UNIVERSITY OF MANITOBA

Rady Faculty of Health Sciences
Rady College of Medicine

Office of Graduate and Advanced Degree Education in Medicine

Student Name: Neelan Sriranjan **Date:** 08/05/16

Project Title: Comparison Between Chronic Lymphocytic Leukemia Patients in India and Canada

Primary Supervisor Name: Dr. James Johnston

Department: Section of Hematology/Oncology, Department of Internal Medicine

Co-Supervisor Name: Dr. Manoranjan Mahapatra

Department: Hematology

Summary (250 words max single spaced):

Chronic lymphocytic leukemia (CLL) is the commonest leukemia in North America, primarily affecting those aged > 65 years. In contrast, CLL is believed to be less common in India. The clinical course of this disease is highly variable, and can be predicted by the Rai stage and a variety of biological prognostic markers. In this study, we have compared all new CLL patients attending Cancer Care Manitoba (CCMB), with those attending the All India Institute of Medical Sciences (AIIMS), New Delhi, between 2008 and 2014. One hundred and six CLL patients attended AIIMS during this time, whereas 391 were seen at CCMB. Patients at AIIMS were younger than at CCMB, with the median age at diagnosis being 60 and 68 years, respectively. In addition, while most patients at AIIMS presented with advanced disease and required therapy at diagnosis, the opposite was true at CCMB. Most patients at CCMB presented with Rai stages 0/I disease, and were diagnosed with routine blood tests at their family doctors. We found that the standard prognostic markers were useful to predict time to treatment and survival in the CCMB population, but these were less frequently performed at AIIMS. In addition, while response and outcome were available at CCMB, monitoring following therapy was rarely carried out in India because of financial difficulties and distance to travel. These data demonstrate differences in the medical systems between India and Canada, highlighting the difficulties in comparing patient populations in the two countries.

Acknowledgments

a) I gratefully acknowledge the support of the following sole sponsor: Dr. Alvin Klady Memorial Fund

b) If not soley funded, I gratefully acknowledge the funding support from one or more of the following sponsors;

H.T. Thorlakson Foundation
Dean, College of Medicine
Research Manitoba
Vice-Dean, Research Rady FHS
Health Sciences Centre Research Foundation
Heart and Stroke Foundation

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MD/PhD MD/MSc. BSc. (MED) MED II Research Program
Joe Doupe Annual Event Undergraduate Medical Student Research Symposium
Canadian National Medical Student Research Symposium

Introduction and Background:

Chronic Lymphocytic Leukemia (CLL) is of great concern in the province of Manitoba. This disease has an incidence of 7.9/100,000 persons (Bieggi et al. 2016). Leukemia is the uncontrolled proliferation and accumulation of abnormal white blood cells in the blood and CLL involves the accumulation of abnormal B-cell Lymphocytes (National Cancer Institute. 2013 and Hallek et al. 2015). To diagnose CLL, the peripheral blood B cell count should be greater than 5 x 10^9/L for at least a three month period and there must be a population of monoclonal (kappa or lambda) B cells (CD19+, CD20+ (weak) and CD23+) that express the T cell marker, CD5 (Hallek et al. 2015).

The CLL cell is a memory B-Cell and there are two main types of CLL, differentiated by whether the variable portion of the immunoglobulin heavy chain gene (IgV_H) for the B cell receptor is mutated or not mutated (Johnston et al. 2013). Mutated IgV_H CLL has a more indolent disease with a better overall survival than unmutated CLL (Cramer et al. 2005). Altered DNA methylation also occurs in CLL and through this selective process, the mutated IgV_H CLL cells have the methylation pattern of memory B cells whereas the unmutated IgV_H cells start to resemble naïve B cells. Regardless of this distinction, both types of CLL have a similar genetic profile, as all CLL cells have a similar propensity for hypomethylation and other epigenetic changes (Johnston et al. 2013).

Chronic Lymphocytic Leukemia is similar to two other disease entities, Small Lymphocytic Lymphoma (SLL) and Monoclonal B-Cell Lymphocytosis (MBL) (Table 1). These disease variants have the same immunophenotype to CLL, but differ in the number of B cells in the blood and in the presence or absence of lymphadenopathy/splenomegaly (Strati et al. 2015). MBL is a precursor to CLL or SLL, where there is no evidence of lymphadenopathy, splenomegaly and the peripheral blood B cell count is <5x10^9/L (Hallek et al. 2015). There is a family predisposition for MBL, as CLL itself has a very strong inherited predisposition. In families with two first degree relatives with CLL, the risk for MBL in unaffected members is two to three fold higher than the general population (Strati et al, 2015).

There are several types of MBL, depending on the type of malignant B cell. However, majority of cases have the CLL immunophenotypes and are precursors to CLL or SLL. Cases with low numbers of monoclonal B cells are unlikely to progress, and the risk of transformation is directly related to the peripheral blood B cell count with the rate of progression being 1-2% per year for high B-cell count MBL. MBL is an area of concern in transfusion medicine, and 3.4% of the donors that were 45 years of age or older had high-count MBL of CLL type. Although the risk of progression to CLL for the donors and recipients was low, it demonstrated the need for a more stringent screening process centred on MBL (Shim et al. 2014).

Chronic lymphocytic leukemia is staged in North America using the Rai staging system, which remains the most important way of determining prognosis. This staging system measures the extent of disease rather than assessing the biology of the disease or rate of disease progression. As the prognosis of patients with early Rai stage CLL is highly variable, it is important to develop biological prognostic markers to determine which of these early stage patients will progress (Cramer et al. 2005, Pflug et al. 2014).

One simple step towards examining the biological characteristics of CLL cells is the use of the lymphocyte doubling time (LDT), which measures how long it takes for the baseline lymphocyte count to double (Abbott et al. 2005). Those patients with a LDT of <12 months have a more aggressive disease than those with a LDT of >12 months.

β2-microglobulin is part of the HLA complex and is expressed on nucleated cell membranes, shed into plasma and excreted by the kidneys. Thus, high levels reflect high tumour burden, renal dysfunction and poor prognosis in CLL (Berrebi et al. 2010, Delgado et al. 2009).

Cytogenetics is important in predicting survival and the likelihood of chemotherapy response in CLL. Fluorescent In-Situ Hybridization (FISH) is a technique that detects chromosomal aberrations. These include gene fusion, aneuploidy, loss of a region on a chromosome, or loss of a whole chromosome. FISH uses fluorescent DNA probes that bind to complementary sequences of DNA, and the presence or absence of these sequences provide information on genomic anomalies. FISH can be performed when a cell is in interphase or when it is in metaphase (Bishop. 2010). As the majority of CLL cells are not dividing, FISH is usually carried out on interphase cells (Alhourani et al. 2015).

Loss of parts of chromosomes 11 and/or 17 have great prognostic significance in CLL. Deletion 17p13.1, which affects the integrity of the *TP53* gene, and deletion 11q22.3, which affects the integrity of the *ATM* gene, have both been associated with unfavourable outcomes in CLL. Both of these are more likely to occur in unmutated IgV_H CLL. The *TP53* and *ATM* genes are involved in the same pathway for DNA damage response (Greipp et al. 2013). The 17p13.1 deletion, with loss of P53, results in resistance to genotoxic chemotherapy, which cannot be overcome with the addition of anti-CD20 antibodies (Hallek et al. 2015). This occurs because P53 is required for the induction of apoptosis with cell damage caused by chemotherapy (Stephens. 2012). Idelalisib and Ibrutinib, which are both B-receptor signalling inhibitors, have acitivity in patients with a deletion 17p13.1 and are thus used for chemotherapy resistant disease (Sanford et al. 2015). The 11q22.3 deletion has been associated with bulky lymphadenopathy in middle-aged inividuals, shorter remission durations and shorter overall survival when compared to patients without this deletion after treatment with conventional chemotherapy. However, ibrutinib and idelalisib may improve survival of these patients (Puiggros et al. 2014).

The CD38 is a transmembrane glycoprotein with signalling function in CLL cells. CD38+ patients (≥20% of the leukemic cells stain positive) had a shorter progression free survival time than their CD38- counterparts (Schroers et al. 2005).

The Zap-70 is a tyrosine kinase that has signalling responsibilities in T cells and natural killer cells, and is associated with high intracellular signalling in CLL cells. Having expression of this protein in ≥20% of leukemic cells is associated with a smaller treatment free survival time (Schroers et al. 2005).

It has also been shown that the number of comorbidities in CLL, as measured by the Cumulative Illness Rating Scale (CIRS), is predictive of survival in CLL and is used to determine if a patient fit for particular therapies (Goede et al. 2014, Eichhorst et al. 2009).

Another measure of treatment fitness is the performance status of the patient (Sagatys and Zhang. 2012). This is assessed using the Eastern Cooperative Oncology Group (ECOG) scoring scale, in which patients are scored between 0 and 4. A zero is given for normal activity, one for close to full ambulation, two for being in bed less than 50% of normal daytime, three for being in bed more than 50% of normal day time, and four if there is an inability to get out of bed (Sorensen et al. 1993). Patients with a high ECOG cannot tolerate aggressive chemotherapy.

Kidney function, another marker for treatment fitness, can be assessed using serum creatinine. As the kidney's ability to filtrate decreases, serum creatinine increases, reflecting a decreased ability to filter toxins. One of the mainstays in chemotherapy regimen, fludarabine, has the ability to cause toxicity if it accumulates in the patient. This drug is renally excreted, and a patient's creatinine can indicate if the kidney can function to excrete the drug adequately (Martell et al. 2002).

Age and gender are also important factors for survival in CLL. For example, CLL is usually more aggressive in the elderly, particularly if they are men (Seftel, 2009). Several trials have shown that women have a more benign disease course than men in CLL, with better overall survival, progression-free survival, and response to treatment (Catovsky et al. 2014).

While CLL is the most common leukemia in Europe and North America, it is less common in Asia (Kawamata et al. 2013, Yang et al. 2014). In India, CLL only accounts for 2-4% of leukemias, which is much lower than the proportion of CLL in Western countries (Gogia et al. 2012).

In addition, it is unclear as to whether the biology of CLL is different in Asia. As an initial step to answering this question, we have compared patients with CLL in Canada with those in India, by studying patients at CancerCare Manitoba (CCMB), Winnipeg, Manitoba and the All India Institute of Medical Sciences (AIIMS), a tertiary care centre in New Delhi, India. The purpose of this study was to compare the clinical and laboratory features of patients at the two centres, their treatments and outcomes.

Methods:

Selection of Study Cohort

This study included CLL and SLL patients diagnosed between 2008 and 2014 at AIIMS and CCMB. The diagnostic criteria for CLL was having expression of CD5, CD19, CD20, and CD23 in monoclonal (kappa or lambda) B cells. Classifying patients as MBL, CLL or SLL and staging of CLL patients was carried out as described in Tables 1 and 2.

Accumulation of the Data at the All India Institute of Medical Sciences

Patients' paper charts were retrieved for CLL patients based on numbers assigned to patients in the existing CLL database at AIIMS. Paper charts were mainly handwritten and rarely had printed lab reports in them. This is a reflection of the medical system in India, which requires individuals to carry their own investigation reports from one institution to another as opposed to the Canadian practice of the institution itself keeping a copy. Therefore, the majority of the data was obtained from the physicians' notes or from the CLL database mentioned above. Data points collected at this centre include, age at diagnosis, sex, Rai stage, comorbid conditions, creatinine, CD38 status, Zap-70 status, and initial treatment. The comorbid condition information was designated a CIRS score based on how much of an impairment the comorbidity caused.

Accumulation of the Data at Cancer Care Manitoba

The data from Manitoba was obtained from the CCMB research database known as CAISIS. CAISIS accumulates CLL patients' information from the CCMB clinical database known as ARIA as well as paper charts when necessary. The data points collected at CCMB include, age at diagnosis, sex, Rai stage, CIRS, ECOG, creatinine, β2-microglobulin, LDT, CD38 status, Zap-70 status, IgV_H mutational status, initial treatment, subsequent treatments, as well as date of death.

Statistical Analysis by SAS

Follow up was calculated as the time between date of CLL diagnosis and date of death or end of study (December 31, 2014), whichever came first. Time to first treatment (TTFT) was calculated as the time between CLL diagnosis and the start of first CLL treatment. Time to second treatment (TTST) was calculated as the time between the start of first treatment and the start of second treatment in patients with at least two lines of CLL treatment. These were only calculated for patients at CCMB, as the data was not available for patients at AIIMS. Kaplan-Meier estimates were used to construct survival curves in order to investigate the effects of CLL clinical features on overall survival and TTFT and to estimate p-values for these associations. p-values < 0.05 were considered statistically significant. Microsoft excel and SAS Studio 3.5 were utilized for data management, chart creation and statistical analyses.

Parameters Analyzed at Both Institutions

Refer to Table 3 for information on threshold values.

Results:

Comparison of Patient Clinical Characteristics

There were 391 patients at CCMB, with a median age of 68 (range 39 – 99), 64% being male, 82% of all CLL patients being diagnosed with Rai stage 0/1 and 19% being diagnosed with SLL. There were 76 patients diagnosed with SLL and 77 with MBL. MBL patients were removed from this cohort to make it comparable with the India cohort. One-third of patients received treatment during the course of this study (Table 3).

There were 106 patients at AIIMS, with a median age of 60 (range 35 – 79), 74% being male, less than half (46%) being diagnosed with Rai stage 0/1 and only one patient (2%) being

diagnosed with SLL. Majority of patients (57%) were treated for CLL at this centre (Table 2). There were not patients with MBL.

Initial treatment

At AIIMS, of the 47 treated patients, 76% were on chlorambucil and only 18% were put on fludarabine based therapy (Figure 1). At CCMB, of the 119 treated patients, 53% were on fludarabine based therapy and only 23% were on chlorambucil based therapy (23%) (Figure 2).

Molecular and Serum Markers

The two molecular markers that were available at baseline at both institutions were Zap-70 and CD38. CD38 was positive in 31% of CCMB patients and 33% of AIIMS patients (Figure 3). Zap-70 was positive in 27% of CCMB patients and 23% of AIIMS patients (Figure 4).

Other Molecular and Serum Markers Only Available at CCMB

In CCMB 49% of patients had high β2-microglobulin (Figure 5), 8% had a LDT of less than six months (Figure 6), and 61% had the mutated IgV_H gene (Figure 7).

Overall Survival at CCMB

Rai stage was associated with overall survival ($p<0.0001$) with Rai stages 0/1 having the longest overall survival followed by SLL. Rai stages II/III had a moderate overall survival and Rai stage IV had the shortest with approximately 40% dying within two years of CLL diagnosis (Figure 8). Mutational status was also associated with overall survival with IgV_H mutated patients having the better outcome ($p=0.0331$) (Figure 9). However, receiving treatment did not appear to be associated with overall survival ($p=0.0723$).

Time to First Treatment at CCMB

Rai stage was also associated with TTFT ($p<0.0001$) with Rai stages 0 having the longest TTFT. Rai stage I/SLL had a moderate TTFT and Rai stages II/III had a moderate-short TTFT. Rai stage IV had the shortest TTFT with almost all patients requiring treatment immediately after CLL diagnosis (Figure 10).

IgV_H mutational status was associated with TTFT with mutated patients having significantly longer TTFT ($p<0.0001$) (Figure 11). LDT was also predictive of TTFT with patients with LDT > 12 having the longest treatment free survival ($p =0.0324$) (Figure 12). Similar pattern was observed with Zap-70 ($p=0.0010$) and CD38 ($p<0.0001$) with Zap 70- and CD38- cases having a longer TTFT than those that were Zap-70+ or CD38+ (Figure 13 and 14). Thus, unmutated IgV_H disease, short LDT, Zap 70+ or CD38+ disease were associated with more rapidly progressive disease.

Discussion:

In a previous study at CCMB, we have demonstrated that, because of referral bias, the age of CLL patients in a clinic may not be representative of the age in the population. Thus, the median age of patients in the CCMB CLL clinic has been consistently 68 years, while the

median age in the Manitoba CLL population is 72 (Bieggi et al. 2016). The cause for this referral bias was unclear, but presumably is because non-referred patients are considered too frail for referral. Certainly, the referred patients are more likely to receive chemotherapy, and, after correcting for age and sex, have a significantly better prognosis than patients who were not referred. In the present study, the median age at AIIMS was 60 years (Table 4), younger than at CCMB (68 years). The reason for the younger age at AAIMS may be related to its referral base, which is from surrounding states in India, like Behar, Harayana, Punjab, Uttarakand, and Uttar Pradesh. Thus, individuals would need to be fairly young and fit to make the long journey to Dehli. In contrast, in Manitoba most of the population resides in Winnipeg or Brandon, and do not have to travel too far to reach CCMB.

A second interesting feature was the greater number of males seen at AIIMS, as compared to CCMB. While 64% of CLL patients at CCMB were males, 74% of the CLL patients were male at AIIMS (Table 4). While it is known that CLL is more common in men than in women, there was no significant difference between the ratio of males : females referred and not-referred to CCMB (Beiggi, 2016). If this holds true in India, then it suggests that there are a disproportionate number of males with CLL in India, indicating a difference in the biology of the disease between the two countries. An alternative explanation is that for cultural reasons, there may be referral bias in India, with young males being preferentially referred for treatment.

Patients at AIIMS had more advanced disease than those at CCMB. Thus, 31% of the patients at AIIMS had high-risk CLL, as compared to 9% at CCMB (Table 4). In addition, approximately 15% of patients at CCMB had MBL, whereas non of the patients at AIIMS had MBL. This difference is directly related to medical practices in the two countries, where routine blood work (often annual) is typically carried in Canada on the healthy population, screening for diabetes, high cholesterol, anemia, etc. As a result, many healthy individuals are being diagnosed with MBL, as they are found to have a persistent slight increase in the peripheral lymphocyte count. As these patients have an increased risk of second malignancies, infections and transformation to CLL/SLL they are routinely followed on an annual basis for evidence of these complications. Routine screening on the general population is not carried out in India.

While the patients in India were more likely to be younger and male, we determined whether there was a difference in their fitness, as measure by the CIRS and ECOG scores. Interestingly, 94% of the patients at AIIMS were considered fit, as they had a CIRS between 0-6, compared to only 77% at CCMB (Table 4). However, the discrepancy in CIRS between AIIMS and CCMB may be to some degree related to how the data was obtained. There were no referral letters in the charts at AIIMS to provide information as to other ongoing medical issues, past history and drug usage. Thus, assessing CIRS depended on whether the physician taking the history had included this information. At CCMB, it was possible to review several notes (referring doctor's letter, nurses note, physician's admitting history and E-health for drug usage). For patients at CCMB, 90% achieved an ECOG score of either "0" or "1", and would therefore be at least almost fully ambulatory (Sorensen et al. 1993) (Table 4). The lower the ECOG score, the more likely the patient is to tolerate chemotherapy, which in turn correlated with prognosis (Shanafelt. 2013). Unfortunately, the ECOG could not be found at all in the CLL database at AIIMS.

The indications for treatment of CLL are rapid disease progression or advanced disease, with the type of treatment depending on the patient's fitness and age. At AIIMS, 57% of the patients were treated, and 72% of treated patients received chlorambucil, primarily because this drug is much cheaper than fludarabine in India (Figure 2). In India, most chemotherapy is generic, as they are not controlled by patent laws, and so the differential in cost of drugs is quite different to North America. Consistent with the fact that the majority of patients at CCMB had low-risk disease, only 30% required treatment (Table 4). In contrast to India, 53% of the patients at CCMB were given a fludarabine-based therapy and 23% a chlorambucil-based therapy (Figure 1). The reason for the difference to India is that fludarabine has traditionally been felt to be the preferred treatment for CLL, in patients who can tolerate this agent. Cost factors have not been so limiting in Canada as in India. However, another factor was the increased incidence of mild uremia in the Indian population. Twenty-six percent of the patients at AIIMS had increased creatinine, compared to only 12% at CCMB. Fludarabine is excreted by the kidneys, and should be avoided or the dose modified in patients with uremia (Martell et al. 2002).

As shown in the data from CCMB, overall survival was lowest in high-risk patients ($p<0.0001$) (Figure 8). The prognostic value of other markers was assessed. As mentioned earlier, CD38 is positively correlated with shorter progression free survival and Zap-70 is positively correlated with a shorter treatment free survival (Schroers et al. 2005). In the data from CCMB, the TTFT was shorter in patients with a Zap-70+ status ($p=0.0010$) (Figure 13) and those with CD38+ status ($p<0.0001$) (Figure 12).

β2–microglobulin, lymphocyte doubling time, and IgV_H mutational status are all also important prognostic markers in CLL. Unfortunately, this data was only obtained at CCMB. AIIMS did not obtain IgV_H mutational status at all and β2-microglobulin and lymphocyte doubling time were both collected in a very small number of patients. Forty nine percent of patients had a high β2–microglobulin serum level, which reflects tumor burden and uremia (Figure 5). Only 8% of the patients at CCMB had a LDT of <6 months, compared to 76% of patients with a time of >12 months (Figure 6). The patients with a LDT of >12 months had a higher survival probability from less than one year onwards ($p=0.0324$) (Figure 14). This is reflected by the 52% of patients with low-risk disease, according to Rai stage, and only 30% of patients requiring treatment (Table 1). Whether LDT or β2-microglobulin provides a more accurate picture of the aggressiveness of disease is subject for further investigation. Sixty one percent of patients were IgV_H mutated, which predicts a more indolent disease course and a better overall survival (Cramer et al. 2005) (Figure 7). This superior outcome is seen in the CCMB data in patients with the mutated IgV_H cohort ($p=0.0331$) (Figure 10). In addition, the mutated cohort also had a much shorter TTFT, augmenting the case for having a more indolent disease ($p<0.0001$) (Figure 11). This again correlates with the Rai Stage data as well as the LDT and number of patients treated.

We determined whether there was a difference in the biological characteristics of CLL cells at CCMB compared to AIIMS. Thirty three percent of the patients at AIIMS were CD38+, compared to 31% at CCMB (Figure 3). Although the difference seems negligible, it is important to mention again that AIIMS required 30% of leukemic cells to express CD38 for the patient to be considered CD38+, as opposed to CCMB, which required 20% of leukemic cells to express CD38. This difference in threshold for positive expression could thus have decreased the number of CD38+ patients diagnosed at AIIMS. The ZAP-70 positivity at AIIMS was 23% but

27% at CCMB (Figure 4). However, it should be pointed out that only 40 patients were tested for Zap-70 at AIIMS, whereas 315 patients were tested at CCMB (Figure 4). Despite this, these data suggest that the two populations were similar using these biological markers.

Although baseline data was completely obtained at CCMB and to a certain degree at AIIMS, follow-up and outcome of patients could not be compared. Outcome data depends on follow-up and many patients at AIIMS were lost to follow-up. This was due to the distance many of the patients had to travel, as some came from surrounding provinces. In addition, some patients could not afford optimal treatment and therefore were lost to follow up. The existence of private hospitals in India allows for patients to leave AIIMS and attend these hospitals. Moreover, patients could leave AIIMS and attend another public institution, especially if they come from another province in India. Although, switching clinics in Manitoba allows for the current doctor to send the requisite files to the new doctor, in India the patients are required to carry their own lab results and present it to the physician they are seeing. The physician then records the information given to them by the patient on the chart at the institution and returns the original files to the patient. Throughout the study, it was found that not everything was recorded in the chart, and reliance on only the chart for the study was inadequate.

Another limitation to the study was the number of patients from AIIMS was much less than the number of patients from CCMB. The manual filing system at AIIMS proved to be a foil in matching patient numbers from the database and patient numbers in the file room. Although there were many more patients in the database than there are in this study, these patients could not be utilized because their corresponding charts could not be found.

Finally, for financial and other reasons, many of the routine baseline parameters, such as β2-microglobulin, LDT, and IgV_H mutational status were not available at AIIMS. Extra funding was required to perform β2-microglobulin measurements, as it was not a routine test done at the institution. Lymphocyte doubling time requires follow up, which is a challenge at AIIMS, in order to gather successive lymphocyte numbers required to calculate this parameter. Finally, funding prevented obtaining IgV_H mutational status, as this was not done at all at AIIMS.

Conclusion:

This comparative study between CCMB and AIIMS demonstrates differences in the medical systems between Canada and India, showing that referred CLL patients at AIIMS were younger, had more advanced disease and required earlier treatment than patients at CCMB. However, the study has opened the door for further collaborations between our two centres. Of particular interest is to determine whether the biology of CLL is different between the two countries, although the incidence of CD38 and Zap-70 positivity between the two populations suggests that they may be similar. Further studies will require the exchange of tumor samples between our centres, to compare the biology of the disease in India and Canada.

Resources:

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Appendix

Table 1. CLL Variants[1]

	MBL	CLL	SLL
B cell count	<5 x 10^9/L	≥5 x 10^9/L	<5 x 10^9/L
Enlarged Lymph Nodes or Spleen	None	Maybe	Yes

[1]This table is adapted from the CCMB Practice Guidelines.

Table 2. Rai Staging for Chronic Lymphocytic Leukemia[1]

Rai Stage		Characteristic	Description	Median Survival (years)
0	Low Risk	Lymphocytosis only (B cells ≥5 x 10^9/L)	>10	
1	Intermediate Risk	Lymphocytosis + Lymphadenopathy	9	
2	Intermediate Risk	Lymphocytosis + Splenomegaly	7	
3	High Risk	Lymphocytosis + Hemoglobin <110 g/L	2-5	
4	High Risk	Lymphocytosis + Platelets <100x10^9/L	2-5	

[1]This table is adapted from the CCMB Practice Guidelines.

Table 3. Threshold Values for Each Parameter

Parameter	CCMB	AIIMS
Zap-70[1]	20%	20%
CD38[1]	30%	30%
Creatinine[2]	40 – 100 μmol/L	40 – 100 μmol/L
β2 – microglobulin[2]	1.1 – 2.4 g/L	N/A

[1]The percent of leukemic cells that need to express the antigen to be considered positive expression. [2]Normal level is between the two numbers.

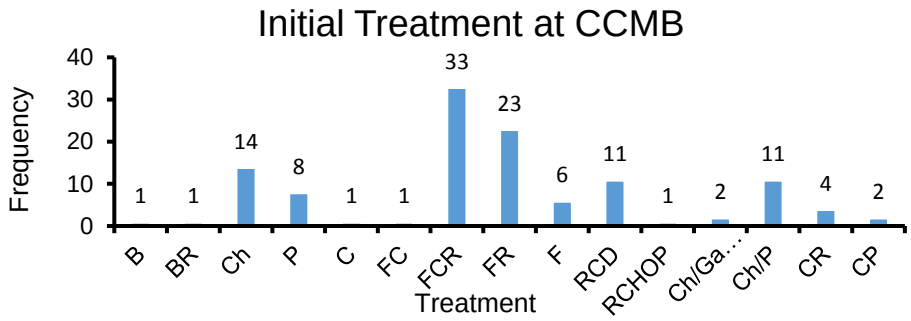

Figure 1. “B” is bendamustine, “R” is rituximab, “Ch” is chlorambucil, “P” is prednisone, “C” is cyclophosphamide, “H” is doxorubicin hydrochloride, “O” is vincristine sulphate, and Ga101 is an anti-CD20 antibody. Combining the designations indicates multiple agents are involved in therapy.

Table 4. Patient Clinical Characteristics

		CCMB (n=391)	AIIMS (n = 106)
Median Age (years)	Median	68	60
	Minimum	39	35
	Maximum	99	79
Sex Distribution (%)	Male	252 (64)	78 (74)
	Female	139 (36)	28 (26)
Rai Stage (%)[1]	Rai 0	165 (52)	23 (33)
	Rai I	94 (30)	9 (13)
	Rai II	27 (9)	16 (23)
	Rai III	18 (6)	20 (29)
	Rai IV	11 (3)	1 (2)
Diagnosis	CLL	315	105
	SLL	76	1
	MBL	77	0
ECOG (%)[2]	0	254 (65.1)	N/A
	1	114 (29.2)	N/A
	2	18 (4.6)	N/A
	3	2 (0.5)	N/A
	4	2 (0.5)	N/A
CIRS (%)[3]	0	301 (77)	87 (94)
	1	85 (22)	5 (5)
	2	3 (1)	1 (1)
Creatinine (%)[4]	High	48 (12)	12 (26)
	Normal	2 (1)	1 (2)
	Low	335 (87)	33 (72)
Treatment Status (%)[5]	Treated	119 (30)	47 (57)
	Untreated	272 (70)	35 (43)

[1]Missing data for 37 patients at AIIMS. [2]Missing data for 1 patient. [3]Missing data for 2 patients at CCMB and 13 patients at AIIMS. [4]Missing data for 6 patients at CCMB and 60 patients at AIIMS. [5]Missing data in 24 patients at AIIMS.

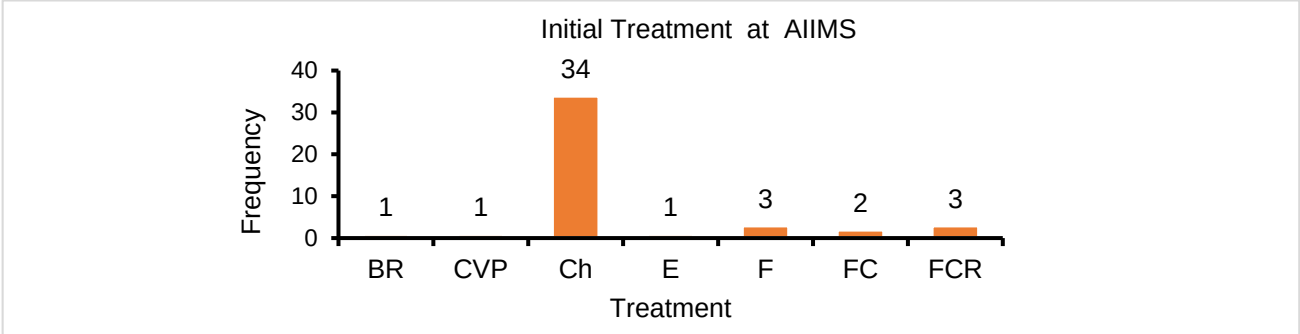

Figure 4. Initial Treatment at AIIMS. “B” is bendamustine, “C” is cyclophosphamide, “V” is vincristine, “P” is prednisone, “Ch” is chlorambucil, “E” is etoposide, “F” is fludarabine, and “R” is rituximab. Combinations of the designations means multiple agents are involved in therapy.

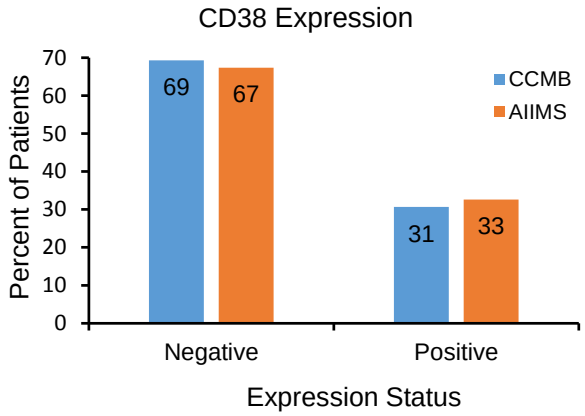

Figure 3. Comparison of CD38 Expression between AIIMS and CCMB.

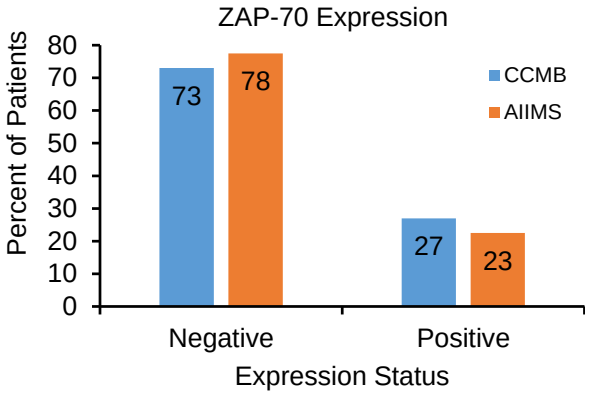

Figure 4. Comparison of Zap-70 Expression between AIIMS and CCMB

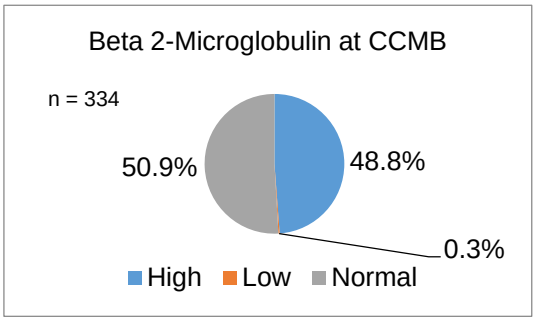

Figure 5. Variance in concentration of Beta 2-Microglobulin between patients at CCMB

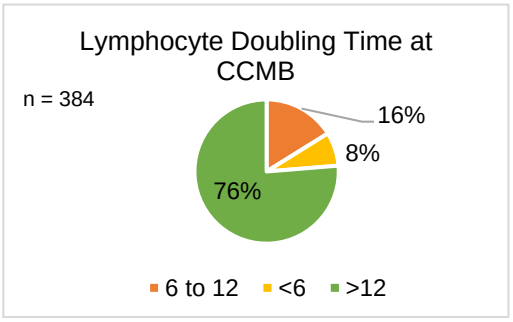

Figure 6. Variance in Lymphocyte Doubling Time between patients at CCMB

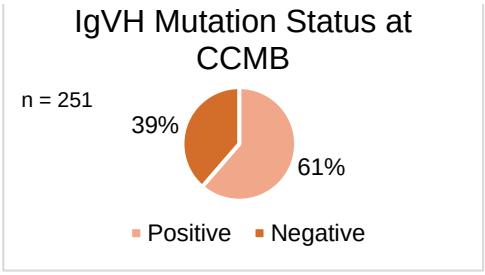

Figure 7. Proportion of patients with and without the mutated IgV_H gene

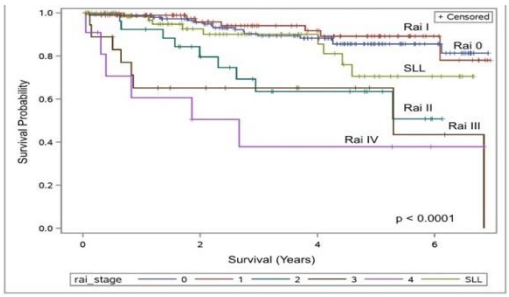

Figure 8. Overall survival comparison between each Rai stage.

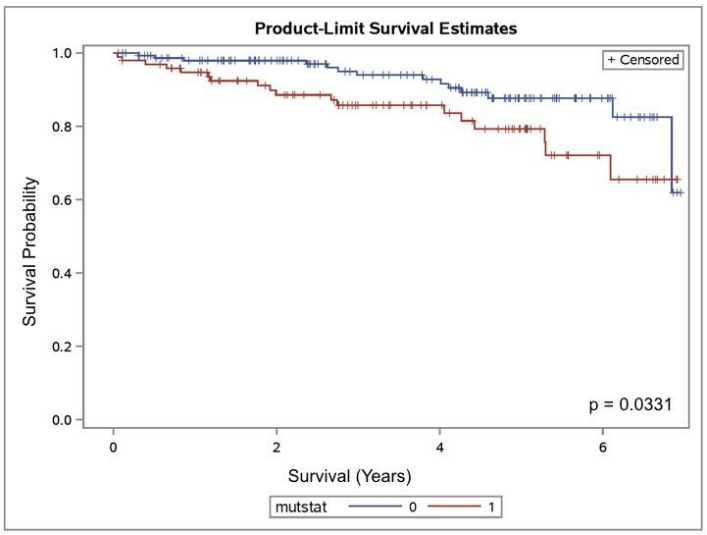

Figure 9. Overall survival comparison between umutated IgV_H status and mutated IgV_H status.

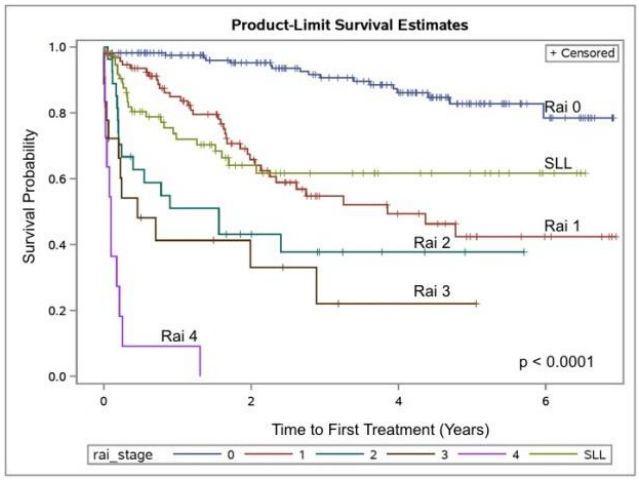

Figure 10. Time to First Treatment comparison between Rai stages.

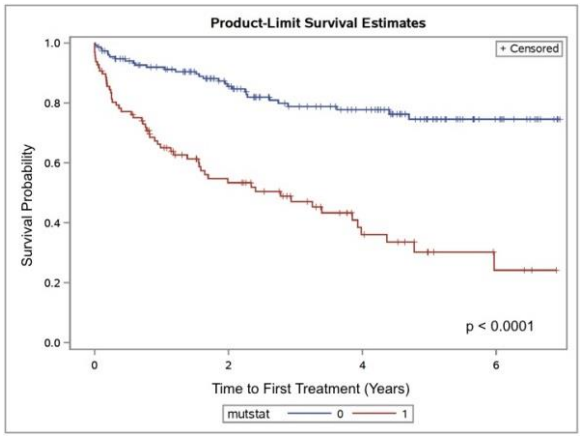

Figure 11. Comparison between patients with the IgV_H mutation and patients without for Time to First Treatment.

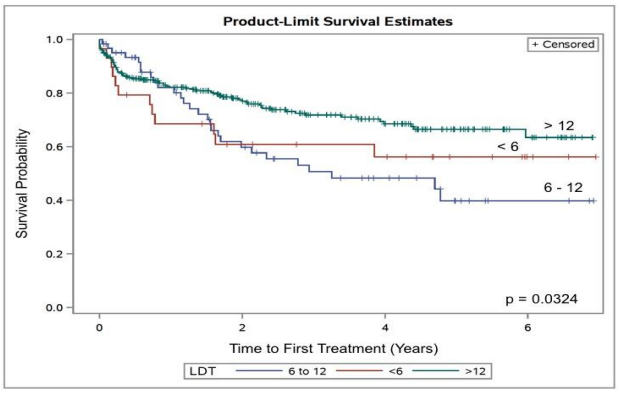

Figure 12. Comparison between patients with varying LDT for Time to First Treatment

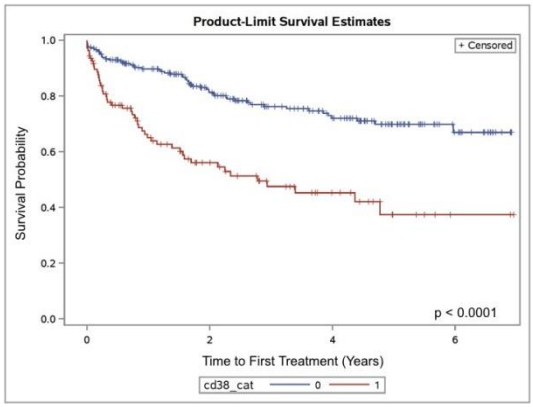

Figure 13. Comparison between CD38+ and CD38- patients for Time to First Treatment.

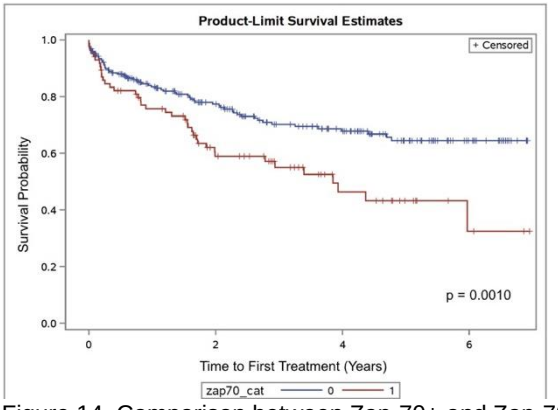

Figure 14. Comparison between Zap-70+ and Zap-70- patients for Time to First Treatment.