The Role of Consolidation in Reduced Intensity Transplantation for Acute Myeloid Leukemia

by

Charles Kristjan Paulson

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Community Health Science

University of Manitoba

Winnipeg

Abstract:

Background: Allogeneic stem cell transplant is the most effective treatment for the majority of patients with acute myeloid leukemia. Unfortunately, this treatment is associated with significant toxicity, with a very real risk of death during the procedure. The risks of transplant are higher in older patients, and historically were considered especially prohibitive in patients older than age 60. Newer transplant protocols (reduced intensity protocols) using lower doses of chemotherapy can safely offer this curative treatment to older adults. However, reduced intensity protocols are associated with a higher risk of relapse. A strategy that combines the reduced toxicity of reduced intensity preparative regimens with the low relapse rate of standard transplant protocols would dramatically improve care for older patients with acute myeloid leukemia. We sought to understand whether giving patients additional chemotherapy prior to transplant might be an effective tool to reduce the risk of relapse after transplant through the use of a large retrospective database.

Methods: A theoretical framework was developed based on previous publications to help guide the project. Potential confounding variables from the database were identified using this theoretical framework. Patients who received consolidation chemotherapy prior to transplant were compared to patients who did not receive consolidation chemotherapy. A multivariable model was used to adjust for confounding variables, to understand if the use of consolidation chemotherapy

influenced overall survival, disease free survival, non-relapse mortality, and relapse rates.

Results: Patients who received consolidation chemotherapy (particularly high dose cytarabine) were more likely to be younger ($p < 0.01$), be transplanted in more recent years ($p < 0.01$), and to have received standard "7+3" as induction chemotherapy, but in other aspects were generally similar to those that did not. In univariate and multivariate analysis, there was no difference between groups in relapse rate. While there was a lower rate of treatment related mortality at one year in the consolidation group in univariate analysis, that difference was no longer significant at three years and in multivariate analysis. Finally, there was also no difference in overall survival between groups. Thus, this study did not find that the administration consolidation chemotherapy was associated with a lower relapse rate or improved overall survival, and consolidation chemotherapy.

Discussion: This study suggests that clinicians should not routinely administer consolidation chemotherapy with the aim of reducing relapse rates and improving outcomes after transplant. As a retrospective observational study, this study is subject to many potential forms of bias, most notably selection bias. We are lacking information on patients in whom consolidation chemotherapy was planned but not given due to relapse or toxicity. However, with no randomized trial planned or likely to be done, this study provides the best available clinical evidence. There are other potential reasons for administering consolidation chemotherapy, most

commonly to ensure disease control while planning for allogeneic transplant. Unfortunately, relapse after transplant is still common; therefore, other methods of reducing the risk of relapse should be a direction for future study.

Acknowledgements:

There are more people who deserve acknowledgement than there is space available. That said, some individuals and groups deserve special mention:

- Dr. Donna Turner, for agreeing to my persistent requests to serve as my supervisor and helping me throughout the extended duration of the project;
- Dr. Mary Eapen, and the team at the Medical College of Wisconsin and the Centre for International Blood and Marrow Transplantation, for their support in making this study possible and building the foundations of my research career;
- Dr. Donna Wall, my supervisor in my Leukemia/BMT fellowship, during which I completed this M.Sc. and for her mentorship over the past 5 years as I've transitioned to practice;
- The team of dedicated clinical research professionals at CancerCare Manitoba and across the world who make this work possible;
- The R. Samuel McLaughlan Foundation, the CancerCare Manitoba Foundation, and Research Manitoba for fellowship funding which allowed me to complete this project; and
- The team of nurses, pharmacists, and physicians I work with in the Leukemia/Bone Marrow Transplant Clinic CancerCare Manitoba and the Health Sciences Centre, for their dedicated care of a critically ill group of patients.

Dedications:

This thesis is dedicated to:

- My parents, for fostering an environment growing up where education was the most important goal;
- My wife, for supporting me every step of the way through my many degrees, fellowships, and exams; and
- My son, who appears to have inherited the same curiosity and love of learning as his parents.

Table of Contents:

List of Tables:

List of Figures:

Introduction:

1.1: Descriptive Epidemiology

Leukemias are blood cancers that are associated with increased numbers of abnormal immature cells in the peripheral blood or bone marrow, and are categorized based on their presentation. Acute leukemias usually develop over weeks to months, while chronic leukemias may take years to develop and typically progress slowly. Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults. In leukemia, the abnormal immature blood cells divide and reproduce rapidly within the bone marrow. These leukemia cells can escape the bone marrow and enter the peripheral blood. In addition, the abnormal leukemia cells crowd out normal hematopoietic cells within the bone marrow, causing anemia (low hemoglobin) and thrombocytopenia (low platelet count). Patients may present initially for medical attention due to either symptoms from the progressively increasing number of abnormal immature white blood cells, or due to the decreased number of normal blood cells.

Acute myeloid leukemia is treated with aggressive, multi-agent chemotherapy. While most patients respond to chemotherapy and achieve an initial remission, relapses after an initial remission are common. Often, patients require a

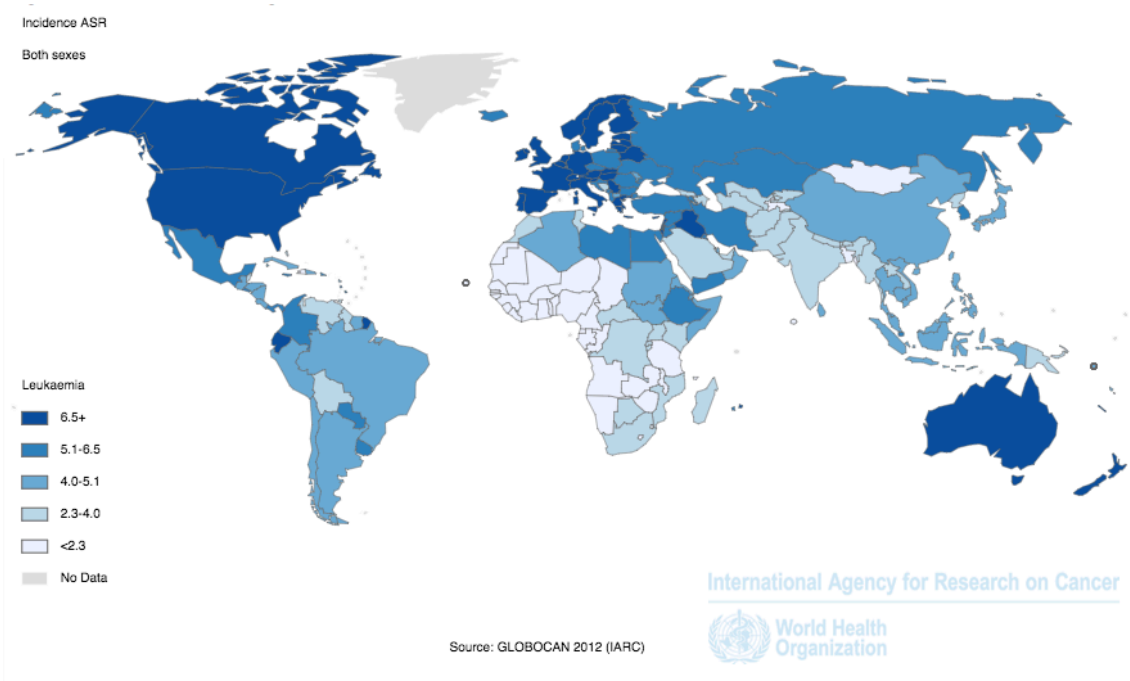
stem cell transplant as curative therapy, which replaces their blood production system with that of a family member or unrelated donor in an effort to remove the abnormal leukemia cells. In younger patients, the combination of multi-agent chemotherapy with stem cell transplant can be curative in many cases. However, as the stem cell transplant itself is associated with significant toxicity, it is associated with unacceptable treatment related mortality in older patients.

Acute myeloid leukemia is a relatively uncommon type of cancer. According to data from the Canadian Cancer Registry, there were 1195 new cases of AML in Canada in 2009, and 1180 new cases in 2010.[1] The age standardized incidence rate of AML was 3.0 new cases per 100,000 population in 2008, and 2.9 new cases per 100,000 population in 2009 (standardized to the 1991 Canadian Census population).[1] AML has a much lower incidence than other common cancers. For comparison, the age standardized incidence of lung cancer was 69.4 cases per 100,000 population, and the age standardized incidence of colorectal cancer was 62.2 cases per 100,000 population.[1] The incidence of AML is also lower than chronic lymphocytic leukemia, the most common leukemia (age standardized incidence of 6.3 cases per 100,000 population), but higher than other leukemias like chronic myeloid leukemia (1.6 cases per 100,000 population) and acute lymphoblastic leukemia (1.2 cases per 100,000 population).[1]

The incidence of all forms of acute leukemia are higher in countries that are more developed, possibly due to improved methods of diagnosis and detection.[2,3]

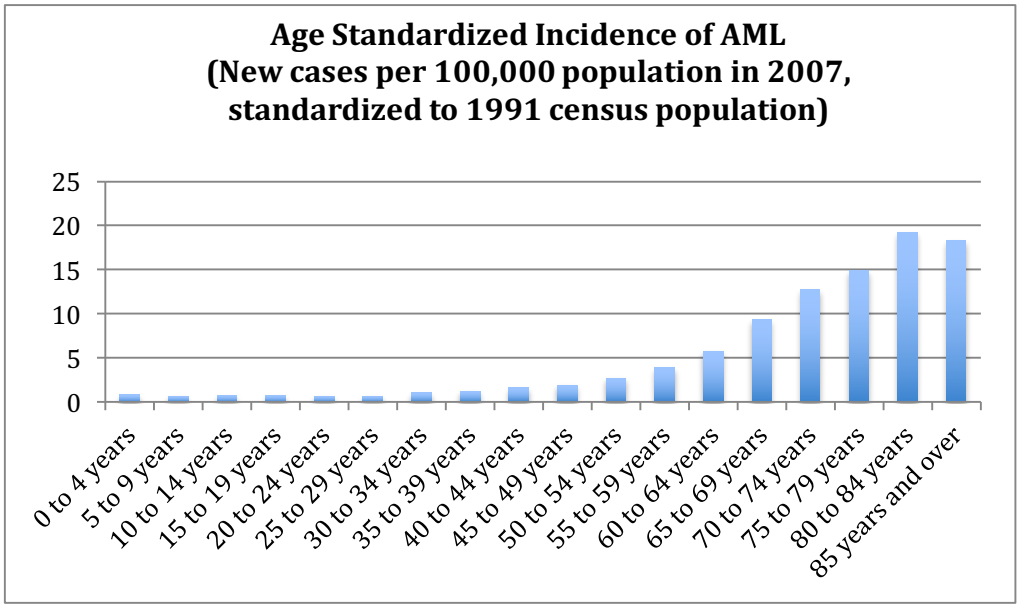
GLOBOCAN is a product produced by the International Agency for Research on Cancer, and attempts to estimate the risk of cancer subtypes worldwide. The data used to generate these estimates vary from data directly provided by cancer registries (high quality), to estimates based on neighboring countries in nations without cancer registries (low quality). GLOBOCAN groups all forms of acute and chronic leukemia together into one category, making detailed country-by-country comparisons of AML difficult. According to GLOBOCAN, the highest incidence of leukemia was in developed nations such as New Zealand, Israel, the United States, Australia, and Canada.[4] The lowest incidence was primarily in developing nations in sub-Saharan Africa. Figure 1 illustrates the differences in the incidence of leukemia in all nations reporting data to GLOBOCAN.

Figure 1: Incidence of Leukemia in GLOBOCAN Reporting Nations



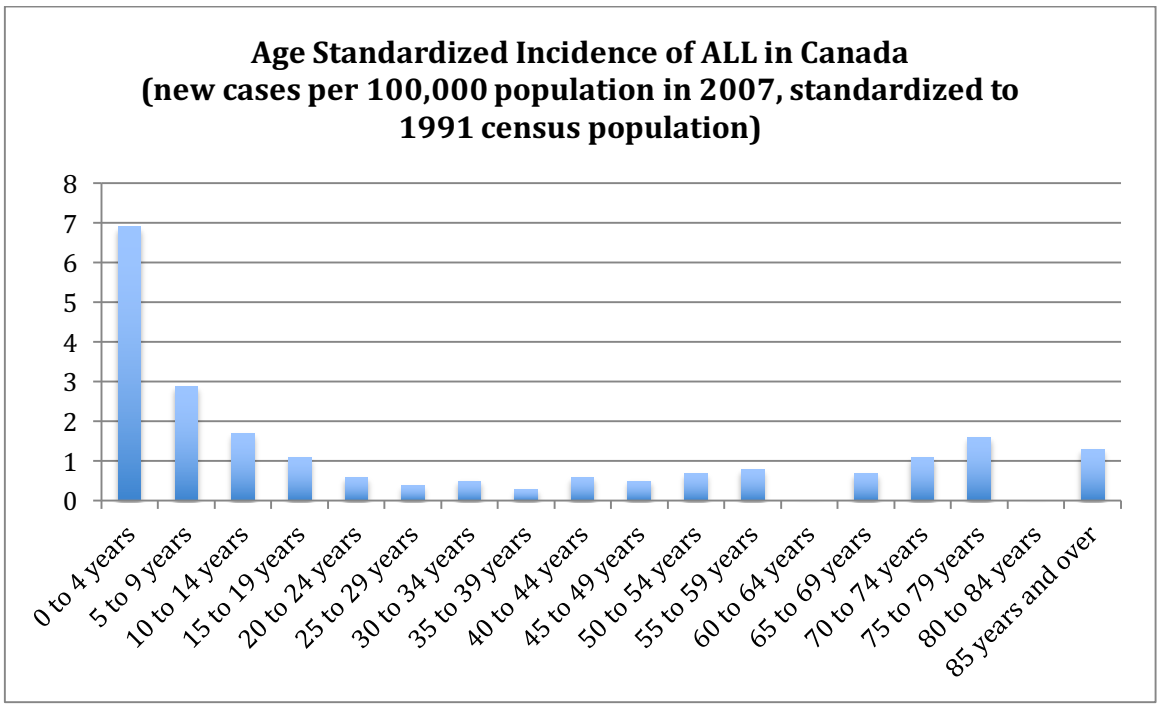
While acute myeloid leukemia can occur at any age, it is more common in the elderly. In Canada, the age-specific incidence of AML is 0.8 cases per 100,000 population in those younger than 15 years, while it is as high as 19.3 cases per 100,000 population in those 80-84 years of age (see Figure 2).[1] The incidence of AML remains relatively unchanged until age 50, at which point it begins to increase. In terms of absolute numbers, 869 (77.9%) new cases of AML were diagnosed in patients over the age of 50 in 2007, as compared to 246 (22.1%) in patients under the age of 50. The age distribution of the incidence of AML is in stark contrast to the other type of acute leukemia, acute lymphoblastic leukemia (ALL), which is much more common in younger age groups, and is less common in adults (Figure 3). Of the new cases of ALL in 2007, 55.6% occurred in patients younger than age 15, with only 44.4% of new cases occurring in patients aged 15 years or older.

Figure 2: Age Standardized Incidence of AML



Source: CANSIM[1]

Figure 3: Age Standardized Incidence of ALL



Source: CANSIM[1]

The incidence of AML in Canada in 2006 and 2007 was similar between males and females. In 2007, the incidence was 3.5 cases per 100,000 population for men, and 3.2 cases per 100,000 population for women, both adjusted to the 1991 census population.

In 2012, there were 72 new cases of AML in Manitoba.[4] The age standardized incidence was 5.36 cases per 100,000 population, standardized to the 2001 Manitoba population.[4] Due to small numbers, data on the incidence of AML in

Manitoba stratified by age group are censored by CANSIM. The *Cancer in Manitoba; 2008 Annual Statistics Report* produced by the Epidemiology and Cancer Registry of CancerCare Manitoba does provide some information on the incidence of AML by age group (Table 1).[4] The most recent year for which data on AML was reported separately from other types of leukemia was 2008. At that time, the incidence of AML in those younger than 29 was 1.46 cases per 100,000 population, as compared to 0.56 in those aged 40 – 59, 5.59 in those aged 50 – 59, 12.20 in those aged 60 – 69, 10.22 in those aged 70 – 79, and 17.56 in those over 80. Due to small numbers, no breakdown in the incidence by region is available. The mortality rate generally paralleled the incidence rate, with a lower mortality rate in younger patients, and a higher mortality rate in older patients (see Table 1). In older patients, the mortality rate was similar to the incidence rate, reflecting the very poor prognosis of this disease in older individuals.

Table 1: Incidence of AML in Manitoba in 2008 [4]

Age Group	New Cases	Crude Rate (per 100,000 population)	Deaths	Crude Rate (per 100,000 population)
0 – 29	7	1.46	0	0
30 – 39	0	0	1	0.64
40 – 49	1	0.56	2	1.12
50 – 59	9	5.59	5	3.10
60 – 69	13	12.20	7	6.57
70 – 79	7	10.22	5	7.30
80+	9	17.56	9	17.56
Total	46	3.82	29	2.41

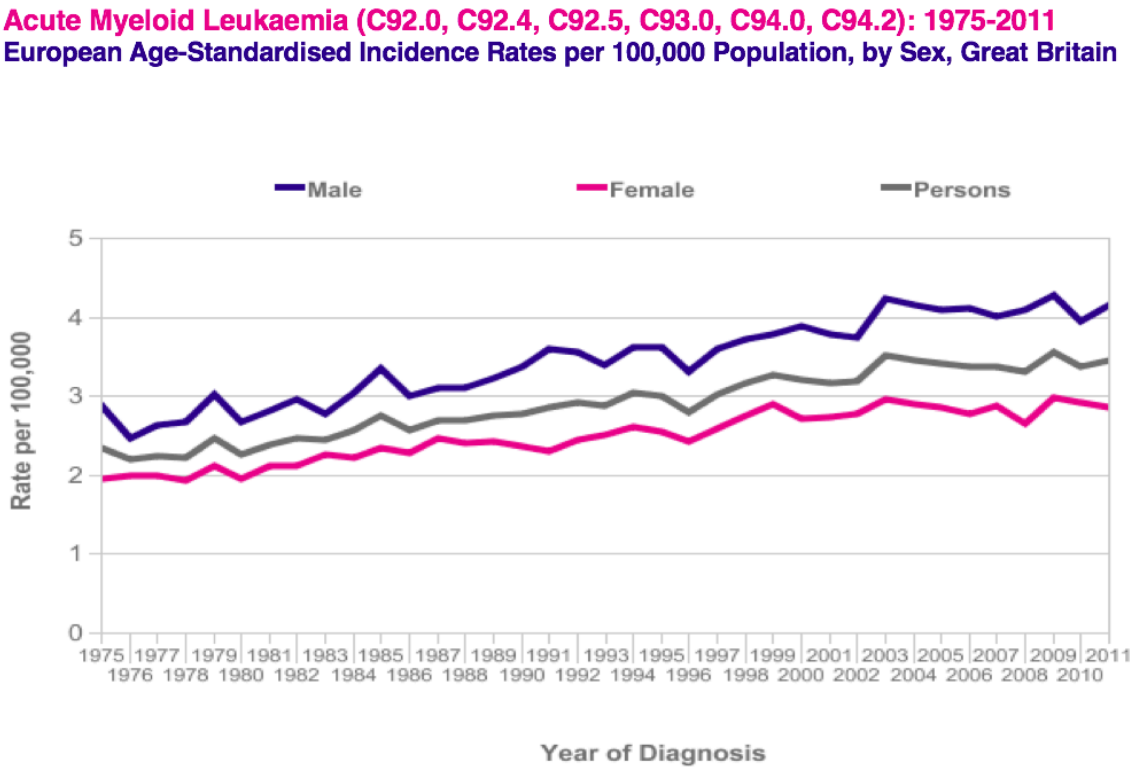
There are no Canadian data describing the difference in incidence of AML among different racial or ethnic groups. The Surveillance, Epidemiology, and End Result registry (SEER), an American population based cancer registry, has data on

the incidence of AML among different ethnic groups. The most recent data available are from 2004 – 2008. The incidence and mortality rates are similar to Canadian data. The incidence of AML in the United States was 3.5 cases per 100,000 population, adjusted to the US 2000 standard population.[5] While different standard populations were used, making direct comparisons impossible, this number is roughly comparable to the Canadian incidence of 2.9 cases per 100,000 population in 2009, standardized to the 1991 Canadian census population.[1] In the SEER data, the incidence was higher in men than women, and of a greater magnitude than seen in Canadian data. The incidence was 4.3 cases per 100,000 population in men, and only 3.0 cases per 100,000 population in women (both age-adjusted to the US 2000 standard population).[5] There were some differences seen between races. White Americans had the highest incidence of AML, with an age standardized incidence of 4.5 cases per 100,000 population for men, as compared to 3.4 cases per 100,000 population for black Americans, and only 2.3 cases per 100,000 American Indian men.[5] A similar pattern was seen in women.

There are limited data on the potential years of life lost due to AML, likely due to the low incidence of the disease. When all leukemias are grouped together, leukemia accounts for a higher number of potential years of life lost than would be expected based on incidence alone, due to the high mortality rate and young age of onset. In Canada, all leukemias represent 3.4% of all potential years of life lost due to cancer, while leukemias account for 2.6% of all cancers.[6] Similarly, according to data from CancerResearch UK, leukemias represent 2.5% of all cancers diagnosed in

the United Kingdom, but account for 3.0% of all cancer deaths, and 3.6% of all potential years of life lost.[7,8] There are no data on the prevalence of AML in the literature, as patients with AML are typically cured rapidly with systemic chemotherapy, or die from their disease within months of diagnosis, leading to a short duration of illness. There are no Canadian data on the change in incidence of AML over time, but no significant change has been seen in other comparable nations. According to SEER data, the incidence of AML decreased by 5.4% between 2001 and 2004, but increased by 1.8% between 2004 and 2008. In the United Kingdom, the incidence of AML increased by 24% for men, and 22% for women between 1975 and 1999, but have largely remained stable since.[5,6]

Figure 4: Incidence of AML over time



Acute myeloid leukemia is the most common form of acute leukemia, and while it is more common in older adults, it remains one of the most common cancers in younger patients due to the relative infrequency of other malignancies in young patients. Due to the relatively high incidence in this age group, and the high mortality rate, leukemia was the second leading cause of cancer deaths in men aged 20-44 in Canada between 1990 and 1999.[9]

1.2: Risk Factors for AML

Risk factors for AML can generally be divided into exposures that are rare, but are strongly associated with AML (such as ionizing radiation, benzene, and chemotherapy), and exposures that are common but are weakly associated with AML (such as alcohol use, smoking, occupational chemical exposures, and dietary risk factors). In addition, although hereditary causes of AML are relatively rare, there are several genetic disorders associated with AML.

Most cases of AML are sporadic, and are not associated with any specific environmental, genetic, or infectious risk factor.[2,10] There are several notable exceptions to this. Firstly, AML is associated with a well-known pre-leukemic state known as myelodysplastic syndrome (MDS). Most patients with AML do not present with antecedent MDS, but a majority of cases of MDS, if followed for a sufficient length of time, will eventually progress to AML.[11,12] In addition, AML (and MDS) are

the most common secondary malignancies in patients who have previously received chemotherapy or radiation therapy. In particular, exposure to alkylating agents or anthracyclines is strongly associated with the development of AML or MDS, with up to 5% of patients on some intense chemotherapy protocols developing secondary AML or MDS.[13] Finally, radiation is a significant risk factor for AML, and among patients exposed to high doses of radiation (Hiroshima or Chornobyl survivors), a significant burden of leukemia disease exists due to radiation exposure.[14-16]

Radiation was the first exposure to be linked to the development of AML. Both Marie Curie and her daughter Irene died of leukemia, most likely AML, due to exposure to ionizing radiation during initial experiments with radiation. Ionizing radiation directly damages DNA, leading to increased mutagenesis.[16] Pioneering work done shortly after the atomic bomb was dropped on Hiroshima demonstrated a dramatic rise in all forms of leukemia, including AML, to those exposed to radiation. The highest risk was in those closest to the epicentre, with a relative risk of 15 for the development of AML among those less than 1 km, compared to those more than 10 km away.[17] Similar work was conducted amongst survivors of the Chernobyl nuclear accident, demonstrating similar findings.[14,15,18] Exposure to large doses of radiation fulfills many of Bradford-Hill's criteria for determining if an association between an exposure and outcome is causally related.[19] Exposure to radiation precedes the development of AML (*temporality*), the association is relatively strong (*strength of association*), a biologic gradient exists, and there is biologic probability present (*damage to DNA by ionizing radiation*).

Fortunately, exposure to large doses of ionizing radiation are quite rare. However, other groups of individuals are chronically exposed to lower doses of radiation over a longer period of time. There is mixed evidence suggesting that chronic exposure to low dose ionizing radiation is associated with an increased risk of leukemia. A Canadian cohort study examined the risk of leukemia among 45,468 nuclear power workers in Canada.[20] A non-statistically significant increased risk of the development of non-chronic lymphocytic leukemia (most likely AML) was seen among workers exposed to higher lifetime doses of radiation, with a relative risk of 3.54 for workers exposed to 50-99 mSv of radiation (95% CI 0.34 – 37.2), and a relative risk of 6.6 for those exposed to > 100 mSv of radiation (95% 0.54 – 79.3). Thus, while a higher rate of AML was seen in exposed workers, and it did increase with increasing exposure, even with the large numbers of patients included, no significant difference was seen.[20] This finding contradicts an American study, which followed 18,883 workers at the Savannah River nuclear research site, and did find an increased risk of death from non-CLL leukemia.[21] Thus, chronic exposure to lower doses of radiation may be associated with an increased risk of AML, but the full effect remains yet to be determined.

The second significant risk factor for AML is exposure to prior chemotherapy. Between 10 and 15% of all cases of AML are associated with prior chemotherapy, commonly alkylating agents and anthracyclines.[22] In addition, secondary AML is typically associated with abnormalities of chromosomes 5 and 7, both of which are

adverse prognostic factors.[23] The rate of secondary AML varies depending on the chemotherapeutic agent, dose, and duration of exposure. Hershman et al. reviewed the rate of secondary AML amongst breast cancer patients using SEER data, and found that 1.2% of patients went on to develop AML.[24] In clinical trials comparing therapies for Hodgkin Lymphoma, the rate of secondary AML/MDS was 3.2%, and in studies of patients with lymphoma undergoing autologous transplantation,[13] the rate of secondary AML was 4.5%.[25] In several cohort studies, the relative risk for the development of AML has ranged from 20 – 80 among patients with Hodgkin Lymphoma treated with chemotherapy.[26-29] In patients with active malignancy, the small increased risk of secondary AML is usually outweighed by the benefits of the treatment, but in some cancers such as Hodgkins Lymphoma, with high cure rates, recent focus has been on titrating therapy to maintain efficacy but minimize long-term toxicity.

In high doses, benzene has been associated with an increased risk of AML. This has best been seen in several occupational cohorts, including a cohort of Turkish shoe workers, and Chinese factory workers.[30] These groups were exposed to doses of benzene much higher than many occupational groups would be today. Benzene may cause direct damage to DNA, and it has several metabolites that have anti-topoisomerase II activity, similar to anthracycline chemotherapeutic agents, and it is through these mechanisms that it may cause AML.[31] Workers in several industries, including the petrochemical industry and the manufacturing industry, have occupational exposures to benzene, albeit at lower doses than previous

cohorts where a definite association was seen. Several studies have focused on the leukemogenic potential of chronic exposure to lower doses of benzene. In a cohort of 1845 workers involved in the manufacture of rubber hydrochloride, a small increase in the mortality due to AML was found, with a relative risk of 1.19 for AML deaths less than 10 years after the exposure (95% C.I. 1.10 – 1.28), but no significant association was found for AML deaths more than 10 years after the exposure.[32] Similarly, in a population exposed to benzene following a large gasoline leak, a small but statistically significant increase in the risk of developing AML was found (standardized incidence ratio 4.40, 95% C.I. 1.09 – 10.24).[33] Thus, some evidence suggests that chronic exposure to lower doses of benzene may also be harmful, although this is an area of ongoing research.

Genetic causes of AML are relatively rare, but several genetic syndromes are associated with the development of AML. Li-Fraumeni syndrome, an autosomal dominant disorder of the p53 tumor suppressor gene, is commonly associated with acute lymphoblastic leukemia (ALL), but has also been rarely associated with AML.[34] Fanconi Anemia is an autosomal recessive disorder of DNA repair, and can present with aplastic anemia, myelodysplastic syndrome, or AML. Up to one third of patients with Fanconi Anemia will eventually develop AML by age 40.[35] AML is particularly challenging to manage in patients with Fanconi Anemia, as the DNA repair defect leads to significant toxicity with standard chemotherapy approaches.[36] Finally, Down syndrome is associated with an increased rate of an unusual subtype of acute myeloid leukemia, acute megakaryoblastic leukemia. Patients with Down

syndrome have a relative risk of 10 – 20 for the incidence of AML, although only about 1% of children with Down syndrome will develop AML.[37,38]

A variety of other potential associations have been explored in the literature, including cigarette smoking, alcohol use, chemical exposures, hormonal differences, and diet. All of these exposures are much more common than chemotherapy or ionizing radiation, but are associated with a much smaller relative risk for the development of AML.

Of these less significant risk factors, the one with the strongest evidence to support a link with the development of AML is cigarette smoking. The exact mechanism is unclear, but cigarettes contain many toxic compounds that are known to be leukemogenic, including benzene.[39] One case control study conducted in Los Angeles county found that cigarette smoking was associated with an increased incidence of several subtypes of AML.[40] In another case control study, increased rates of paternal, but not maternal, smoking was found to be associated with an increased incidence of childhood leukemia.[41] In a cohort study, cigarette smoking was found to be an adverse prognostic factor, with a shorter event free survival and overall survival amongst cigarette smokers.[42] A meta-analysis was conducted, combining seven prospective cohort studies, and eight case-control studies. In the prospective studies, a small increase in the incidence of AML was found, with a relative risk of 1.3 (95% C.I. 1.3 – 1.4). A smaller increase was found in the case-control studies, with a relative risk of 1.1 (95% C.I. 1.0 – 1.2). Thus, the risk of AML

associated with cigarette smoking is of relatively small magnitude, but given the common nature of the exposure, the population-attributable risk was calculated to be 17% (or, 17% of cases of AML would be prevented if all cigarette smoking could be stopped).[42]

Many other chemicals have been hypothesized to be associated with the development of AML. There has been intense focus on the possible association between pesticide use and the development of cancer, and numerous cohort and case-control studies have been conducted. A systematic review and meta-analysis was conducted by Maele-Fabry et al. to determine if occupational exposure to pesticides was associated with the development of AML or chronic myeloid leukemia (CML).[43] Most studies reviewing the incidence of AML amongst manufacturing workers and pesticide applicators were cohort or case-control studies. A non-significant increase in the incidence of AML was found amongst those exposed to high doses of pesticides through occupational exposures (meta-RR 1.55, 95% C.I. 1.02 – 2.34). Lower doses of pesticides have not been studied, so the potential impact of casual exposure to pesticides is unclear. It is uncertain whether casual, low dose exposure to pesticides used in lawn and garden applications is associated with an increased risk of AML.

The immunomodulatory effect of alcohol may increase the incidence of AML, while the antioxidants contained in some types of alcohol (red wine) may reduce the risk of AML.[44] An American study, conducted in Iowa and Minnesota, found a no

significant increase in the incidence of AML (OR 1.3, p = NS), and no dose-response curve.[45] A similar case-control study, conducted in Los Angeles, found a no significant effect (OR 0.7, 95% C.I. 0.3 – 1.5). Again, there was no dose-response curve, with a similar odds ratios for those with the highest and lowest alcohol consumption.[46] An Italian study found a no significant change in the incidence of all types of leukemia amongst the heaviest drinkers (OR 1.15, 95% C.I. 0.82 – 1.63). There have been no prospective studies conducted in this area, and no retrospective cohort studies have been performed. Thus, on the basis of three contradictory case-control studies, one must conclude there is insufficient evidence to support or refute a potential association between alcohol use and the development of AML.

As the incidence of AML appears to be slightly lower in women than in men, the role of gonadal hormones in AML has also been explored. For example, in Canada, using data from the Canadian Cancer Registry, the incidence of AML in 2007 was 3.5 cases per 100,000 population for men, and 3.2 cases per 100,000 population for women.[1] The reason for this difference is unclear, and some authors have postulated that it may be due to the immunomodulatory effects of pregnancy. A cohort study was conducted in Sweden combining the population-based Fertility Register, which includes on the number of children each women has had (or none, as the case may be), and the Swedish Cancer Registry. Among women who had ever been pregnant, there was a slightly reduced risk of the development of AML, with an odds ratio of 0.85 (95% C.I. 0.69 – 1.0). Having been pregnant more than once did not appear to be associated with a lower risk of developing AML than being

pregnant only once. The authors concluded that pregnancy might be associated with a lower risk of developing AML, potentially due to hormones, although this remains uncertain.[47]

The relationship between diet, lifestyle, and the incidence of AML has also been studied. In the NIH-AARP Diet and Health Study, a detailed dietary questionnaire was sent to more than 3.5 million members of the American Association of Retired Persons in six states in the United States in 1995 and 1996. 491,163 responses were obtained. Incident cancer cases were determined through linkage with various state cancer registry databases, and a total of 338 cases of AML were identified. Cancer status was determined in 2003. Higher meat intake was found to be associated with an increased risk of AML (hazard ratio, comparing highest and lowest quintile = 1.45, 95% C.I. 1.02 – 2.07). As some concern had existed that overcooked meat might be associated with cancer, subjects were asked about how they usually prepared their meat. There was no association between overcooked meat and the development of AML. Those that did not drink coffee had a higher rate of AML, indicating a potentially protective effect of coffee intake. Compared to those that did not drink coffee, subjects who consumed moderate amounts of coffee had a hazard ratio for the development of AML of 0.72 (95% C.I. 0.50 – 1.02). There was no association between fruit and vegetable intake and the development of AML.[48]

Due to the antioxidant effect, green tea has been hypothesized as having a potentially protective effect on the development of AML. A case-control study was conducted in China to determine if decreased consumption of green tea was a risk factor for the development of AML.[49] 107 adults hospitalized with leukemia (67% AML) were compared with 110 controls hospitalized for orthopedic problems. Detailed in-person interviews were conducted with each case and control. Fewer cases than controls reported regular consumption of green tea, with an odds ratio of 0.51 for the development of AML (95% C.I. 0.27 – 0.96). There also appeared to be a biologic gradient, with a lower incidence of AML among those that had been drinking green tea for longer periods, and those that consumed higher amounts per day. No other studies have reviewed this question, but the presence of a significant association, biologic gradient, and biologic plausibility suggest that green tea may have a protective effect.

In summary, while there are many potential risk factors for AML, most are either uncommon (ionizing radiation, chemotherapy, benzene), or are associated with a small relative risk (smoking, diet, alcohol use). Most cases of AML occur without a defined risk factor.

Table 2: Risk Factors for AML

Risk Factor	**Reported Relative Risk**
Uncommon Exposure, High Risk	
Chemotherapy	20 – 80
Ionizing Radiation (Hiroshima survivor)	70

Ionizing Radiation (chronic exposure to low dose)	3-6
Benzene (chronic exposure)	1.1 – 4
Down Syndrome	10 – 20
Common Exposure, Low Risk	
Smoking	1.1 – 1.3
Alcohol Use	0.7 – 1.2
Male Gender	1.2
High Meat Diet	1.4
Tea Consumption	0.5

1.3: Current Treatment Options for Acute Myeloid Leukemia

AML is a treatable and potentially curable cancer. Surgery is not a treatment option for leukemia. Radiation is occasionally used. The primary treatment used is chemotherapy. High dose combination chemotherapy can often lead to a complete remission. Around 70%-80% of patients with AML will achieve a remission with initial chemotherapy.[50] However, without additional treatment, relapse is common. Thus, after the achievement of an initial remission, additional treatment must be given to reduce the risk of relapse. Two major treatment options are considered – additional chemotherapy (consolidation chemotherapy), or an allogeneic blood and marrow transplant.

Allogeneic blood and marrow transplantation (AlloBMT) replaces the blood production system and immune system of the patient with that of a suitable donor. Initially, stem cells are collected from a donor, which depending on availability can either be a family member or an unrelated donor. The human leukocyte antigen (HLA) system is the major way for the immune system to recognize "self" from "non-

self", and the donor and recipient must be matched at major HLA antigens. Stem cells are collected from the donor through either through a bone marrow harvest (performed in the operating room), or an apheresis procedure (performed in a medical day unit).

Once a donor is identified, the patient is given chemotherapy or radiation therapy aimed at conditioning the host to prevent initial graft rejection and destroy remaining malignant cells (conditioning regimen). The stem cells are then infused into the patient through intravenous access. The stem cells migrate from the peripheral blood into the bone marrow, and begin to produce new blood cells. The time from infusion of donor stem cells until new blood cell production and immune recovery can take several weeks. During this time, the patient is at high risk for infection, and requires extensive supportive care.

In the treatment of AML, AlloBMT has several advantages over chemotherapy alone. The high doses of chemotherapy (conditioning regimen) used to prevent rejection are an effective treatment for the underlying leukemia. Donor stem cells provide additional benefit. The donor immune system replaces the host immune system, and should recognize and control infections. The donor immune system can also recognize residual leukemia cells as foreign, and mount an attack on these cells. This is called a "graft versus leukemia" effect. These two advantages result in much lower relapse rates and improved overall survival in patients who undergo a AlloBMT, compared to those that do not. [51]

There are also several disadvantages of AlloBMT. The conditioning chemotherapy is associated with significant toxicity, which can lead to cardiac, lung, liver, and kidney damage. In addition, the patient will have low blood counts for weeks after AlloBMT, which places them at increased risk for infection. Finally, despite optimal HLA matching, the new donor immune system can recognize major organs as foreign, leading to graft versus host disease (GVHD). GVHD is similar in nature to many other autoimmune diseases, and results from the donor immune system attacking host tissues. Thus, while AlloBMT is associated with much lower relapse rates, it is also associated with much higher toxicity.[52] In patients with a higher risk of relapse, the reduced risk of relapse associated with AlloBMT is greater than the increased risk of toxicity, resulting in a net overall benefit, while no benefit has been seen in patients at lower risk for relapse. [53]

Traditional stem cell transplantation required very high doses of chemotherapy or total body radiation as a part of the conditioning regimen. The doses of chemotherapy and radiation given were enough to destroy most blood making cells in the body (myeloablative). This high dose chemotherapy results in a significant risk of mortality during the transplantation process, making the treatment prohibitive in older adults. [54] Given that AML is much more common in older adults, this toxicity created a problematic paradox: the most effective treatment option is not a viable choice for the majority of patients with AML.

New ways of performing blood and marrow transplant have been developed to address the toxicity associated with more traditional AlloBMT. [55,56] Rather than delivering sufficient chemotherapy to destroy all of the patient's blood making system (myeloablative AlloBMT), newer protocols target specific parts of the immune system thought to be responsible for graft rejection. Protocols known as "reduced intensity regimens" or "non-myeloablative" (NMA-AlloBMT) require much lower doses of chemotherapy. Traditional myeloablative and novel non-myeloablative transplants differ in the dose of chemotherapy used in the transplant process. In myeloablative transplantation, the dose of chemotherapy is sufficiently large that normal blood cell production would not recover for months, if at all. In non-myeloablative transplantation, normal blood cell production would recover within a few weeks of transplant. Reduced intensity AlloBMT is associated with much less toxicity than traditional myeloablative AlloBMT, and is feasible in older adults or in patients with more comorbidities.[57]

However, the relapse rate after reduced intensity AlloBMT is higher than with full intensity transplants. This is potentially due to the lower doses of chemotherapy used, which results in less effective leukemia treatment prior to transplant. The increased relapse rate has prevented the broader utilization of reduced intensity AlloBMT, and relapse remains the most common cause of treatment failure.[57,58] A strategy that is able to reduce the relapse rate following reduced intensity AlloBMT would allow AlloBMT (the most effective treatment option for AML) to be performed with greater efficacy in more patients.

One potential option to reduce the relapse rate may be to administer several cycles of chemotherapy prior to transplant (consolidation chemotherapy). Even if patients are in remission following initial chemotherapy, with newer analysis techniques, some residual leukemia cells can usually be detected (minimal residual disease). It is also known that the amount of minimal residual disease correlates strongly with the risk of relapse. [59]

Consolidation chemotherapy, given after the achievement of remission, but before transplant, may lower the burden of remaining leukemia cells going into transplant, and lead to lower relapse rates after transplant. The consolidation approach has been studied in patients receiving myeloablative conditioning regimens, and was not shown to be beneficial. One possible explanation for this finding with myeloablative regimens is that the very high doses of chemotherapy may mask any potential benefit given by consolidation chemotherapy. Thus, consolidation chemotherapy might be of greater benefit in patients receiving non-myeloablative AlloBMT. The role of consolidation chemotherapy had not been revisited with reduced intensity conditioning regimens.

Clinicians might elect to give consolidation chemotherapy for several reasons, including reduction of leukemia burden before transplant, and thus reduce the relapse rate after transplant. However, it is important to note that this has not previously been studied in the non-myeloablative setting, and when previously

studied in myeloablative transplantation, consolidation chemotherapy was not effective at reducing relapse rate. Thus, we anticipate this would not be the most common reason for administering cytarabine consolidation chemotherapy. A more likely reason for administration of consolidation chemotherapy would be to ensure disease control while a transplant is planned. The preparation for AlloBMT can take up to several months. Important steps include identification of a potential donor, the pre-transplant workup for both the donor and the patient, and the collection of stem cells from the donor. During the time while the transplant is being planned, the patient is at risk for relapse. Consolidation chemotherapy is often given to preserve disease control while planning a transplant. It is standard practice at CancerCare Manitoba to give consolidation chemotherapy if there expected to be a delay of more than 2-3 weeks between the time of remission and transplant.

While a randomized control trial would be able to answer the question of whether consolidation chemotherapy before transplant results in lower relapse rates and improved overall survival after transplant, no such trial has been conducted or is being planned. By using retrospective data, comparing relapse rates and survival between patients who received consolidation chemotherapy prior to transplant to those that did not, some answers to this important question might be obtained.

1.4: Role of Disease Specific Registries in Clinical Research

Disease or procedure specific registries are useful tools in the evaluation of health care interventions. These registries capture clinical information on a defined cohort with a specific disease, or who are undergoing a specific procedure.[60] Registries aim to capture a comprehensive view of the management and outcomes of patients in a real-world clinical setting. Registry data differ from clinical trial data in several important ways. Clinical trials often have rigorous selection criteria, which means that patients enrolled on clinical trials may be different than the typical patient. For example, patients in clinical trials may be less likely to have significant comorbidities than typical patients, an effect that been termed the 'healthy user bias'. One study found that nearly 80% of patients taking bisphosphonates for osteoporosis would not have been eligible for randomized controlled trials.[61] Clinical trials can be time consuming, expensive, and the results of a treatment in a homogenous clinical trial population may not be applicable to the more heterogeneous general population.[62] Thus, observational data from disease specific registries can address issues of relevance to a population's health that may not be fully captured in a clinical trial. [63,64]

Compared to administrative databases, disease or procedure specific registries have several advantages. While research using administrative databases must rely on data collected for another purpose, disease specific registries are often designed

with research as the primary goal. This allows for data elements to be designed specifically for the needs of researchers. In addition, the availability and quality of administrative databases typically vary from jurisdiction, which makes national and international collaboration challenging. This might be of particular benefit in rare diseases, where one jurisdiction might be unable to have sufficient numbers of patients to conduct a study independently.

In contrast, administrative databases have several strengths as well. Firstly, administrative databases typically do not require any additional data entry, as data are collected as part of the administrative aspects of health care delivery. Disease specific registries usually do require additional data capture and entry. Administrative databases are also population based, and typically include all patients in that jurisdiction. In contrast, disease specific registries might not include all available patients. Not all health care centres might elect to collect and submit data on patients to a disease specific registry, due to limited resources or lack of interest in research. This might lead to selection bias, if patients included in the disease specific registry are different than patients in the general population.

The Centre for International Blood and Marrow Transplant Research (CIBMTR) database has elements of both an administrative database and a disease/procedure specific clinical registry. In the United States, submission of data on all transplant patients to the CIBMTR is mandatory, and data are used to track centre outcomes. Transplant outcomes are analyzed, and are made available to the

general public on the Health Resources and Services Administration (HRSA) website. Participation in Canada is not legally mandated, but the majority of centres participate as part of quality control programs, to benchmark outcomes, to meet accreditation requirements, and to further transplant research. A basic set of data elements is captured on all patients (the Transplant Essential Data, or TED, form). More detailed data are captured on a randomly selected subset of patients (the Comprehensive Report Form, or CRF). This study primarily uses the data included in the Comprehensive Report Forms. Previous CIBMTR studies have shown that outcomes are similar to patients on the TED track compared to the CRF track. Data elements are designed specifically with the intent for future use in research. Thus, complete information is available on most important variables. Detailed information on the patient, including underlying comorbidities and overall health, underlying disease (including important prognostic factors), and transplant (such as donor source and conditioning regimen) is collected prospectively on consecutive patients. This database has proved invaluable to the BMT community, and many important clinical decisions are made based on the results of retrospective studies conducted through this resource.

1.5: Theoretical Framework

This study is an example of comparative effectiveness research, using an established disease-specific registry. Comparative effectiveness research aims to guide future patient care by understanding how different clinical interventions

perform in the real-world clinical setting. Comparative effectiveness research can take many forms. In some cases, the data are collected for the primary purpose of secondary analysis for research projects, as is the case with CIBMTR data. In other cases, the data might be collected as part of the administration process, with research being a secondary endpoint. Typically, the information is captured prospectively, but analyzed retrospectively; however, other models can be used. Some prospective randomized clinical trials can also be considered comparative effectiveness research, although many trials are designed to answer questions about the efficacy of a treatment rather than the effectiveness.[65]

The Institute of Medicine produced a consensus report on comparative effectiveness research in 2009.[65] They defined comparative effectiveness research as "the generation and synthesis of evidence that compares the benefits and harms of alternate methods to prevent, treat, and monitor a clinical condition". In another section of the report, the authors attempted to describe the type of clinical question best answered through comparative effectiveness research. Comparative effectiveness research is subject to bias not found in clinical trials. For example, patients are often analyzed according to the treatment they received (per-protocol analysis), rather than the treatment the treating clinician intended for them to receive (intention-to-treat analysis). In some settings, "bias or confounders might limit the reliability of data collected through retrospective means".[65] In this case, retrospective studies might not produce useful results. However, in settings where important confounding variables are captured and can be adjusted for, and where

prospective randomized clinical trials would be prohibitively expensive or time consuming, comparative effectiveness research fills an important need.

There are a number of critical principles that underline comparative effectiveness research. First, comparative effectiveness research aims to look at the real-world utility of a treatment, and as such, the population studied must be representative of clinical practice. Prospective randomized clinical trials often focus on well-chosen segments of the general patient population, which may or may not be translatable to everyday clinical practice. In addition to the patient population in the study being representative of the typical patient, the clinical setting must also be generalizable to routine clinical practice. Clinical trials often include more rigorous monitoring than might be feasible in the typical clinical environment. Finally, comparative effectiveness research aims to study both the benefits and potential harms of treatment that are important to patients.

The current study meets these criteria. The aim of this study is to understand if the addition of consolidation chemotherapy prior to transplant reduces the risk of relapse without adding significant toxicity. Both the patient population and treatment intent do reflect the real world clinical setting, as all patients in the United States and a majority of patients in Canada are included in the CIBMTR database, and administration of consolidation chemotherapy is relatively commonly done. The study (to understand relapse rates and overall survival after

transplant) focuses on very important outcomes to patients. Thus, this clinical question seems ideal to be addressed using comparative effectiveness research.

We attempted to construct a theoretical framework for comparative effectiveness research in BMT, incorporating patient related factors, including comorbidities, disease related factors, and treatment related factors into a model to predict patient outcome. No such model has previously been published in the context of blood and marrow transplant. However, Carpenter et al aimed to establish a theoretical framework for understanding comparative effectiveness research for all types of cancer research.[66] They initially conducted a literature review to understand existing theoretical frameworks for cancer research and found one conceptual framework that seemed to apply well to comparative effectiveness research. This model, developed by Zapka et al, titled the *Quality in the Continuum of Cancer Care*, presented an approach to understand how various factors influence the types of cancer care and the transitions between them.[67] A unique feature of this model is that the authors argued that the treatment of cancer should be viewed as a continuum rather than as an acute event.

Using this conceptual framework as a foundation, Carpenter et al conducted semi-structured interviews with 76 researchers in the field to determine the needs of researchers, and the broader goals of comparative effectiveness research in cancer. [66] Initially, a convenience sample of local researchers was used, but snowball sampling was also applied to provide broader input. These researchers

were asked to discuss critical measures and outcomes in cancer research databases, as well as gaps in available resources.

As a result, this team developed a proposed new model to guide data needs for comparative effectiveness research in cancer. This model incorporates environmental factors, patient characteristics, provider characteristics, disease prognostic indicators and diagnostic results, treatment factors, as well as intermediate and long-term outcomes. Critically, this model viewed cancer treatment from a "patient-centred, longitudinal chronic care perspective, rather than a single-episode, acute care perspective".

The theoretical framework formed by Carpenter et al was modified for this study, incorporating data available as well as treatment and outcomes unique to AlloBMT (Figure 5: Theoretical Framework for Outcomes Research in BMT). Provider and patient characteristics, present at the time of diagnosis, influence transplant and treatment related factors. For example, some patient characteristics such as age are correlated with disease or treatment factors. Older patients are more likely to have pre-existing myelodysplastic syndrome, or abnormal cytogenetic (disease). In addition, some transplant centres (provider characteristics) might be more likely to use a particular graft source or GVHD prophylaxis regimen (transplant factors). All of these factors (patient, provider, disease, and transplant characteristics) influence intermediate outcomes such as relapse or toxicity. Lastly, these intermediate outcomes predict final outcomes such

as overall survival. This longitudinal model serves as the theoretical framework for this study.

For most patients, three states are possible following transplant – alive and free of leukemia, relapse and death from leukemia, or death from complications related to transplant. Some characteristics are more likely to be predictive of relapse, and other characteristics are more likely to be predictive of toxicity. Together, the intermediate outcomes of relapse and toxicity influence overall survival. Based on previous research, we would expect that disease factors (Figure 5, blue boxes) would more strongly relate to relapse, while patient and provider characteristics and treatment factors (Figure 5, red boxes) would more strongly relate to toxicity from treatment. There is likely to be some degree of overlap, as some patient demographic features like age are known to be associated with relapse, and some disease features, like induction regimen might be associated with toxicity. However, this framework provides a useful construct which can be used to help understand the complicated patient, disease, and transplant features which all interact to predict outcomes.

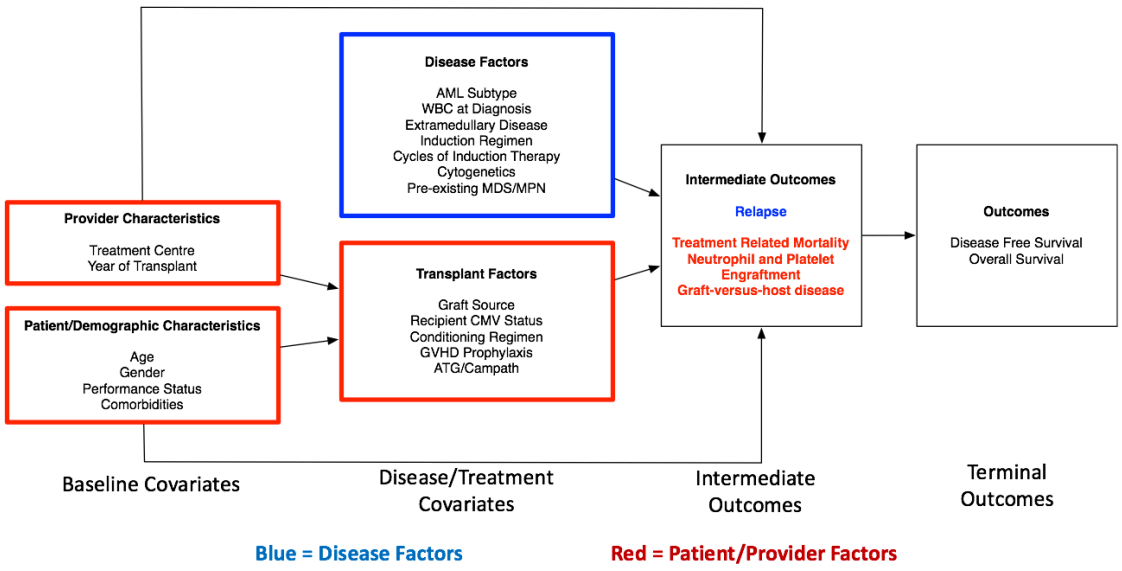


Figure 5: Theoretical Framework for Outcomes Research in BMT

1.6: Research Questions

Reduced intensity transplant is the most effective treatment for older patients with acute myeloid leukemia. However, relapse after transplant is the most common cause of treatment failure. Consolidation chemotherapy prior to transplant might lower the disease burden prior to transplant, leading to reduced relapse rates and eventually improved overall survival after transplant.

Other specific sub-questions include investigating:

(i) The impact of consolidation chemotherapy on relapse after AlloBMT

(ii) The impact of other potential patient, disease, and transplant related variables on outcomes following AlloBMT

(iii) The feasibility of reduced intensity transplant in older patients

We hypothesize that consolidation chemotherapy will result in lower relapse rates following AlloSCT, due to a lower disease burden going into transplant. This decrease in relapse rates will result in improved overall survival.

Methods:

2.1: Data Source

The Centre for International Blood and Marrow Transplant Research (CIBMTR) is a network of 450 transplant centres in 50 countries that collaborate and share clinical data on patients undergoing blood and marrow transplantation.[68] A major component of this collaboration is the development and maintenance of a clinical data registry. The CIBMTR maintains a statistical centre, in Milwaukee, Wisconsin, that coordinates and manages the scientific activities of the registry. The CIBMTR collects data on approximately 18,000 patients undergoing transplant annually. Each one of the 450 transplant centres obtains informed consent from patients, and collects and submits data electronically to the CIBMTR. Data are contained in the Stem Cell Transplantation Outcomes Database (SCTOD). Data submission is mandatory for centres based in the United States, and is optional for centres worldwide. Currently, 90% of Canadian transplant centres submit data to the CIBMTR, capturing data on approximately 80% of all Canadian patients who received a transplant over the period of the study. The only major AlloBMT programs in Canada that do not submit to CIBMTR are the British Columbia and Saskatchewan programs.

There are two levels of data submission to the CIBMTR. Basic registration information on diagnosis and relapse is available on all patients in the database

(Transplant Essential Data). More detailed forms are completed on a subset of patients (Comprehensive Report Forms, or CRF). Transplant programs can elect to send only registration level data or complete the optional research level data (Comprehensive Report Forms, or CRF) as well. Centres are reimbursed for the completion of CRF data through funding provided by the National Institutes of Health (NIH) and the Health Resources and Services Administration (HRSA). The CRF forms contain much more detailed information than the basic registration forms. Resource issues make completion of CRF forms on all patients unfeasible. Hence, CRF forms are only available on a subset of all transplant recipients. At centres that have chosen to complete CRF forms, an algorithm assigns patients to the TED (basic) track or to the CRF (comprehensive) track. This process is not entirely random, as patients with rare diseases are more likely to be chosen for a CRF, for example. However, for a given patient group at a centre that sends CRF forms, the probability of being assigned to the CRF track is random.

At the end of 2011, for patients with AML, Transplant Essential Data (TED) were available on 49,328 patients, and Comprehensive Report Form data were available on 22,655 patients.[68] The Manitoba Blood and Marrow Transplant program (MBMT), based at CancerCare Manitoba and the Health Sciences Centre, is an active participant in the CIBMTR, and submits the more detailed Comprehensive Report Forms.

In the United States, data submission to the CIBMTR is mandatory as part of

the transplant centre accreditation process to monitor quality of care. Patients who have their data included in the database have the option of providing consent to participate in research studies. In other countries, with no mandatory reporting requirement, only patients who have consented for research have their data submitted to the CIBMTR. Only data on patients who provided informed consent for research were used in this protocol.

Data are collected on patients at the time of transplant, at day 100 post-transplant, every six months for the first two years, and yearly after two years. TED baseline data forms include questions regarding demographics, underlying disease, transplant characteristics (donor source, HLA matching), and conditioning regimen. The more detailed CRF forms cover similar topics, but with substantially more detailed information in all areas. Both the TED and CRF follow-up forms include questions regarding current vital status, disease status (relapsed, or in remission), and overall health status.

Cytarabine is the only medication is commonly used for consolidation chemotherapy. Indeed, most debate within the hematology community and clinical trials has focused on the dose of cytarabine used, rather than comparing cytarabine to other medications. Cytarabine is a nucleoside analogue that interferes with DNA synthesis. Cytarabine is a critical medication in both induction and consolidation chemotherapy for AML. Other medications have been studied and compared to cytarabine in numerous trials, and no other chemotherapy medications have been

shown to have superior efficacy.[69] Thus, this study was restricted to considering only patients who received cytarabine as consolidation chemotherapy, compared to those that did not.

Questions on consolidation chemotherapy prior to transplant are included on the more detailed CRF forms, but not on the TED forms. Thus, only patients on whom CRF level data are available were eligible for this study. In addition, this study was limited to patients who received a reduced intensity transplant for AML between 2000 and 2010, as reduced intensity transplant was relatively rare prior to 2000. Patients who received a transplant after 2010 were excluded to allow for sufficient follow-up. The study was restricted to adults, as reduced intensity transplantation is not common in pediatric patients, and the management of AML is somewhat different in children.

The CRF form for AML was reviewed in the context of the theoretical framework to identify patient, provider, disease, and treatment related characteristics of interest. Table 3 lists all variables retrieved from the database (Table 3: Variables Included). The variables chosen are described in more detail in later sections, but generally can be grouped into three categories based on our theoretical framework. Some variables relate specifically to the patient before the time of transplant, including patient age, gender, and the year of transplant. Other variables relate more specifically to the underlying leukemia. These variables include the more specific pathologic subtype, cytogenetic or mutational

abnormalities present in the leukemia cells, the previous treatment of the disease prior to transplant, white blood cell count at the time of diagnosis, the presence of extramedullary disease (disease outside the bone marrow or peripheral blood), and the presence of pre-existing myelodysplastic or myeloproliferative diseases. Finally, other variables attempt to describe the transplant process itself. These variables include information on the stem cell donor (matched sibling donor, unrelated donor, or cord blood donor), the degree of matching between the host and recipient, the drugs used as a part of the conditioning process, and recipient antibodies to cytomegalovirus. The relevant questions describing all of these variables were abstracted from the database.

Table 3: Variables Included

Variable	Description
Main effect variable	
Consolidation therapy	Binary (yes, no)
Consolidation therapy	Categorical (standard dose, high dose, none)
Patient Characteristics	
Age	Continuous
Age	Categorical (18-29; 30-39; 40-49; 50-59; 60;69; 70-79)
Gender	Binary (Male, Female)
Karnofsky Performance Status	Binary (< 80%, ≥80%)
Provider Characteristics	
Year of Transplant	Continuous
Year of Transplant	Categorical (2000-2005; 2006-2008; 2009 – 2011)
Transplant Centre	Categorical
Disease Characteristics	
AML Subtype	Categorical (FAB M0-3; FAB M4-7; AML NOS; WHO Categories of: AML with Abnormal Eosinophils, AML with 11q23 Abnormalities, AML with Multilineage

	Dysplasia; Other/Missing
White blood cell count at diagnosis	Continuous
White blood cell count at diagnosis	Binary (< 5, ≥5)
Extramedullary Disease	Binary (Present, absent)
Induction Chemotherapy	Binary (7+3 or similar, other)
Number of lines of induction therapy	Binary (1, 2)
Drugs used in consolidation therapy	Binary (cytarabine only, cytarabine and other drugs)
Cycles of consolidation therapy	Categorical (1, 2 or missing)
Cytogenetics (by Southwest Oncology Group criteria)	Categorical (Intermediate Risk, Unfavorable Risk, other)
Pre-existing Myelodysplastic/Myeloproliferative disease	Binary (yes, no)
Transplant Characteristics	
Graft Type	Categorical (Matched Sibling Donor, Matched Unrelated Donor, Mismatched Unrelated Donor, Cord Blood)
Donor Sex	Binary (yes, no)
Recipient CMV Status	Binary (positive, negative)
Conditioning Regimen	

2.2: Data Analysis:

All adults who underwent a reduced intensity allogeneic stem cell transplant for acute myeloid leukemia in first complete remission between 2000 and 2010 were identified. Patients were categorized based on the use of cytarabine as consolidation chemotherapy prior to stem cell transplant. A secondary sub-analysis was conducted to understand the relationship of cytarabine dose and transplant outcomes (either standard dose cytarabine (less than 3 grams/m^2), or high dose cytarabine (greater than or equal to 3 grams/m^2). Relevant patient, disease, and transplant characteristics were compared between patients who received

consolidation chemotherapy, and those that did not.

Categorical variables were compared using chi-square testing, and continuous variables will be compared using either the Wilcoxan signed rank test, or the Student's t-test, depending on the distribution of the variable. Data transformation was considered for non-normally distributed variables. A list of all potentially relevant covariates was listed in Table 3 above (Table 3: Variables Included).

The completeness index was calculated for all patients meeting eligibility criteria, by comparing the ratio of the observed person-time of follow-up to the potential person-time of follow-up.[70] It is a requirement that follow-up forms are completed at regular intervals post-transplant, but as is the case in other voluntary registries, compliance with this requirement varies. The completeness index attempts to understand how complete follow-up data are for patients included in this cohort. For example, if a patient received a transplant five years ago, but only had the one-year follow up form completed, and no subsequent data submitted, the completeness index for this patient would be 0.2. If all five years of data submission were completed, the completeness index for this patient would be 1. Based on previous studies, it is anticipated that most outcomes will occur within two years of transplant, so the completeness index was calculated at 1 year and 2 years post-transplant.

Overall survival was chosen as the primary endpoint for several reasons. While disease-free survival is used as the primary endpoint in some other oncology studies, overall survival more accurately reflects the goal of therapy – to prolong the life of patients with a life threatening malignancy. In addition, disease-free survival differs from overall survival only in that patients who relapse without eventually dying from their disease are not recorded as events, and the date of the event is defined as the date of relapse and not the date of death. Disease free and overall survival can be quite different if relapse after treatment is associated with a reasonable prognosis. Unfortunately, outcomes for patients who relapse after allogeneic stem cell transplant for AML are very poor, with median survival typically less than 6 months, and with long-term survivors rare.[71] Thus, one could expect that overall survival and disease free survival would be quite similar. In that case, it might be preferred to use overall survival as the primary endpoint, as it has a more clear and distinct meaning to the general medical community and to patients themselves. Thus, overall survival was the primary endpoint, and disease free survival was a secondary endpoint.

Several other secondary endpoints were evaluated, including relapse rate, non-relapse mortality, and the development of acute or chronic graft versus host disease. Relapse was said to have occurred if the patient developed any evidence of leukemia after transplant. Specific questions on the data collection forms asked about clinical, morphologic, cytogenetic, or molecular evidence of relapse, as well as the date of that assessment. A patient who had evidence of relapse using any one of

these methods was considered to have relapsed disease. The date of the first abnormal lab test was defined as the date of relapse. Patients who died without evidence of relapsed disease or patients who were alive without disease at the time of last contact were censored for this endpoint.

Non-relapse mortality, defined as deaths in patients who had not relapsed, was considered a secondary endpoint as a measure of toxicity of therapy. Events were defined as patients who died without evidence of relapsed disease. Patients were censored at the time of relapse, or at the time of last contact if they were alive and disease free at that time. Complete details on cause of death were not available on all patients. It is possible that in some cases, the cause of death may have been unrelated to the stem cell transplant. However, it is likely that a significant majority of these deaths were due to a short or long term complication of the stem cell transplant process. Causes of death were reviewed, where available, to ensure that the cause of death was not relapsed disease (in which case they were categorized as having relapse prior to death). For disease free survival, events will be either deaths from any cause, or relapses, with patients censored if they were alive and had not relapsed at the most recent evaluation.

Several additional complications of transplant were also captured to provide more background information. Acute and chronic graft versus host disease are relatively common after transplant, although the addition of pre-transplant therapy was not hypothesized to influence the development of either of these complications.

Both of these complications are similar manifestations of the new donor immune system reacting against vital organs in the recipient. Acute and chronic graft versus host disease differ somewhat in pathogenesis, pattern of organ involvement, and time course, but are both associated with significant morbidity and mortality.

Acute and chronic graft versus host disease are also graded somewhat differently. Acute graft versus host disease (aGVHD) is categorized as Grade 1-4, based on the severity of illness in the most affected organ (cutaneous, hepatic, gastrointestinal).[72] Patients with Grade 3 or 4 aGVHD have been shown to have inferior overall survival, and these patients were identified. All patients who were diagnosed with chronic GVHD were identified.

For all endpoints, the time interval chosen was date of transplant until date of event (or date of last contact, if the patient was censored). While interval from date of diagnosis or date of achievement of first complete remission would be acceptable alternatives, this was chosen as this study primarily focused on a population undergoing transplant. Interval from date of diagnosis until transplant and interval from date of date of achievement of first complete remission until transplant were both considered as covariates, but were expected to correlate strongly with consolidation chemotherapy status (our main effect variable).

As patients who received consolidation chemotherapy might be different than patients who did not, a model adjusting for all significant covariates was required.

Multivariable models were created for overall survival, disease free survival, relapse rate, and non-relapse mortality. Cox multivariable proportional hazard models were created using the reverse stepwise technique. As consolidation chemotherapy is our primary variable of interest, it was included in the model at all times. All covariates with a p value less than 0.20 in univariate testing were included in the initial multivariable model. Variables with a p value greater than 0.05 were removed one by one until a final multivariable model was constructed containing the clinically significant covariate age, the main effect variable consolidation chemotherapy, and all other statistically significant covariates.

Interaction terms were tested between the main effect variable consolidation chemotherapy and all other significant covariates. Statistically significant interaction terms ($p < 0.05$) were included in our model. For all variables, the Suprenum test was conducted to ensure that the proportional hazards assumption holds. If the proportional hazards assumption is violated, time dependent analyses were conducted. The linearity of all continuous covariates was tested using the method of fractional polynomials. The final model was evaluated by reviewing the Martingale residuals and the deviance residuals, to look for any potential outliers. Finally, the goodness of fit of the model was reviewed by calculating the measure of explained variation and the measure of explained randomness.

Competing risks were considered for both relapse rate and non-relapse mortality, with cumulative incidence used as the primary univariate analysis

method. Patients who relapse are not at risk for non-relapse mortality, and vice versa. Several methods exist to account for competing risks in multivariate models. The most widely used method is the analysis technique proposed by Fine and Gray.[73] This model accounts for identified competing risks in a similar fashion to the cumulative incidence model, but allows for accounting for important covariates. Using the covariates identified in the Cox proportional hazard models for relapse and non-relapse mortality, Fine and Gray models were created to account for competing risks. These models were compared to the Cox models to determine if the adjustment for competing risks resulted in significant changes.

While there is no standard accepted way of determining power in a Cox model, a generally accepted rule is 10 events per variable included in the final multivariable model. Unfortunately, based on previous studies of allogeneic transplant for AML, we expected approximately 50% of the subjects enrolled to suffer either from relapse or treatment related mortality. Given the numbers of patients included, the power of this study was felt to be adequate.

With our final model, adjusted probability of disease free and overall survival was determined, adjusting for age and all other statistically significant covariates. A plot of the estimated adjusted survival function was created for our main effects variable, consolidation chemotherapy, in an attempt to graphically represent the influence of consolidation chemotherapy on overall survival and disease free survival. Based on the final model, an attempt was made to determine whether

patients who received either standard or high dose consolidation chemotherapy, after adjusting for age and other statistically significant covariates, had different overall survival or disease free survival than patients who did not receive consolidation chemotherapy prior to reduced intensity transplantation.

SAS software version 9.2 was used for all analyses.

2.3: Potential Sources of Bias

Several potential sources of bias were considered. Firstly, selection bias could be present. Patients who received consolidation chemotherapy might be different than others. This effect could work in several different directions. Centre physicians might choose to give consolidation chemotherapy to patients who they felt were at high risk for relapse, and not give it to patients they felt were at lower risk for relapse. Thus, patients who received consolidation chemotherapy might have a higher risk of relapse, and have inferior outcomes regardless of any treatment effect. Physicians might not choose to give consolidation chemotherapy to patients they felt were at higher risk for complications from chemotherapy, to avoid excess toxicity. This might lead to patients who received consolidation chemotherapy having better outcomes due to better overall health and fewer comorbidities. Informed consent is required to have patient data used for research purposes. It is possible that this might also lead to selection bias. For example, patients that were more unwell or had more comorbidities might be less likely to

provide consent. However, the consent rate typically approaches 99%, making any potential bias from this source likely small.

In addition, patients who receive several cycles of consolidation chemotherapy are likely to have a longer interval between the time they achieve complete remission and the time of transplant. This could result in attrition bias. These patients would have a longer period where they would be at risk for relapse. If patients relapsed before they received a transplant, they would not have been included in this cohort. Similarly, patients who received several cycles of chemotherapy prior to transplant would be at increased risk for complications such as organ toxicity or significant infections that might result in them no longer being a candidate for transplant. Data are only captured on patients who received a transplant (per protocol basis), and not on patients in whom a transplant was planned but not completed (as might be captured on an intention to treat basis in a prospective study).

Other potential sources of bias in retrospective cohort studies include information bias and misclassification bias, although these might be of less concern in this study. There should not be any significant differences in data capture or follow-up for patients based on whether they received consolidation chemotherapy, minimizing the risk of information bias. Administration of consolidation chemotherapy is well captured within the data collection form, diminishing the threat of misclassification bias. As is always the case, there is the potential the

forms might be completed incorrectly by centre staff. However, the CIBMTR has an ongoing audit program, and visits sites on a regular basis to review source documentation. Centres that have issues identified (such incomplete or incorrect data) are flagged, and remediation programs are developed. A flagged centre is then revisited by CIBMTR staff to ensure that the issues identified have been resolved. In rare cases where the issue persists, the data from the involved centre are not used as a part of research studies.

Another potential source of bias would be a centre effect (participation bias). While patients from over 100 transplant centres are included in this cohort, some larger centres contributed more patients. If routine practice at some centres was to include or omit pre-transplant consolidation chemotherapy, then any effect found for the main effect variable consolidation chemotherapy status might partially reflect other practices as this centre. To exclude the possibility of a centre effect, a frailty model was fitted using the covariates identified in the main model, as well as transplant centre.

As compared to the gold standard of medical evidence, the randomized control trial, retrospective cohort studies are subject to numerous potential sources of bias. The underlying patient populations might be different, and while multivariable analysis can adjust for measured covariates, unmeasured covariates cannot be adjusted for. Attrition bias might result in patients who were intended to proceed to transplant but did not due to toxicity or relapse not being included in this cohort.

While information bias and misclassification bias should not be significant issues in this study, they are important to consider. While a randomized control trial studied on an intent to treat basis might minimize selection bias and attrition bias, no such study has been conducted nor is it likely to be conducted. Thus, this study is likely best positioned to answer this clinical question, despite the multiple potential sources of bias that need to be considered.

Results:

3.1 – Cohort Development

The CIBMTR database contains data on over 300,000 patients. The first step in this study was the establishment of a retrospective cohort of patients who met the defined inclusion and exclusion criteria, and in which complete information was obtained on important covariates and outcome variables.

A total of 11,399 patients were reported to the CIBMTR for an allogeneic transplant for AML between 2000 and 2010, with CRF level data available. Of these patients, only 604 (5.3%) were included in the final study population (Table 4: Exclusion Criteria) (Figure 6: Cohort Development). This cohort was refined and developed by applying uniform inclusion and exclusion criteria, and by reviewing the data set to ensure that complete information was available for critical variables.

Table 4: Exclusion Criteria

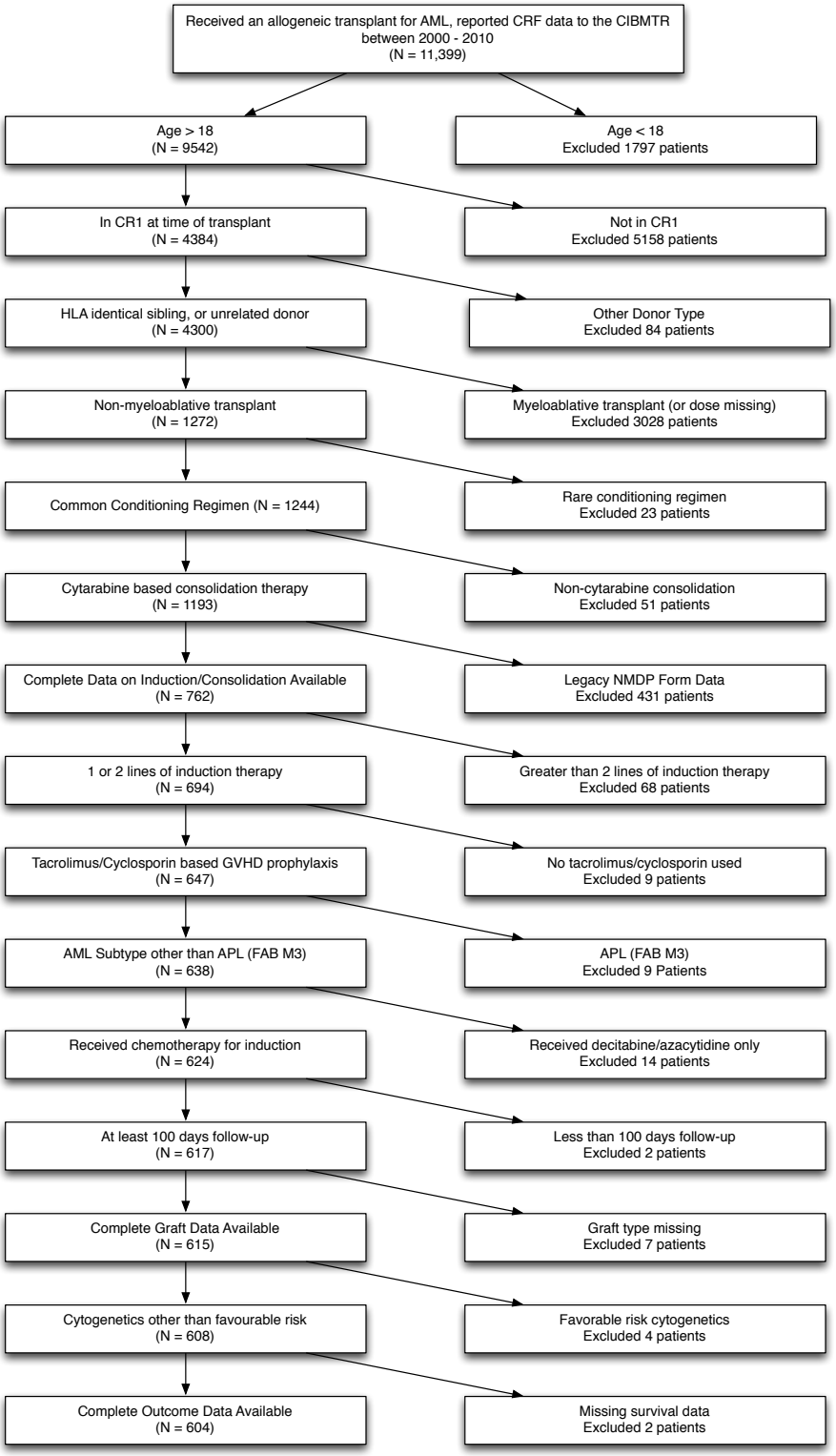
Selection criteria	exclusion	N
Patients who underwent first allogeneic HCT for AML and reported to CIBMTR between 2000 and 2010		11339
Adult patients with age ≥ 18	Age<18 (N=1797)	9542 (84.1%)
Transplant done in first complete remission (CR1)	PIF (N=1324) ≥CR2 (N=2076) Relapse (N=1575) No treatment (N=56) (Predominantly MDS) Missing (N=127)	4384 (38.6%)
HLA-identical sibling or unrelated donor	Identical twin (N=16)	4300 (37.9%)

	Related donor (N=62) Multiple donor (n=2) Missing (N=3)	
Exclude Myeloablative Transplants	Myeloablative (2897) Missing (131)	1272 (11.2%)
Common used RIC conditioning regimens	ATG+ARAC +/- others (7) Bu +/- Others (7) Cy+/- Others (5) Mel+/- Others (4)	1244 (10.9%)
Non-cytarabine based consolidation	Other consolidation (N=51)[1]	1193 (10.5%)
Induction and consolidation data available	Legacy NMDP Forms (431)	762 (6.7%)
1 or 2 lines of induction therapy	5 lines of induction therapy (N=2) 4 lines of induction therapy (N=8) 3 lines of induction therapy (N=25) Missing (33)	694 (6.1%)
FK506/CSA contained GVHD prophylaxis	Others (28)[2] Missing (19)	647 (5.7%)
Subtype of AML other than Acute Promyelocytic Leukemia (APL)	Subtype is APL (N=9)	638 (5.6%)
Received cytotoxic therapy for induction	Received azacytidine/decitabine (14)	624 (5.4%)
At least 100 days follow-up	Not 100 days follow up (1) No day 100 form (N=1)	617 (5.4%)
Complete details on graft type	Missing (2)	615 (5.4%)
Removed Favorable Risk Cytogenetics	7 patients	608 (5.4%
Incomplete Outcome Data	4 patients	604 (5.3%)

[1] Idarubicin (14), mitoxantrone (3), 6-thioguanine (2), daunorubicin (2), others (not specified)

[2] T cell depletion alone (4), T-cell depletion with immunosuppression (2), CD34 selection alone (6), CD34 selection with immunosuppression (2), MMF+sirolimus (6), others (cyclophosphamide alone, MMF + cyclophosphamide, MMF + corticosteroid, MTX + corticosteroid)

Figure 6: Cohort Development



Initially, 1797 pediatric patients (15.8%) were excluded as this study focused on adult patients. The management of AML is substantially different in younger patients. In addition, this study focused on patients in first complete remission (CR1) at the time of transplant, so an additional 5158 patients (45.2%) were excluded as they were not in remission, were in second or third remission, or details on disease status at the time of transplant were missing. Finally, the study population was restricted to patients receiving matched sibling donors and matched or mismatched unrelated donors. Other donor types (such as identical twin donors (also known as syngeneic donors)) were excluded.

Previous studies have reviewed the impact of consolidation chemotherapy in patients receiving myeloablative conditioning regimens. A variety of drug combinations, and drug doses, have been used in conditioning regimens. These combinations range in spectrum to very intense (traditional myeloablative regimens), to minimally intense protocols (still leading to significant immunosuppression, but with minimal myeloablation). A consensus meeting in 2009 attempted to develop common definitions to ensure agreement between clinical research projects. They defined myeloablative regimens as those containing more than 5 Gray of total body irradiation (TBI) in one dose, or more than 8 Gray in multiple doses, or those regimens containing more than 8 mg/m^2 of busulfan. All other regimens were defined as non-myeloablative or reduced intensity. We applied the consensus definitions to our study population, calculating the dose of busulfan based on body weight as recorded pre-transplant. There was incomplete

dosing information (either dose or weight missing) on 131 patients (1.1%) and these patients were excluded. 2897 patients (25.4%) were excluded as they received myeloablative conditioning regimens. An additional 23 patients received unusual or rare conditioning regimens, and these patients were excluded as well.

As the study question related to the effect of cytarabine consolidation on outcomes following transplant, complete information on consolidation chemotherapy was required. 431 patients (3.8%) had data submitted to the CIBMTR on older forms that did not capture information on consolidation chemotherapy, and these patients were excluded. Prior to 2007, separate forms were used for patients who received an unrelated donor transplant. This form did not include questions on consolidation chemotherapy. After 2007, one single form was used for all transplant recipients. Thus, patients with information on consolidation chemotherapy were primarily related donor transplant recipients prior to 2007, and a mixture of related and unrelated donor transplant recipients after 2007. Due to the exclusion of unrelated donor transplant recipients prior to 2007, this cohort had relatively more matched sibling donor patients compared to the general transplant population. In order to exclude the possibility this would lead to bias, all prognostic variables were compared between the excluded patients (unrelated donor recipients prior to 2007), and the remaining patients. There were no significant differences in the distribution of prognostic variables between these groups.

An additional 51 patients (0.4%) received some form of consolidation chemotherapy, but did not receive cytarabine. Common other agents used were idarubicin, mitoxantrone, 6-thioguanine, and daunorubicin. These patients were excluded as, compared to cytarabine, these agents use different mechanisms of action and might have different effects.

Finally, patients were excluded if they received more than two lines of induction therapy prior to being in complete remission. Most patients enter remission after one cycle of induction therapy.[74] Requiring three or more lines of induction therapy to enter CR is a poor prognostic sign, and these patients are likely to have significantly worse outcomes than other patients in CR1.[75] 35 patients (0.3%) required three or more cycles of induction therapy, and the number of cycles of induction therapy was missing for an additional 33 patients (0.3%). All of these patients were excluded.

In addition, several patients were excluded to produce more homogeneous cohort for analysis. Most patients received either tacrolimus-based or cyclosporine based graft versus host disease (GVHD) prophylaxis, so the small number of patients that received neither of these drugs were excluded (47 patients, 0.4%). Acute promyelocytic leukemia (APL, also known as FAB M3 AML) is a distinct subtype of AML with different management, for which AlloSCT is not typically required in CR1. There were 9 patients included in our cohort with APL, and they were excluded (0.1%). In addition, small numbers of patients did not receive traditional cytotoxic

chemotherapy agents for induction, but received novel hypomethylating agents.[76,77] These patients (14, 0.2%) were also excluded for this reason. Seven patients had favorable risk cytogenetics. Several studies have shown that AlloSCT is not of benefit in patients with favorable risk cytogenetics, and these patients were excluded from the cohort.[51]

Lastly, patients were excluded if they had missing or incomplete data on several important variables. Two patients had only the pre-transplant form submitted, with no post-transplant follow-up forms available. These patients were excluded, as they did not contribute any outcome information. In addition, graft type was missing for two patients, and four other patients had some follow-up data available, but with missing vital status (alive/dead). These six patients were also excluded. In total, 8 patients were excluded due to incomplete outcome data (0.1% of final cohort).

The final cohort thus consisted of 604 patients (5.3% of initial cohort), all of whom had provided consent to participate in research studies.

3.2 – Baseline Variables and Data Cleaning

As mentioned previously, patients were categorized based on whether they had received cytarabine as consolidation chemotherapy prior to transplant (main effect variable). In total, 402 patients received cytarabine consolidation chemotherapy, and 202 did not. Among recipients of consolidation chemotherapy, 64% received cytarabine alone, and 35% received cytarabine in combination with other agents. 42% received only one cycle of cytarabine, 33% received two or more cycles, and 25% did receive consolidation but the precise number of cycles was unavailable. Of the 402 patients who received cytarabine consolidation, 226 received high dose cytarabine (greater than or equal to 3 grams/m^2), and 176 received standard dose cytarabine (less than 3 grams/m^2).

Again, potential covariates were identified using the established theoretical framework. These variables were compared between those who received consolidation therapy, and those that did not to see if there were any significant differences between patients that received consolidation and patients that did not. Our theoretical framework identified four categories of covariates to be included in the model. The following section outlines each reviewed prognostic variable in each of these four categories (provider characteristics, patient/demographic characteristics, disease factors, and transplant factors).

3.2.1 – Provider Characteristics

Two provider characteristics were reviewed – treatment centre and year of transplant (Table 5: Provider Characteristics). The patients who did not receive cytarabine consolidation were from 90 different transplant centres, and the patients that did receive cytarabine consolidation were from 75 different transplant centres worldwide. A frailty model was used to identify the presence of a potential centre effect, and none was detected. [78] When year of transplant was reviewed as a continuous variable, there was no difference between groups. When year of transplant was compared as a categorical variable, there some slight differences between groups ($p < 0.01$, chi-square test). More patients who received consolidation therapy had a transplant done between 2008 and 2010 (37%, vs. 32%).

Table 5: Provider Characteristics

Characteristics of Patients	**No Cytarabine Consolidation**	**Cytarabine Consolidation**	**P-value**
Number of Patients	202	402	
Number of Centres	90	75	
Year of Transplant (Median, range)			0.34
	2008 (2000-2010)	2008 (2000 - 2010)	
Year of Transplant (by Group)			< 0.01
2000-2005	53 (26%)	106 (26%)	
2006-2007	84 (42%)	148 (37%)	
2008-2010	65 (32%)	148 (37%)	

3.2.2 – Patient/Demographic Characteristics

We then moved on to review patient related characteristics. Findings are summarized in Table 6: Patient Characteristics. There was no difference in age between patient groups, nor was there a difference in gender balance (p = NS, chi-square test for both). Performance status, as a marker of overall health and comorbidities, was included. There are several validated measures of performance status, but the most commonly used measure in transplant is the Karnofsky Performance Status (KPS).[79] Patients are scored from 10 – 100, with 100 representing normal health, without any significant functional limitation, and 10 representing a moribund patient near death. There was no difference in KPS between groups, with most patients having excellent performance status.

Table 6: Patient Characteristics

Characteristics of Patients	No Cytarabine Consolidation	Cytarabine Consolidation	P-value
Patient Related:			
Age at Transplant (Median, range)			0.18
	60 (18-75)	59 (19-76)	
Age Group			0.29
Less than 45	21 (10%)	41 (10%)	
45 - 60	76 (38%)	177 (44%)	
Greater than 60	105 (52%)	184 (46%)	
Gender			0.16
Male	125 (62%)	225 (55%)	

Characteristics of Patients	No Cytarabine Consolidation	Cytarabine Consolidation	P-value
Female	77 (38%)	177 (45%)	
Karnofsky Performance Status			0.84
>=90%	133 (66%)	268 (67%)	
<90%	60 (30%)	120 (30%)	
Missing	9 (4%)	14 (3%)	

3.2.3 – Disease Characteristics

Having detected no significant differences in provider or patient characteristics between the two groups, we moved on to review disease factors (Table 7: Disease Characteristics). AML is sub-categorized into many different subtypes. Aside from Acute Promyelocytic Leukemia (APL), the management of these subtypes is similar, but some subtypes have been associated with more favorable prognosis.[75] Thus, AML subtype was considered as a covariate. The standard classification system for AML changed during the study period. The French-American-British (FAB) classification system, based primarily on histologic appearance of the leukemia cells, was introduced in 1976.[80] The World Health Organization (WHO) classification system, with more significant focus on molecular features, was introduced in 2002.[81] The pre-transplant form allowed centres to classify patients based on either system. There were some differences in AML subtype between patients who received consolidation chemotherapy and those that did not ($p < 0.01$, Chi-Square test). *Acute Myeloid Leukemia, Not Otherwise Specified* was a more common diagnosis among patients who did not receive consolidation

chemotherapy (25% vs. 18%), and the FAB categories of M4 – M7 were more common among patients who did receive consolidation chemotherapy (32% vs. 20%).

White blood cell count at the time of diagnosis has been shown to have prognostic significance in some studies.[82] White blood cell count was evaluated both as a continuous, and as a categorical variable. For the categorical variable, a cutoff of 5 x 10^9/L was chosen, as it was quite close to the median value. As a continuous variable, there were some differences between groups, with a higher WBC found in patients who received consolidation chemotherapy ($p = 0.02$, Kruskal-Wallis). However, when WBC was evaluated as a categorical variable, there was no difference between groups.

Extramedullary disease (disease in organs other than the peripheral blood and bone marrow; most commonly in the central nervous system or skin) is associated with a poor prognosis.[83] There was no difference in the proportion of patients with extramedullary disease between groups, and the rate of extramedullary disease in both groups was comparable to previously published numbers.

Other important prognostic variables related to the underlying disease include response to initial therapy, cytogenetic risk status, and pre-existing myelodysplastic syndrome. Patients who required more than two cycles of

induction therapy to achieve remission were excluded. These patients are usually defined as having refractory leukemia, and the ideal management of these patients is uncertain.[58] However, patients who enter complete remission after one line of induction therapy are likely to have a more favorable prognosis than those that require two lines of induction therapy. There were more patients who entered CR after one line of induction therapy in the group of patients who did not receive consolidation chemotherapy (81%, as compared to 67%, $p < 0.01$, Chi-Square test). The most common induction chemotherapy regimen is seven days of low-dose cytarabine (200 mg/m^2), in combination with three days of daunorubicin or idarubicin (known colloquially as "7+3" chemotherapy). This chemotherapy regimen was the most common in both groups, but other options were slightly more common in the no-consolidation group (22%, versus 11%, $p < 0.01$).

While AML usually arises de novo, it can develop from a previous hematologic disorder (most commonly myeloproliferative neoplasms (MPN) or myelodysplastic syndromes (MDS)). Again, these patients have a worse prognosis than other patients with de novo AML.[75] Patients with pre-existing MPN or MDS were more common in the no-consolidation group (34% versus 19%, $p < 0.01$).

AML, like most other malignancies, is caused by mutations in DNA. The mutation present can be identified through a variety of techniques, including conventional cytogenetics, florescence in-situ hybridization, and molecular techniques such as reverse transcription polymerase chain reaction.[84] The specific

mutation present often has strong prognostic significance, and is one of the most important prognostic markers. A variety of classification strategies have been used to classify mutations into several risk categories. We used one of the most common risk groupings, originally proposed by the Southwest Oncology Group.[85] Patients were grouped into favorable risk, intermediate risk, unfavorable risk, and other/missing according to this classification strategy. As mentioned previously, AlloBMT is not recommended for patients with favorable risk mutations.[51] These patients have a good prognosis, and the toxicity associated with AlloBMT outweighs any potential benefit. Thus, patients with favorable risk mutations were excluded. Intermediate risk mutations were more common in patients who did not receive consolidation therapy (48% vs. 38%, p = 0.03), but there was no difference in the presence of unfavorable risk mutations (31% vs. 30%).

Table 7: Disease Characteristics

Characteristics of Patients	**No Cytarabine Consolidation**	**Cytarabine Consolidation**	**P-value**
Disease Related:			
AML Subtype			< 0.01
FAB M0/M1/M2	64 (32%)	139 (35%)	
FAB M4/M5/M6/M7	40 (20%)	127 (32%)	
AML NOS	51 (25%)	74 (18%)	
WHO Categories of: AML with Abnormal Eosinophils, AML with 11q23 Abnormalities, AML with Multilineage Dysplasia	21 (10%)	31 (7%)	
Other /Missing	26 (13%)	31 (8%)	
WBC at Diagnosis			0.02
Median (Range)	4.7 (0 – 600)	5.0 (0 – 831)	
WBC at Diagnosis			0.16

Characteristics of Patients	No Cytarabine Consolidation	Cytarabine Consolidation	P-value
Greater than 5	110 (54%)	195 (49%)	
Less than 5	92 (46%)	207 (51%)	
Extramedullary Disease			0.67
Absent	193 (96%)	382 (95%)	
Present	8 (4%)	44 (5%)	
Induction Regimen			< 0.01
7+3 or Similar	157 (78%)	358 (89%)	
Other	45 (22%)	44 (11%)	
Drugs used in Consolidation Therapy			
Cytarabine Only	N/A	259 (64%)	
Cytarabine with other Drugs	N/A	143 (35%)	
Cycles of Consolidation Chemotherapy			
1 cycle	N/A	168 (42%)	
2 or more cycles	N/A	134 (33%)	
Missing	N/A	100 (25%)	
Cycles of Induction Chemotherapy			< 0.01
1 cycle	136 (67%)	326 (81%)	
2 cycles	66 (33%)	76 (19%)	
Pre-existing MDS/MPN			<0.01
No	134 (66%)	327 (81%)	
Yes	68 (34%)	75 (19%)	
Cytogenetics (SWOG classification)			
Intermediate	77 (38%)	193 (48%)	
Unfavorable	63 (31%)	119 (30%)	
Other/Missing	62 (31%)	90 (22%)	0.03

3.2.4 – Transplant Characteristics

Finally, again reviewing covariates according to the theoretical framework, we reviewed transplant related variables (Table 8: Transplant Characteristics).

These included information on the donor, drugs used as a part of the conditioning regimen, and graft-versus-host disease prophylaxis.

Approximately half of all transplants recorded were done using matched sibling donors. Approximately one quarter of patients received a transplant from a fully matched unrelated donor. This contrasts with the general transplant population, as throughout the time period studied, unrelated donor transplants were more common. The higher prevalence of matched sibling donors likely relates to differences in data capture before 2007. Prior to 2007, different forms were used for matched sibling donors and matched unrelated donors. The matched sibling donor form was more likely to be complete and these patients were more likely to be included in the cohort. Importantly, there was no significant difference in donor type between the consolidation and no-consolidation groups, meaning both consolidation chemotherapy groups were equally affected by incomplete data. After 2007, the same form was used for all donor sources. Smaller numbers received a transplant from partially matched (usually one HLA mismatch) unrelated donor or received umbilical cord blood stem cells. Again, there was no difference between the patients that received consolidation chemotherapy and those that did not in terms of stem cell source.

While this study focused on conditioning regimens that are less intense than traditional myeloablative transplant, even the lesser intense protocols have been stratified further into either reduced intensity (RI) or non-myeloablative. The

distinction between these two groups was defined by a consensus meeting in 2009.[86] There were slightly more patients receiving NMA regimens in the consolidation chemotherapy group, as compared to the non-consolidation chemotherapy group (37% vs. 26%, $p < 0.01$). In addition, patients were grouped according to the drugs used as a part of the conditioning protocol. There were some differences again, with patients in the no consolidation chemotherapy group more likely to have received fludarabine and melphalan, and less likely to have received a TBI-based conditioning protocol ($p = 0.01$).

Graft-versus-host disease (GVHD) is a common complication of AlloSCT, and is caused by an attack by the donor immune system on major body organs. In the immediate post transplant period, all patients receive some form of immune suppression in an attempt to reduce the incidence and severity of GVHD. Again, there is no convincing evidence that any one agent is preferred, but the two most common drugs used are cyclosporine and tacrolimus. There was no significant difference in the utilization of cyclosporine or tacrolimus between the groups.

In addition, anti-thymocyte globulin (ATG) and alemtuzumab (Campath) are sometimes used as a part of the conditioning regimen. Both medications impair lymphocyte response/recovery following transplant. This has a few important effects. In general, the use of ATG or Campath is associated with lower rates of GVHD, but higher rates of relapse and infection. Thus, the administration of ATG or alemtuzumab might have uncertain short and long term effects, and the impact on

outcomes might be time dependent. There was no significant difference in ATG or alemtuzumab use between patients who received cytarabine consolidation and those that did not, although there was a non-significant trend (p = 0.06) towards less use in patients who did not receive cytarabine consolidation. 34% of patients in the no-consolidation group received either ATG or alemtuzumab, as compared to 42% in the cytarabine consolidation group.

Finally, for patients who did receive cytarabine consolidation, information was gathered about the total dose and dose intensity. Most patients received cytarabine as monotherapy, although 35% received additional chemotherapy drugs. The most common additional drugs received were idarubicin (18%), daunorubicin (10%), and etoposide (7%). 42% of patients received only one cycle of consolidation chemotherapy, with 33% receiving two or more cycles. The number of cycles of consolidation chemotherapy was missing in 25% of patients. Finally, 176 patients received standard dose cytarabine (≤2 grams/m^2/day), while 226 patients received high dose cytarabine (> 2 grams/m^2/day).

Table 8: Transplant Characteristics

Characteristics of Patients	No Cytarabine Consolidation	Cytarabine Consolidation	P-value
Graft Source			0.51
Matched Sibling Donor	106 (52%)	185 (46%)	
Matched Unrelated Donor	52 (26%)	117 (29%)	
Other Unrelated Donor	16 (8%)	38 (9%)	
Cord Blood	28 (14%)	62 (15%)	
Recipient CMV Status			0.29

Characteristics of Patients	No Cytarabine Consolidation	Cytarabine Consolidation	P-value
Negative	61 (30%)	128 (32%)	
Positive	141 (70%)	269 (67%)	
Missing	0 (0%)	5 (1%)	
Conditioning Regimen			< 0.01
FLU/BU	89 (44%)	173 (43%)	
FLU/Mel	51 (25%)	54 (13%)	
FLU/Other	39 (19%)	104 (29%)	
TBI-based	23 (12%)	71 (18%)	
Conditioning Regimen (2)			0.01
Reduced Intensity			
FLU/BU	89 (44%)	173 (43%)	
FLU/Mel	51 (25%)	54 (13%)	
Flu/TBI (300-500 cGy)	3 (1%)	9 (2%)	
Other	3 (1%)	22 (5%)	
Non-Myeloablative			
Flu/Cy	33 (16%)	75 (19%)	
Flu/ATG	3 (1%)	7 (2%)	
Flu/TBI (200 cGy)	20 (10%)	62 (15%)	
GVHD Prophylaxis			0.32
Tacrolimus-based	115 (57%)	212 (53%)	
Cyclosporin-based	87 (43%)	190 (47%)	
ATG/CAMPATH			0.06
None	133 (66%)	235 (58%)	
ATG or CAMPATH	69 (34%)	167 (42%)	
Cytogenetics (SWOG classification)			0.03
Intermediate	77 (38%)	193 (48%)	
Unfavorable	63 (31%)	119 (30%)	
Other/Missing	62 (31%)	90 (22%)	

3.3 – Outcome Variables

In the theoretical framework, we postulated that provider, patient, transplant, and disease factors would influence intermediate outcomes (relapse,

treatment related mortality, neutrophil and platelet engraftment, and graft versus host disease). These intermediate outcomes would in turn predict overall survival and disease free survival. Overall survival, and all secondary endpoints (relapse rate, non-relapse mortality, disease free survival, development of acute and chronic graft versus host disease, and neutrophil engraftment) were determined using the techniques described in the data analysis section. Table 9: Outcome Variables summarizes all outcome variables measured.

Table 9: Outcome Variables

Outcome	N (%)
Relapse	236 (39.1%)
Grade III / IV Acute GVHD	102 (16.9%)
Chronic GVHD	242 (40.1%)
Neutrophil Engraftment	575 (95.2%)
Disease Free Survival	220 (36.4%)
Overall Survival	239 (40.0%)

Among all patients, 236 patients had relapsed (39.1%), with most relapses occurring early (median time of 3.9 months from transplant to date of diagnosis of relapse). Next, non-relapse mortality was reviewed, as a marker for the toxicity of treatment. There were a total of 150 deaths among patients who were in persistent remission (24.8%). Again, most events of non-relapse mortality occurred early, with a median time of 6.1 months from transplant until date of non-relapse death.

In total, 102 patients (16.9%) developed Grade 3 or 4 aGVHD. In contrast, there is no well-established grading system for cGVHD, aside from categorizing it as

either limited or extensive based on the number of organs involved and the potential need for treatment. As there is no clearly established grading system for cGVHD, all patients with cGVHD were identified. 242 patients developed cGVHD (40%). The rates of severe aGVHD and total cGVHD were similar to previously published results.[87]

In addition, the rate of neutrophil engraftment was also recorded. Neutrophils are white blood cells designed primarily to defend against bacterial infection. In contrast to the red blood cells and platelets, neutrophil transfusions are not commonly available. Thus, the date when the new donor marrow begins to produce adequate quantities of neutrophils (engrafts) is used as a marker of marrow recovery, and the patient's progress through the initial phase of transplant. Neutrophil engraftment was achieved in 95% of patients (575/604), at a median of day 16. In patients who achieved neutrophil engraftment, all but two had done so by day 100 post-transplant.

Disease free survival was also reviewed. For this endpoint, events were defined as either relapses or deaths, and patients were censored if they were alive without evidence of leukemia at the time of last contact with the registry. A total of 384 patients (63.5%) had either relapsed or died over the course of available follow-up. This number is roughly comparable to the total number of deaths (365). Thus, for the 236 patients who relapsed after transplant, only 19 were still alive at

the completion of available follow-up (8.1% of all relapses), reflecting the poor prognosis of patients with relapsed disease after transplant.

Finally, the primary endpoint of this study was overall survival. In total, 365 patients were deceased, with the remaining 239 alive and well at the time of last contact. For survivors, the median duration of follow-up was 35.9 months.

3.3 – Univariate Analysis

The primary outcome variable (consolidation status) was analyzed to determine if it predicted differences in intermediate outcomes (relapse rate, non relapse mortality, neutrophil and platelet engraftment, graft versus host disease) and final outcomes (disease free survival and overall survival). For overall survival and disease free survival, Kaplan Meier graphs were produced, and log-rank statistics were used to compare differences in survival. Relapse rate and non-relapse mortality were considered competing risks, so cumulative incidence was used instead of Kaplan Meier analysis.[88] For relapse rate, the competing risk was non-relapse mortality, and vice versa. Similarly, the development of Grade III-IV aGVHD and cGVHD were analyzed using cumulative incidence as well. Death without aGVHD and death without cGVHD were considered the competing risks. For cumulative incidence analysis, pointwise comparisons were made at 1 and 3

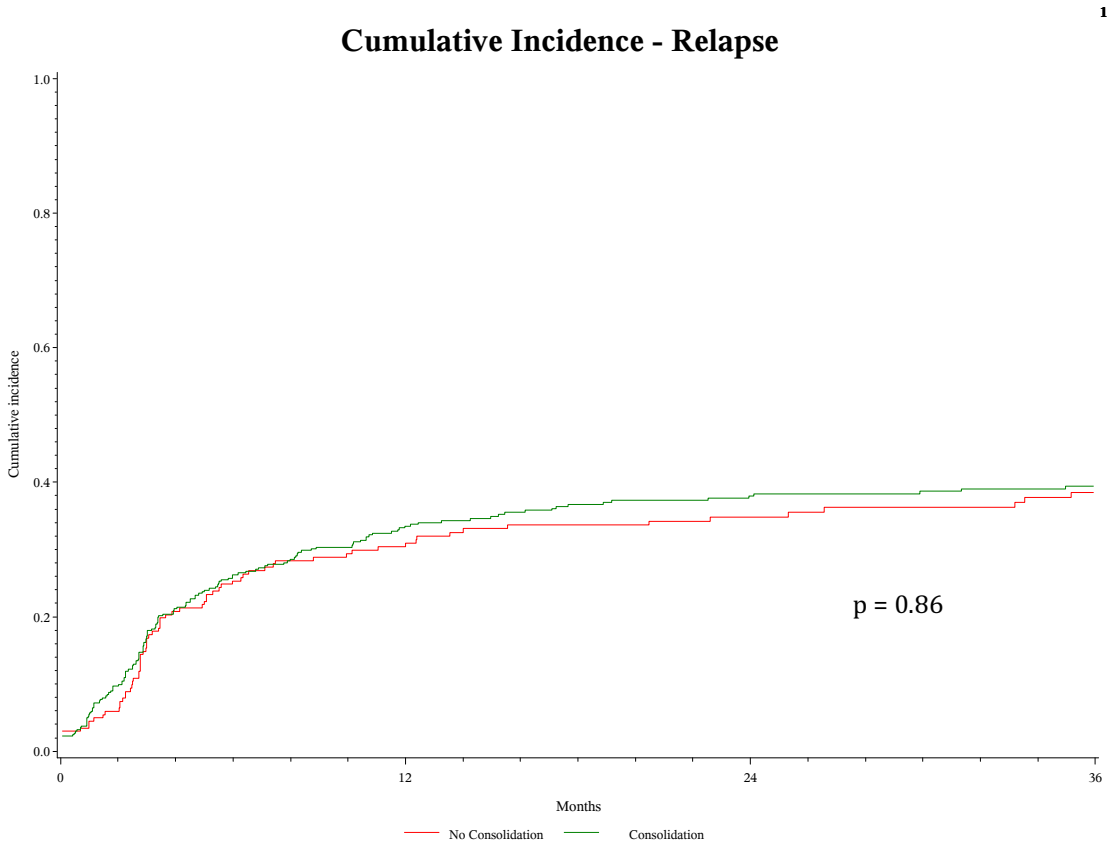
years post transplant to determine if there were differences between groups.[89] The results of the univariate analysis are described below, and can be found in Table 10.

Table 10: Univariate Analysis

	No Cytarabine Consolidation (N=202)		**Cytarabine Consolidation (N=402)**		p-value
Outcome	N	Prob (95% CI)	N	Prob (95% CI)	p-value
Neutrophil engraftment	201		400		
28-day		86 (77-92)		82 (76-87)	0.3846
100-day		95 (86-99)		95 (89-99)	0.9581
Grade 3-4 Acute Graft Versus Host Disease	202		402		
100-day		16 (12-22)		13 (10-16)	0.2647
Chronic Graft versus Host Disease	195		395		
1-year		38 (31-45)		35(30-41)	0.5447
3-year		41 (34 -49)		41(36-47)	0.9569
Non Relapse Mortality	197		393		
1-year		22 (17-29)		15 (12-19)	0.0407
3-year		28 (22-35)		21 (17-25)	0.0630
Relapse	197		393		
1-year		30 (24 -37)		33 (29-38)	0.4631
3-year		38 (31-46)		39 (34-45)	0.8387
Disease Free Survival	197		393		
1-year		48 (41-55)		52 (47-57)	0.3660
3-year		34 (27-41)		41 (35-46)	0.1489
Overall Survival	202		402		
1-year		55 (49-62)%		60 (55-65)%	0.3110
3-year		36 (29-43)%		42 (37-47)%	0.1525

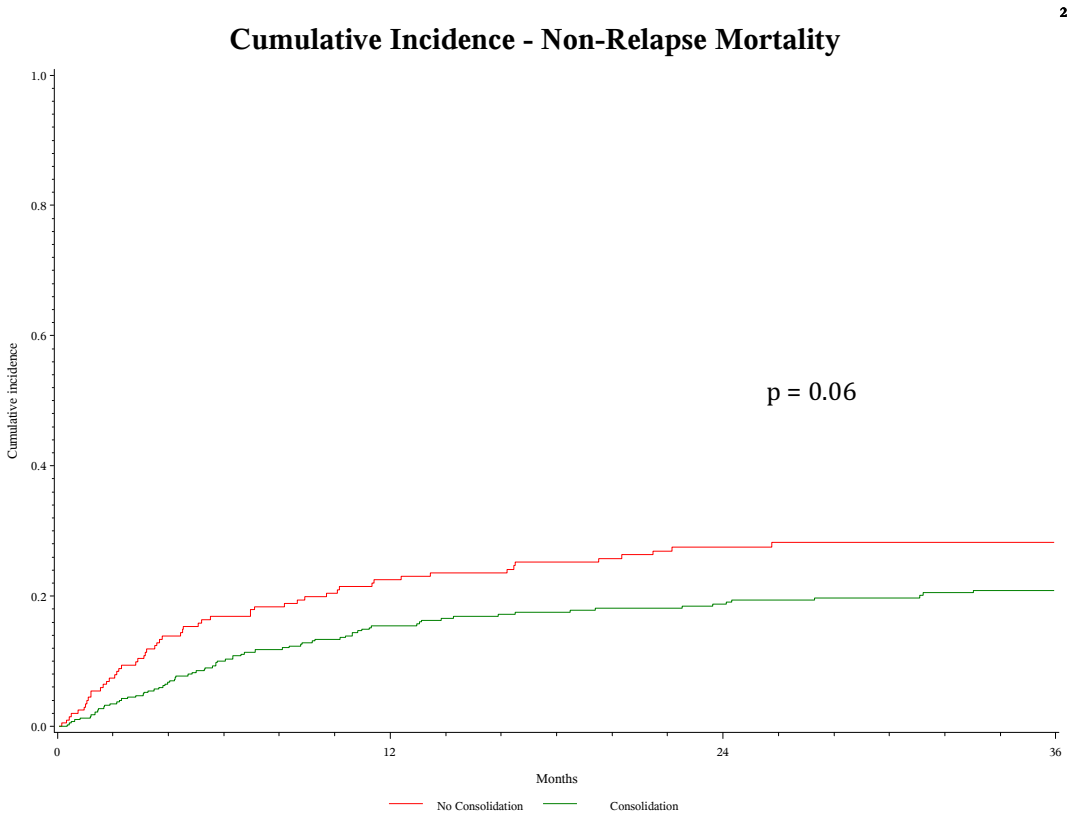
There was no significant difference in relapse rate between patients who received cytarabine consolidation therapy and those that did not, either at one year or at three years (p=0.86). Cumulative incidence estimation was used, with treatment related mortality considered the competing risk. At one year, the relapse rate was 30% (95% CI 24-37%) in patients who did not receive cytarabine consolidation, and it was 33% (95% CI 29-38%) in those that did not receive consolidation. Results were similar at three years (38% vs. 39%). This is illustrated graphically in Figure 7: Cumulative Incidence of Relapse.

Figure 7: Cumulative Incidence of Relapse



In contrast, there was a significant increase in non-relapse mortality among patients who did not receive cytarabine consolidation therapy. The 1-year non-relapse mortality was 30% in patients who did not receive cytarabine consolidation (95% CI 17-29%), as compared to 15% in those that did receive consolidation chemotherapy (95% CI 12-19%). This difference was statistically significant ($p = 0.04$). This difference was somewhat less pronounced at 3 years, and no longer met statistical significance, but there still was a trend towards lower non-relapse mortality in patients who did not receive consolidation chemotherapy (28%, versus 21%, $p = 0.06$). The cumulative incidence graph comparing the rates of non-relapse mortality can be found in Figure 8.

Figure 8: Cumulative Incidence of Non-Relapse Mortality

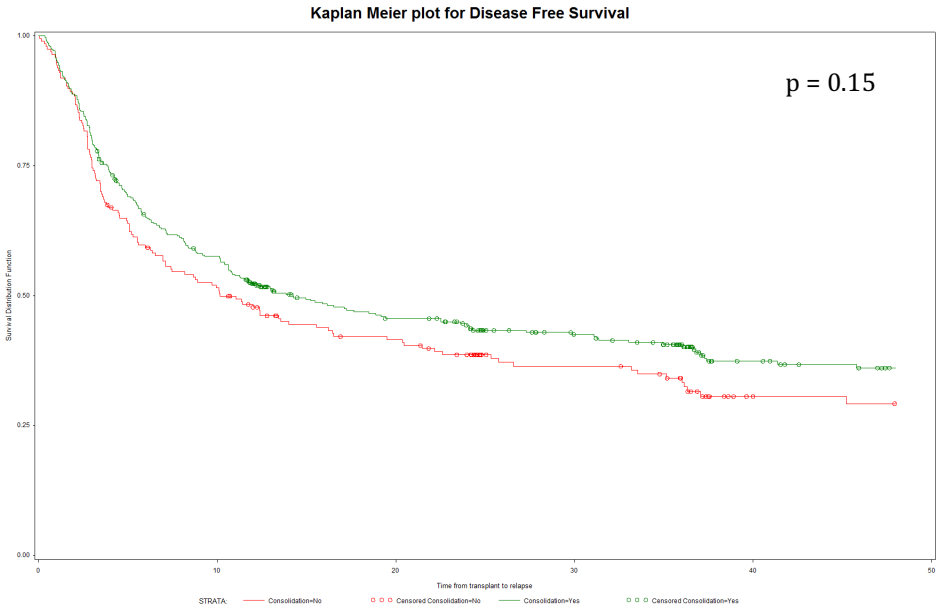


There was no difference in the rates of acute or chronic graft versus host disease between the group that received cytarabine and the group that did not. 16% of patients who did not receive consolidation developed Grade 3-4 aGVHD, which was not significantly different than the 13% of patients who did receive consolidation chemotherapy (p = 0.26). At one year, 38% of patients that did not receive consolidation had developed chronic graft versus host disease, as compared to 35% of patients who did not receive consolidation (p = 0.55). Reflecting the fact that most cases of chronic GVHD present within the first year after transplant, the cumulative incidence of cGVHD was similar at two years (41% in both groups).

Finally, there was also no difference in neutrophil engraftment between groups. 86% of patients in the no consolidation group achieved neutrophil engraftment by day 28 post transplant, as compared to 82% in the consolidation group (p = 0.39).

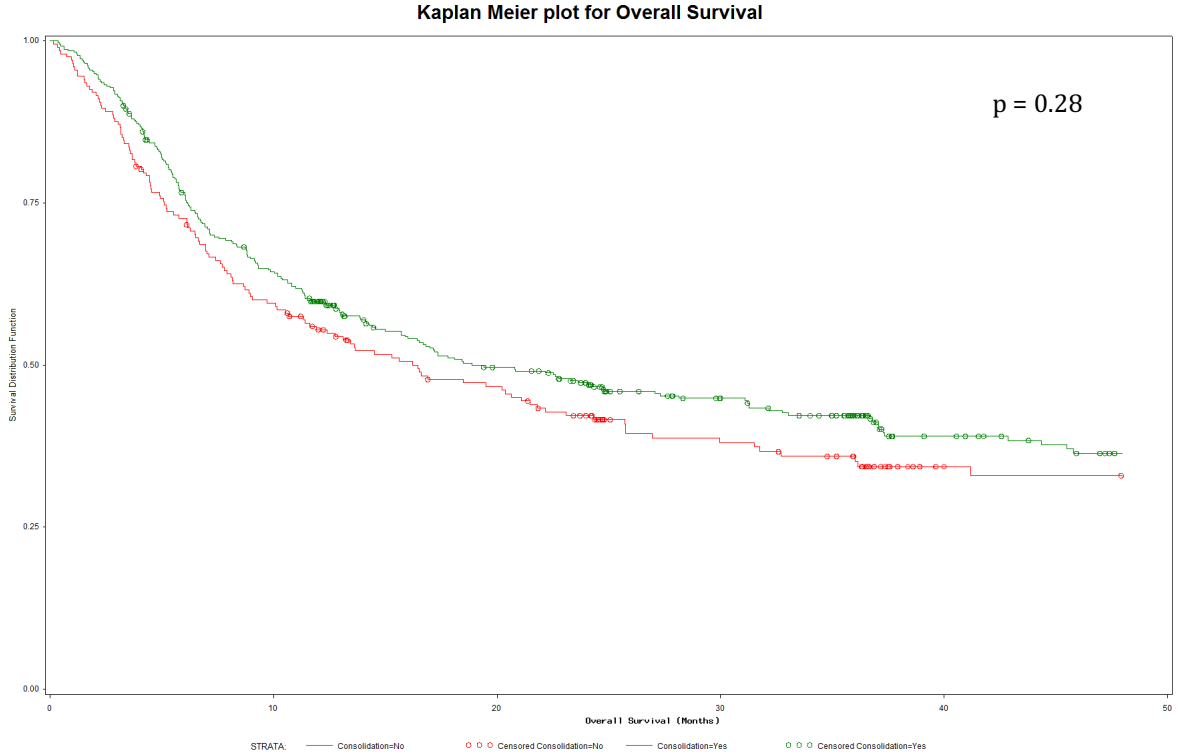
There was no statistically significant difference in disease free survival between groups at one year or at three years (p = 0.15, log rank) (Figure 9: Disease Free Survival). The one-year disease free survival for patients who did not receive cytarabine consolidation chemotherapy was 48% (95% CI 41 – 55), and was 52% in those that did receive cytarabine consolidation (95% CI 47-57). There was a slight decline in disease free survival by three years, but again, there was no significant difference between groups (34%, versus 41%, p = 0.15).

Figure 9: Disease Free Survival



Finally, there was also no statistically significant difference in univariate analysis of overall survival between groups (p = 0.28, log-rank) (Figure 10: Overall Survival). Overall survival was 55% at one year for patients who did not receive consolidation chemotherapy (95% CI 49 – 62%), and 60% in patients who did receive consolidation chemotherapy (95% CI 55 – 65%). Similarly, there was also no difference in three year overall survival (36% in patients who did not receive consolidation chemotherapy, and 42% in those that did (p = 0.15).

Figure 10: Overall Survival



3.5 – Multivariable Analysis

Multivariable models were built for overall survival, disease free survival, relapse rate, and non-relapse mortality. Model building techniques were similar for each outcome, using the reverse stepwise technique. All prognostic variables were tested univariately to see if they predicted outcome, and all significant variables were entered into a model. For variables available both as a continuous and categorical value (age, year of transplant, WBC), both the continuous and categorical were tested, and if results were similar, the categorical was included for ease of interpretation. Variables that were not significant in multivariable analysis were removed one by one until a final model was created. Consolidation status was included in all models as it was the primary variable of interest.

For overall survival, only graft source, patient age, cytogenetic status, and patient gender had prognostic significance in the multivariable model (Table 11: Multivariable Model for Overall Survival). There was no significant difference in overall survival between patients who received cytarabine consolidation and those that did not, after adjusting for graft source, patient age, gender, and cytogenetic status (HR = 0.89, p = 0.28). Graft source was significantly associated with mortality (p = 0.004). When comparing different graft types, the difference was primarily observed between cord blood grafts and unrelated donor grafts. There was inferior overall survival for patients who received a cord blood transplant, compared to those that received a graft from a matched sibling donor (HR = 1.60, p = 0.002).

There was no difference between matched sibling donors, matched unrelated donors, and mismatched unrelated donors. Patient age was also associated with changes in overall survival ($p = 0.04$ for the categorical variable). In pairwise testing, there was no significant difference in mortality between patients aged less than 45 and patients aged 45-60 (HR 0.88, $p = 0.30$). However, there was a significant increase in mortality for patients over the age of 60 compared to patients under the age of 45 (HR = 1.51, $p = 0.03$). Cytogenetic abnormalities also predicted difference in overall survival ($p < 0.0001$). Perhaps not surprisingly, patients with unfavorable risk cytogenetic abnormalities had inferior overall survival to those with intermediate risk cytogenetic abnormalities (HR = 1.74, $p < 0.0001$). There was no difference between patients with intermediate risk cytogenetic abnormalities and those with unclassifiable abnormalities, missing data on cytogenetic abnormalities, and those patients who did not have cytogenetic abnormalities tested. Finally, female gender was associated with slightly better overall survival, with a hazard ratio of 0.78 ($p = 0.023$). Interaction terms were tested between all variables that were included in the final model, and there were no significant interactions found. Similarly, none of the covariates were found to be time-dependent.

Table 11: Multivariable Model for Overall Survival

Variable	Category	N	Hazard Ratio	95% C.I.	Variable P-value	Pairwise P Value
Consolidation	No	202	1.000	.	0.28	-
	Yes	402	0.886	0.71 – 1.10		0.28

Graft Source	Matched sibling	291	1.000	.	0.01	-
	Matched unrelated	169	0.882	0.68 – 1.14		0.34
	Mismatched unrelated	54	1.033	0.71 – 1.51		0.87
	Cord blood	90	1.598	1.18 – 2.16		0.01
Cytogenetics	Intermediate	270	1.000	.	< 0.0001	<.0001
	Unfavorable	182	1.740	1.36 – 2.22		<.0001
	Other / Missing / Not done	152	1.200	0.9 – 1.56		0.18
Patient Age	<45	62	1.000	.	0.04	-
	45-60	253	1.222	0.83 – 1.79		0.31
	>60	289	1.507	1.03 – 2.20		0.03
Patient Gender	Male	350	1.000	.	0.02	-
	Female	254	0.780	0.63 – 0.97		0.02

A similar process was repeated for disease free survival. In general, the model built for disease free survival was similar to that of overall survival, with two exceptions. Patient age was not significantly associated with disease free survival after adjusting for other covariates, and was not included in the final model. The use of ATG or Alemtuzumab (Campath) was associated with differences in disease free in a time dependent manner. There was no difference in short term disease free survival, but there was inferior long term disease free survival for patients that did not receive ATG or Alemtuzumab. Cut-off times were explored, and it appeared that a cut-off of approximately 15 months appeared to best differentiate outcomes. Lack of use of ATG or Alemtuzumab use was not associated with inferior survival before 15 months, but was significantly associated with inferior survival after 15 months.

A variety of options were considered, including a partitioned model. However, as we were not interested in the magnitude of the effect of use of either of these medications, a stratified Cox model was created for ease of interpretation. This stratified model did not include any time dependent covariates, and the proportional hazards assumption was verified.

The stratified model was similar to the model for overall survival, although, as mentioned, age did not influence disease free survival (Table 12: Multivariable Model for Disease Free Survival). Again, there was no difference in disease free survival for patients who received cytarabine as consolidation chemotherapy, as compared to patients that did not (HR 0.87, $p = 0.19$). As in the overall survival multivariate model, patients who received a cord blood transplant had inferior disease free survival compared to those with a matched sibling donor (HR 1.38, $p = 0.04$). There was no difference in disease free survival between patients who received a matched sibling donor transplant, a matched unrelated donor transplant, and a mismatched unrelated donor transplant. As was the case with overall survival, patients with unfavorable risk cytogenetics also had inferior disease free survival (HR 1.65, $p < 0.001$) compared to patients with intermediate risk cytogenetics. There was no difference in disease free survival between intermediate risk cytogenetics and patients with other unclassifiable cytogenetic abnormalities, patients with missing data on cytogenetics, or patients in whom cytogenetics was not performed. Finally, there was better disease free survival for female patients, compared to male patients (HR 0.75, $p = 0.007$).

Table 12: Multivariable Model for Disease Free Survival

Variable	Category	N	Hazard Ratio	95% C.I.	Variable P Value	Pairwise P Value
Consolidation	No	197	1.000	.	0.19	-
	Yes	393	0.865	0.70 – 1.07		0.19
Graft Source	Matched sibling	286	1.000	.	0.04	-
	Matched unrelated	163	0.842	0.65 – 1.10		0.20
	Mismatched unrelated	54	0.935	0.64 – 1.36		0.72
	Cord blood	87	1.369	1.01 – 1.85		0.04
Cytogenetics	Intermediate	264	1.000	.	0.0003	-
	Unfavorable	179	1.645	1.29 – 2.10		<.0001
	Other / Missing / Not done	147	1.194	0.92 – 1.55		0.19
Patient Gender	Male	342	1.000	.	0.02	-
	Female	248	0.747	0.60 – 0.92		0.02

Next, a multivariable model was created for relapse. Again, the use of ATG or alemtuzumab was associated with differences in relapse rate in a time-dependent manner. Lack of use of ATG or alemtuzumab was associated with late relapses, but not early relapses. A variety of cutpoints were explored, as with disease free survival, 15 months again best able to differentiate between outcomes. Lack of use of ATG or alemtuzumab was not associated with higher relapse rates prior to 15 months, but was strongly associated with higher relapse rates after 15 months. A stratified Cox model was again used (stratified based on use of ATG or

alemtuzumab). This model did not include any other time dependent covariates, and was used as the final multivariable model for relapse.

The multivariable model for relapse included only three variables – consolidation chemotherapy status, cytogenetic risk group, and white blood cell count (Table 13: Multivariable Model for Relapse). As was the case in all other models, the administration of consolidation chemotherapy prior to transplant had no impact on the relapse rate, after adjusting for cytogenetic risk group and white blood cell count (HR = 1.027, p = 0.86). Unfavorable risk cytogenetics was again associated with a higher relapse rate (HR 1.865, p < 0.0001), compared to patients with intermediate risk cytogenetics. Patients with intermediate risk cytogenetics had similar relapse rates to patients with other abnormalities, patients with missing data, or patients in whom testing was not performed. Finally, white blood cell count also predicted relapse rates, with patients presenting with white blood cell counts higher than 5 having lower relapse rates (HR 0.765 for relapse, p = 0.049). Of note, this is the only model that did not find inferior outcomes for patients receiving cord blood transplantation, as cell source was not significantly associated with differences in the relapse rate, and was not included in the final model.

Table 13: Multivariable Model for Relapse

Variable	Category	N	Hazard Ratio	95% C.I.	Variable P Value	Pairwise P Value
Consolidation	No	197	1.000	.	0.19	-
	Yes	393	1.027	0.77 – 1.36		0.19

Cytogenetics	Intermediate	264	1.000	.	0.0002	-
	Unfavorable	179	1.865	1.37 – 2.53		<.0001
	Other / Missing / Not done	147	1.170	0.83 – 1.67		0.37
White blood cell count	> 5 x 10^6/L	342	1.000	.	0.049	-
	< 5 x 10^6/L	248	0.765	0.59 – 0.99		0.049

* Model stratified for use of either ATG or alemtuzumab

The last multivariable model created was for non-relapse mortality (presumed treatment-related mortality) (Table 14: Multivariable Model for Treatment Related Mortality). There were no time dependent covariates found, and the proportional hazards assumption was verified. Thus, contrary to the models for relapse and disease free survival, no stratification was necessary. This model was similar to other models, with several exceptions. One additional variable, conditioning regimen, had prognostic significance for non-relapse mortality. Patients who received a combination of fludarabine and melphalan as the conditioning regimen had higher treatment related mortality than other groups (HR 1.59, p = 0.03, compared to the baseline fludarabine/busulfan group). There was no difference in treatment related mortality between the baseline fludarabine/busulfan group, the fludarabine with other drugs group, or the total-body irradiation based regimen group. There may have been a slight trend towards lower treatment related mortality in patients who received consolidation chemotherapy, but it was not statistically significant (HR = 0.74, p = 0.08). Patient age and gender did influence treatment related mortality. Higher treatment related mortality was seen

in older patients (HR 1.96, p = 0.04), comparing patients over the age of 60 to patients under the age of 45. There was no difference in treatment related mortality comparing patients under the age of 45 to patients aged 45 – 60. Finally, there was lower treatment related mortality in women compared to men (HR 0.65, p = 0.01).

Table 14: Multivariable Model for Treatment Related Mortality

Variable	Category	N	Hazard Ratio	95% C.I.	Variable P Value	Pairwise P Value
Consolidation	No	197	1.000	.	0.08	-
	Yes	393	0.742	0.53 – 1.04		0.08
Graft Source	Matched sibling	286	1.000	.	< 0.001	-
	Matched unrelated	163	0.998	0.65 – 1.52		0.99
	Mismatched unrelated	54	1.371	0.79 – 2.38		0.26
	Cord blood	87	3.834	2.24 – 6.54		<.0001
Conditioning Regimen	Fludarabine/Busulfan	87	3.834		< 0.001	-
	Fludarabine/Melphalan	257	1.000	1.05 – 2.43		0.01
	Fludarabine/Other	101	1.594	0.38 – 1.14		0.03
	TBI-based	141	0.653	0.57 – 1.56		0.13
Patient Age	<45	61	1.000	.	0.008	-
	45-60	246	1.196	0.63 – 2.28		0.59
	>60	283	1.958	1.04 – 3.68		0.04
Patient Gender	Male	342	1.000	.	0.01	-
	Female	248	0.646	0.46 – 0.91		0.01

Thus, after adjusting for other significant covariates, there did not appear to be an impact of administration of consolidation chemotherapy on overall survival, disease free survival, non-relapse mortality, or relapse rate. To exclude the possibility that the beneficial effect of consolidation chemotherapy might be seen

only in patients receiving higher doses of cytarabine, the analysis was repeated with a dose-dependent categorization of cytarabine dose. Patients were grouped into one of three categories – no cytarabine consolidation, standard dose cytarabine (less than 3 grams/m^2), or high dose cytarabine (greater than or equal to 3 grams/m^2). All four multivariable models were created again using this new categorical variable.

In univariate analysis, there was a slight decrease in non-relapse mortality for patients who received high dose. However, this difference was not significant in multivariable analysis after adjusting for other significant covariates. Again, there was no difference in overall survival, disease free survival, or relapse rate between patients who received no consolidation chemotherapy, standard dose cytarabine consolidation chemotherapy, or high dose cytarabine consolidation chemotherapy in either univariate or multivariate analysis. Thus, there did not appear to be a dose-dependent benefit to cytarabine consolidation chemotherapy prior to reduced intensity stem cell transplant.

Discussion:

The primary aim of this study was to understand the impact on administration of pre-transplant chemotherapy on overall survival after reduced intensity transplant. It was hypothesized that consolidation chemotherapy after remission, but prior to transplant, would result in a decreased burden of disease going into transplant, and would lead to a lower relapse rate and improved overall survival. However, the results of this study showed no difference in outcome in patients who received pre-transplant chemotherapy compared to those that did not. Thus, based on the results of this study, this therapy should not be expected to improve outcomes after transplant. There remain many reasons to consider administration of chemotherapy prior to transplant, most commonly to provide disease control while a transplant is being planned. Planning for allogeneic transplant can take several months. This study did not show any increased toxicity in patients who received several cycles of chemotherapy prior to transplant, reassuring clinicians that at a minimum this strategy appears to be safe.

A theoretical framework was constructed to understand the numerous factors that influence outcomes. The results of this study show again that numerous variables influence outcomes after transplant, and while consolidation chemotherapy alone cannot improve outcomes, we again identified numerous factors that are strongly associated with outcomes following transplant. These included disease factors (cytogenetic status), patient factors (age, gender), and

transplant factors (graft source). As predicted by our theoretical framework, disease factors were more predictive of relapse, and patient and transplant factors were predictive of toxicity (treatment related mortality). Thus, our theoretical framework appeared to perform well. A simplified version of our theoretical framework, with only variables significant in our multivariate models, is included below (Figure 11: Final Theoretical Framework).

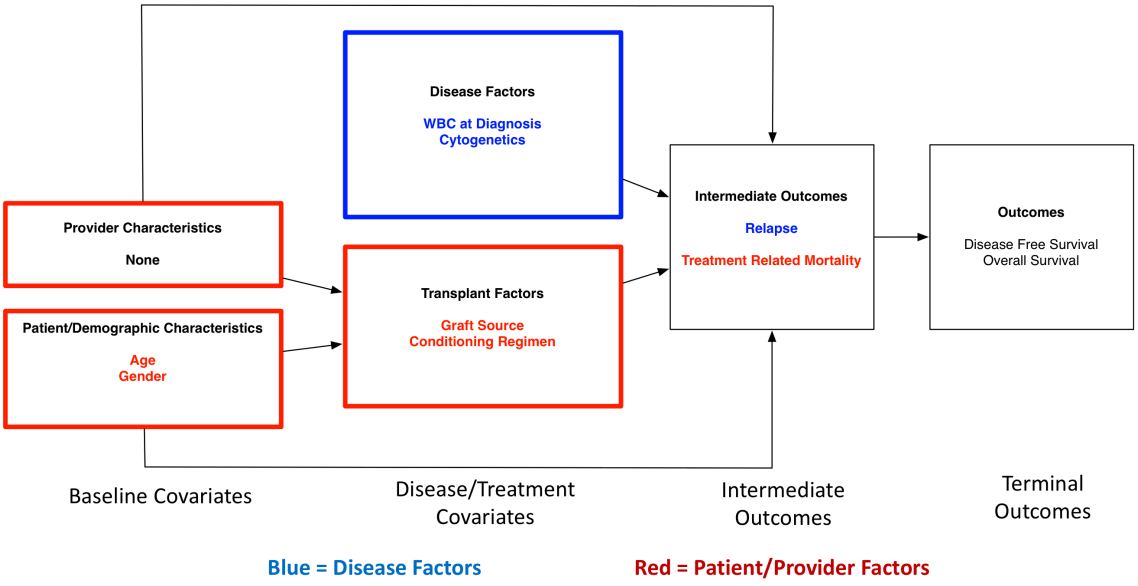


Figure 11: Final Theoretical Framework

These results generally agree with the published literature. While the role of consolidation chemotherapy has never been studied in patients undergoing reduced intensity transplant, previous studies have shown no impact of consolidation in other transplant settings.[92] All of the patients included in this study were in remission at the time of transplant, and all received some chemotherapy as a part of

their transplant conditioning regimen. Thus, perhaps it is not surprising that giving more chemotherapy to patients in remission was not of significant benefit.

This study has several weaknesses. First, it is not a randomized controlled trial, and understanding the precise impact of cytarabine consolidation chemotherapy is not possible. Compared to a prospective randomized trial, this study design (a retrospective cohort) has several potential sources of bias. There were several differences in important baseline variables between groups, and likely differences in unmeasured covariates as well. A randomized control trial would help ensure that the groups are balanced in both measured and unmeasured covariates. The reason for administration of cytarabine consolidation was not available. There could be some sampling bias, where physicians were more likely to administer consolidation chemotherapy to patients in which they were more concerned about relapse, or avoid chemotherapy in patients they considered at high risk of toxicity. A randomized trial would avoid some of these potential sources of bias.

Unfortunately, no such randomized trial is likely to be performed for several reasons. Allogeneic transplant is a rare intervention, and AML is not a common disease. A potential randomized trial would be very expensive, and as cytarabine is a relatively inexpensive generic medication, industry support would likely not be forthcoming. Moreover, this study did not suggest a benefit to consolidation chemotherapy, and limited resources for clinical trials are likely to be directed to

more promising areas of study. This study remains the largest to address this very important and relevant clinical question, and was conducted with the largest available data source for this patient population. As such, is likely to be the primary study upon which clinical decisions are made.

This study was analyzed on a per-protocol basis, not an intention-to-treat basis. This has several implications. Data were only captured on patients who actually received a transplant, not on patients who were intended to receive a transplant. If large numbers of patients were intended to receive a transplant but did not, attrition bias results. This bias could skew in both directions. It is possible that both the relapse rate and the treatment related mortality rates reported here on a per-protocol basis are smaller than would be the case if data were available on patients who were intended to receive a transplant and did not due to reasons of relapse or toxicity. For example, if a large cohort of patients did not receive consolidation chemotherapy and subsequently relapsed prior to receiving a transplant, the beneficial impact of consolidation chemotherapy could be missed. Similarly, if consolidation chemotherapy was very toxic, and patients who were intended to receive a transplant did not because they were not well enough to proceed with transplant, a potential harmful impact of consolidation chemotherapy could not be missed. Unfortunately, this bias is not easily addressed with this study methodology.

Although it was not statistically significant, there was a trend towards lower treatment related mortality in patients who received cytarabine consolidation (HR 0.74, p = 0.08). As there is no biologic rationale why more chemotherapy should lead to less toxicity, this might suggest some component of attrition bias. One possible explanation might be that only patients who were healthier and fitter were still well enough to proceed with transplant after the administration of several cycles of consolidation chemotherapy. Additional patients who were planned to receive a transplant but became too unwell after consolidation chemotherapy to proceed with transplant would not have been included in this cohort, as data are only available on patients who actually underwent the transplant. Patients who went directly to transplant would not have been subject to this selection bias, and the less fit patients would not have been "selected out" by the administration of consolidation chemotherapy. Another possibility might be that clinicians only chose to proceed with consolidation chemotherapy in fitter patients, concerned that less fit patients might not be able to tolerate additional chemotherapy prior to transplant.

However, as mentioned, clinicians can be reassured that there was no signal that consolidation chemotherapy is harmful, even if there are reasons to be skeptical of a potential benefit. One concern could be that patients might develop new comorbidities and toxicity while going through additional chemotherapy prior to transplant, resulting in higher treatment related mortality. Physicians should be reassured by these results, in that patients who had been exposed to several

additional cycles of chemotherapy and went on to receive a transplant did not seem to be at higher risk than those that did not receive consolidation chemotherapy.

Other common sources of bias in retrospective cohort studies are information bias and misclassification bias. The impact of both of these sources of bias should be relatively small. In terms of misclassification bias, it is relatively easy to determine whether a patient received consolidation chemotherapy or not, and there is a separate section on the standard case report form that asks this question specifically. Thus, patients should be correctly allocated to each group. In addition, all outcome variables are easily captured. A relapse of AML is easily diagnosed, and captured well in the forms completed. With regards to information bias, there was no observed difference in completeness of form reporting between groups, and the completeness index measured was good. The completeness index was 97% at one year, and 86% at two years. The number of patients lost to follow-up was small in each arm, and was not different between groups.

While this study did not show an improvement in outcomes with consolidation chemotherapy, several questions remain. This study looked at the impact of cytarabine consolidation on outcomes after transplant. Cytarabine was chosen, as it is almost exclusively the only drug used for consolidation chemotherapy. Previous randomized trials done outside the setting of allogeneic transplant have shown that cytarabine is the most effective chemotherapy drug in AML consolidation.[90-93] Without randomized trials in patients undergoing

transplant, clinicians have extrapolated this evidence. However, other drugs might be more effective then cytarabine in patients undergoing transplant. Similarly, it is possible that the doses used were not optimal. These remain open questions.

There were several important differences between groups. Patients who received consolidation chemotherapy were more likely to be transplanted in more recent years. The reason for this difference is uncertain. One hypothesis is that this might be related to differences in donor type. The use of unrelated donor transplant has increased significantly over time, and unrelated donor transplants take considerably longer to plan than matched sibling donors. While a matched sibling donor transplant can take only a few weeks to plan, a matched unrelated donor transplant can take several months, with additional time required for donor typing and donor consent. This need for additional time to plan for transplant might lead to an increased need for consolidation chemotherapy. Importantly, year of transplant was not significantly associated with any outcome in the multivariable models.

There were four disease characteristics that were different between consolidation and no-consolidation groups –number of cycles of induction chemotherapy, history of MDS, induction chemotherapy regimen, and cytogenetic status. Several of these differences might be explained by the transplant planning process. A history of MDS is an indication for transplant, and the transplant

planning process might begin earlier, resulting in less need for consolidation chemotherapy. Similarly, patients needing two cycles of induction chemotherapy to achieve remission will also usually have a donor search started earlier, and might be less likely to need consolidation chemotherapy. One hypothesis as to why regimens other than "7+3" were more common in the no consolidation group might be the drugs used in these regimens. "7+3" includes cytarabine, and so patients that responded and achieved a remission with a cytarabine containing regimen might be more likely to be selected to receive cytarabine as part of consolidation.

Cytogenetic status has a strong association with outcome in AML, and in most studies is the only risk stratification technique used.[51] However, the difference between groups primarily related to the higher number of patients with missing data in the no consolidation group. The reason for this is unclear, but there was no significant difference in outcome between patients with missing data and patients in the intermediate risk group. There was a similar prevalence of poor risk cytogenetic mutations in both groups in this study.

Finally, there were some differences in conditioning regimens between groups; however, this is of uncertain significance. The most common regimen in both groups was fludarabine and busulfan, which is the primary conditioning regimen used at CancerCare Manitoba . There was slightly higher use of fludarabine and melphalan in the no consolidation group (25% versus 13%). The current literature does not support any one conditioning regimen as the standard, and the

choice of conditioning regimen is often based more on centre experience and local practice. [4] Interestingly, fludarabine and busulfan was associated with a higher risk of treatment related mortality (HR 3.83, p = 0.01). The relevance of this finding is uncertain, as previous studies have not shown significantly higher toxicity in patients who received busulfan.[95]

In multivariable models, there were no interaction terms found, suggesting that there was no differential impact of cytarabine in any specific patient population. However, one recent important development in the field of acute leukemia has been the creation of specialized tests to measure the presence of minimal residual disease. While this study included only patients in remission, it is now recognized that the depth of remission is important. Traditional definitions of remission would define patients with less than 1% leukemia cells in the bone marrow at the completion of chemotherapy as being in remission. New specialized tests (advanced molecular tests such as polymerase chain reactions or multiparameter flow cytometry) can detect the presence of leukemia cells at a concentration as low as 0.001%. Studies have shown that patients with measurable minimal residual disease (less than 1% leukemia cells, but more than 0.001%) have much worse outcomes compared to patients with no minimal residual disease (less than 0.001% leukemia cells).[59] Perhaps additional consolidation chemotherapy would be of greater benefit to those patients with minimal residual disease, but this hypothesis has not been tested, and is not something that can be answered with the data presented here.

Our primary endpoint was overall survival. With 202 subjects in the no-consolidation group, and an overall survival of 40% at 3 years, there were well over 100 events in this group. With only 5 variables in the final multivariable model for overall survival, we expect this study to have adequate power. There were smaller numbers of events in the non-relapse mortality endpoint (40 events in the no-consolidation group), and again 5 variables included in the final multivariable model. Thus, inadequate power might have been an issue in this secondary endpoint.

Relapse after allogeneic transplant remains a significant problem. Although patients who have an allogeneic transplant have lower rates of relapse than patients that do not, relapse remains the most common cause of treatment failure, and outcomes after relapse are poor. While additional treatment prior to transplant has not shown here to be an effective strategy, there remain several questions. Medications other than cytarabine might be more effective, and it is possible that there is a dose dependent effect that this study could not address. It is also possible that the effect of cytarabine consolidation might only be seen in patients with minimal residual disease. These questions should be directions for future research. Another option currently under study is maintenance treatment after transplant, commonly with hypomethylating agents like decitabine or azacytidine.[96,97] Initial results have been promising, and this is an area of active research.

There were several other specific questions we sought to address (see 1.6: Research Questions). One goal was to understand if reduced intensity transplant was feasible in older adults with AML. In this population, with an average age of 60, the non-relapse mortality was 24.8%, which is consistent with other published studies.[58,98,99] One previous study attempting to understand the role of AlloBMT in older patients with AML in CR1 had still shown a benefit despite a non-relapse mortality of 36%, due to a reduction in relapse rate from 81% to 32% in patients who received a transplant.[100] This study was not specifically designed to determine if proceeding to AlloBMT in all patients with AML in CR1 was beneficial. However, with non-relapse mortality at least comparable to, and perhaps lower than previous studies, this data would suggest that AlloSCT is feasible.

We also sought to understand the impact of other baseline, disease, and treatment factors on outcomes after reduced intensity allogeneic transplant. There appeared to be worse outcomes for patients who underwent transplant from a cord blood donor, with inferior overall survival and disease free survival (HR 1.60 for overall survival in multivariable analysis, $p = 0.01$). This appeared to be mainly driven by increased treatment related mortality. In multivariable analysis, after accounting for consolidation chemotherapy, conditioning regimen, age, and gender, cord blood recipients had a much higher risk of non-relapse mortality (HR 3.83, $p < 0.001$). While this study was not designed to specifically address the role of reduced intensity cord blood transplant in AML, this finding is certainly interesting. Important variables that are known to have prognostic significance in cord blood

transplant were not captured here. For example, the cell dose and degree of HLA matching were not captured in detail. Other studies designed specifically to address the role of cord blood transplant have shown outcomes that are comparable to recipients of matched sibling donors, provided the cord blood unit or units chosen have an acceptable cell dose and are suitably matched.[99] Hence, this finding should be interpreted skeptically, as other studies with complete data on essential variables related to cord blood transplant have not shown inferior survival after cord blood transplant.

The impact of gender on treatment related mortality was somewhat unexpected, as this has not been noted in previous studies. We found that women were less likely to experience treatment-related mortality than men (HR 0.65 for female gender, $p = 0.01$). As mentioned in the introduction, there is a higher incidence of AML in women, for uncertain reasons. Unfortunately, there is no detailed information on comorbidities available, and men are more likely to suffer from chronic diseases such as ischemic heart disease and chronic pulmonary disease, so this is one possible explanation. This finding should be a question for future research.

As mentioned, our theoretical model appeared to hold well. One limitation of this model was the difficulty in incorporating the interplay between treatment and disease factors, and how this interaction might influence outcomes. Transplant physicians might choose a different conditioning regimen for patients with higher

risk disease, for example. However, in general, our theoretical model proved quite useful in understanding this study and could serve as a framework for future transplant studies.

Allogeneic blood and marrow transplant is a potentially curative treatment option for patients with blood cancers such as leukemia and lymphoma. In nearly every setting, it is more effective than chemotherapy alone at reducing the risk of disease relapse. However, it is also a toxic procedure, with a very real risk of life threatening complications. Historically, this risk was prohibitive in older adults. Reduced intensity allogeneic transplant offers the potential to expand access to transplant to older patients and patients with more comorbidities, and reduce toxicity in younger patients. However, relapse rates are higher after non-myeloablative transplant, and relapse is the most common cause of treatment failure. This study did not show that cytarabine consolidation chemotherapy was effective at reducing the risk of relapse following transplant.

This study includes one of the largest cohorts of patients undergoing reduced intensity allogeneic transplant for patients with AML in first complete remission. Outcomes remain disappointing, with less than half of patients alive at 3 years after transplant. All large retrospective studies to date have shown that in older adults, transplant can reduce the risk of relapse and improve overall survival compared to chemotherapy alone.[100,101] Reduced intensity transplant is an effective platform upon which to build future treatment strategies. Given that the primary cause for

treatment failure is relapse, new ways of reducing relapse following transplant are urgently needed. While treating older patients with aggressive cancers such as AML is challenging, this is a patient population that is not as well studied in clinical trials, and one in which new treatment strategies are urgently needed. Outcomes in older adults with AML remain much worse than younger adults, and this population should be a high priority for future research.

The results of this research are similar to previous research conducted in patients undergoing myeloablative stem cell transplants, contributing to the body of evidence suggesting consolidation chemotherapy is a safe strategy to preserve remission prior to transplant, but does not improve outcomes following transplant.[102] While the lack of a benefit is disappointing, it is perhaps not unexpected. Our theoretical framework identified 18 different factors that might influence outcomes after transplant, in four broad categories (patient, disease, provider, and transplant). Consolidation chemotherapy is just one small part of the overall picture, and one that has been shown in other settings to not be effective at reducing relapse. Allogeneic transplant is now a viable treatment option for older adults with AML with the development and refinement of reduced intensity transplant. Our theoretical framework offers numerous other opportunities for future studies to try to improve outcomes in this high need patient population.

Bibliography:

1. Table 103-0553 - New cases and age-standardized rate for ICD-O-3 primary sites of cancer (based on the July 2011 CCR tabulation file), by sex, Canada, provinces and territories, annual. Statistics Canada CANSIM Database. 2011;http://www5.statcan.gc.ca/cansim/a01?lang=eng.
2. Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology. Cancer. 2006;107:2099-2107.
3. Redaelli A, Lee JM, Stephens JM, Pashos CL. Epidemiology and clinical burden of acute myeloid leukemia. Expert Review of Anticancer Therapy. 2003;3:695-710.
4. Epidemiology and Cancer Registry. Cancer in Manitoba: 2008 Annual Statistical Report. Winnipeg, MB: CancerCare Manitoba; 2011.
5. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) Research Data (1973-2008), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011, based on the November 2010 submission. .
6. Canadian Cancer Society's Steering Committee on Cancer Statistics. Canadian Cancer Statistics 2015. Toronto, ON: Canadian Cancer Society; 2015. 2011.
7. CancerResearch UK. Leukemia Fact Sheet. 2003;CancerStats Leukaemia - UK.

8. Burnet NG, Jefferies SJ, Benson RJ, Hunt DP, Treasure FP. Years of life lost (YLL) from cancer is an important measure of population burden--and should be considered when allocating research funds. Br J Cancer. 2005;92:241-245.

9. "Cancer Care Ontario: Cancer in Young Adults in Canada**,** Toronto, Canada, 2006." May 2006, ISBN 0-921325-10-X (print), ISBN 0-921325-11-8 (pdf). .

10. Sandler DP, Collman GW. Cytogenetic and environmental factors in the etiology of the acute leukemias in adults. American journal of epidemiology. 1987;126:1017.

11. Malcovati L, Della Porta MG, Cazzola M. Predicting survival and leukemic evolution in patients with myelodysplastic syndrome. Haematologica. 2006;91:1588-1590.

12. Tefferi A, Vardiman JW. Myelodysplastic syndromes. N Engl J Med. 2009;361:1872-1885.

13. Engert A, Diehl V, Franklin J, Lohri A, Dörken B, et al. Escalated-dose BEACOPP in the treatment of patients with advanced-stage Hodgkin's lymphoma: 10 years of follow-up of the GHSG HD9 study. J Clin Oncol. 2009;27:4548-4554.

14. Gluzman D, Imamura N, Sklyarenko L, Nadgornaya V, Zavelevich M, Machilo V. Malignant diseases of hematopoietic and lymphoid tissues in Chernobyl clean-up workers. Hematol J. 2005;5:565-571.

15. Noshchenko AG, Zamostyan PV, Bondar OY, Drozdova VD. Radiation-induced leukemia risk among those aged 0-20 at the time of the Chernobyl accident: a case-control study in the Ukraine. Int J Cancer. 2002;99:609-618.

16. Kamada N, Tanaka K, Oguma N, Mabuchi K. Cytogenetic and molecular changes in leukemia among atomic bomb survivors. J Radiat Res (Tokyo). 1991;32 Suppl 2:257-265.

17. Heyssel R, Brill AB, Woodbury LA, Nishimura ET, Ghose T, et al. Leukemia in Hiroshima atomic bomb survivors. Blood. 1960;15:313.

18. Romanenko AY, Finch SC, Hatch M, Lubin JH, Bebeshko VG, et al. The Ukrainian-American study of leukemia and related disorders among Chornobyl cleanup workers from Ukraine: III. Radiation risks. Radiat Res. 2008;170:711-720.

19. Hill AB. The Environment and Disease: Association Or Causation? Proc R Soc Med. 1965;58:295-300.

20. Zablotska LB, Ashmore JP, Howe GR. Analysis of mortality among Canadian nuclear power industry workers after chronic low-dose exposure to ionizing radiation. Radiat Res. 2004;161:633-641.

21. Richardson DB, Wing S. Leukemia mortality among workers at the Savannah River Site. Am J Epidemiol. 2007;166:1015-1022.

22. Leone G, Pagano L, Ben-Yehuda D, Voso MT. Therapy-related leukemia and myelodysplasia: susceptibility and incidence. Haematologica. 2007;92:1389-1398.

23. Larson RA. Etiology and management of therapy-related myeloid leukemia. ASH Education Program Book. 2007;2007:453-459.

24. Hershman D, Neugut AI, Jacobson JS, Wang J, Tsai WY, et al. Acute myeloid leukemia or myelodysplastic syndrome following use of granulocyte colony-stimulating factors during breast cancer adjuvant chemotherapy. J Natl Cancer Inst. 2007;99:196-205.

25. Tarella C, Passera R, Magni M, Benedetti F, Rossi A, et al. Risk factors for the development of secondary malignancy after high-dose chemotherapy and autograft, with or without rituximab: a 20-year retrospective follow-up study in patients with lymphoma. J Clin Oncol. 2011;29:814-824.

26. Mauch PM, Kalish LA, Marcus KC, Coleman CN, Shulman LN, et al. Second malignancies after treatment for laparotomy staged IA-IIIB Hodgkin's disease: long-term analysis of risk factors and outcome. Blood. 1996;87:3625-3632.

27. Ng AK, Bernardo MV, Weller E, Backstrand K, Silver B, et al. Second malignancy after Hodgkin disease treated with radiation therapy with or without chemotherapy: long-term risks and risk factors. Blood. 2002;100:1989-1996.

28. Swerdlow AJ, Douglas AJ, Hudson GV, Hudson BV, Bennett MH, MacLennan KA. Risk of second primary cancers after Hodgkin's disease by type of treatment: analysis of 2846 patients in the British National Lymphoma Investigation. BMJ. 1992;304:1137-1143.

29. van Leeuwen FE, Klokman WJ, Veer MB, Hagenbeek A, Krol ADG, et al. Long-term risk of second malignancy in survivors of Hodgkin's disease treated during adolescence or young adulthood. Journal of Clinical Oncology. 2000;18:487.

30. Yaris F, Dikici M, Akbulut T, Yaris E, Sabuncu H. Story of benzene and leukemia: epidemiologic approach of Muzaffer Aksoy. J Occup Health. 2004;46:244-247.

31. Natelson EA. Benzene-induced acute myeloid leukemia: a clinician's perspective. Am J Hematol. 2007;82:826-830.

32. Richardson DB. Temporal variation in the association between benzene and leukemia mortality. Environ Health Perspect. 2008;116:370-374.

33. Patel AS, Talbott EO, Zborowski JV, Rycheck JA, Dell D, et al. Risk of cancer as a result of community exposure to gasoline vapors. Arch Environ Health. 2004;59:497-503.

34. Ånensen N, Skavland J, Stapnes C, Ryningen A, Børresen-Dale AL, et al. Acute myelogenous leukemia in a patient with Li--Fraumeni syndrome treated with valproic acid, theophyllamine and all-trans retinoic acid: a case report. Leukemia. 2006;20:734-736.

35. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, et al. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood. 2003;101:1249-1256.

36. Mehta PA, Ileri T, Harris RE, Williams DA, Mo J, et al. Chemotherapy for myeloid malignancy in children with Fanconi anemia. Pediatr Blood Cancer. 2007;48:668-672.

37. Taub JW. Relationship of chromosome 21 and acute leukemia in children with Down syndrome. J Pediatr Hematol Oncol. 2001;23:175-178.

38. Fong CT, Brodeur GM. Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. Cancer Genet Cytogenet. 1987;28:55-76.

39. Thomas X, Chelghoum Y. Cigarette smoking and acute leukemia. Leuk Lymphoma. 2004;45:1103-1109.

40. Pogoda JM, Preston-Martin S, Nichols PW, Ross RK. Smoking and risk of acute myeloid leukemia: results from a Los Angeles County case-control study. Am J Epidemiol. 2002;155:546-553.

41. Chang JS, Selvin S, Metayer C, Crouse V, Golembesky A, Buffler PA. Parental smoking and the risk of childhood leukemia. Am J Epidemiol. 2006;163:1091-1100.

42. Chelghoum Y, Danaila C, Belhabri A, Charrin C, Le QH, et al. Influence of cigarette smoking on the presentation and course of acute myeloid leukemia. Annals of oncology. 2002;13:1621.

43. Van Maele-Fabry G, Duhayon S, Lison D. A systematic review of myeloid leukemias and occupational pesticide exposure. Cancer Causes Control. 2007;18:457-478.

44. Gorini G, Stagnaro E, Fontana V, Miligi L, Ramazzotti V, et al. Alcohol consumption and risk of leukemia: A multicenter case-control study. Leuk Res. 2007;31:379-386.

45. Brown LM, Gibson R, Burmeister LF, Schuman LM, Everett GD, Blair A. Alcohol consumption and risk of leukemia, non-Hodgkin's lymphoma, and multiple myeloma. Leuk Res. 1992;16:979-984.

46. Pogoda JM, Nichols PW, Preston-Martin S. Alcohol consumption and risk of adult-onset acute myeloid leukemia: results from a Los Angeles County case-control study. Leuk Res. 2004;28:927-931.

47. Ekström K, Wuu J, Hsieh CC, Glimelius B, Lambe M. Childbearing and the risk of leukemia in Sweden. Cancer Causes Control. 2002;13:47-53.

48. Ma X, Park Y, Mayne ST, Wang R, Sinha R, et al. Diet, lifestyle, and acute myeloid leukemia in the NIH-AARP cohort. Am J Epidemiol. 2010;171:312-322.

49. Zhang M, Zhao X, Zhang X, Holman CD. Possible protective effect of green tea intake on risk of adult leukaemia. Br J Cancer. 2008;98:168-170.

50. Fernandez HF, Sun Z, Yao X, Litzow MR, Luger SM, et al. Anthracycline dose intensification in acute myeloid leukemia. N Engl J Med. 2009;361:1249-1259.

51. Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. JAMA. 2009;301:2349-2361.

52. Cornelissen JJ, van Putten WL, Verdonck LF, Theobald M, Jacky E, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? Blood. 2007;109:3658-3666.

53. Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. JAMA. 2009;301:2349-2361.

54. Klingemann HG, Storb R, Fefer A, Deeg HJ, Appelbaum FR, et al. Bone marrow transplantation in patients aged 45 years and older. Blood. 1986;67:770-776.

55. Blaise DP, Michel Boiron J, Faucher C, Mohty M, Bay JO, et al. Reduced intensity conditioning prior to allogeneic stem cell transplantation for patients with acute myeloblastic leukemia as a first-line treatment. Cancer. 2005;104:1931-1938.

56. Hegenbart U, Niederwieser D, Sandmaier BM, Maris MB, Shizuru JA, et al. Treatment for acute myelogenous leukemia by low-dose, total-body, irradiation-based conditioning and hematopoietic cell transplantation from related and unrelated donors. J Clin Oncol. 2006;24:444-453.

57. Magenau J, Couriel DR. Hematopoietic stem cell transplantation for acute myeloid leukemia: to whom, when, and how. Curr Oncol Rep. 2013;15:436-444.

58. Gupta V, Tallman MS, Weisdorf DJ. Allogeneic hematopoietic cell transplantation for adults with acute myeloid leukemia: myths, controversies, and unknowns. Blood. 2011;117:2307-2318.

59. Hourigan CS, Karp JE. Minimal residual disease in acute myeloid leukaemia. Nat Rev Clin Oncol. 2013;10:460-471.

60. Nancy AAD, Nancy AAD. Registries for Evaluating Patient Outcomes: A User's GuideAHRQ publication. U.S. Department of Health and Human Services, Public Health Service, Agency for Healthcare Research and Quality, 2010.

61. Silverman SL. From randomized controlled trials to observational studies. Am J Med. 2009;122:114-120.

62. Sullivan P, Goldmann D. The promise of comparative effectiveness research. JAMA: The Journal of the American Medical Association. 2011;305:400-401.

63. Benson K, Hartz AJ. A comparison of observational studies and randomized, controlled trials. N Engl J Med. 2000;342:1878-1886.

64. Concato J. Observational versus experimental studies: what's the evidence for a hierarchy? NeuroRx. 2004;1:341-347.

65. Initial national priorities for comparative effectiveness research. Washington, D.C.: National Academies Press; 2009.

66. Initial national priorities for comparative effectiveness research. Washington, D.C.: National Academies Press; 2009.

67. Carpenter WR, Meyer AM, Abernethy AP, Stürmer T, Kosorok MR. A framework for understanding cancer comparative effectiveness research data needs. J Clin Epidemiol. 2012;65:1150-1158.

68. Zapka JG, Taplin SH, Solberg LI, Manos MM. A Framework for Improving the Quality of Cancer Care The Case of Breast and Cervical Cancer Screening. Cancer Epidemiology Biomarkers & Prevention. 2003;12:4-13.

69. Center for International Blood and Marrow Transplant Research. CIBMTR Annual Progress Report. Milwaukee, WI:; 2011.

70. Rowe JM. Optimal induction and post-remission therapy for AML in first remission. ASH Education Program Book. 2009;2009:396-405.

71. Clark TG, Altman DG, De Stavola BL. Quantification of the completeness of follow-up. Lancet. 2002;359:1309-1310.

72. Savani BN, Mielke S, Reddy N, Goodman S, Jagasia M, Rezvani K. Management of relapse after allo-SCT for AML and the role of second transplantation. Bone Marrow Transplant. 2009;44:769-777.

73. Rowlings PA, Przepiorka D, Klein JP, Gale RP, Passweg JR, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol. 1997;97:855-864.

74. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. Journal of the American Statistical Association. 1999;94:496-509.

75. Löwenberg B, Ossenkoppele GJ, van Putten W, Schouten HC, Graux C, et al. High-dose daunorubicin in older patients with acute myeloid leukemia. N Engl J Med. 2009;361:1235-1248.

76. Dohner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel,on behalf of the European LeukemiaNet. Blood. 2009.

77. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10:223-232.

78. Fenaux P. Inhibitors of DNA methylation: beyond myelodysplastic syndromes. Nature Clinical Practice Oncology. 2005;2:S36-S44.

79. Wintrebert C, Putter H, Zwinderman AH, Van Houwelingen JC. Centre-effect on Survival after Bone Marrow Transplantation: Application of Time-dependent Frailty Models. Biometrical journal. 2004;46:512-525.

80. Péus D, Newcomb N, Hofer S. Appraisal of the Karnofsky Performance Status and proposal of a simple algorithmic system for its evaluation. BMC Med Inform Decis Mak. 2013;13:72.

81. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976;33:451-458.

82. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100:2292-2302.

83. de Jonge HJ, Valk PJ, de Bont ES, Schuringa JJ, Ossenkoppele G, et al. Prognostic impact of white blood cell count in intermediate risk acute myeloid leukemia: relevance of mutated NPM1 and FLT3-ITD. Haematologica. 2011;96:1310-1317.

84. Bakst RL, Tallman MS, Douer D, Yahalom J. How I treat extramedullary acute myeloid leukemia. Blood. 2011;118:3785-3793.

85. Morrissette JJ, Bagg A. Acute myeloid leukemia: conventional cytogenetics, FISH, and moleculocentric methodologies. Clin Lab Med. 2011;31:659-86, x.

86. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood. 2000;96:4075-4083.

87. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. 2009;15:1628-1633.

88. Gupta V, Tallman MS, He W, Logan BR, Copelan E, et al. Comparable survival after HLA-well-matched unrelated or matched sibling donor transplantation for acute myeloid leukemia in first remission with unfavorable cytogenetics at diagnosis. Blood. 2010;116:1839-1848.

89. Satagopan JM, Ben-Porat L, Berwick M, Robson M, Kutler D, Auerbach AD. A note on competing risks in survival data analysis. Br J Cancer. 2004;91:1229-1235.

90. Lin G. Analyzing Survival Data with Competing Risks Using SAS® Software. Proceedings of the SAS® Global Forum 2012 Conference. 2012.

91. Tallman MS, Pérez WS, Lazarus HM, Gale RP, Maziarz RT, et al. Pretransplantation consolidation chemotherapy decreases leukemia relapse after autologous blood and bone marrow transplants for acute myelogenous leukemia in first remission. Biol Blood Marrow Transplant. 2006;12:204-216.

92. Bloomfield CD, Lawrence D, Byrd JC, Carroll A, Pettenati MJ, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. 1998;58:4173-4179.

93. Kern W, Estey EH. High-dose cytosine arabinoside in the treatment of acute myeloid leukemia: Review of three randomized trials. Cancer. 2006;107:116-124.

94. Thomas X, Elhamri M, Raffoux E, Renneville A, Pautas C, et al. Comparison of high-dose cytarabine and timed-sequential chemotherapy as consolidation for younger adults with AML in first remission: the ALFA-9802 study. Blood. 2011;118:1754-1762.

95. Schaich M, Parmentier S, Kramer M, Illmer T, Stölzel F, et al. High-dose cytarabine consolidation with or without additional amsacrine and

mitoxantrone in acute myeloid leukemia: results of the prospective randomized AML2003 trial. J Clin Oncol. 2013;31:2094-2102.

96. Schiffer CA. Clinical issues in the management of patients with myelodysplasia. Hematology Am Soc Hematol Educ Program. 2006:205-210.

97. Blaise D, Vey N, Faucher C, Mohty M. Current status of reduced intensity conditioning allogeneic stem cell transplantation for acute myeloid leukemia. haematologica. 2007;92:533-541.

98. Shimoni A, Hardan I, Shem-Tov N, Rand A, Herscovici C, et al. Comparison between two fludarabine-based reduced-intensity conditioning regimens before allogeneic hematopoietic stem-cell transplantation: fludarabine/melphalan is associated with higher incidence of acute graft-versus-host disease and non-relapse mortality and lower incidence of relapse than fludarabine/busulfan. Leukemia. 2007;21:2109-2116.

99. Choi J, Bernabe N, Abboud CN, Westervelt P, Vij R, et al. Maintenance therapy with decitabine after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia and high-risk myelodysplastic syndrome. Blood. 2013;122:4638-4638.

100. de Lima M, Giralt S, Thall PF, de Padua Silva L, Jones RB, et al. Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study. Cancer. 2010;116:5420-5431.

101. Chen YB, Aldridge J, Kim HT, Ballen KK, Cutler C, et al. Reduced-intensity conditioning stem cell transplantation: comparison of double umbilical cord blood and unrelated donor grafts. Biol Blood Marrow Transplant. 2012;18:805-812.

102. Brunstein CG, Eapen M, Ahn KW, Appelbaum FR, Ballen KK, et al. Reduced-intensity conditioning transplantation in acute leukemia: the effect of source of unrelated donor stem cells on outcomes. Blood. 2012;119:5591-5598.

103. Farag SS, Maharry K, Zhang MJ, Pérez WS, George SL, et al. Comparison of reduced-intensity hematopoietic cell transplantation with chemotherapy in patients age 60-70 years with acute myelogenous leukemia in first remission. Biol Blood Marrow Transplant. 2011;17:1796-1803.

104. Kurosawa S, Yamaguchi T, Miyawaki S, Uchida N, Kanamori H, et al. A Markov decision analysis of allogeneic hematopoietic cell transplantation versus chemotherapy in patients with acute myeloid leukemia in first remission. Blood. 2011;117:2113-2120.