ABSTRACTS

1. **Amrein, L, Larroque AL, Peyrard L, Borrelli D, Jean-Claude B, Panasci L**

   Src/c-Abl-directed molecular re-engineering of chlorambucil and bendamustine offer new therapeutic opportunities for CLL treatment

2. **Beiggi A, Johnston JB, Seftel MD, Pitz MW, Banerji V, Griffith EJ, Gibson SB.**

   Second malignancies in patients with chronic lymphocytic leukemia: A Canadian population based study

3. **Brown, M, Beiggi S, Banerji V, Gibson SB, Johnston JB**

   Mutational status and VH gene family usage in chronic lymphocytic leukemia (CLL) patients at CancerCare Manitoba CLL clinic

4. **Gehrke I, Bouchard E, Poepple A, Johnston J, Gibson SB, Banerji V**

   Highly efficient apoptosis-mediated killing of leukemic cells from fludarabine-resistant, relapsed and del17p high-risk CLL patients through inhibition of NAMPT-mediated NAD-generation


   Comprehensive assessment of clinical outcomes for CLL patients with trisomy 12 (+12): Results of a population-based analysis of 822 CLL patients in British Columbia (BC), Canada


   Genetic epidemiology of chronic lymphocytic leukemia (CLL)

7. **Huang, SJT, Gillan T, Gerrie AS, Toze CL, Bruyere**

   Influence of clone and deletion size on CLL outcome in BC patients with an isolated deletion 13q

Re-purposing an antiviral for the treatment of chronic lymphocytic leukemia

9. **Quest GR, Larratt LM** ................................................................. 12

Clinical and laboratory epidemiology of hairy cell leukemia in Northern Alberta

10. **Streu E** ...................................................................................... 13

Cases of second malignancy from the CLL clinic at CancerCare Manitoba

11. **Yang L, Bieggi S, Zhang Y, Gibson SB, Johnston JB** ................. 14

Shortened telomere length is associated with poor prognostic features in chronic lymphocytic leukemia

12. **Zhang Y, Ishdorj G, Mai S, Banerji V, Gibson SB, Johnston JB** ........ 15

Correlation between telomere shortening in CLL with abnormalities in the shelterin proteins and DNA oxidation

13. **Zhu F, Spaner D, Gorczynski R** .................................................. 16

A tumor cell based vaccine against CLL
Src/c-Abl-directed molecular re-engineering of chlorambucil and bendamustine offer new therapeutic opportunities for CLL treatment

Lilian Amrein, Anne-Laure Larroque, Lisa Peyrard, Daniel Borrelli, Bertrand Jean-Claude and Lawrence Panasci

B-cell chronic lymphocytic leukemia (CLL) is characterized by actively dividing B-lymphocytes in the lymph nodes and bone marrow, associated with the accumulation of quiescent lymphocytes in the peripheral blood of affected patients. Current treatments for this disease include chemotherapeutic (chlorambucil (CLB), cyclophosphamide, fludarabine) and immunotherapeutic agents (Rituximab, Alemtuzumab) or the combination of immunotherapy with chemotherapeutics drugs. In 2008, the FDA approved bendamustine, a hybrid agent with a nitrogen mustard moiety and a purine analog, for the treatment of patient with CLL. During treatment, the enzyme-mediated repair of DNA damage can induce resistance to chemotherapeutic drugs. We have previously shown that small molecule inhibitors of c-abl (a key protein of the homologous recombinational repair pathway) such as imatinib, nilotinib or dasatinib, sensitizes primary CLL lymphocytes to CLB. We also demonstrated that ZRF4, combi-molecule designed to target c-abl (through an imatinib moiety) and to induce DNA damage (through a nitrogen mustard moiety), has a more potent cytotoxic effect than the combination of CLB plus imatinib in CLL lymphocytes. Here we report the anticancer effect of three different combi-molecules composed of a chlorambucil moiety and a dasatinib (a dual Src/c-abl inhibitor) moiety compare to the individual components in primary CLL lymphocytes in-vitro. Using the MTT assays on CLL lymphocytes from 40 CLL patients, we found that the IC_{50} (concentration which kill 50% of the cells) of AL748, AL758 and AL816 are significantly lower than the IC_{50} of CLB when used alone and the combination of CLB with 0.1 μM dasatinib (median value = 1.7 μM, 0.6 μM, 0.9 μM, and 11 μM and 3.1 μM respectively). Moreover, our results suggest that the mechanism of action of AL758, AL816, CLB and CLB plus dasatinib share common downstream targets including inhibition of Src kinase, induction of DNA damage and apoptosis. Using western blot analysis of a small number of samples, the drugs induced similar changes in early marker of DNA damage (γH2AX, p53 and 21) and effectors of apoptosis (AnnexinV and cleaved caspase-3). We also determined, by MTT assay, the cytotoxic effect of a combi-molecule composed of a bendamustine moiety and a dasatinib moiety compare to the individual components in-vitro in primary B-lymphocytes from 20 CLL patients. Our results demonstrated that this combimolecule has a better anticancer activity than bendamustine in combination with 0.1 μM dasatinib (p<0.0005). Dose-limiting toxic effects and drugs pharmacokinetic are important and limiting factors to take into account for the development of chemotherapy and will be tested in a CLL mice model. Our results suggest that the combi-molecules AL758, AL816, and AL887 may be alternative for treatment of CLL patients.
Second malignancies in patients with chronic lymphocytic leukemia: A Canadian population based study

Sara Beiggi, MSc, James B. Johnston, Matthew D. Seftel, Marshall W. Pitz, Versha Banerji, E. Jane Griffith, Spencer B. Gibson.

Background
Chronic Lymphocytic Leukemia (CLL) patients are reported to have an increased risk of other malignancies. However, it is unclear whether the increased risk of second malignancies is due to genetic or environmental factors that predispose patients to second cancers, immunosuppression associated with CLL, surveillance bias due to close lifetime monitoring, or a consequence of therapy.

Methods
All immunophenotypically confirmed CLL and FL diagnoses in Manitoba between January 1998 and December 2003 were obtained from the Manitoba Cancer Risk of Registry and followed to end of 2009. The relative risk of second cancers and its association with treatment was estimated.

Results
The incidence of second malignancies was higher in CLL patients than in FL patients, another indolent B-cell malignancy (Tables 1 and 2). Non-melanoma skin cancer (NMSC) was the most common type of second cancers (37%) followed by cancers of digestive organs (16%), prostate (12%), breast (10%) and lung (9%). Second cancers in FL and the general population followed the same general pattern.

Untreated CLL patients had an increased incidence of second malignancies compared to untreated FL patients. Treated CLL patients had a further increased risk of second cancers compared to untreated CLL patients. Neither CLL nor FL patients had an increased probability of preceding cancers.

Conclusions
We have observed that the risk of second cancers in CLL patients is not only greater than the general population, but is also greater than a closely related lymphoproliferative disorder, FL. This increased risk is independent of treatment or surveillance bias. Of the second malignancies, skin cancers were the most common type. Heightened awareness about risks of second cancers should be communicated to improve the care and outcome of CLL patients.

Reference
This work is published in the British Journal of Cancer
Mutational status and $V_{H}$ gene family usage in chronic lymphocytic leukemia (CLL) patients at CancerCare Manitoba CLL clinic

Michelle Brown, Sara Beiggi, Versha Banerji, Spencer B. Gibson, James B. Johnston

Background
In this study we evaluated IgV$_{H}$ gene usage in patients attending the CancerCare Manitoba (CCMB) CLL clinic in Winnipeg. Similar studies are underway to study CLL patients attending the All India Institute of Medical Sciences (AIIMS) in New Delhi, India, to determine if there are racial differences in CLL IgV$_{H}$.

The correlation between IgV$_{H}$ gene mutational status and other prognostic factors was also assessed. Furthermore, we looked at the effects of IgV$_{H}$ gene status on treatment-free survival (TFS) of the CLL patients.

Methods
IgV$_{H}$ gene analysis was carried out by converting RNA to cDNA, as described by Jelinek et al (JCI, 118:306, 2008). $5 \times 10^6$ – $1 \times 10^8$ cells were isolated from leftover blood in CBC tubes (EDTA); cells were obtained from as little as 2 mL of blood.

Association was investigated using $\chi^2$ test. TFS was analyzed using Kaplan-Meier methods and Cox proportional hazard and competing risk regression models.

Results
Of 930 patients seen at the CLL clinic, 821 (88%) consented for tumor banking. Currently, we have IgV$_{H}$ mutational status in 649 (94%) samples.

Distribution of gene family usage (Figure 1 and 2) was not random among mutated and unmutated patients. Unmutated IgV$_{H}$ status was associated with shorter time to first treatment (TTFT) (Figure 3 and Table 1). In addition, Zap70 expression was not randomly distributed among mutated and unmutated patients (p<0.0001) (Table 2). TFS of unmutated patients is reduced, regardless of Zap70 status (Figure 4).

In a multivariable model, after adjusting for age, Zap70 expression, Lymphocytic Doubling Time (LDT) and Rai stage, mutational status was still significantly associated with shorter TTFT (Table 3). Furthermore, IgV$_{H}$ mutational status appears to be a powerful predictor of TFS in low-stage CLL patients (Figure 5 and Table 4).
Highly efficient apoptosis-mediated killing of leukemic cells from fludarabine-resistant, relapsed and del17p high-risk CLL patients through inhibition of NAMPT-mediated NAD-generation

Iris Gehrke, Eric Bouchard, Armando Poepll, James Johnston, Spencer B Gibson, Versha Banerji

Chronic lymphocytic leukemia (CLL) remains without curative therapy. New therapeutic options are especially warranted for rapidly progressing and treatment-refractory patients, particularly characterized by del17p. Dependent on external stimuli, CLL-cells can either be quiescent or active. Active CLL-cells feature increased metabolic rates and are suggested to be critical for disease aggressiveness, drug-resistance and relapse. Consequently, metabolism-associated processes may present rational therapeutic targets for CLL. Nicotinamide phosphoryltransferase NAMPT is the rate-limiting enzyme for nicotinamide-salvaging to nicotinamide adenine dinucleotide (NAD), thereby crucially involved in cellular energy generation. Further, NAD has been described to serve as coenzyme for several cancer-relevant proteins. NAMPT is up-regulated in lymph node–associated activated CLL-cell populations demonstrated by GEP. FK866 is a selective inhibitor of NAMPT with promising activity and good tolerance in several cancers demonstrated in preclinical studies.

We hypothesize NAMPT-inhibition may be a promising strategy for treatment of patients, especially patients with advanced, aggressive and treatment-refractory/relapsed disease.

FK866 treatment lead to concentration- and time-dependent decrease in intracellular energy-content based on NAD- and ATP. CLL-cells over-expressed NAMPT and showed superior response to FK866 than healthy peripheral blood mononuclear cells (PBMCs) assessed by AnnexinV/PI-status with lethal dose 50 (LD50) of 21.5+/-3.8nM for CLL-cells and 307.1+/-89.3nM (n=4) for healthy PBMCs. FK866 induced apoptosis indicated by externalization of phosphatidylserine, caspase 3- and PARP-cleavage, downregulation of the anti-apoptotic proteins Mcl1 and XIAP, reduction of the mitochondrial membrane potential (MMP), increased generation of reactive oxygen species (ROS) and inhibition of Akt- and Erk-survival-signaling. FK866 was effective independent of age, gender and prognostic markers, ZAP70, LDT, beta-2-microglobulin and Rai-stage. Patients with positive CD38 status were significantly less sensitive to FK866 compared to patients negative for CD38. As CD38 is a NADase itself, it can be hypothesized that these cells are adapted to reduced NAD-content making them less susceptible to FK866. FK866 was effective in CLL-cells from patients with in vitro Fludarabine-resistance (n=5, LD50:30.7+/-5.4nM), del17p (n=4, LD50:23.5+/-7.4nM) and treatment-refractory/relapsed patients (n=5, LD50:38.7+/-2.1nM). CLL-cells from some in vitro Fludarabine-resistant patients could be sensitized to Fludarabine by FK866-pretreatment (10nM). Both, inhibition of the major consumer of NAD, poly-ADP ribose polymerase (PARP) as well as exogenous NAD resulted in increased intracellular NAD-levels, which was sufficient to rescue FK866-induced decrease in cellular energy-content (ATP and NAD), viability reduction, caspase 3 cleavage and down-regulation of anti-apoptotic proteins.

We conclude, NAMPT-inhibition through FK866 may be a promising strategy for treatment of CLL-patients, especially high-risk and relapsed CLL-patients and/or to complement treatment with standard CLL-drugs.
Comprehensive assessment of clinical outcomes for CLL patients with trisomy 12 (+12): Results of a population-based analysis of 822 CLL patients in British Columbia (BC), Canada.

Alina S. Gerrie, MD, FRCPC1, Steven J.T. Huang, BMLSc2; Helene Bruyere, MD, FCCMG3, Chimnay Dalal, MD4, Monica Hrynchak, MD, FRCPC, FCCMG3, Aly Karsan, MD, FRCPC4, Adam C. Smith, MSc, PhD, FCCMG, FACMG4, Khaled Ramadan, MD, FRCPC5, Tanya L. Gillan, PhD, FCCMG2, and Cynthia L. Toze, MD, FRCPC1

1Hematology, Leukemia/BMT Program of BC, University of British Columbia, Vancouver, BC, Canada; 2Pathology and Laboratory Medicine, Vancouver General Hospital, University of British Columbia, Vancouver, BC; 3Cytogenetics Laboratory, Royal Columbian Hospital, New Westminster, BC, Canada; 4Cancer Genetics Laboratory, Pathology and Laboratory Medicine, British Columbia Cancer Agency, University of British Columbia, Vancouver, BC, Canada; 5Division of Hematology, St. Paul’s Hospital, University of British Columbia, Vancouver, BC

Background: Important advances in the understanding of CLL pathogenesis include the discovery that NOTCH1 mutations are present in ~28% of pts harboring +12. There is a need for improved understanding of the clinical outcomes of CLL patients (pts) with +12 on a population-level, as this subgroup is rapidly becoming the focus of biologic studies evaluating pathogenesis of disease and clinical trials investigating novel targeted therapies. In the province of BC, population 4.5 million, CLL pts receive uniform evaluation and therapy based on centrally derived protocols with FISH testing implemented since 2004. We sought to characterize the clinical outcomes of +12 in this large unselected population-based cohort of CLL pts.

Methods: Clinical and laboratory data on all pts referred for CLL FISH testing at 1 of 3 BC cytogenetic labs from 2004-2011 were entered into the BC Provincial CLL Database and included in this analysis. Pts without a confirmed diagnostic date were excluded. Baseline features of pts with and without +12 were compared using Fisher’s exact test for categorical and Wilcoxon rank sum test for continuous (cnts) variables. Primary and secondary endpoints were OS and TFS (defined as time from diagnosis [dx] to first therapy). Percent of abnormal (%abn) nuclei harboring +12 was evaluated for association with OS/TFS. Cox proportional hazard (PH) models were constructed to determine predictors of OS/TFS for the +12 cohort, including age at dx, sex, Rai stage (0, 1-2, 3-4), WBC at dx, CD38 positivity and concomitant 17p-, 11q- or deletion 13q (13q-). Cox PH models were also constructed to determine effect of +12 on TFS/OS for the entire cohort.

Results: As of Dec. 2011, 882 pts had CLL FISH testing in BC of which 164 (19%) had +12 on their 1st FISH test: 8 (5%) with concomitant 17p-; 14 (9%) with 11q-; 142 (86%) without either 17p- or 11q-, of which 43 (30%) had 13q-; 16/124 tested (13%) had an IGH translocation [t(IGH)]. Of the 164 +12 pts, median age at dx was 60 yrs (range 35-93), 70% were male, 10% had Rai stage 3-4. At median follow-up of 4.5 yrs (range 0-19), 95 pts (59%) received treatment, 31 (19%) died. For the +12 cohort, median OS was 14.7 yrs (95% CI 9.8-19.0) and median TFS 3.7 yrs (95% CI 2.7-5.4).
Of the 658 non +12 CLL pts (N12CPs), prevalence of recurrent cytogenetic abnormalities (RCA) were: 17p-, 10%; 11q-, 11%; 13q-, 60%; t(IGH) 7%. Significant differences between +12 and N12CPs included more CD38+ pts (66% vs 28%, P<0.001), higher t(IGH) incidence (13% vs 7%, P=0.04) and fewer 17p- (5% vs 10%, P=0.03) or 13q- (26% vs 60%, P<0.001) abn among +12 pts. When pts were grouped by hierarchical FISH abn, +12 pts retained an intermediate OS (median 15.9 yrs) and TFS (median 4.2 yrs) when compared to other RCAs (Fig 1A). Multivariate analysis (MVA) for the whole cohort (n=822) demonstrated no significant effect of +12 on OS (HR 0.72, 95% CI 0.36-1.43, P=.35) or TFS (HR 0.86, 95% CI 0.69-1.36, P=.86) after adjustment for covariates.

For the +12 cohort (n=162), univariate analysis demonstrated shorter OS associated with age (P=.001), Rai stage (P=.01) and 17p- (P=.07). A longer OS was associated with presence of 13q- (median OS 11.6 vs 18.7 yrs, P=.04), Fig 1B. Shorter TFS was associated with Rai stage (P<.001), WBC at dx (P=.01) and 17p- (P=.04). %abn nuclei harboring +12 was not predictive of OS (P=.33) or TFS (P=.25) as a cnts variable; however those with <20% vs ≥20% abn had a significant improvement in OS (P=.02). MVA for the +12 cohort demonstrated Rai stage (HR 3.26, 95% CI 1.23-8.63, P=.02) and 11q- (HR 9.07, 95% CI 1.44-57.02, P=.02) as independent risk factors for OS, while 13q- did not retain its protective effect (P=.98). For TFS, MVA found Rai stage (HR 2.92, 95% CI 1.78-4.78, P<.001) and 17p- (HR 5.44, 95% CI 1.52-19.43, P=.01) as negative predictors while 13q- (HR 2.01, 95% CI 1.08-3.75, P=.03) again had a positive effect.

**Conclusion:** We report the largest, population-based cohort of CLL pts with FISH testing and confirm that +12 occurs in 19% of CLL pts and in the absence of 17p- or 11q-, confers an intermediate prognosis. The presence of 13q- had a protective effect on TFS and a trend towards improved OS, thus improving the prognosis of a subset of +12 pts. This finding is consistent with recent observations that NOTCH1 mutations and 13q- are mutually exclusive in +12 pts and may explain the clinical heterogeneity seen in this subgroup. Further research into these distinct subsets of +12 pts is warranted.
Molecular epidemiology is an important tool to further understand populations. CLL patients are an ideal population to study as new diagnoses are catalogued locally by flow cytometry and clinical features. There is a strong suggestion that in CLL; genetics contributes to the etiology with an 8 fold risk of CLL in first degree relatives. The Manitoba Blood and Marrow disorder Bank is collaborating with the Mayo Clinic who have developed a study to investigate this genetic basis through the use of biological materials from high risk CLL families. This study aims to understand the role of genetics in susceptibility and describe the interrelation of genetics and environment in this disease. It also has the potential to identify precursor states of malignancy in relatives of CLL patients through the use of flow cytometry. In the second year of our participation in this study we obtained funding which allowed us to have dedicated project staff as well as further develop our infrastructure. This allowed us to increase our family participation from 3 to 18 families.
Influence of clone and deletion size on CLL outcome in BC patients with an isolated deletion 13q

Steven J.T. Huang1, Tanya L. Gillan2, Alina S. Gerrie1, Cynthia L. Toze1, Helene Bruyere2

1Hematology, Leukemia/BMT Program of BC, University of British Columbia, Vancouver, BC; 2Pathology and Laboratory Medicine, Vancouver General Hospital, University of British Columbia, Vancouver, BC

Background: Cytogenetic abnormalities are one of the factors influencing the variable clinical course of chronic lymphocytic leukemia (CLL). Although it is well described that an isolated deletion 13q represents a factor of good prognosis when detected by fluorescence in situ hybridization, there is still outcome heterogeneity within this subgroup. We sought to investigate whether the load of the malignant clone with isolated deletion 13q and the size of the deletion influence patient outcome, as previous studies have reported conflicting results.

Methods: We reviewed the BC CLL database to identify patients with isolated deletion 13q and to record the percentage of abnormal cells. We performed fluorescence in situ hybridization with an RB1 probe located at 13q14.2 on 114 patients to investigate for the presence or absence of this locus. Kaplan-Meier analyses and log-rank tests were performed to estimate and compare treatment free and overall survivals of the different groups.

Results: 456/815 (56%) patients had a deletion 13q (del 13q) identified with a D13S319-D13S25 probe. 292 (36%) patients had an isolated deletion, 217 monoallelic, 51 mixed mono- and biallelic and 24 biallelic only. A longer treatment free survival (TFS) for patients with less than 60% of nuclei with a del 13q was observed compared to patients with 60% or more irrespective of deletion size (median TFS not reached vs 10.3 years, p=0.013). There was no difference in overall survival. 56/114 (49%) patients showed a deletion encompassing the RB1 locus. The presence or absence of a RB1 deletion did not influence the outcome when evaluating deletion size alone. However, when deletion and clone size were compared together, patients with no RB1 deletion and a smaller clone size (<80% del 13q) had a significantly longer TFS than patients with no RB1 deletion but with a larger clone size (≥80% del 13q) and patients with a RB1 deletion irrespective of clone size (p=0.004); overall survival was not significantly different. There was no difference when a 60% or 70% cutoff was used.

Conclusion: In our BC cohort, patients with an isolated del 13q require treatment sooner if they have high disease burden (≥60%) but there is no difference in overall survival. RB1 deletion was detected in 49% of cases with isolated del 13q and the absence of a RB1 deletion is associated with a longer TFS if the clone size of deletion 13q is less than 80%.
Re-purposing an antiviral for the treatment of chronic lymphocytic leukemia.

Veronica Martinez-Marignac, May Shawi, Edgar Pinedo-Carpio, Lawrence Panasci, Wilson Miller, Filippa Pettersson and Raquel Aloyz*

* Lady Davis Institute for Medical Research & Department of Oncology, McGill University, Montreal, Quebec, Canada

Ribavirin is a well-characterized antiviral small molecule that has been shown to target the eukaryotic translation initiation factor 4E (eIF4E) in a variety of systems, down-regulating eIF4E targets, affecting eIF4E subcellular localization, decreasing AKT phosphorylation and hinder the pro-survival signaling in human cancer cells and in patients with acute myeloid leukemia (AML). It is also known that eIF4E is over-expressed in many cancers, including hematological malignancies, and has been reported to play important roles in development, progression, as well as chemoresistance of cancer. We report for the first time that eIF4E is highly expressed and variably phosphorylated in primary chronic lymphocytic leukemia (CLL) lymphocytes. Furthermore, in these leukemic cells, two inhibitors of eIF4E functions, Ribavirin and the MNK inhibitor CGP57380, decreased drug resistance ex vivo. Ribavirin sensitization to Fludarabine (FLU) preferentially occurred in samples with unmutated IgVH and high TCL-1 expression, both associated with bad prognosis in CLL patients. CGP5738 sensitized only unmutated CLL lymphocytes, which we showed express higher basal levels of phosphorylated eIF4E. We found that Ribavirin can abrogate FLU-induced changes to eIF4E cellular localization and reduce homeostatic pro-survival signals induced by FLU. Importantly, when CLL lymphocytes are co-cultured with stromal cells, which stimulate pro-survival signals, eIF4E targeting can decrease these micro-environmental clues and cause cell death. Biologically, our results highlight the contribution of eIF4E to the survival of quiescent primary human malignant cells.
Clinical and laboratory epidemiology of hairy cell leukemia in Northern Alberta

Graeme R. Quest, Loree M. Larratt

Background:
Hairy cell leukemia (HCL) is a chronic lymphoproliferative disorder characterized by pancytopenia, splenomegaly, marrow fibrosis and a distinctive immunophenotype. Advances in the diagnosis of HCL have utilized molecular diagnostics, flow cytometry, and immunophenotyping, allowing for increasingly specific diagnosis and differentiation from its mimics. The presenting clinical and laboratory features of HCL have not been evaluated in nearly 20 years, and has never been thoroughly examined in the Canadian population.

Objectives: To characterize the clinical and laboratory epidemiology of HCL in Northern Alberta, and identify variances from previously published studies.

Methods: A retrospective audit of all bone marrow biopsy and flow cytometry, reports from January 1, 1992 to August 1, 2013 was performed using the CoPath database. All cases which included “Hairy Cell Leukemia” in the diagnosis or case comments were reviewed, with available data extracted and correlated with other clinical and laboratory features by chart review.

Results: The database search identified 115 cases of HCL, with 100 cases at initial diagnosis. This data set corroborates many of the classical features of HCL, including gender bias, age of presentation, and presentation with pancytopenia. Interestingly, monocytopenia was absent in 30% of cases, while circulating leukemic cells were not morphologically apparent in approximately 18% of cases. Splenomegaly was somewhat underrepresented, seen in 76% of cases, with hepatomegaly and lymphadenopathy seen in only 10% of cases each. While the classical immunophenotype (CD20, CD11c, CD25, CD103) is seen in nearly 100% of the cases tested, a surprising number of diagnoses were made without assessment of these markers (~25% of cases). Aberrant coexpression of CD5 or CD10 was seen in 7% and 8% of cases, respectively. Prognosis of patients diagnosed with HCL appears generally excellent, with only two deaths directly related to the disease.

Conclusions: The clinical and laboratory features of HCL in Northern Alberta appear largely consistent with previously published presenting features, though some discrepancies are seen. It is unclear if this is a result of changes in clinical practice, diagnostic misclassification, or simply bias from the sample set obtained. Further investigation of past cases with modern diagnostic markers may help elucidate the root cause.
Cases of second malignancy from the CLL clinic at CancerCare Manitoba

Erin Streu RN MN CON(C), Clinical Nurse Specialist, Lymphoma Disease Site, CCMB

The literature estimates a 2-3 fold overall increase risk for patients with chronic lymphocytic lymphoma (CLL) to be diagnosed with a second cancer. The prevalence of skin cancer in the CLL patient population is also significantly higher compared with an age-matched population. The CLL clinic at CancerCare Manitoba actively follows over 600 patients from across the province providing the most current treatments and education to patients and family members. Due to the increased risk of second cancers, the scope and breadth of practice in the clinic has evolved over the past year. Health histories and review of systems are now more focused and detailed; routine screening practices for malignancies are encouraged, and vague or uncertain symptoms are investigated more closely.

This poster will highlight some interesting cases of second malignancies from our clinic at CancerCare Manitoba including cases of lung cancer in non-smokers, melanoma, squamous and basal cell carcinomas of the head and neck and prostate cancer. Because early assessment and detection of cancers may impact patient outcomes and improve quality of life, specific nursing interventions and patient education strategies aimed at risk reduction and health promotion have also been incorporated into clinic. Specific nursing-based health promotion and risk reduction strategies will also be provided in this presentation.
Shortened telomere length is associated with poor prognostic features in chronic lymphocytic leukemia

Yang L¹,², Bieggi S¹, Zhang Y¹, Gibson SB¹, and Johnston JB¹,²

¹Manitoba Institute of Cell Biology, Winnipeg, MB, Canada
²Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada

Introduction: Chronic lymphocytic leukemia (CLL) is a common and incurable B cell malignancy. Its variable clinical course has led to the utility of several prognostic markers to risk stratify those whose disease will progress and/or respond to treatment. An emerging area of interest are telomeres; repetitive sequences found at the end of chromosomes that protects against genomic instability. In CLL, patients who have short telomeres tend to have poorer clinical features and worse overall survival. The use of telomere length, however, as an independent predictor of disease progression has not been well elucidated.

Objectives: (1) To correlate telomere length with known prognostic factors and (2) to characterize telomere length with disease progression and/or treatment response.

Methods: The CancerCare Manitoba CLL tumor bank contains about 200 newly diagnosed CLL patients; one third have required chemotherapy and half have subsequently died. Genomic DNA was extracted from 48 of these cryo preserved patient samples collected at the time of diagnosis. Telomere length was established using telomere specific primers in multiplex quantitative real-time PCR. t/s value was calculated against the human beta-globulin gene. Statistical analysis was performed using Statistical Analysis Software (SAS).

Results: Using a median telomere t/s level of 0.87, short telomere length is significantly associated with unmutated IVGH status (p<0.0001), CD38 positivity (p=0.0328) and LDT of less than 12 months (p=0.0031). Although sample size remains small, there was a statistically significant correlation between short telomere length and secondary malignancy (p=0.0790). There was a trend in patients with shorter telomeres to have higher Rai staging and an earlier time to treatment, although this did not reach statistical significance likely reflecting the low sample number. Using multi-variable log regression adjusting for IVGH mutational status, the only significant parameters was LDT with OR=6.05 CI 95%(1.09-33.6).

Conclusions: Our results conforms to published data that short telomeres are significantly associated with IVGH mutation, CD38 positivity and LDT of less than 12 months in CLL patients. Significantly, CLL patients with secondary malignancies are also more likely to have short telomere lengths which may represent the genomic instability in such patients. Further studies of the CLL patient cohort, prior and during disease course, will provide insight into the role of telomere length as an independent prognostic feature of CLL.
Correlation between telomere shortening in CLL with abnormalities in the shelterin proteins and DNA oxidation

Zhang Y, Ishdorj G, Mai S, Banerji V, Gibson SB and Johnston JB

Telomeres are important for maintaining the integrity of chromosomes. The shelterin proteins (TRF1, TRF2, RAP1, POT1, TIPP1 and TIN2) are involved in the maintenance and synthesis of telomeres. TIN2 is particularly important as it links TRF1, TRF2 and TPP1. In addition, it plays a role in mitochondrial respiration. In chronic lymphocytic leukemia (CLL), the telomeres are shorter than in normal B cells, with very short telomeres being associated with poor survival. The mechanism for this telomere shortening has been evaluated in the present study. We demonstrated in 150 CLL patients using a Q-RT-PCR assay that the median telomere length was similar in mutated IgVH (Mu-IgVH) cases as in normal B cells, but was significantly shorter in unmutated (Un-IgVH) cases. However, there was considerable variation and overlap in telomere length between the groups. In addition, telomere length was found to shorten in both IgVH mutated and unmutated groups when patients were sequentially followed over years indicating that telomere shortening was an ongoing process. When lymphocytes were stimulated and the telomeres evaluated in metaphase by Q-FISH, telomere length shortening affected all chromosomes equally. Compared to normal B cells, there was increased lipid peroxidation and hydroxyguanosine formation in CLL cells, particularly unmutated cells. Protein levels of the shelterins were increased in the CLL cells, except for TIN2 which was primarily present as a 35 KD form rather than the native 40 KD form. TIN2 mRNA was present as the full length (6 exon) form and a spliced (missing exon 2) form in normal B cells, but primarily present as the spliced form in CLL cells. TRF2 protein was mainly localized to the nucleus in normal B cells but to the cytoplasm in CLL cells, possibly because exon 2 is required for the binding of TRF2 to TIN2.

In summary, these studies suggest that the short telomeres in CLL may be related to increased oxidative stress and abnormalities in the shelterins. TIN2 may play a key role as it is primarily present in the spliced form in CLL. Ongoing transfection studies are evaluating the activity of the spliced form of TIN2, as compared to the full-length form, on telomere and mitochondrial function.
A tumor cell based vaccine against CLL

Fang Zhu, David Spaner, Reginald Gorczynski

Malignant B cells in CLL are antigen-presenting cells (APCs) themselves and are ideal targets of immunotherapy. However, primary CLL B cells have low levels of costimulatory molecules and high levels of immunosuppressive molecules, such as CD200.

In this study, CLL cells were treated with PMA, IL-2 and the TLR-7 agonist Imiquimod (P2I), as well as Ionomycin, all of which represent B cell activators. The phenotype of treated cell was analyzed by flow cytometry and these treated cells were tested in CLL animal models (NOD.SCID.IL-2γ−/− mice) as tumor vaccine.

Costimulatory molecules include CD80 and CD86 were elevated on cells treated with P2I. CD54 and CD83 are markers of activated APC. The expression of both molecules was upregulated on CLL B cells after treatment too. Addition of ionomycin further increased the expression of both molecules as well as CD200. CLL B cells treated with P2I+Ionomycin induced more CLL killing than CLL cells treated with P2I in CTL assay. CD200 blockade improve the immunogenicity of cells treated with P2I+Ionomycin further. In vivo, CLL cells engrafted less well in both PC and SP of vaccinated mice, especially those given cells treated with P2I+Ionomycin and anti-CD200 antibody. CD8+ T cells were increased along with decreased CLL B cell engraftment both in PC and SP.

Our data suggest that Ionomycin increases the immunogenicity of CLL B cells treated with P2I, especially after CD200 blockade. Tumor vaccine with such treated cells can activate CD8+ T cells and diminish CLL engraftment in vivo.