Effect of lymphocyte count and AML prognosis

Hanahlyn Park

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Nursing

University of Washington

2014

Committee:

Kathleen Shannon Dorcy

Elihu Estey

Program Authorized to Offer Degree:

Nursing University of Washington Tacoma

University of Washington

Abstract

Effect of lymphocyte count and AML prognosis

Hanahlyn Park

Chair of the Supervisory Committee:
Professor Kathleen Shannon Dorcy
Department of Nursing

Studies revealed a relationship between lymphocyte count and prognosis of certain cancers. Some studies found that decreased absolute lymphocyte count (ALC) correlates to poorer outcomes of the cancers. Many studies were done and revealed important prognostic factors of acute myeloid leukemia (AML), but prognosis of AML is not always promising. In order to improve prognosis of AML, other unknown independent prognostic factors should be evaluated and studied. The purpose of this study was to evaluate the effect of ALC on prognosis of AML. A total of 259 AML patients were selected for the final analysis. The patients were >18 year-old newly diagnosed AML patients, received induction treatment between January 2008 to June 2013 and got partial or complete responses from the induction therapy.

Both univariate and multivariate analyses revealed that higher-than-normal ALC at diagnosis has a positive correlation with poorer AML outcomes. This study concluded that higher-than-normal ALC at diagnosis of the disease was associated with shorter remission, relapse-free survival and overall survival durations. Lower-than-normal ALC at remission was associated with a longer overall survival duration while ALC at remission was not statistically significant in remission and relapse-free survival durations. Further studies are needed to evaluate frequency of Treg cells, a subpopulation of T cells that is known as immune activation inhibitor, since high frequency of Treg cells may result in increased ALC.

ACKNOWLEDGEMENTS

First, I would like to express my sincere gratitude and thank to my mentor and my chair of committee, Kathleen Shannon Dorcy. She showed me her continuous support, encouragement, and patience throughout my Master in Nursing study and research. I also want to express sincere appreciation to my committee member, Dr. Elihu Estey, for his continuous support and his assistance in the preparation of this project. My sincere thanks also go to Vicky Sandhu and Megan Othus, for their great assistance and insightful thoughts. Vicky Sandhu provided a great support throughout this project and Megan Othus helped with statistical analysis. Lastly, I also want to thank to Merav Bar, for bringing such great ideas into my thesis project. This thesis would not have been completed without all the help.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I: Introduction

Despite the many advances in medical care and treatment modalities for cancer patients, there is not a single treatment that will guarantee a cure. Nor are there singular tests for which oncology patients' prognoses can be made with certitude. In this context, leukemia is no exception; indeed, leukemia is one of the leading causes of cancer deaths in the United States. Leukemia was reported as the fifth most common cause of cancer deaths in men and the sixth most common cause of cancer deaths in women in 2006-2010. The incidence of leukemia is 12-fold higher in adults than in children and adolescents. Thus it is wells advised to target the adult population for identification of contributing factors that may improve prognosis of the disease and perhaps improve outcomes (Leukemia & Lymphoma Society [LLS], 2014).

Statistics reported by the LLS show survival rates in four subtypes of leukemia, and the statistics of acute myeloid leukemia (AML), one of the subtypes of leukemia, stood out for two reasons: 1) significantly low survival rate in adults (24.9%) compared to all other subtypes of leukemia, and 2) significantly large gap of survival rates between adults and children/adolescents (difference of 39.9%). The statistics concluded that even with the best current practice in treating AML, survival rate is significantly low compared to other leukemic subgroups. Some studies identified lymphocyte counts as one of the prognostic factors in certain cancers' outcomes, but limited studies were done to look at the relationship between lymphocyte counts and AML outcomes. Thus, this research question was designed to analyze the relationship between lymphocyte counts and the prognosis of AML (LLS, 2014).

Purpose

The purpose of this study was to evaluate and analyze the relationship between absolute lymphocyte count (ALC) and the prognosis of adult patients diagnosed with AML. Finding

another key contributing factor may influence the current AML treatment plans and improve patient care. The population for this study were patients who received chemotherapy for AML and achieved a partial or complete remission between January 2008 and June 2013 at a large comprehensive cancer center in the northwest United States. The Institutional Research Board (IRB) of the institution granted a waiver of consent for retrospective chart review from an existing data respository. Following the IRB approval, a total of 259 patients' data were selected and evaluated retrospectively. The AML patients' lymphocyte count at the point of original diagnosis and at remission were assessed to evaluate an association between lymphocyte count and AML outcomes.

CHAPTER II: Background and Literature Review

Leukemia

Hematopoiesis is the process of formation, multiplication, and differentiation of blood cellular components in the bone marrow. Primarily, blood stem cells are produced from bone marrow, and the blood stem cells then become either myeloid or lymphoid stem cells. Both myeloid and lymphoid stem cells produce several types of immature cells, which eventually become specialized mature cells and function differently to maintain health of the human body. Myeloid stem cells are differentiated into several functioning mature cells: Red blood cell (RBC), platelets, neutrophil, monocyte, eosinophil, basophil and mast cell. Lymphoid stem cells are differentiated into three types of mature cells, aka lymphocytes: T-lymphocyte, B-lymphocyte, and natural killer (NK) cell. The mature cells are released into the blood stream and function in their own unique ways to protect the human body from invasive microorganisms. If there is a disruption of development of normal blood cells, it is called leukemia. Leukemia is a clonal malignant disorder of the bone marrow, which causes uncontrolled proliferation of

malignant leukocytes (CRUK, 2014; Huether, McCanece, Brasher, & Rote, 2012; National Cancer Institute [NCI], 2014).

Generally, leukemia can be divided into four sub-groups: Acute myeloid leukemia (AML), acute lymphatic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphatic leukemia (CLL). The classification of leukemia is determined by two factors: 1) the predominant cell of origin (myeloid or lymphoid) and 2) the degree of differentiation that took place before a cell became malignant (acute or chronic). Acute leukemia is characterized by a rapid growth of undifferentiated cells whereas chronic leukemia is characterized by a slow growth of more differentiated cells. The onset of acute leukemia is abrupt and rapid while the onset of chronic leukemia is slow (Huether et al., 2012; NCI, 2014).

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is defined as a malignancy of the hematopoietic stem cells that affect myeloid blood stem cells, which alters the early growth phase of the hematopoietic system and proliferation of the normal cell. Etiology of AML is unknown, but current medicine suggests the benzene exposure and prior chemotherapeutic or radiation therapies as possible causes of a genetic predisposition that may alter the nuclear deoxyribonucleic acid (DNA) of a single cell. The leukemic cells are unable to mature and respond to normal regulation of the cell proliferation process; as a result, the leukemic cells remain in the immature stage and proliferate quickly in the bone marrow. As the number of immature cells increases, the number of mature cells decreases partly due to uncontrolled multiplication of immature cells and the limited space of the bone marrow. An increase of the immature cells in the bone marrow will eventually infiltrate into the blood stream and can cause some life-threatening complications (Huether et al., 2012; NCI, 2014).

A decrease in production of the myeloid lineage mature cells directly causes a reduction in quantity and quality of RBCs, WBCs and platelets, resulting in anemia, leukopenia, and thrombocytopenia. Anemia happens due to lack of healthy erythrocytes, also known as RBCs. A reduction in the total number of the erythrocyte can be interpreted as a reduction in the hemoglobin, which carries oxygen to the tissues from the lung. As a result, hypoxemia (not enough oxygen in the blood) and hypoxia (not enough oxygen in the tissue) can happen. Individuals with severe or sudden anemia may experience fatigue, palpitation, dyspnea, and heart dysfunction. Thrombocytopenia is a term used when there are not enough platelets. The platelet is vital to coagulation and abnormal bleeding problems can result with thrombocytopenia. Leukopenia, or lowered white blood count, (WBCs), includes key cells of the immune system (e.g. monocyte, neutrophil, eosinophil, basophil, lymphocyte and natural killer cell). Individuals with low WBC are more likely to be at risk for febrile episodes, and infections because they are unable to mount an immune response to fight microorganisms (Huether et al., 2012; NCI, 2014).

AML is known as the most common type of acute leukemia in older adults. The American Cancer Society's (ACS) estimates, there will be about 18,860 new cases of AML in 2014. Additionally, the ACS projects about 10,460 deaths in 2014 will be associated with AML. The average age of AML patients is about 66 years and it is slightly more common among men than women (American Cancer Society [ACS], 2014).

For AML, overall survival (OS), complete remission (CR) and relapse-free survival (RFS) durations after the treatment are important metrics to understand because they are a direct way to measure the AML outcomes. Patients with <5% of blasts, and a recovery of platelet (>100 thou/μL) and neutrophil counts (>1.0 thou/μL) are considered to be in CR. In a review of the literature, there are different factors that can predict the duration of remission, RFS and OS.

Currently, some of the well-known prognostic factors, which help to predict outcomes of AML, include the following: age, AML status (*de novo* or secondary), cytogenetic, molecular studies and transplant status. In addition, the platelet and absolute neutrophil counts at first remission are important prognostic factors to consider since more patients died due to complications of AML (e.g. infections and hemmorrhages) rather than the disease itself (Cheson et al., 2003; Huether et al, 2012).

Even with the current best practices relatively few AML patients survive greater than five years. The Cancer Research United Kingdom (CRUK) (2014) and the LLS (2014) estimated that only 25% of adults with AML survive greater than five years. In addition, five-year survival rate in AML patients is relatively low when compared with other types of leukemia and other common cancers such as breast or prostate cancers. The LLS (2014) reported that 58.6% of chronic myeloid leukemia patients, 68.8% of acute lymphocytic leukemia patients, and 83.1% of chronic lymphocytic leukemia patients survive more than five years. In addition, National Cancer Institute (NCI) (2014) reported that 89.2% of breast cancer patients and 98.9% of prostate cancer patients survive more than five years. These statistics clearly support the need for additonal study to discover new and to date, yet unidentified independent prognostic factors that could serve as metrics correlated with AML survival rates.

Literature Review

Recently, some studies have examined lymphocyte count and the possible correlation to the risk of relapse cancer. One such study suggested there is a direct association between elevated peripheral blood lymphocyte-to-monocyte ratio with favorable prognosis in nasopharayngeal carcinoma (Li et al., 2013). In another study, these authors also concluded that lower peripheral blood lymphocyte and monocyte ratio predicts higher relapse rate in patients

with diffuse large B-cell lymphoma (Li, Gu, Pan, Jiao, & Zhai, 2014). Two most recent studies also suggested that higher absolute lymphocyte count and lymphocyte-to-monocyte ratio predict longer relapse-free-duration in diffuse large B-cell lymphoma and longer survival length in ovarian cancer (Wei et al., 2014; Williams, 2014). Other investigators looked at the relationship between ALC and outcomes of ALL and AML; these authors concluded that the ALC is a key indicator that can be used to predict the outcomes of ALL and AML (De Angulo, Yuen, Palla, Anderson & Zweidler-McKay, 2008). It is not known if the lymphocyte count at the time of diagnosis or recovery is an independent predictor of AML outcomes while lymphocyte count recovery is considered as one of the key predictive factors of immune reconstitution after transplant for AML patients (Behl et al., 2006; Kumar et al., 2001; Porrata et al., 2002).

There are some diagnostic procedures available to diagnose AML. A bone marrow aspirate and biopsy is an essential diagnostic work-up to diagnose AML. Blood and marrow smears are morphologically examined, and at least 200 leukocytes in blood smears and 500 nucleated cells on marrow smears are recommended for the accurate diagnosis of AML. Generally, a marrow or blood blast count of 20% or more is required to diagnose AML. However, AML can be diagnosed with some chromosome abnormalities such as t (15;17), t(8;21), inv(16), and t(16;16) even without 20% of blast count by morphology.

Flow cytometry also can be done in a blood or bone marrow sample, to confirm blasts using specific markers. Flow cytometry is done to assess immaturity of cells and determine the types of cells in the sample. Flow cytometry in AML assists healthcare providers to identify minimal residual disease (MRD), which is a significant prognostic factor in AML. However, morphology is the most reliable method for blast enumeration. Follow up bone marrow is often done to evaluate the response to the chemotherapeutic therapy, and percentages of blasts are

evaluated by morphology and flow cytometry. Ideally, <5% of blasts by morphology and no blast by flow cytometry are expected status post chemotherapeutic therapy (Dohner et al., 2010; Mayoclinic, 2012; National Institutes of Health [NIH], 2012; Seattle Cancer Care Alliance [SCCA], n.d.).

Independent Prognostic Factors

Age. The incidence of AML diagnosis increases with age. The LLS (2014) reported that the risk of developing AML in the age group of 65 to 69 is about 10-fold higher compared to the age group of 35 to 59. Statistics showed that <20% AML diagnosed patients are under 45 year-old (NCI, 2014). Age is also inversely related to survival rate and cure rate and age at the time of AML diagnosis is suggested as the strongest prognostic indicator (Liersch, Muller-Tidow, Berdel & Krug, 2014; CRUK, 2014). While cure rate for adult AML patients is well below 25%, cure rate of children with AML is just below 50%. In addition, if patients are diagnosed with AML >65 years of age, overall 5-year survival rate is only 5.2% while if patients are diagnosed with AML <65, overall 5-year survival rate is 39.6%. Overall, 5-year survival rate is a lot better in children under 15 years-old compared to adults (60.9%) (LLS, 2014; NCI, 2014).

Cytogenetics. Cytogenetics are done in current practice as the test provides some of important prognostic information that could predict overall outcomes of AML as well as pre-remission and post-remission prognosis of AML (Ferrant, et al, 1995; Grimwade et al., 1998; Grimwade et al., 2010; Mrozek et al., 1997; Samuels et al., 1988; Slovak et al., 2000). Most of the chromosomal abnormalities can be detected by cytogenetic analysis and about 55% of AML patients have cytogenetic abnormalities at diagnosis (Mrozek, Heinonnen, & Bloomfield, 2000; Mrozek, Heinonen, & Bloomfield, 2001). The importance of cytogenetic characteristics has been

recognized by the World Health Organization (WHO) as it recommends classifying AML into several subsets depending on the cytogenetic characteristics.

Most commonly, there are three different categories that are differentiated by cytogenetic results: Favorable, Intermediate and Unfavorable. The Southwest Oncology Group (SWOG) cytogenetic risk classification is widely used. According to the SWOG category, favorable cytogenetic risk group includes t (15; 17), t (8; 21), inv (16)/t (6; 16)/del (16q). Intermediate cytogenetic risk group includes normal cytogenetic, +8, +6, -Y and del (12p). Unfavorable cytogenetic risk group includes all other abnormalities including complex cytogenetic (Mrozek, & Bloomfield, 2006).

Favorable and Intermediate karyotype groups tend to achieve better outcomes compared to Unfavorable karyotype (Byrd et al., 2002; Griwade et al., 2010). Slovak et al. concluded that there is a direct association between CR and cytogenetic risk groups. Among three different cytogenetic risk groups, 84% of the Favorable group patients and 76% of the Intermediate group patients achieved CR while only 55% of the Unfavorable group patients achieved CR (Slovak et al., 2000).

Molecular markers. Molecular markers are also important contributing factors to consider (Helbig et al., 2014). In the last ten years, many studies were done validating the importance of the molecular marker mutation status. Molecular marker tests are done to identify some key markers such as Nucleophosmin (NPM1), frms-like tyrosine kinase 3 (FLT3) and CCAAT enhancer binding protein alpha (CEBPA) mutation status have been identified as important prognostic factors. The clinical and genetic prognostic markers now are used to guide physicians to come up with the best treatment and research interventions for patients with AML.

Recent studies suggested the importance of mutations of NPM1. Some studies concluded that about 50-60% of AML patients with normal cytogenetic have NPM1 mutation (Chen, Rassidakis & Medeiros, 2006; Verhaak et al., 2005). Patients with NPM1 mutation tend to have higher CR rate and longer OS and RFS durations (Dohner et al., 2005; Schnittger et al., 2005; Thiede et al., 2006). Thied et al. (2006) and Verhaak et al. (2005) suggested that AML patients with NPM1 gene mutation tend to have lower incidence of relapse. Furthermore, other studies concluded that AML patients with NPM1 gene mutation and without FLT3 gene mutation tend to have favorable clinical outcomes (Chen, Rassidakis, & Medeiros, 2006; Schnittger et al., 2005; Verhaak et al., 2005). In current practice, the NPM1 mutation status is used to monitor minimal residual disease (Falini, Nicholetti, Martelli & Mecucci, 2007).

The FLT3 is known as the most common gene mutation in AML. About 30-40% of AML patients have the FLT3 gene mutations (Kiyoi & Naoe, 2006). Of many different types of the FLT3 gene mutations, the most common subtype of the FLT3 gene mutation consists of internal tandem duplication, aka FLT3-ITD. The AML patients with the FLT3-ITD gene mutation tend to have poorer clinical outcomes. Several studies confirmed that the presence of the FLT3-ITD gene mutation in AML patients with normal karyotype showed an adverse prognosis for RFS and OS durations (Kainz et al., 2002; Ciollis et al., 2004; Bienz et al., 2005). In addition, the size of the FLT3-ITD was inversely related to OS duration (Stirewalt et al., 2006).

Several studies report approximately 15-20% of AML patients have CEBPA. These studies have shown that the CEBPA gene mutation positively correlates with durations of CR, RFS and OS (Boissel et al., 2005; Bienz et al., 2005; Barjesteh van Waalwijk van Doorn-khosrovani et al., 2003; Frohling et al., 2004). Other studies also support that the CEBPA is an independent prognostic factor for the OS duration regardless of age and the FLT3-ITD gene

mutation status (Preudhomme et al, 2002). Schlenk et al. (2008) suggested that the FLT3, NPM1 and CEBPA mutation analysis is important to know in order to improve risk stratification in normal cytogenetic AML patients.

Transplant. Transplant status is a well-known prognostic factor. Often times, stem cell transplant is done to increase the duration of RFS and OS as well as CR rate. The LLS (2014) stated the remission and cure rates of AML have been improved over years partly due to successful stem cell transplantation. Hematopoietic stem cell transplantation (HSCT) is also known as stem cell transplant (SCT) and it is widely used in current practice. In the past 50 years, new and innovative approaches have been proposed in order to decrease the mortality rate and adverse events of HSCT. Initially, HSCT was developed for two different purposes: 1) HSCT replaces the abnormal hematopoietic system with healthy one and 2) it allows the delivery of the intense chemotherapeutic and radiation agents in order to cure the hematologic disease (Peccatori & Ciceri, 2010). Because HSCT involves intense chemotherapy and total body irradiation (TBI), a high mortality rate was associated with HSCT historically. Although, the mortality rate has been significantly reduced over the years, death is still a possible adverse effect that of the HSCT (Hahn et al., 2013).

Regardless of the known adverse effects, the transplantation is one of the effective treatments for AML. A report by Gooley et al. (2010) clearly found that mortality rate of the HSCT has been significantly reduced while OS duration has been increased compared to the period of 1993 to 1997. There has been a significant reduction of death related to the STC (Gooley et al., 2010). Other significant improvements include the reductions in major adverse events and side effects of the STC such as organ damage, infection and sever acute graft versus host disease (GVHD). Hahn et al. (2013) concluded that OS at day 100 and survival duration

have been significantly improved for AML patients in the first CR after the allogeneic HSCT regardless of increased of median age and unrelated allogeneic SCT rates. Furthermore, Koreth et al (2009) compared RFS and OS survival duration in non-allogeneic HSCT and allogeneic SCT. Koreth et al. (2009) found that allogeneic HSCT has longer RFS and OS durations for intermediate – and poor-risk AML groups. Transplantation also results in lowest incidence of relapse (NCI, 2014). Orozco and Appelbaum (2012) concluded that HSCT is positively correlated with CR after induction therapy and consolidation with allogeneic HSCT.

***De novo* and secondary AML**. There are two presentations of AML when newly diagnosed: *de novo* AML and secondary AML. *De novo* AML refers to development of AML without any other causes associated. Secondary AML refers to development of AML due to history of previous diseases (e.g. myelodysplastic syndrome), exposure of carcinogens, or other chemotherapeutic agents or radiation therapy for other malignancies. Studies have concluded that the prognosis of AML is poorer in secondary AML group and AML group with the history of previous antecedent hematologic disorders compared to the *de novo* AML group (Shiffer, Larson & Connor, 2014).

Laboratory values. Platelet and absolute neutrophil count (ANC) are important laboratory values as it reflects in response code (e.g. CR). Response to the induction therapy criteria include both ANC and platelet recovery information. Response description will be described in detail later in the paper. Studies suggested that the platelet and ANC recovery predict prognosis of the AML. Without recovery of platelet and neutrophil counts, the patients are at risk of complications. Neutrophils are generated from myeloid precursors and function as the body's primary line of defense mechanism by eliminating foreign microorganisms or invaders through phagocyte process. An inadequate number of neutrophils can cause severe

immunodeficiency, which can lead to death eventually (Huether et al., 2012; Kolaczkowska & Kubes, 2013). Platelets are also one of the important blood components that are generated from myeloid precursors. They function as a clotting factor and recognize damages of blood vessels. Without the recovery of platelet counts, complications such as abnormal bleeding may occur (Huether et al., 2012).

Current Practice

Aggressive treatment is needed for AML patients to achieve CR (Mayoclinic, 2012). Generally, a combination of an anthracycline (e.g. idarubicin, daunoruicin) and cytarabine is used, often known as 3+7 or standard therapy. Treatment of the disease consists of two phases: Induction and consolidation. The first phase, also known as *induction therapy phase*, focuses on reducing the number of blasts. The goal of the first phase is to achieve CR, which is defined as marrow blasts <5% and neutrophil and platelet recovery. Upon the success of the induction therapy, the consolidation therapy, also known as *second phase,* aims to prevent relapses. Then, the third phase, also known as *maintenance therapy*, could follow, which involves a low dose of a chemotherapeutic agent after the completion of the consolidation therapy to help the patients to stay in the remission stage (ACS, 2014).

The diagnosis of AML is made with a diagnostic bone marrow (BM) aspiration or biopsy evaluation. Although it is possible to make the diagnosis of AML with the peripheral blood examination itself, current practice still recommends a BM aspiration or biopsy evaluation over peripheral blood examination to make a diagnosis of AML. AML is diagnosed with the >20% of blasts in BM. Evaluation of BM followed by the induction therapy reveals the response to the therapy. Clinically, CR refers to blasts <5% by morphology and no blast by flow cytometry in BM with >1.0 thou/μL neutrophil and >100 thou/μL platelet count. The flow cytometry can be

done on peripheral blood or bone marrow sample. It is a method used to assess the antigen expression of the cell, which allows quantification and identification of subpopulations and abnormal populations of the cell. Follow-up flow cytometry is often done in both peripheral blood and bone marrow samples after chemotherapeutic treatment to assess and evaluate any abnormal populations, which can detect the presence of minimal residual disease (MRD) (ACS, 2014; Cheson et al., 2003; NCI, 2014; University of Washington [UW], 2010).

The OS duration is measured from the date of the initial diagnosis of AML to the date of death regardless of cause of death. If patients are lost contact of the patients, or 'lost follow-up', or date of death is unknown, the OS duration is censored on the date of the last contact. The RFS duration is measured for the patients who achieve any types of CRs (e.g. CR, CRi, CRp and MRDs) and is measured from the date of the leukemia-free state (e.g. BM <5% blasts) to the date of relapse or date of death. If patients are lost follow-up and date of relapse is uncertain or unknown, the RFS duration is censored on the date of the last contact for the purpose of the study. The CR duration is measured for the patients with the CR and is measured from the date of the CR, BM <5% and ANC and platelet recovery, to the relapse date. If patients are lost follow-up or deceased, remission duration is censored on the date of death or date of last contact. Relapse is defined as the recurrence of the blasts in the PB or >5% of blasts in the BM examination (ACS, 2014; Cheson et al., 2003; NCI, 2014).

CHAPTER III: Methodology

Study Cohort

The study population was selected from the AML database at the Fred Hutchinson Cancer Research Center (FHCRC). The AML database contains data about the AML patients who are seen at the Seattle Cancer Care Alliance (SCCA) and the University of Washington

Medical Center (UWMC). The FHCRC is an alliance facility of SCCA, UWMC and Seattle Children's Hospital. The study was approved by the FHCRC Institutional Review Board (IRB) on May 2012 prior to the data collection and waiver of consent was granted for data was collection from the data base retrospectively.

Initially, a total number of 259 AML patients, age 18 and older, were selected for this study. The study cohort was treated at the Fred Hutchinson Cancer Research Center (FHCRC) between January 2008 and June 2013. All the patients were newly diagnosed AML patients and got their first induction therapy at SCCA/UWMC. The diagnosis of AML was made with the bone marrow study that revealed >20% of blasts by morphology. The follow-up bone marrow biopsy and Complete Blood Count (CBC) were done to precisely evaluate the response to the initial therapy.

Selection of Subjects

A formal query of the AML database was created to select a sample with the diagnosis of Acute Myeloid Leukemia (AML). The inclusion criteria were: (1) all ages; (2) newly diagnosed patients (both *de novo* AML and secondary AML); (3) patients who achieved a partial or complete remission post-therapy; and (4) patients who get treated at SCCA/UWMC between January 1, 2008 and June 31, 2013.

Exclusion criteria were: (1) patients whose disease response to the therapy is resistant, unknown or who are dead; and (2) patients with acute promyelocytic leukemia (APL) or cerebral spinal fluid (CSF) relapse patients. All the patient responses were double checked by the AML data coordinators for accuracy and inter-rater reliability. The patients' gender, age, treatment start date, treatment intensity (low, intermediate and high intensity), transplant information, AML status, response to the therapy, relapse status, survival status, CR duration, pre-treatment

and post-treatment laboratory values (lymphocyte, monocyte, platelet, absolute neutrophil), transplant status, cytogenetic and molecular markers (FLT3, NPM), were collected for all patients for the final analysis.

A total of 919 patients visited SCCA/UWMC between January 2008 and June 2013. Among them 303 patients were excluded since they were relapse or refractory patients and 36 acute promyelocytic leukemia (APL), a subtype of AML, patients were excluded. Thus, a total of 579 AML patients were initially selected. Among 579 patients, only 473 patients got induction therapy at the SCCA/UWMC. Of those 473 patients, 275 patients achieved partial or complete CRs. Among them, 16 patients were excluded from the final analysis due to unknown laboratory values (n=15) and unknown date of death (n=1). Thus, a total of 259 patients were selected for the final analysis (*Figure1*).

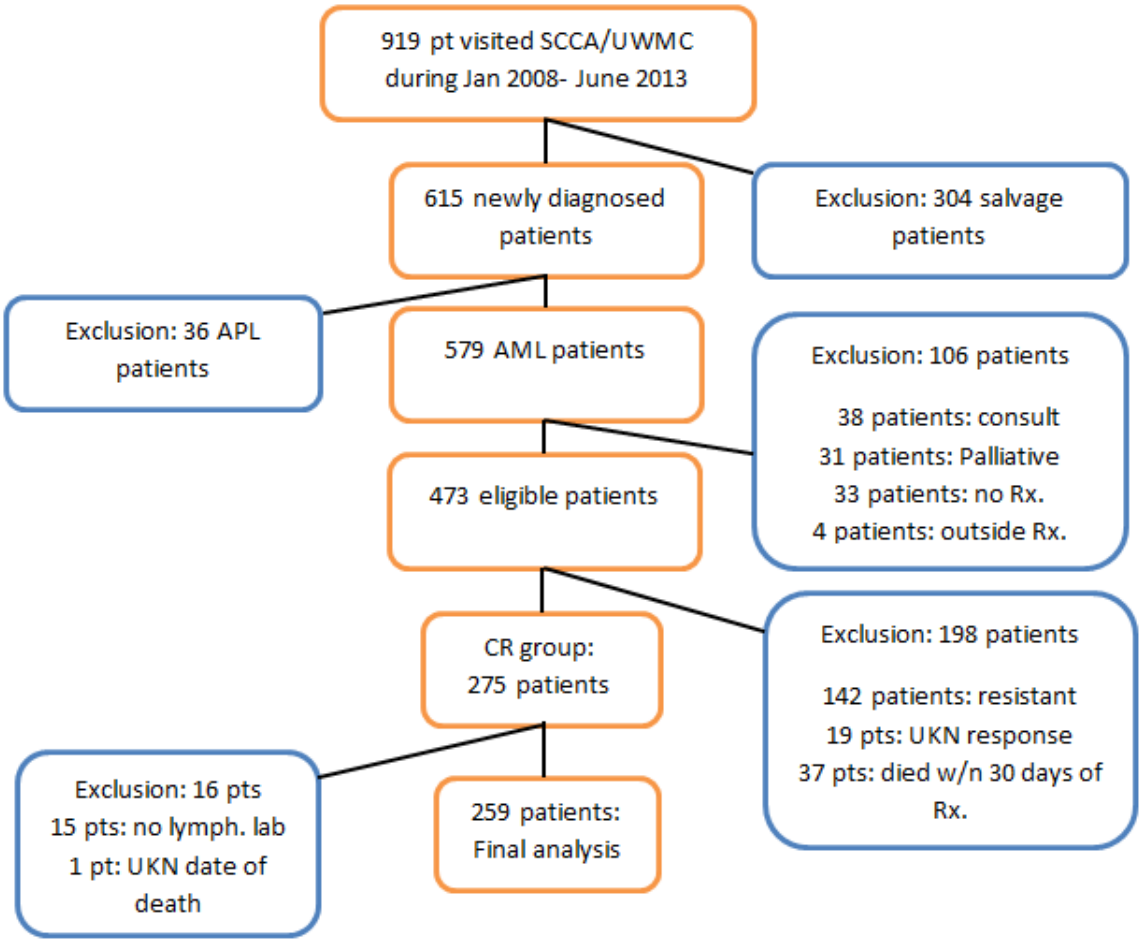

Figure 1. Patient selection criteria

Patient Characteristics

The patients were divided into three different groups: low lymphocyte count group (lymphocyte count 0-1 thou/μL), normal lymphocyte count group (lymphocyte count > 1-4.8 thou/μL) and high lymphocyte count group (lymphocyte count > 4.8 thou/μL). The median age of the cohort was 56 (range18-84). One hundred and eighty patients (69%) achieved CR, and 103 patients (47%) underwent HSCT for AML. One hundred and sixty patients (62%) were in favorable or intermediate cytogenetic groups at the time of diagnosis. The median WBC at diagnosis was 6 (range 0.26-296.48 thou/μL), median ALC was 1.65 (range 0.04-11.97 thou/μL),

median neutrophil count was 0.91 (range 0-101.19 thou/μL) and median platelet count at diagnosis was 54 (range 5-1698 thou/μL). Median follow-up time of censored patient was 1.1 years (407 days). Patient characteristics of the study population are summarized in *Table 1*.

Table 1.

Patient Characteristics

ALC $x10^3$/μL at Dx.	Low (0-1)	Normal (>1-4.8)	High (>4.8)
Total # of patients (n=259)	71	157	31
ALC (median, range)	0.71 (0.04-1)	1.83 (1.01-4.71)	6.15 (4.84-11.97)
Age (years)			
Range (18-84)	23-84	18-81	19-70
< 60 (n=150)	37	88	25
= or > 60 (n=109)	34	69	6
Median (56) (n=259)	56	58	51
Gender			
Male (n=151)	44	94	13
Female (n=108)	27	63	18
AML Status ND1/ND2			
ND1 (n= 143) – *de novo*	24	93	26
ND2 (n=116)- secondary	47	64	5
Pre-treatment lab values			
WBC $x10^3$/μL	1.52 (0.26-119.89)	7.79(1.3-226.94)	40.5(22.39-296.48)
Platelet $x10^3$/μL	51 (5-249)	57 (6-1698)	48 (12-104)
Neutrophils $x10^3$/μL	0.52 (0.01-22.11)	1.12 (0-101.19)	4.31 (0.15-72.8)
Monocyte $x10^3$/μL	0.04 (0-40.11)	0.27 (0-15.57)	7.16 (0-64.85)
Treatment Intensity			
Low (n=52)	20	32	0
Intermediate (n=100)	19	65	16
High (n=107)	32	60	15
Response Code			
CR (n=180)	42	113	25
CR-MRD (n=38)	9	26	3
CRp (n=24)	12	10	2
CRp-MRD (n=10)	5	5	0
Cri (n=4)	1	2	1
Cri-MRD (n=3)	2	1	0
HSCT Status			
HSCT Yes (n=123)	44	64	15
HSCT No (n=136)	27	93	16

SWOG cytogenetic category			
Favorable (=29)	1	18	10
Intermediate (n=131)	33	83	15
Unfavorable (n=75)	28	43	4
Miscellaneous (n= 19)	7	10	2
Not Done or Unknown (n=5)	2	3	0
Relapse Status			
Yes (n=86)	22	46	18
No (n=173)	49	111	13
Current Status			
# of patients alive (n=124)	35	79	10
# of patients died (n=94)	29	49	16
# of LTFU patients (date of last contact >1yr) (n=41)	7	29	5
Response ALC ($x10^3/\mu L$)			
ALC (median, range)	0.74 (0.07-2.73)	0.89 (0-4.4)	0.78 (0.15-4.03)
Low (0-1 $x10^3/\mu L$) (n=156)	50	86	20
Normal (>1-4.8 $x10^3/\mu L$) (n=103)	21	71	11
Other post-treatment lab values (platelet, ANC and monocyte)			
WBC $x10^3/\mu L$	4.39 (0.11-27.65)	4.41(0.11-38.34)	4.84(0.28-21.06)
Platelet $x10^3/\mu L$	144 (7-764)	168 (6-1436)	241 (37-546)
Neutrophils $x10^3/\mu L$	2.19 (0-11.1)	2.2 (0.01-24.61)	2.13 (0.15-23.95)
Monocyte $x10^3/\mu L$	0.44 (0-2)	0.7 (0-10.78)	0.68 (0.18-4.72)
Molecular studies			
FLT3 positive (n=33)	9	15	9
NPM1+/FLT3- (n=30)	7	20	3

Variables

Several variables were selected for analysis. Some known independent variables were age, AML status (e.g. *de novo,* secondary), SWOG cytogenetic, response to the initial induction, relapse status, transplant status, FLT3, NPM1 and laboratory values which included neutrophils, platelet and white blood cell counts. For the purpose of the study, monocyte and lymphocyte count were also included in the final analysis.

Age of the patient was collected at the time of diagnosis of AML. All patients were divided into two different categories: AML ND1 (*de novo)* and AML ND2. AML ND1 is defined as a spontaneous AML presentation that is not related to other diseases or agents. AML ND2 is

known as secondary AML, which means the disease was developed after exposure to chemotherapy agents or as progression of other cancers or antecedent disease (Cheson et al., 2003). SWOG cytogenetic data was divided into five different groups: Favorable, Intermediate, Unfavorable, Miscellaneous and Unknown. The Intermediate risk group included normal cytogenetic patients, +8, +6, -Y and del (12p). Unfavorable risk group included del(5q)/-5, -7/del(7q), abn 3q,9q,qq1,20q,21q,17p, t (6;9), t (9;22) and complex karyotypes but limited to up to three unrelated abnormal karyotypes. The Miscellaneous risk group included all other abnormalities and more than four unrelated abnormal karyotypes. Treatment intensity was also categorized into three groups: Low, Intermediate and High.

CHAPTER IV: Results

Statistical Analysis

Tables 2 and 3 summarize the risk factors for length of CR, tables 4 and 5 summarize the risk factors for the duration of OS, and tables 6 and 7 summarize the risk factors for the duration of RFS. The univariate analysis, in Table 2, showed that higher-than-normal ALC at the time of AML diagnosis was associated with a shorter duration of CR (hazard ration [HR] 2.2; 95% CI, 1.37-3.54; P=0.0011) while there was no relationship between ALC at remission and the CR duration. The multivariate analysis also revealed that there was a statistically significant relationship between higher-than-normal ALC at the time of AML diagnosis and a shorter duration of CR (HR, 4.06; 95% CI, 1.85-8.01; P<0.001) (*Table 3*). Table 3 also shows that the low-intensity treatment (HR, 0.37; 95% CI, 0.16-0.89; P=0.027) and transplantation (HR, 0.51, 95% CI, 0.3-0.85, P=0.01) were associated with a longer CR duration.

Table 4, univariate analysis, shows that lower-than-normal ALC at remission suggested a longer duration of OS (HR, 0.46; 95% CI, 0.29-0.73; P<0.001). In Table 5, the

multivariate analysis confirmed that lower-than-normal ALC at remission was associated with a longer duration of OS (HR, 0.56, 95% CI, 0.33-0.96, *P*=0.036). Furthermore, the multivariate analysis also confirmed that higher-than-normal ALC at AML diagnosis was correlated with a shorter duration of OS (HR, 3.85; 95% CI, 1.85-8.01; *P*<0.001) (Table 5; Figure 2) and secondary AML (ref= *de novo)* was associated with a shorter duration of OS (HR, 1.78; 95% CI, 1.03-3.07; *P*=0.038) (*table 5*). In addition, table 5 also shows that the partial responses, CR-MRD and CRi, to the induction therapy were correlated (*P* <0.001) with a shorter duration of OS while the CR response and transplantation suggested a longer duration of OS (HR, 0.31; 95% CI, 0.19-0.52, *P*<0.001).

Table 6 shows that the number of pre-treatment ALC was not associated with the RFS duration while lower-than-normal ALC at remission was associated with a longer duration of RFS (HR, 0.68; 95% CI, 0.47-0.98; *P*=0.04). The multivariate analysis, Table 7, confirmed the statistical significance of the relationship between higher-than-normal ALC and a shorter RFS duration (HR, 3.47, 95% CI, 1.78-6.77; *P*<0.001). Similar to previous findings, CR-MRD (HR, 4.29; 95% CI, 2.43-7.58; P<0.001) and CRi-MRD (HR, 5.72; 95% CI, 2.55-12.79; P<0.001) responses were statistically significant to a shorter RFS duration (*Table 7*).

As a result, there was no statistically significant relationship between ALC at the time of diagnosis or remission. However, there was an association between ALC at diagnosis and the durations of CR, RFS and OS. Both univariate and multivariate analyses revealed that higher-than-normal ALC at the time of diagnosis was associated with shorter CR, OS, and RFS durations. Furthermore, there was no statistical significance between lower-than-normal ALC and CR and RFS durations; but, there was a statistical significant relationship between lower-than-normal ALC and a longer OS duration (*P*<0.05).

Table 2.

Univariate Analysis for Duration of Remission, n=259

Covariate	**HR**	**95% CI**	***P-value***
Low pre-treatment lymphocytes (ref=normal)	1.11	(0.66-1.87)	0.69
High pre-treatment lymphocytes (ref=normal)	2.2	(1.37-3.54)	0.0011
Low remission lymphocytes (ref=normal)	0.93	(0.61-1.41)	0.73

Table 3.

Multivariate Analysis for Duration of Remission, n=259

Covariate	**HR**	**95% CI**	***P-value***
Low pre-treatment lymphocytes (ref=normal)	1.15	(0.61, 2.19)	0.67
High pre-treatment lymphocytes (ref=normal)	**4.06**	**(2.29, 7.21)**	**<0.001**
Low remission lymphocytes (ref=normal)	1.56	(0.91, 2.69)	0.11
Age at diagnosis (years)	1.02	(1, 1.04)	0.088
Secondary AML (ref = de novo AML)	1.51	(0.87, 2.64)	0.14
Intermediate intensity treatment (ref=high)	0.79	(0.43, 1.44)	0.44
Low intensity treatment (ref = high)	0.37	(0.16, 0.89)	0.027
Favorable cytogenetics (ref = intermediate)	0.59	(0.28, 1.27)	0.18
Miscellaneous cytogenetics (ref = intermediate)	1.33	(0.5, 3.52)	0.56
Unfavorable cytogenetics (ref = intermediate)	1.28	(0.7, 2.33)	0.43
Unknown cytogenetics (ref = intermediate)	3.32	(0.87, 12.61)	0.078
CR-MRD (ref = CR)	4.69	(2.34, 9.42)	<0.001
CRi (ref = CR)	2.28	(0.91, 5.68)	0.077
CRi-MRD (ref = CR)	4.63	(1.39, 15.41)	0.013
HCT (ref = no HCT)	0.51	(0.3, 0.85)	0.01
Other NPM1/FLT3 (ref = NPM1+/FLT3-)	1.26	(0.65, 2.45)	0.49
Missing NPM1/FLT3 (ref = NPM1+/FLT3-	0.83	(0.36, 1.9)	0.66
Pre-treatment platelets	1	(0.99, 1)	0.26
Pre-treatment ANC	1.01	(0.99, 1.03)	0.26
Pre-treatment monocytes	1.01	(1, 1.03)	0.11
Response platelets	1	(1, 1)	0.32
Response ANC	0.97	(0.94, 1.01)	0.14
Response monocytes	1.13	(0.96, 1.32)	0.14

Table 4.

Univariate Analysis for Duration of Overall Survival (OS), n=259

Covariate	**HR**	**95% CI**	***P-value***
Low pre-treatment lymphocytes (ref=normal)	1.21	(0.76-1.92)	0.42
High pre-treatment lymphocytes (ref=normal)	1.62	(0.92-2.86)	0.0093
Low remission lymphocytes (ref=normal)	0.46	(0.29-0.73)	<0.001

Table 5.

Multivariate Analysis for Duration of Overall Survival (OS), n=259

Covariate	**HR**	**95% CI**	***P-value***
Low pre-treatment lymphocytes (ref=normal)	0.92	(0.54, 1.57)	0.75
High pre-treatment lymphocytes (ref=normal)	**3.85**	**(1.85, 8.01)**	**<0.001**
Low remission lymphocytes (ref=normal)	0.56	(0.33, 0.96)	0.036
Age at diagnosis (years)	1.01	(0.99, 1.03)	0.17
Secondary AML (ref = de novo AML)	1.78	(1.03, 3.07)	0.038
Intermediate intensity treatment (ref=high)	0.96	(0.56, 1.62)	0.87
Low intensity treatment (ref = high)	0.62	(0.31, 1.25)	0.18
Favorable cytogenetics (ref = intermediate)	0.42	(0.16, 1.07)	0.068
Miscellaneous cytogenetics (ref = intermediate)	1.08	(0.41, 2.83)	0.87
Unfavorable cytogenetics (ref = intermediate)	1.31	(0.79, 2.18)	0.29
Unknown cytogenetics (ref = intermediate)	2.55	(0.71, 9.11)	0.15
CR-MRD (ref = CR)	3.62	(1.93, 6.79)	<0.001
CRi (ref = CR)	2.41	(1.13, 5.17)	0.023
CRi-MRD (ref = CR)	5.03	(2.11, 11.98)	<0.001
HCT (ref = no HCT)	0.31	(0.19, 0.52)	<0.001
Other NPM1/FLT3 (ref = NPM1+/FLT3-)	3.39	(1.15, 9.94)	0.026
Missing NPM1/FLT3 (ref = NPM1+/FLT3-	3.92	(1.28, 12)	0.017
Pre-treatment platelets	1	(0.99, 1)	0.17
Pre-treatment ANC	1.01	(0.98, 1.03)	0.54
Pre-treatment monocytes	0.99	(0.97, 1.02)	0.66
Response platelets	1	(1, 1)	0.78
Response ANC	0.93	(0.87, 1.01)	0.075
Response monocytes	1.15	(0.88, 1.51)	0.29

Table 6.

Univariate Analysis for Duration of Relapse-Free-Survival (RFS), n=259

Covariate	HR	95% CI	*P-value*
Low pre-treatment lymphocytes (ref=normal)	1.1	(0.73-1.66)	0.65
High pre-treatment lymphocytes (ref=normal)	1.43	(0.86-2.36)	0.17
Low remission lymphocytes (ref=normal)	0.68	(0.47-0.98)	0.04

Table 7.

Multivariate Analysis for Duration of Relapse-Free-Survival (RFS), n=259

Covariate	**HR**	**95% CI**	***P-value***
Low pre-treatment lymphocytes (ref=normal)	0.95	(0.6, 1.52)	0.84
High pre-treatment lymphocytes (ref=normal)	**3.47**	**(1.78, 6.77)**	**<0.001**
Low remission lymphocytes (ref=normal)	0.83	(0.53, 1.28)	0.4
Age at diagnosis (years)	1.02	(1, 1.04)	0.033
Secondary AML (ref = de novo AML)	1.56	(0.98, 2.5)	0.063
Intermediate intensity treatment (ref=high)	0.85	(0.53, 1.37)	0.51
Low intensity treatment (ref = high)	0.64	(0.35, 1.17)	0.15
Favorable cytogenetics (ref = intermediate)	0.52	(0.24, 1.12)	0.093
Miscellaneous cytogenetics (ref = intermediate)	1.49	(0.69, 3.23)	0.31
Unfavorable cytogenetics (ref = intermediate)	1.14	(0.72, 1.8)	0.57
Unknown cytogenetics (ref = intermediate)	2.53	(0.72, 8.93)	0.15
CR-MRD (ref = CR)	4.29	(2.43, 7.58)	<0.001
CRi (ref = CR)	2.67	(1.36, 5.27)	0.0045
CRi-MRD (ref = CR)	5.72	(2.55, 12.79)	<0.001
HCT (ref = no HCT)	0.32	(0.21, 0.49)	<0.001
Other NPM1/FLT3 (ref = NPM1+/FLT3-)	1.75	(0.84, 3.66)	0.13
Missing NPM1/FLT3 (ref = NPM1+/FLT3-	1.99	(0.91, 4.33)	0.084
Pre-treatment platelets	1	(0.99, 1)	0.12
Pre-treatment ANC	1.01	(0.98, 1.03)	0.6
Pre-treatment monocytes	0.99	(0.97, 1.02)	0.63
Response platelets	1	(1, 1)	0.79
Response ANC	0.97	(0.92, 1.02)	0.24
Response monocytes	1.17	(0.96, 1.42)	0.13

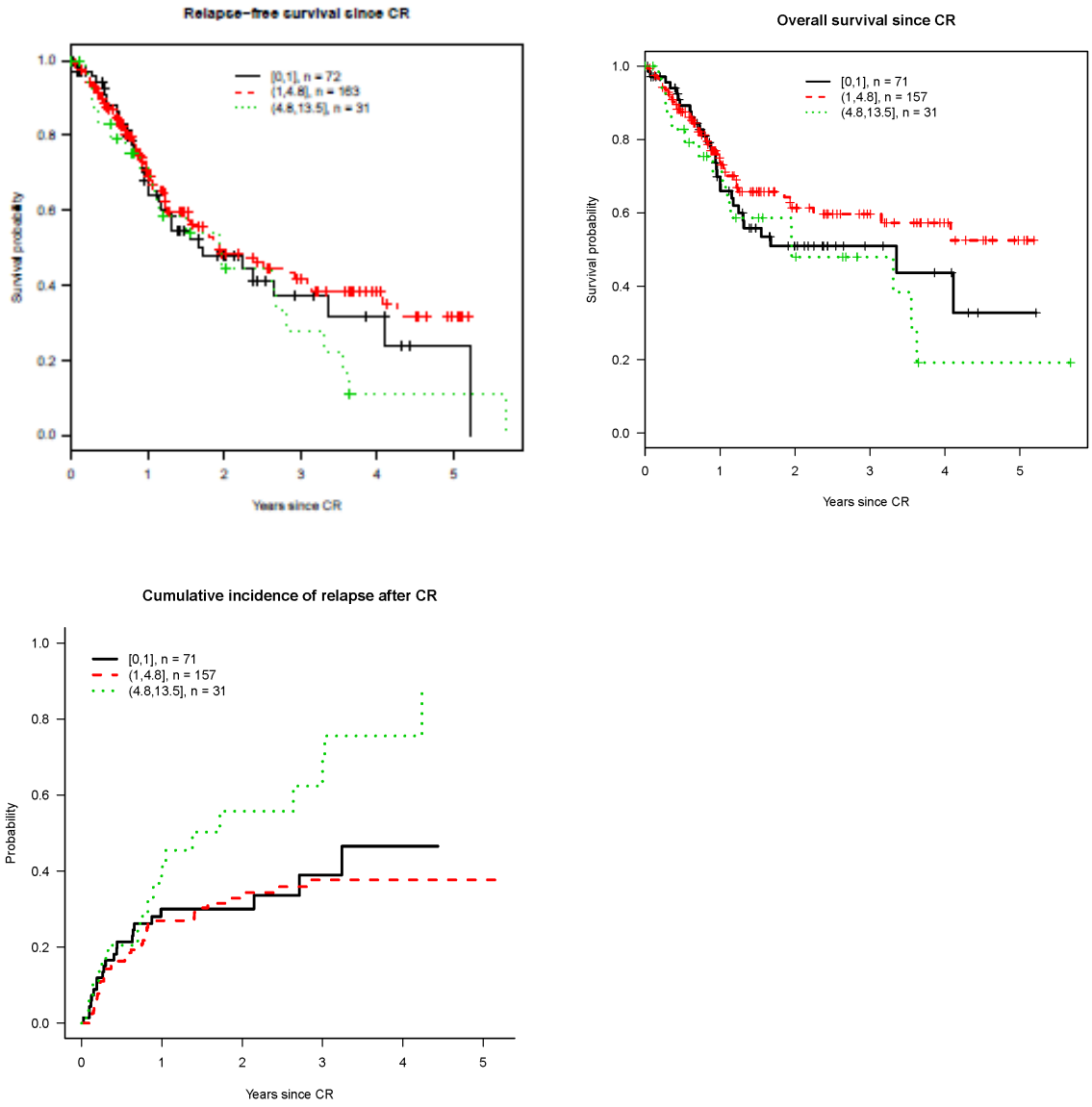

Figure 2. Cumulative incidence of relapse after CR, RFS and OS (related to ALC at diagnosis).

CHAPTER V: Discussion and Limitations

Discussion

This study suggests a strong correlation between higher-than-normal lymphocyte count at diagnosis of AML and poorer prognosis. Similar findings from other investigators concluded that an association exists between the high ALC at diagnosis of AML and poorer outcomes (Le Jeune

et al. 2014). Reasons for this association may be due to subpopulations of lymphocytes that may be responsible for the effect of lymphocyte count at the time of diagnosis and remission. As mentioned previously, there are three different types of lymphocyte and it can be broken down into numerous subpopulations of lymphocytes (e.g. CD3, CD4).

CD4+ T cell is a subpopulation of T-lymphocytes and it is composed of regulatory T (Treg) cells and conventional T helper (Th) cells. Treg cells are known as an immune activation inhibitor. Some studies concluded that the Treg frequencies were significantly increased in newly diagnosed, relapse, and refractory AML patients compared to healthy individuals. Furthermore, the studies suggested that the increase of Treg cells in BM and PB were directly associated with poorer prognosis of AML. In other words, a greater frequency of Treg cells predicted the poor response to the induction therapy (Buggins et al., 2001; Gasteiger & Kastenmuller., 2012; Orleans-Lindsay, Barber, Prentice, & Lowell, 2001; Shenghui et al., 2011; Szczepanski et al., 2009; Yang & Wu, 2013; Zhou et al., 2009). Because the high frequency of Treg cells may still results in increase quantity of lymphocytes it is possible that higher-than-normal lymphocyte group had increased number of Treg cells (Bachanova et al., 2014). If this is a case, high lymphocyte count can predict poorer AML outcomes. This hypothesis may further explain why higher-than-normal lymphocyte group had poorer AML outcomes in this study. In order to evaluate this hypothesis, frequencies of lymphocyte subpopulations in bone marrow (BM) and peripheral blood (PB) samples should be evaluated.

Limitations

This study has a number of limitations. The data was collected retrospectively, and patients were treated with different protocols and the methods of collecting of some data (e.g. cytogenetic, molecular markers) were not standardized since only certain number of patients had

cytogenetic and molecular studies. Also, these studies were done at different times (e.g. at diagnosis, during the treatment, or after the remission). And finally the methods of tracking follow-up responses were not standardized and chemotherapeutic regimen was subjectively categorized into three different intensities: High, Intermediate and Low.

CHAPTER V: Conclusion

There are established and well-known contributing factors that play an important role in AML prognosis. The mortality rate of adult patients with AML is very high and the survival rate is very low compare to other cancers and leukemic subgroups, and suggesting continuous new researches in prognostic factors could be identified. Some studies already validated that ALC is another possible key factor that contributes in outcomes of other malignancies; this study was designed to evaluate the contribution of ALC in prognosis of AML in adult populations.

This study's findings were not consistent with the studies that revealed a positive correlation between ALC and OS, RFS, and CR durations of other cancers; rather, the statistical analysis of this study concluded exactly opposite results from other studies. In conclusion, this study revealed that there was an inverse correlation between ALC and AML outcomes. Further studies are needed to evaluate other key unknown variables to improve AML patient care and outcomes.

References

American Cancer Society (2014). *Chemotherapy for acute myeloid leukemia.* Retrieved from http://www.cancer.org/cancer/leukemia-acutemyeloidaml/overviewguide/leukemia-aml-overview-treating-chemotherapy

Bachanova, V., Cooley, S., Defor, T. E., Verneris, M. R., Zhang, B., McKenna, D. H., … Miller, J.S. (2014). Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. *Blood, 123* (25), 3855-3863. doi: 10.1182/blood-2013-10-532531

Barjesteh van Waalwijk van Doorn-Kkhosrovani, S., Erpelinck, C., Meijer, J., van Oosterhoud, S., van Putten, W. L., Valk, P. J, … Delwel, R. (2003). Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. *Hematology Journal, 4*(1): 31-40. doi: http://dx.doi.org/10.1182/blood-2008-09-179895

Behl, D., Porrata, L.F., Markovic, S.N., Letendre, L., Pruthi, R.K., Hook, C.C., … Litzow, M.R. (2006). Absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in acute myelogenous leukemia. *Leukemia, 20* (1), 29-34.

Bienz, M., Ludwig, M., Leibundgut, E.O., Mueller, B.U., Ratschiller, D., Solenthaler, M., … Pabst, T. (2005). Risk assessment in patients with acute myeloid leukemia and a normal karyotype. *Clinical Cancer Research, 11*(4), 1416-1424. doi: 10.1158/1078-0432.CCR-04-1552

Boissel, N., Renneville, A., Biggio, V., Phillippe, N., Thomas, X., Cayuela, J.M., … Preudhomme, C., (2005). Prevalence, clinical profile, and prognosis of NPM mutations in AML with normal karyotype. *Blood, 106*(10), 4011-4020. doi: http://dx.doi.org/10.1182/blood-2005-05-2174

Buggins, A.G., Milojkovic, D., Arno, M.J., Lea, N.C., Mufti, G.J., Thomas, N.S., & Hirst, W.J. (2001). Microenvironment produced by acute myeloid leukemia cells prevents T cell activation and proliferation by inhibition of NF-kappaB, c-Myc, and pRb pathways. *Journal of Immunology, 167*, 6021-6030. doi: 10.4049/jimmunol.167.10.6021

Byrd, J. C., Mrozek, K., Dodge, R. K., Carroll, A.J., Edwards, C. G., Arthur, D.C., … Cancer and Leukemia Group B. (2002). Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood, 100,* 4325-4336. doi: http://dx.doi.org/10.1182/blood-2002-03-0772

Chen, W.G., Rassidakis, G.Z. & Medeiros, L.J. (2006). Nucelophosmin gene mutations in acute myeloid leukemia. *Achieves of Pathology and Laboratory Medicine, 130*(11), 1687-1692.

Cheson, B. D., Bennett, J. M., Kopecky, K. J., Buchner, T., Willman, C. L., Estey, E. H., … Bloomfield, C. D. (2003). Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *Journal of Clinical Oncology, 21*(24), 4642-649. doi: 10.1200/JCO.2003.04.036

Ciollis, S., Vannucchi, A. M., Leoni, F., Nozzoli, C., Longo, G., Salati, A., … Bosi, A. (2004). Internal tandem duplications of FLT3 gene (FLT3/ITD) predicts a poor postremission outcome in adult patients with acute non-promyelocytic leukemia. *Leukemia Lymphoma, 45*(1), 73-78. doi: 10.1080/1042819031000151851

De Angulo, G., Yuen, C., Palla, S. L., Anderson, P. M., & Zweidler-McKay, P. A. (2008).

Absolute lymphocyte count is a novel prognostic indicator in ALL and AML. *Cancer, 112*(2), 407-415. doi: 10.1002/cncr.23168

Dohner, K., Schienk, R.F., Habdank, M., Scholl, C., Rucker, F.G., Corbacioglu, A.,… Dohner, H. (2005). Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. *Blood, 106*(12), 3740-3746. doi: http://dx.doi.org/10.1182/blood-2005-05-2164

Falini, B., Nicholetti, I., Martelli, M.F., & Mecucci, C. (2007). Acute myeloid leukemia carrying cytoplasmic/mutated nucleophosmin (NPMc+ AML): biological and clinical features. *Blood, 109*(3), 493-505. doi: http://dx.doi.org/10.1182/blood-2006-07-012252

Ferrant A, Doyen C, Delannoy A, Straetmans, N., Martiat, P., Mineur, P., …Michaux, J. L. (1995). Karyotype in acute myeloblastic leukemia: prognostic significance in a prospective study assessing bone marrow transplantation in first remission. *Bone Marrow Transplant, 15,* 685-690.

Frohling S., Sclenk, R. F., Stoize, I. Bihlmayr, J., Benner, A., Kreitmeier, S., … Dohner, K. (2004). CEBPA mutations in younger adults with acute myeloid leukemia and normal cytogenetics: prognostic relevance and analysis of cooperating mutations. *Journal of Clinical Oncology, 22*(4), 624-633. doi: 10.1200/JCO.2004.06.060

Gasteiger, G., & Kastenmuller, W. (2012). Foxp3+ Regulatory T-cells and IL-2: The Moirai of T-cell Fates? *Frontiers in Immunoogy 3*:179. doi: 10.3389/fimmu.2012.00179

Gooley, T. A., Cchien, J. W., Pergam, S. A., Hingorani, S., Sorror, M., Boeckh, M., …

McDonald, G. B. (2010). Reduced mortality after allogeneic hematopoietic cell transplantation. *New England Journal of Medicine, 363*, 2091-2101. doi: 10.1056/NEJMoa1004383

Grimwade, D., Hills, R. K., Moorman, A. V., Walker, H., Chatters, S., Goldstone, A. H., … Burnett, A. K.v. (2010). Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger patients treated in the United Kingdom Medical Research Council trials. *Blood, 116*(3), 354-365. doi: http://dx.doi.org/10.1182/blood-2009-11-254441

Grimwade D, Walker H, Oliver F, Wheatley, K., Harrison, C., Harrison, G., …Goldstone, A. (1998). The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. *Blood, 92*, 2322-2333.

Hahn, T., McCarthy, P.L., Hassebroek, A., Bredeson, C., Gajewski, J.L., Hale, G.A., … Majhail, N.S. (2013). Significant improvement in survival after allogeneic hematopoietic cell transplantation during a period of significantly increased use, older recipient age, and use of unrelated donors. *Journal of Clinical Oncology, 31*(19), 2437-2449. doi:10.1200/JCO.2012.46.6193

Helbig, G., Wozniczka, K., Wieclawek, A., Soja, A., Bartkowska-Chrobok, A., & Kyrcz-Krzemien, S. (2014). Clinical relevance of mutant NPM1 and CEBPA in patients with acute myeloid leukemia-preliminary report. *Contemporary Oncology, 18*(4), 241-245. doi: 10.5114/wo.2014.43490

Huether, S.E., McCance, K.L., Brashers, V.L., & Rote, N.S. (2012). Understanding pathophysiology (5th ed.). St. Louis, MO: Mosby.

Kainz, B., Heintel, D., Marculescu, R., Schwarzinger, I., Sperr, W., Le, T., Weltermann, A., … Jaeger, U. (2002). Variable prognostic value of FLT3 internal tandem duplications in patients with de novo AML and a normal karyotype, T(15;17), T(8;21), or inv(16). *Hematology Journal, 3*(6), 283-289. doi: 10.1038/sj.thj.6200196

Koreth, J., Schlenk, R., Kopecky, K. J., Honda, S., Sierra, J., Djulbegovic, B. J., … Culter, C. (2009). Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systemic review and meta-analysis of prospective clinical trials. *The Journal of American Medical Association, 301*(22), 2349-2361. doi: 10.1001/jama.2009.813

Kiyoi, H., & Naoe, T (2006). Biology, clinical relevance, and molecularly targeted therapy in acute leukemia with FLT3 mutation. *International Journal of Hematology, 83*(4), 301-308.

Kumar, S., Chen, M.G., Gastineau, D.A. Gertz, M.A. Inwards, D.J., Lacy, M.Q., …Litzow, M.R. (2001). Effect of slow lymphocyte recovery and type of graft-versus-host disease prophylaxis on relapse after allogeneic bone marrow transplantation for acute myelogenous leukemia. *Bone Marrow Transplantation, 28*(10), 951-956. doi: 10.1038/sj.bmt.1703262

Le Jeune, C., Bertoli, S., Elhamri, M., Vergez, F., Borel, C., Huguet, F., …Thomas, X. (2014). Initial absolute lymphocyte count as a prognostic factor for outcome in acute myeloid leukemia. *Leukimea Lymphoma, 55*, 855-862. doi:10.3109/10428194.2013.813504

Leukemia and Lymphoma Society (2014). *Disease information and support: treatment Outcomes.* Retrieved from http://www.lls.org/diseaseinformation/leukemia/acutemyeloidleukemia/treatment/outcomes/

Leukemia and Lymphoma Society (2014). *Facts and Statistics*. Retrieved from http://www.lls.org/diseaseinformation/getinformationsupport/factsstatistics/

Li, J., Jiang, R., Liu, W., Liu, Q., Su, M., Feng, Q., …Zeng, Y. (2013). A large cohort study reveals the association of elevated peripheral blood lymphocyte-to-monocyte ratio with favorable prognosis in nasopharyngeal carcinoma. *Public Library of Science, 8*(12), 1-10. doi: 10.1371/journal.pone.0083069

Li, Y., Gu, K., Pan, Y., Jiao, Y., & Zhai, Z. (2014). The lower peripheral blood lymphocyte/monocyte ratio assessed during routine follow-up after standard first-line chemotherapy is a risk factor for predicting relapse in patients with diffuse large B-cell lymphoma. *Leukemia Research, 38* (3), 323-328. doi: 10.1016/j.leukres.2013.12.005

Liersch, R., Muller-Tidow, C., Berdel, W., & Krug, U. (2014). Prognostic factors for acute myeloid leukemia in adults-biological significance and clinical use. *British Journal of Haematology, 165*(1), 17-38. doi: 10.1111/bjh.12750

Mayoclinic. (2012*). Acute myeloid leukemia: treatments and drugs*. Retrieved from http://www.mayoclinic.org/diseases-conditions/acute-myelogenous-leukemia/basics/treatment/con-20043431

Mayoclinic. (2012). *Acute myelogenous leukemia (AML)*. Retrieved from http://www.mayoclinic.org/diseases-conditions/acute-myelogenous leukemia/basics/definition/con-20043431

Mrozek, K., & Bloomfield, C.D. (2006). Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. *Hematology, 2006*(1), 169-177. doi: 10.1182/asheducation-2006.1.169

Mrozek, K., Heinonen, K., de la Chapelle, A., & Bloomfield, C. D. (1997). Clinical significance

of cytogenetics in acute myeloid leukemia. *Seminars in Oncology, 24*, 17-31.

Mrozek, K., Heinonen, K., & Bloomfield, C. D. (2000). Prognostic value of cytogenetic findings in adults with acute myeloid leukemia. *International Journal Hematology 72*: 261-271.

Mrozek, K., Heinonen, K., & Bloomfield, C. D. (2001). Clinical importance of cytogenetics in acute myeloid leukemia. *Best Practice Result Clinical Hematology, 14*, 19-47.

National Cancer Institute. (2014). *General information about adult acute myeloid leukemia.* Retrieved from http://www.cancer.gov/cancertopics/pdq/treatment/adultAML/Patient/page1

National Cancer Institute. (2014). *Surveillance, epidemiology, and end results program (SEER) stat fact sheets: Leukemia.* Retrieved from http://www.seer.cancer.gov/statfacts/html/leuks.html

National Cancer Society. (2014). *Adult acute myeloid leukemia treatment: adult acute myeloid leukemia in remission.* Retrieved from http://www.cancer.gov/cancertopics/pdq/treatment/adultAML/healthprofessional/page6

National Cancer Institute (2014). *Adult acute myeloid leukemia treatment: stages of adult acute myeloid leukemia*. Retrieved from http://www.cancer.gov/cancertopics/pdq/treatment/adultAML/Patient/page2

National Institutes of Health (2012). *Acute myeloid leukemia.* Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000542.htm

Orleans-Lindsay, J. K., Barber, L. D., Prentice, H. G., & Lowdell, M. W. (2001). Acute myeloid leukaemia cells secrete a soluble factor that inhibits T and NK cell proliferation but not cytolytic function--implications for the adoptive immunotherapy of leukaemia. *Clinical and Experimental Immunology, 126*, 403-411. doi: 10.1046/j.1365-2249.2001.01692.x

Orozco, J.J. & Appelbaum, F.R. (2012). *Unfavorable, complex, and monosomal karyotypes: the most challenging forms of acute myeloid leukemia.* Physicians Practice. Retrieved from http://www.physicianspractice.com/login?referrer=http%3A//www.physicianspractice.com%2Funfavorable-complex-and-monosomal-karyotypes-most-challenging-forms-acute-myeloid-leukemia

Peccatori, J., & Ciceri, F. (2010). Allogeneic stem cell transplantation for acute myeloid leukemia. *Hematologica, 95*(6), 857-859. doi:10.3324/haematol.2010.023184

Porrata, L. F., Litzow, M. R, Jefferi, A., Letendre, L., Kumar, S., Geyer, S. M., & Markovic, S. N. (2002). Early lymphocyte recovery is a predictive factor prolonged survival after autologous hematopoietic stem cell transplantation for acute myelogenous leukemia. *Leukemia, 16*(7), 1311-1318.

Preudhomme, C., Sagot, C., Boissel, N., Cayuela, J.M., Tigaud, I., De Botton, S., …Dombret, H. (2002). Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). *Blood, 100*(8), 2717-2723. doi: http://dx.doi.org/10.1182/blood-2002-03-0990

Samuels, B.L., Larson, R.A., Le Beau, M.M., Daly, K.M., Bitter, M.A., Vardiman, J.W., … Golomb, H.M. (1998). Specific chromosomal abnormalities in acute nonlymphocytic leukemia correlate with drug susceptibility in vivo. *Leukemia, 2* (2), 79-83.

Schlenk, R.F., Dohner, K., Kruter, J., Frohling, St., Corbaciouglu, A., Bulilnger, L., … Dohner, H. (2008). Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *New England Journal of Medicine, 358*, 1909-1918. doi: 10.1056/NEJMoa074306

Schnittger, S., Schoch, C., Kern, W., Mecucci, C., Tschulik, C., Martelli, M.F., … Falini, B.

(2005). Neucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. *Blood, 106*(12), 3733-3739. doi: http://dx.doi.org/10.1182/blood-2005-06-2248

Seattle Cancer Care Alliance (n.d.). *Acute myelogenous leukemia (AML): Diagnosing AML.* Retrieved from http://www.seattlecca.org/diseases/adult-aml-diagnosingaml.cfm

Shenghui, Z., Yixiang, H., Jianbo, W., Kang, Y., Laixi, B., Yan, Z., & Xi, X. (2011). Elevated frequencies of CD4(+) CD25(+) CD127 low regulatory T cells is associated to poor prognosis in patients with acute myeloid leukemia. *Inernationalt Journal of Cancer, 129*, 1373-1381. doi: 10.1002/ijc.25791

Slovak, M. L., Kkopecky, K. J., Cassileth, P. A. Harrington, D. H., Theil, K. S., Mohamed, A., … Appelbaum, F. R. (2000). Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: A Southwest Oncology Goup/Eastern Cooperative Oncology Group Study. *Blood, 96*(13), 4075-4083.

Stirewalt, D. L., Kopecky, K. J., Meshinchi, S., Engle, J. H., Pogosova-Agadjanyan, E. L., Linsley, J., … Radich, J. P. (2005). Size of FLT3 internal tandem duplication has prognostic significance in patients with acute myeloid leukemia. *Blood, 107*(9), 3724-3726. doi: http://dx.doi.org/10.1182/blood-2005-08-3453

Szczepanski, M.J., Szajnik, M., Czystowska, M., Mandapathil, M., Strauss, L., Welsh, A., … Boyiadzis, M. (2009). Increased frequency and suppression by regulatory T cells in patients with acute myelogenous leukemia. *Clinical Cancer Research, 15*, 3325-3332. doi: 10.1158/1078-0432.CCR-08-3010.

Thiede, C., Koch, S., Creutzig, E., Steudel, C., Illmer, T., Schaich, M.m & Ehninger, G. (2006). Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute

myeloid leukemia (AML). *Blood, 107*(10), 4011-4020. doi: http://dx.doi.org/10.1182/blood-2005-08-3167

University of Washington: Department of Laboratory Medicine (2010). *Hematopathology Section.* Retrieved from http://depts.washington.edu/labweb/Divisions/Hema/Flow.htm

Verhaak, R.G. Goudswaard, C.S., Van Putten, W., Bijl, M.A. Sanders, M.A., Hugens, W., … Valk, P.J. (2005). Mutations in nuclosphosmin (NPM1) in acute myeloid leukemia (AML): Association with other gene abnormalities and previous established gene expression signatures and their favorable prognostic significance. *Blood, 106*(12), 3747-3754. doi: http://dx.doi.org/10.1182/blood-2005-05-2168

Williams, K. A., Labidi-Galy, S. I., Terry, K. L., Vitonis, A. F., Welch, W. R., Goodman, A., & Cramer, D. W. (2014). Prognostic significance and predictors of the neutrophil-to-lymphocyte ratio in ovarian cancer. *Gynecologic Oncology, 132*(3), 542-550. doi: 10.1016/j.ygyno.2014.01.02

Yang, W., & Xu, Y. (2013). Clinical significance of Treg cell frequency in acute myeloid leukemia. *International Journal of Hematology, 98*(5), 558-562. doi: 10.1007/s12185-013-1436-3

Zhou, Q., Bucher, C., Munger, M. E., Highfill, S. L., Tolar, J., Munn, D. H.,…Blazar, B.R. (2009). Depletion of endogenous tumor-associated regulatory T cells improves the efficacy of adoptive cytotoxic T-cell immunotherapy in murine acute myeloid leukemia. *Blood, 114*, 3793-3802. doi: 10.1182/blood-2009-03-208181