSCT in our setting as treatment option for patents who achieved CR has a potential negative impact on the overall outcome of our patients.

The overall outcome for this study was poor with mortality reaching 88.88% with only 5.55% of participants still alive at the end of the study. Of note, among the 5 that are still alive, 3 are on maintenance therapy while 2 are under surveillance. This result confirms the rationale for combining chemotherapy and maintenance on promoting long remission and reducing the high relapse and mortality rates in AML (25) (Table 7 & Table 8).

In our cohort, under the 7+3 regimen, recognised worldwide as an international standard (5), the survival time was short with majority of patients dying within 3 months after diagnosis. Even though there were proportionally more females than males in this cohort, the difference in death occurring between the two genders was not statistically significant (p:0.777).

Infection status had no significance in the death rate in the present cohort (p:0.5217). From a general therapeutic point of view, this finding points only to the urgency of vigorously treating any infection.

Other factors are the cytogenetic features and molecular characteristics. At a glance, one may think that unfavourable cytogenetics lead inevitably to rapid death. Nevertheless, an observation made from the results in this study shows, from Cox analysis, that patients with unfavourable cytogenetics, there were 55% less likely to die than those who were in the intermediate category. The patient's health status, age and wide type of mutations may probably altogether explain this result.

Lastly, 88.88% (n=80) of participants later died, 5.55% (n=5) lost to follow-up, and only 5.55% (n=5) were still alive at the end of the study (figure 4).

CHAPTER 7: CONCLUSION, LIMITATIONS and RECOMMENDATIONS

7.1 Conclusion

This study is a 5-year retrospective, cross-sectional review of patients with a confirmed diagnosis of AML admitted to a tertiary institution in Johannesburg from January 2017 to 31 July 2021. A mix cohort of 90 patients, including one group with de novo AML patients (n=82) and the other with t-AML patients (n=8), was reviewed in line with the clinical and laboratory findings, cytogenetics, and molecular features as well as treatment plans and outcome. There was, in addition, a second cohort (B) of 10 year-period with treatment related AML patients (t-AML).

The researcher recorded more females than males (54.44% vs. 45.46%), but the difference was statistically insignificant. The majority of patients were between 19-40 years (52.22%) with the median age of 40 years (IQR: 32-59 years), with the rest distributed as 25% in the age group between 41-60 years and 22.22% above 60 years. Both de novo-AML and t-AML patients had bone marrow suppression manifested with anaemia, bicytopenia and / or pancytopenia. But altogether, this sample remains a small sample, reducing the power of the findings.

overall, there were more normal molecular karyotypes in 54.44% (n=49) and abnormal features detected as RUNX1-RUNX1T1(20.0%, n=18), PML-RARA(13.33%, n=12), and 4.44% NMP1.

Furth more, in the t-AML patients, the cytogenic features were normal karyotypes in 25.0% and abnormal mutations found in 75.0%. Common FAB types were 12.5% for M3 and M4. The molecular types were PLM-RARA and RUNX1 (12.5%) each; the cytogenetic features were normal in 25% and 25% deletion 5 and 7 in the second group. The rest of subtypes represented 12.5% [(t(11,7), t(15,17), t(8,21)].

Treatment was in line with the recommended standard protocols based on induction therapy,

known as the 7+3 regimen, followed by consolidation therapy. From this approach, 24,44% (n=22) went into complete remission vs. 52.22% (n=47) had refractory disease. Among those who achieved CR most of them relapsed and died later on.

Managing comorbidities plays a crucial role in achieving better outcomes, but none of these comorbidities (i.e., HIV status) negatively impacted the patients' outcomes in this cohort. The HIV status did not play any role in the prognosis.

As for the mortality rate, the older the patients, the more risk of death they had, which was statistically significant (p=0.0091), and most deaths occurred during the first year of the diagnosis.

7.2 Limitations

The sample size was small compared to cohorts from Asia or the US and could not be used for any generalisation.

New recent drugs were not prescribed, probably due to their unavailability in state hospitals (e.g., Midostaurin).

7.3 Recommendations

Larger samples will yield higher power and meaningful generalisations in cytogenetic profile and molecular features.

Further research is needed to identify local incidence of mutations.

Adequate Kaplan-Meier analysis in prospective studies will ascertain the adequacy of treatment and treatment outcome.

CHAPTER 8: REFERENCES

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CHAPTER 8: APPENDICES

A: Timing of events

TIMESCHEDULE: **Project Activity**	February 2021	March 2021	April 2021	May 2021	June 2021	July 2021	August 2021	September 2021	October 2021	November 2021	December 2021	January 2022	February2022	March 2022	April 2022	May 2022
Submit 1st Draft proposal to supervisor																
Corrections and final proposal write up																
Submission to HREC																
Data collection																
Data analysis																
Dissertations write up																
Submit a draft to supervisors for editing.																
Submit final dissertation for examination.																

B :Proposed study budget

ITEM	DETAILS	COST
Stationery	Paper, pencil, pens	R1 000.00
Statistician	In house Dept Internal Medicine	R0.00
Printing and binding		R1 000.00
Hardcover binding		R2 000.00
	Total Cost	**R4 000.00**

This was a self-funded study.

C :Data collection sheet

Patient case number:_____Age: _____

Gender___M_____F_____Ethnic Group:____BW____C____I_

Date of disease first documented blasts (Peripheral smear/BM/Flow cytometry) Year

_____Month_____

Clinical presentation

	Yes	**No**	**If yes, Provide details**
Bleeding (Provide site)			
Infection (Provide site)			
Other presentation			
Previous Malignancy			
Previous Cytotoxic drug exposure			
Previous Radiation exposure			

HIV positive patients

Date of diagnosis: Day_____Month_____Year_____

Baseline CD4 count:_____ Viral load

WHO stage:_____

On ART: Y N Regimen1st 2nd 3rd

If yes, Duration on therapy (months) <6m6-12m 12-24m 24-36m 36-48m 48-60m >60m

Regimen changed: Y N

If yes: detail_____

Clinical Findings (at diagnosis)

GENERAL	**Yes**	**No**	**If yes, provide details**
Pallor			
Lymphadenopathy			
BLEEDING			
Petechia/Purpura/EcchymosisMenorrhagia/hematuria/Gums			
Extramedullary			
Gum hypertrophy/Leukemiacutis/myeloid sarcoma			
Infections: if yes provide site			
Positive blood cultures			
HIV associated opportunistic infections			
TB/PJP/CMV/OTHER			

Results flowchart

BMAT	At Diagnosis	At Remission	End Consolidation	Relapse	Maintenance
Cellularity (I/N/D)					
Blasts%					
Cellular dysplasia (Y/N)					
FAB subtypes (M0-M7)					
Flow cytometry CD45/13/14/15/19 / 34/38/16/66. HLADR					
Cytogenetics (Specify)					
Blood results					
WCC					
Hb					
Hct					
MCV					
PLT					
Neutro					
Mono					
Lymphs					
Eos					

Baso					
Ferritin					
Vit B12					
Red cell folate					
Na					
K					
Chl					
Urea					
Creatinine					
LDH					
Uric acid					
Ca^{2+}					
Mg^{2+}					
PO_4					
T.Bil					
Cong. Bil					
T.prot					
Alb					
ALP					
GGT					
AST					
ALT					
HIV(Pos/Neg/U)					
CD4					
HIVVL					
Other					

Treatment of AML

Induction	**YES**	**NO**
Induction chemotherapy		
Consolidation		
Maintenance therapy		

Outcome

Alive: Y N

If no, DOD_____OR lost to follow up Y N Current

disease status: CR_Y_____N__PD: Y N If

PD: date of recurrency_____

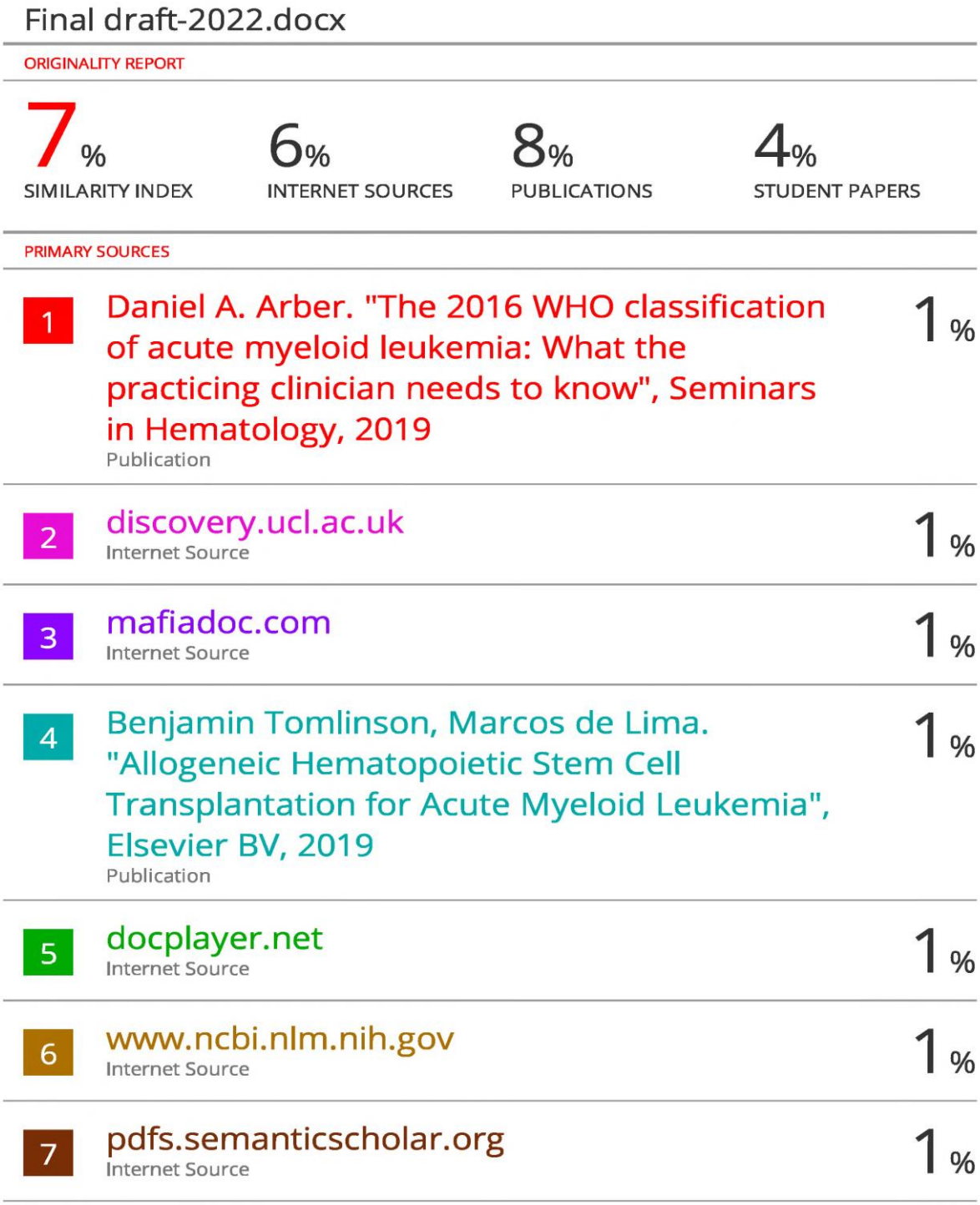
Documented cause/s of death:_____

Therapy-related AML (t-AML)

	Year of diagnosis	Year treatment completed	Regimen used	Molecular studies	Cytogenetic profile
Primary tumour					
t-AML					

D: Flow Diagram of participants

E: TurnIt In Report



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ORIGINALITY REPORT

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F: Clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr MV Mpanza

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210820

NAME: (Principal Investigator)	Dr MV Mpanza
DEPARTMENT:	School of Clinical Medicine Department of Medicine Division of Internal Medicine Medical School University
PROJECT TITLE:	*A retrospective audit of the cytogenetic profile and management outcome in Acute Myeloid Leukemia patients treated at Charlotte Maxeke Johannesburg Academic Hospital (2017-2021)*
DATE CONSIDERED:	2021/08/27
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professor P Ruff and Dr D Tshabalala
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/09/16

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **August** and therefore reports and re-certification will be due in the month of **August** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____