Anti-Malarial Drugs Disrupt Lysosomes in Chronic Lymphocytic Leukemia (CLL) Cells
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Lysosomes are the most acidic vesicles within mammalian cells, and are promising targets in cancer therapy. The disruption of lysosomes leading to cell death was shown to be effective in breast cancer and Acute Myeloid Leukemia (AML), and we were the first to find that this approach was also effective in Chronic Lymphocytic Leukemia (CLL). Our results showed that CLL cells were selectively sensitive to lysosome disruption and cell death, which corresponded to increased levels of the enzyme sphingosine 1-phosphate phosphatase (SPP1) and increased levels of its product sphingosine. The addition of extra sphingosine permeabilized lysosomes and killed CLL cells, but not healthy B cells. To further this study and find a lysosomotropic drug candidate to test clinically, we screened FDA-approved anti-malarial drugs mefloquine, atovaquone, primaquine, and tafenoquine. One of the original anti-malarial drugs chloroquine was shown to be effective in CLL, and mefloquine was shown to be effective in AML. Results show that only mefloquine and tafenoquine could permeabilize lysosomes and kill primary human CLL cells at doses that were clinically-achievable. Tafenoquine was the most effective at the lowest dose, and thus was investigated further. This drug permeabilized lysosomes immediately after addition, and killed CLL cells regardless of previous treatments or poor prognostic factors. Further studies are focused on testing drug combinations and \textit{in vivo} efficacy. Targeting lysosomes may be an effective way to kill CLL cells, even aggressive cells that have failed previous treatment regimens.
**Investigating the Treatment of CLL Using Glycogen Synthase Kinase-3 (GSK-3)**

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Glycogen Synthase Kinase-3 (GSK-3) is an important target in acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL). GSK-3 exists as two highly homologous isoforms: GSK-3α and GSK-3β. As part of the β-catenin destruction complex, GSK-3 phosphorylates β-catenin, which is then degraded via ubiquitination. Pan GSK-3 inhibition, leads to β-catenin stabilization and translocation to the nucleus to activate TCF/LEF-1 and ultimately AML cell survival *in vivo* versus *in vitro*. If either isoform is inhibited, β-catenin is degraded and AML cells differentiate and die. In collaboration, with the Broad Institute we have tested novel GSK-3 inhibitors (PCT/US2013/064716) for isoform selectivity in CLL.

We aim to determine the on target effect of these novel compounds, their impact on cell viability and the mechanism of cell death in CLL.

CLL-cells from CLL patients were treated with 2 pan or 3 isoform selective GSK-3 inhibitors. We observed on target GSK-3α inhibition with GSK-3α-selective inhibitors with no loss in cell viability. Pan-GSK-3 inhibitors lead to loss of viability and induction of apoptosis and DNA damage. They also induced stabilization of β-catenin. The GSK-3β selective inhibitor was not selective for GSK-3β and performed as a pan-GSK-3 inhibitor. Compared to other GSK-3 pan inhibitors the degree of β-catenin stabilization was less. The AML cell line, U937, was used for comparison and was treated the same as the patient cells.

Future direction is to test the impact of β-catenin stabilization in microenvironmental models to determine impact on CLL cell survival and eventually in a murine model.
In Vitro Analysis of NAMPT Inhibition-Based Drug Combinations Yields Promising Therapeutic Strategies for CLL

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Background: Chronic Lymphocytic Leukemia (CLL) is primarily a disease of the elderly. As such, patients often present with significant comorbidities and poor clinical fitness. Since the standard of care remains chemotherapy, it is poorly tolerated by these patients. Therefore, new targets are required to treat them. One promising target is Nicotinamide Phosphoribosyl Transferase (NAMPT), the rate limiting enzyme in Nicotinamide Adenine Dinucleotide (NAD) generation, which is upregulated in CLL. The NAMPT inhibitor FK866 has completed phase II clinical trials, but its use is limited by bone marrow toxicity. As with current CLL treatments, combination therapies may lower required doses, increase the specificity of NAMPT inhibition and lower treatment toxicity.

In this study we evaluated NAMPT inhibition in combination with standard of care chemotherapeutics, novel targeted agents and other metabolic inhibitors. We also validated GMX-1778, a novel, orally administered NAMPT inhibitor.

Methods: CLL cells were isolated from the peripheral blood of consenting donors by density gradient centrifugation. When patients presented with white blood cell counts < 40,000 cells/µL, b-lymphocytes were negatively selected using Rosette Sep antibody cocktail. Cells were treated with several doses of the NAMPT inhibitors, FK866 and GMX-1778, alone or in combination with fludarabine, bendamustine, chlorambucil, ibrutinib, idelalisib, 2-deoxy-d-glucose (2-DG), or FCCP for three days. Then, cell viability was assessed by flow cytometry using annexin V-FITC/PI or Annexin V-FITC/7AAD staining. Finally, drug synergy was determined according to the Loewe additivity model using Combenifit software.

Results: Combination of NAMPT inhibition with either alkylating agents or nucleoside analogs achieved additivity. Moreover, combination with the novel tyrosine kinase inhibitors as well as the glucose analogue achieved mild synergy. However, when NAMPT inhibition was combined with the mitochondrial uncoupler FCCP, significant synergy was observed in the majority of doses evaluated.

Conclusions: FK866 and GMX-1778 produced analogous effects in many of the drug combinations, providing further evidence that the newer agent acts similarly to FK866 in CLL. No antagonism was observed between NAMPT inhibitors and either current
chemotherapeutics or targeted agents. As such, NAMPT inhibition warrants further study as a strategy for lowering required dosage and reducing toxicity in current regiments. The significant synergy observed between NAMPT inhibition and mitochondrial uncoupling is particularly promising and currently under further investigation.
Overexpression of Activation-Induced Deaminase in TCL1 Mice Leads to the Development of IGHV-Mutated and -Unmutated CLL Clones That Resemble Unique Subsets of Human CLL

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Somatic hypermutation (SHM) and class-switch recombination (CSR) are critical physiologic events in an effective normal B-cell immune response, and both are initiated by activation-induced cytidine deaminase (AID). In CLL, IGHV clonal mutations correlate strongly with better clinical outcomes. Eμ-T-cell leukemia-1 (TCL1) transgenic (Tg) mice are a valuable model of CLL. However because SHM and CSR occur rarely in these animals, they mimic only IGHV-unmutated CLL and do not provide an understanding of the roles of SHM and CSR in disease evolution. To address these issues, we developed two new TCL1 strains by interbreeding mice over-expressing AID in all cells (Eμ-TCL1xActin-AID) or only in B lymphocytes (Eμ-TCL1xVκ-AID).

B-cell clonal expansions were identified in spleen cells from 22 TCL1 and 33 TCL1xAID Tg (10 Eμ-TCL1xActin-AID and 23 Eμ-TCL1xVκ-AID) mice at 10-20 months of age by amplifying cDNAs by PCR using consensus FR and IgM, IgG, and IgA primers. DNA sequences were compared to murine germline IGHVs and IGHV-D-J rearrangements by IMGT V-Quest. Because there were no major differences in the parameters listed below for the two TCL1xAID Tg mouse strains, data were combined.

Monoclonal/oligoclonal expansions were detected in all TCL1 mice; these used only μ H chains. Similar expansions were detected in 26 of 33 TCL1xAID mice; each animal bore an IgM+ clone and 7 also an IgG+ clone.

IGHV use did not differ significantly between IgM+ TCL1 and IgM+ TCL1xAID clones. Approximately 50% used Vh1-55, Vh11-2, or Vh12-3, some of which encoded stereotyped anti-phosphatidylcholine antibodies.

IgM+ TCL1 clones exhibited a mutation frequency of 0.05%, which was considerably less than that in TCL1xAID mice (0.47% for IgM+ and 3.0% for IgG+ clones). Mutations localized 10 times more frequently in AID hotspots than coldspots.

However, SHM did not affect all clones equally. Mutation frequency in Vh12-3 and Vh11-2 clones was only 0.38% (range: 0-1.9%), and no mutations were detected in Vh1-55 clones. None of these genes were found in IgG-expressing clones.

Notably, in only 2 of 9 instances was the same IGHV-D-J rearrangement found in IgM+ and IgG+ clones; these used Vh5 genes. For the remaining 7, only the IgG+ version was detected; all but one of these used a Vh1 gene. Also, within the IgG-only group, IGHV1-47 was used by 2 different clones that were highly mutated (8.9%).

Thus, over-expression of AID in TCL1 mice led to markedly increased SHM and CSR. However, SHM was not equivalent for all IGHV's since despite AID over-expression, certain genes appeared resistant to major increases in SHM and CSR. This property resembles some
human CLL *IGHVs* that rarely develop SHMs or undergo CSR despite the B-cell’s ability to synthesize AID (e.g., *IGHV*1-69). AID overexpression also led to IgG⁺ clones for which an IgM precursor was not found. This resembles those human stereotyped CLL clones that are only found as IgGs (e.g., stereotyped subsets 4 and 8). Finally, the two new TCL1xAID mouse strains described provide new models to study *IGHV*-mutated and *IGHV*-unmutated CLL and represent novel tools to evaluate the role of AID in leukemic progression.
Subcutaneous Immunoglobulin replacement in patients with secondary hypogammaglobulinemia: A feasibility study

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ABSTRACT

CLL is the most common type of leukemia in the Western world and represents the most common cause of secondary immunodeficiency which can benefit from immunoglobulin replacement therapy (IgRT). While immunoglobulin replacement therapy is usually administered intravenously in a hospital or outpatient facility, the availability of subcutaneous (SCIg) administration is often very appreciated by patients, for it offers greater flexibility and can lead to improved independence and quality of life.

We conducted a pilot study to assess the feasibility of subcutaneous immunoglobulin replacement (SCIg) in patients with secondary hypogammaglobulinemia due to B lymphocyte-derived malignancies. Five patients were transitioned from intravenous replacement (IVIG) to SCIg. Mean patients’ serum levels of IgG on IVIg were 7.32 g/L while they increased to 11.39 g/L on SCIg. For IVIg, infections occurred almost once a month on average, while they were almost reduced to none after the SCIg switch. Moreover, no adverse effects were manifest after the SCIg change, as opposed to some serious discomfort in the case of 2 patients under the IVIg regime. This, in turn, contributed to an important reduction in the number of hospital visits related to the treatment.

This pilot trial suggests that SCIg may represent a feasible alternative to IVIG for the management of patients with secondary hypogammaglobulinemia
A Canadian Perspective on the Use of Immunoglobulin Therapy to Reduce Infectious Complications in Chronic Lymphocytic Leukemia

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ABSTRACT
Infections are a major cause of morbidity and mortality in patients with chronic lymphocytic leukemia (CLL), who typically have an increased susceptibility because of hypogammaglobulinemia (HGG) related to the disease and its treatment. Immunoglobulin replacement therapy (IgRT) has been shown to reduce the frequency of bacterial infections and associated hospitalizations in patients with HGG and/or a history of infection. However, its use in CLL is contentious. Studies examining IgRT in CLL were largely conducted prior to the use of newer chemo-immunotherapies, which may extend lifespan but do not correct the HGG inherent to the disease; thus, the utility of IgRT needs to be re-evaluated in the present setting. The use of immunoglobulin replacement therapy reduces the frequency of bacterial infections and associated hospitalizations in patients with hypogammaglobulinemia and/or a history of infection. Patients with CLL should therefore be monitored to determine the potential benefit of immunoglobulin replacement therapy in reducing the risk of infection. Here we discuss the evidence for the use of IgRT in CLL and provide a practical approach for its use in the prevention and management of infections.
Keywords: chronic lymphocytic leukemia, hypogammaglobulinemia, immunoglobulin, immunoglobulin replacement therapy, infection, IVIG, SCIG, immunodeficiency
The emerging role of the UGT2B17 metabolic pathway in CLL.

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**Background:** We previously observed mRNA overexpression of *UGT2B17* gene in CLL patients with poor prognosis. UGT2B17 is an enzyme responsible for the metabolic inactivation of numerous drugs and endogenous substrates such as androgens. Our goal is to better understand the role of UGT2B17 in CLL, and as a first objective, to identify CLL molecular pathways associated with UGT2B17 overexpression. We hypothesized that UGT2B17 oncogenic effects are possibly explained by a role in regulating intracellular levels of UGT2B17 substrates that in turn influence hematopoietic malignant pathways.

**Methods:** We developed CLL-derived cell lines, MEC1 overexpressing UGT2B17 (MEC1-2B17) or depleted in UGT2B17 using shRNA (MEC1-KD). Cell proliferation assays were initially conducted whereas RNA sequencing and stable isotope labeling by amino acids in cell culture (SILAC) coupled to mass spectrometry (MS) were performed to identify gene and protein expression pathways modulated by UGT2B17 expression levels.

**Results:** Compared to MEC1-KD, cells overexpressing UGT2B17 displayed a significant proliferative advantage (30% vs. KD, \( p = 0.03 \)). Gene expression analysis followed by an Ingenuity Pathway Analysis (IPA) revealed that high UGT2B17 levels predominantly affects genes involved in regulation of proliferation, intracellular trafficking and immunological pathways. In line with these observations, we used SILAC to identify multiple differentially expressed proteins in MEC1 cell lines critical for hematopoietic development, viral response and cell motility (1.5 fold change, FDR<0.01). Validation of top candidates is currently ongoing and will be discussed. We also plan to address whether these effects are mediated through UGT2B17-dependent glucuronidation, by producing a MEC1 cellular model that expresses a catalytically inactive UGT2B17 enzyme.

**Conclusion:** Data are consistent with altered gene and protein expression associated with UGT2B17 overexpression that likely influences CLL cell behaviour. *Funded by CRS and LLSC.*
IL-17/IL-6 axis is a therapeutic target for Chronic Lymphocytic Leukemia

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The tumor microenvironment (TME) is critical to the longevity of tumor B cells in chronic lymphocytic leukemia (CLL). Bone marrow mesenchymal stem cells (BMMSCs) and the cytokines they produce including IL-6 are important components of the TME in CLL. We found BMMSCs supported the survival of CLL cells in vitro and the IL-6 antagonist Actemra abolished this effect. BMMSCs produce high levels of IL-6 and the direct interaction between BMMSCs and CLL cells induced increased IL-6 production by CLL cells. IL-17 which induces IL-6 generation in a variety of cells induced production of IL-6 both in CLL cells and BMMSCs in vitro. In a xenograft CLL mouse model, BMMSCs and the culture supernatant of BMMSCs increased engraftment of CLL cells through an IL-6 mediated mechanism with human recombinant IL-6 showing similar effects in vivo. Human recombinant IL-17 treatment also increased CLL engraftment in mice through a IL-6 mediated mechanism. Plasma of CLL patients showed elevated levels of both IL-6 and IL-17 by ELISA compared to healthy controls with levels of IL-6 linearly correlated with IL-17 levels. CLL patients receiving ongoing therapy expressed higher levels of IL-6 and IL-17, while CLL patients with the lower levels of IgA/IgM had higher levels of IL-6, but not IL-17. These data imply an important role for the IL-17/IL-6 axis in CLL which could be therapeutic targets.
Identify genetic and non-genetic factors associated with non-Hodgkin lymphoma in a sample from Shanghai, China

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\textbf{Introduction:} Non-Hodgkin lymphoma (NHL) is one of the most common cancers. The risk factors causing NHL are still poorly understood. Previous epidemiologic and genetic studies have identified a few genetic and non-genetic factors associated with incidence risk of NHL. However, the findings have been rarely replicated, particularly in Asian population.

\textbf{Methods:} We undertook a hospital-based case-control study in Shanghai, China to examine the associations between non-genetic factors, single nucleotide polymorphisms (SNPs) in selected immunoregulation genes and NHL risk. One hundred and sixty-nine NHL patients diagnosed according to the World Health Organization 2001 standard and four hundred and twenty-one controls were recruited. Family history of the cancer, body mass index (BMI), smoking, environmental exposures, and nine SNPs in four genes (TNF-\textalpha, IL1RN, IL10, and IL4) were included in the association analysis. Genetic association analysis was performed using the Cochran-Armitage trend test and logistic regression models by adjusting multiple epidemiologic factors.

\textbf{Results:} Three SNPs were significantly associated with increased risk of NHL overall and subtypes, i.e., IL10 rs1800893 (OR=2.64, 95% CI 1.75-3.98, \textit{P-value}=3.54 \times 10^{-6}), IL1RN rs4251961 (OR=2.67, 95% CI 1.72-4.16, \textit{P-value}=1.26 \times 10^{-5}), and TNF-\textalpha rs1800630 (OR=1.80, 95% CI 1.24-2.63, \textit{P-value}=2.15 \times 10^{-3}). Non-genetic factors, including family history, smoking, environmental exposures and BMI, are also significantly associated with NHL risk.

\textbf{Conclusion:} Our analysis found several non-genetic risk factors and genetic variants in immunoregulation genes for NHL risk in a Chinese population.
Reduced Catalase Expression Results in Accumulation of Reactive Oxygen Species in Chronic Lymphocytic Leukemia B Cells Leading to Activation of Axl: An Escape Strategy?

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**Background:** A challenge for novel therapeutic strategies will be the fine tuning of intracellular reactive oxygen species (ROS) signaling to effectively deprive cells from ROS-induced tumor promoting events, towards tipping the balance to ROS-induced apoptotic signaling. ROS plays a critical role in regulation of the pro-survival receptor tyrosine kinase (RTK) signaling pathways in human cancers. Studies have identified mitochondrial metabolism as the key source for abundant ROS in chronic lymphocytic leukemia (CLL). Unlike in other malignant cells, increased oxidative phosphorylation but not increased aerobic glycolysis has been found in CLL B-cells.

**Methods:** CLL B-cells and normal B-cells were purified from peripheral blood of previously untreated CLL patients and normal, healthy individuals, respectively using RosetteSep B-Cell enrichment kit. Purified CLL B-cells were exposed to H$_2$O$_2$ for 5 min and cell lysates were analyzed for activation of RTKs including Axl by immunoprecipitation/Western blots. We also examined expression status of SIRT3, acetylated-dismutase (SOD)2 and catalase in CLL B-cells by Western blot analyses. Catalase mRNA levels in CLL B-/normal B-cells were determined by qRT-PCR. Finally, accumulation of O$_2^-$ and H$_2$O$_2$ in CLL B- and normal B-cells were measured by flow cytometry after treating the cells with DHE and DCFDA, respectively.

**Results:** Here we report that enforced induction of ROS significantly increases tyrosine phosphorylation levels on multiple cellular proteins in CLL B-cells. Further analysis finds that increased ROS activates Axl and its downstream signaling mediators AKT and Erk1/2 MAPK. Interestingly, phosphorylation level on fibroblast growth factor receptor (FGFR) was also enhanced which we recently defined as a downstream target of Axl, but not on cMET or IGFR1, in response to increased ROS in CLL B-cells.

Finally, the histone deacetylase SIRT3 which activates mitochondrial SOD2 via deacetylation, we found, was overexpressed in CLL B-cells as compared to normal B-cells indicating more efficient conversion of O$_2^-$ into H$_2$O$_2$ in the leukemic B-cells (Fig.1). However, expression of catalase which converts H$_2$O$_2$ into O$_2$ and H$_2$O, was reduced significantly in CLL B-cells as compared to normal B-cells both at mRNA and protein levels. Together, these findings suggest that although SOD2 remains highly active, increased accumulation of H$_2$O$_2$ may occur in CLL B-cells due to reduced catalase expression/activity (Fig.1). Indeed, flow cytometric analysis finds lower levels of O$_2^-$ but accumulation of H$_2$O$_2$ in CLL B-cells as compared to normal B-cells.

**Conclusion:** These observations may explain, at least in part, expression of constitutively active Axl/FGFR signaling pathways in CLL B-cells. Thus, increased accumulation of H$_2$O$_2$ in CLL B-cells may account for the prolonged survival and/or apoptotic resistance of the leukemic B-cells.
Title: Src Family Kinase Feline Gardner-Rasheed is Overexpressed in ZAP-70 Positive Chronic Lymphocytic Leukemia

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Abstract: Expression of Zeta-chan Associated Protein 70 Kinase (ZAP-70) is associated with aggressive disease course and poor prognosis in Chronic Lymphocytic Leukemia (CLL). RNA-seq was done comparing mRNA levels between ZAP-70 (+) and (-) primary patient samples. Of the targets identified, only Feline Gardner-Rasheed (FGR), a Src Family Kinase known to be expressed in CLL but not healthy peripheral B-cells, was found to be differentially expressed at the protein level, with 2-fold higher expression in ZAP-70 (+) cells. Its function in CLL is unknown. To investigate this, fluorescence microscopy was used to determine Fgr’s localization in the RAJI cell line and primary patient samples. FGR is cytoplasmic with partial localization to the mitochondria in unstimulated patient samples. ZAP-70 (+) RAJI cells are more susceptible to treatment with Shikonin, an inhibitor of glycolysis, suggesting altered metabolism.
Retrospective Review of Immunoglobulin Levels in a Cohort of Patients Diagnosed with Chronic Lymphocytic Leukemia (CLL) at CancerCare Manitoba

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While hypogammaglobulinemia is a known complication of chronic lymphocytic leukemia (CLL) and its treatments, little is known about the state of immunoglobulins (Ig) at the time of diagnosis, their trajectory of over time, the clinical characteristics correlated with low levels, and the impact hypogammaglobulinemia has on patient outcomes and clinical practice. Beiggi et al. (2014) found that 25% of patients referred to the CLL clinic at CCMB have low IgG levels at the time of referral but our understanding of immunoglobulin trends over time and the clinical impact of hypogammaglobulinemia is lacking. This research project aims to address the following clinic and research questions:

1. What number of patients who have low level Ig levels at the time of diagnosis and what is the trend over a 5 year period of time?

2. What are the clinical characteristics and chemotherapy treatment histories of patients who have low levels of Ig at the time of diagnosis or who develop hypogammaglobulinemia over time?

3. Is the presence of low IgG levels at diagnosis predictive of the need for replacement therapy?

4. What were the clinical characteristics and outcomes of the patients who received immunoglobulin replacement therapy?

5. Is the presence of low immunoglobulin levels predictive of second cancers?

Patients assessed in the CLL clinic at CancerCare Manitoba have Ig levels (IgA, IgG, IgM) measured at the time of referral as part of their baseline assessment which are repeated annually. We are conducting a retrospective chart review of 293 patients who were referred to the CLL clinic between 2007-2011 to better understand this complication of CLL. The data is currently being analyzed and the findings will be presented.
The Evolution of Patient/Caregiver Information Sessions for CLL at CancerCare Manitoba: From Patient Education to Patient Engagement

Donna Hewitt RN, Research Nurse, University of Manitoba and Erin Streu RN MN CON(C), Clinical Nurse Specialist, CancerCare Manitoba

Each year in conjunction with the Patient and Family Resource Centre at CancerCare Manitoba (CCMB) the chronic lymphocytic leukemia (CLL) clinical team offers an information evening for newly diagnosed patients and their family members. What began 10 years ago as a session aimed at better explaining CLL to patients through a basic disease overview and psychosocial presentations has evolved into an information evening focused on engaging patients and family members in their care and encouraging them to become active members of their healthcare team. Presentations now focus sharing the latest research findings and explaining the implications in practical terms relevant to the patient’s health and cancer journey.

Presentations focus on modifiable risks factors for cancer prevention, cancer screening practices, and health promotion to ensure patients are aware of how optimal fitness can impact their disease and potential treatment options. Sessions have always included a research component which allows us to share with our patient research volunteers how their contributions have made a positive impact on CLL care at CCMB.

This poster presentation will highlight the evolution of patient education using a multidisciplinary team approach to host a session that is not only informative in keeping patient’s abreast of the latest research findings but that is also meaningful for patients and their families.
Subcutaneous Immunoglobulin (SCIG) Therapy for CLL at CancerCare Manitoba: Outcomes from the Pilot Program

Erin Streu RNMN CON(C), Clinical Nurse Specialist, CancerCare Manitoba

Patients with hypogammaglobulinemia experience morbidity from recurrent infection and require replacement therapy with donor immunoglobulin. Subcutaneous immunoglobulin (SCIG) is a well-established route of replacement therapy in primary immune deficiency but relatively novel in immune deficiency secondary to malignancy and/or treatment (secondary deficiency). Patients on SCIG experience fewer side effects while attaining similar clinical benefit. SCIG is safe and less resource intensive. The literature demonstrates that SCIG provides patients/caregivers more autonomy, independence, and better quality of life while saving the healthcare system costs related to infusion of intravenous immunoglobulin (IVIG).

In September 2014, CancerCare Manitoba developed a pilot program offering patients with chronic lymphocytic leukemia (CLL) currently receiving, or starting IVIG the opportunity to transition to a home-based, self-administered, subcutaneous immunoglobulin (Ig) replacement therapy. The goal was to transition/start 20 patients on SCIG and collect data on treatment satisfaction, quality of life data and to determine overall savings and benefits to the patient, institution, and government. A baseline analysis predicted a savings of 734 chemotherapy chairs hours and 940 nursing hours (0.5 EFT) if 20 patients could be transitioned out of the chemotherapy treatment area. A total of 35 patients have been registered in the first year exceeding the target goal. This poster presentation will highlight our program and present preliminary data including a cost/benefit analysis.

The pilot program has demonstrated marked benefits including significant labor/resource savings, better regulation and monitoring of Ig use in the CLL population and most importantly a significant improvement in quality of life for patients.
ZAP-70-dependent alteration of PI3Kγ signaling enhances microenvironmental interaction and survival of CLL cells

**Background:** Chronic Lymphocytic Leukemia (CLL) is the most prevalent hematologic malignancy in the Western world. Despite the efforts to understand CLL biology and the available treatments options, CLL remains largely incurable. It is characterized by the accumulation of mature CD5+/CD19+/CD23+ B lymphocytes in the peripheral blood and, to varying degrees, in lymphoid tissues, spleen, and bone marrow. CLL cells rely on chronic activation signals triggered via B-cell receptor (BCR) to potentiate their survival. Furthermore, CLL cells interact with and shape a microenvironment favourable to their survival and proliferation. The importance of the microenvironment is highlighted by the fact that CLL cells grown *in vitro* undergo spontaneous apoptosis unless co-cultured with bone marrow-derived stromal cells or supplemented with T-cell-derived cytokines. CLL cells migrate to favourable niches (lymph nodes and bone marrow) in response to chemotactic soluble factors, such as the chemokine SDF-1, where they interact with resident stromal cells that anchor and provide them with survival and proliferative stimuli through direct cell-cell contact and soluble factors. The protective microenvironment shields CLL cells from the deleterious effects of therapeutics, therefore conferring a resistant phenotype.

CLL is a heterogeneous disease, with several prognostic biomarkers including the expression of the Syk kinase family member zeta-chain T-cell receptor-associated protein kinase 70 kDa (ZAP-70). ZAP-70 plays an important role in T lymphocyte development through transducing signals downstream of TCR. Normal B cells do not express ZAP-70 but rather express Syk, which is critical for BCR signaling. Abnormal expression of ZAP-70 in the context of CLL is correlated with rapid disease progression and significant decrease in patient survival. Moreover, ZAP-70 modulates the expression and of molecules regulating motility and adhesion in CLL cells, potentially through altered signaling downstream of BCR. While interactions with lymphoid tissue microenvironments are critical for CLL survival and disease progression, the underlying molecular mechanisms and relation to disease heterogeneity are not completely understood.

Improper activation of the phosphoinositide-3 kinase (PI3K) survival pathway has been implicated in tumorigenesis in several tissue types. PI3K is a phospholipid kinase which phosphorylates the 3’ hydroxyl group of the inositol ring of phosphoinositide lipids. PI3Ks are subdivided into 3 classes, however, only class 1 PI3Ks have been implicated in oncogenic transformation. The class 1A PI3K p110δ has established functions in normal and malignant B cell signaling, and p110δ inhibitors have recently been shown to be effective in treatment of CLL. Class 1B PI3K p110g has not been extensively studied in B cells, despite their well-established functions in chemokine receptor signaling in other cell types; however, p110γ inhibitors are now in clinical development for B cell malignancies. Class 1B PI3K consists of a catalytic subunit (p110γ) and two regulatory subunits (p84, p101 and its splice variant p55). The mere overexpression of p101 alone
significantly inhibited UV-induced apoptosis in Jurkat cells. Furthermore, p55 overexpression has been shown to promote proliferation, invasion and metastasis, and angiogenesis of colorectal cancer cells. In xenograft experiments using leukemic cells (AML and CML), p55 blockage significantly reduced tumor burden due to decreased cell proliferation. In the same study, it has been demonstrated that p55 expression, both mRNA and protein, is elevated in leukemic cells isolated from patients. Together current data suggesting levels of class 1B PI3K adaptor subunits may be a critical factor controlling p110γ activity and cell invasiveness.

**Hypothesis:** ZAP-70 dependent alteration in expression and membrane targeting of class 1B adaptor subunits p101, p84 and p55 enhances p110γ activity, CLL survival and disease progression by modulating interactions with lymphoid tissue stromal cells.

**Preliminary results:** We performed RNAseq analysis to search for molecules differentially expressed in ZAP-70-positive CLL that might account for enhanced motility and adhesion of these cells. Expression of the class 1B PI3K adaptor subunit p101 was found to be significantly increased in ZAP-70-positive CLL relative to ZAP-70 negative. Moreover, I have found that p101 protein expression is increased upon BCR stimulation and shows a distinct membrane localization pattern in ZAP-70+ CLL.
Bendamustine is an alkylating agent that synergizes with nucleoside analogues in chronic lymphocytic leukemia.

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Bendamustine (BEN) is a promising new treatment for chronic lymphocytic leukemia (CLL) and is believed to function as an alkylating agent (eg, chlorambucil, CLB) while sharing structural similarities to a nucleoside analogue (eg, fludarabine, FLU). Enhanced cell death has been observed with the combination of BEN and FLU in primary CLL cells in vitro, likely due to the inhibition of repair of BEN DNA cross-linking by FLU. However, both BEN and FLU are marrow-toxic, limiting the use of this combination in the clinic. The nucleoside analog, pentostatin (PEN), is an adenosine deaminase inhibitor that causes the accumulation of deoxyadenosine (dADO) and is less marrow-suppressive than FLU. However, the cytotoxic effectiveness of dADO/PEN in combination with BEN is not known.

Flow cytometry analysis using annexin V/7-ADD and the MTT assay were performed to determine the effectiveness of BEN treatment in primary CLL cells as compared to or in combination with CLB, FLU, or dADO/PEN at varying drug concentrations and time points. In vitro treatment of CLL cells with BEN, CLB, FLU, or dADO/PEN induced apoptosis, the degree being time- and concentration-dependent. However, the sensitivity of CLL cells to BEN or CLB varied, suggesting different mechanisms of action. Enhanced cell kill was seen with the combination of BEN or CLB with FLU or dADO/PEN, with the extent of increased cytotoxicity being similar for FLU or dADO/PEN. Cell death mechanisms were analyzed via staining for surface expression of death receptors (DR4 and DR5) and mitochondrial stress (DICO6 and DHE). γ-H2AX staining was used to measure DNA double-strand breaks (DSBs) and the alkaline comet assay was used to measure both DNA DSBs and single-strand breaks (SSBs). An increase in DR5, but not DR4 surface expression, loss of mitochondrial membrane potential, and increased reactive oxygen species production was observed following BEN, CLB, and FLU treatment. There was an increase in DNA DSB following FLU, as compared to BEN and CLB. Thus, BEN has the properties of an alkylating agent, rather than a nucleoside analog, but has a different spectrum of antitumor activity to CLB. BEN induces apoptosis through both the death receptor and mitochondrial pathways. Increased cell kill is seen on combining BEN with dADO/PEN suggesting that this might be a useful combination in the clinic.
Abstract;

Chronic Lymphocytic Leukaemia (CLL) is a cancer of the B-lymphocytes that primarily affects people over the age of 60. Throughout our evolutionary history, the immune system has become a complex system to battle invading pathogens and foreign molecules. One of the cornerstones of the immune system is the generation of antibodies, which also act as receptors for the B-lymphocytes. Through various mechanisms, each human being is capable of producing approximately $10^{12}$ different antibodies, which means it is almost improbable that two antibodies would have identical sequences, yet almost 30% of CLL patients can be classed into stereotypic groups. These stereotypic groups of patients possess almost identical Complementary Determining Region 3’s (CDR3s), the region primarily associated with the antibody’s specificity for an antigen. We decided to look into the sequences of our 28 patients within the VH3-21 family, a family that has been shown to demonstrate an aggressive disease in the presence of stereotypy, irrespective of traditional prognostic markers. We were able to determine the presence of stereotypy in just over 50% of our VH3-21 patients following criteria previously published, however a larger sample size is needed to determine any significance to clinical outcome.
Clinical Impact of Telomere Shortening in Normal and Leukemia Cells in Chronic Lymphocytic Leukemia
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Introduction: Prognosis in CLL is heterogeneous with the elderly, males and those with multiple co-morbidities having the highest risk of poorer outcomes. Whether this reflects differences in the biology of CLL or the result of altered immunosuppression is unknown. In normal individuals, the telomeres in somatic cells shorten with age, predisposing those with shorter telomeres to death from infections, cancer and cardiovascular disease. In chronic lymphocytic leukemia (CLL), short telomere length in the leukemia cells predicts poor prognosis. The contribution of un-modifiable factors of age, sex or comorbidities to telomere shortening in CLL has not been elucidated. In the present study, we evaluated buccal cell (BC) telomere length in patients with CLL, to determine if this is predictive of survival, and can enhance the predictive value of leukemia cell telomere length.

Methods: The Manitoba CLL tumor bank contains 168 samples from newly diagnosed CLL patients between 2007-2011; with a median follow-up of 4 years. Half of these patients required chemotherapy and one-tenth have died. Genomic DNA was extracted from purified CLL cells and buccal cells (BC) collected at diagnosis. Telomere length was established by multiplex quantitative real-time PCR. Telomere/standard (t/s) ratio was calculated using the beta-globulin gene as the standard. Statistical analysis was performed using Statistical Analysis Software (SAS) and Prism software.

Results: The median adjusted telomere length was shorter in CLL cells than in BCs being 0.53 and 2.01, respectively. In BCs, telomere length significantly shortened with increasing age (p=0.01) but was not reflective of the number of comorbidities or survival. In contrast, telomere length in CLL cells was independent of age (p=0.44) and sex (p=0.75). Short CLL telomere length correlated with other biological and clinical markers of poorer prognosis including un-mutated IGHV status (p<0.0001), Zap70 positivity (p=0.05), CD38 positivity (p=0.003), short lymphocyte doubling time (p=0.004), higher Rai stage (p=0.02) and an earlier time to treatment (p<0.0001). Patients with short CLL telomeres had also decreased survival at four years (p=0.05); living 22 months less than those with normal telomeres. Adjusting for BC telomere length improved the prognostic value of CLL telomere length as an independent predictor of survival.

Conclusions: These results demonstrate that BC telomere length in CLL patients shorten with age independent of co-morbidities, secondary malignancies and the respective CLL telomere length. CLL telomere shortening is strongly associated with biochemical and clinical markers of disease progression and survival. The predictive value for overall survival is enhanced by correcting for changes in buccal cell telomere length.
Progression of Chronic Lymphocytic Leukemia in a Patient on Dasatinib Therapy for Chronic Myeloid Leukemia
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The co-existence of chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) is a rare event and infrequently described in the literature. The standard treatment for CML is either imatinib or dasatinib, which are tyrosine kinase inhibitors directed at Abl. We, and others, have described patients with concomitant CML/CLL where both diseases have responded to imatinib or dasatinib¹. However, the mechanism of action of these agents in CLL has been unclear. In the case of imatinib, its activity in CLL has been ascribed to a direct affect on Abl while dasatinib could also exert its activity through bystander effects on Lyn or Syk. In the present report, we describe an 83-year-old man with CML who developed imatinib and dasatinib resistant CLL. He was initially diagnosed with IgM kappa marginal zone lymphoma and sixteen years later, was diagnosed with Bcr-Abl positive CML in chronic phase. Despite imatinib, six years later the patient’s CML had progressed to accelerated phase and he was concomitantly diagnosed with CLL. The patient was started on dasatinib and had a prompt resolution of his CML and CLL. However, two years later, while his CML entered molecular remission with undetectable Bcr-Abl transcripts, his CLL had progressed to Rai stage IV disease with profound lymphocytosis. FISH studies showed a deletion 13q and the patient had an excellent initial response to chlorambucil /obinutuzumab with normalization of his lymphocyte count. In vitro studies on his CLL cells showed that they were resistant to dasatinib, but not to ibrutinib (inhibits BTK) or gefitinib (inhibits Syk), as measured by annexin V/7AAD staining. This suggests that the downstream BCR pathway is intact in these cells and ongoing studies are determining if mutations in Lyn, Abl or another molecule may explain the acquired resistance of the CLL cells to imatinib/dasatinib.

Choice and timing of therapy for patients attending the CLL Clinic at CancerCare Manitoba
Mandy Squires, Erin Streu, Sara Beiggi, Angela Deneka, Spencer Gibson, Dhali Dhaliwal, Versha Banerji, James Johnston

There are various treatment options in CLL, the choice of therapy depending on the patients’ fitness, as measured by the cumulative illness rating scale (CIRS), ECOG performance status and renal function. Thus, patients receiving FCR (Fludarabine, Cyclophosphamide, Rituximab) or BR (Bendamustine, Rituximab) should have a CIRS of ≤6, an ECOG of 0/I and a creatinine clearance of >60 ml/min; patients not eligible to receive FCR or BR are generally treated with FR (Fludarabine, Rituximab), Chlorambucil/Obinutuzumab, Chlorambucil alone or RCD (Rituximab, Cyclophosphamide, Dexamethasone). Patients with immune cytopenias receive initially prednisone, followed usually by RCD or Cyclosporine. In the present study, we have evaluated the time of treatments and the fitness of all patients with newly diagnosed patients seen at the CLL Clinic at CancerCare Manitoba. We assessed 293 new patients seen from 1st January 2007 until the 31st December 2011. The data is currently being analyzed and the findings will be presented.
ROLE OF PI3K ENZYMES IN CHRONIC LYMPHOCYTIC LEUKEMIA AND INTERACTION WITH LYMPHOID TISSUE MICROENVIRONMENT

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Chronic Lymphocytic Leukemia (CLL) is a lymphoproliferative disorder of mature B cells. A common feature of the disease is abnormal accumulation of malignant B cells in bone marrow and peripheral lymphoid tissues. Mesenchymal stromal cells present in the lymphoid tissue and bone marrow microenvironments can enhance the survival, proliferation and drug resistance of CLL cells through direct contact, thus selective disruption of this cell-cell interaction has been proposed as an effective therapy. Recently approved breakthrough therapies Ibrutinib and Idelalisib are kinase inhibitors targeting signaling enzymes in the phosphoinositide 3-kinase (PI3K) pathway. Recently, work done in our lab indicate that blocking the PI3K pathway impairs malignant B cell adhesion to stromal cells and migration in response to chemotactic factors produced by stromal cells. We hypothesize that targeting the PI3K pathway will effectively impair malignant B cell adhesion and migration functions required to access and colonize protective niches in lymphoid tissues. The mechanisms by which the PI3K pathway regulates CLL cell migration and interactions with stromal cells are still unclear. Here we propose several experiments to define the roles of individual PI3K enzymes on different aspects of this process, namely overall cell motility, chemotactic gradient sensing, cell-cell interaction and chemokine receptor trafficking. This study will apply novel technologies including live cell imaging and microfluidic devices to understand a critical aspect of malignant B cell biology. Preliminary data showed PI3K inhibitors can inhibit migration of CLL cells by SDF-1α in Transwell. Idelalisib (PI3K δ isoform specific inhibitor) can inhibit SDF-1α induced chemokinesis. CLL cells migration on the surface of stromal cells can be directly observed in co-culture system. Migratory capacities of the cells as well as cell-cell interactions were further analyzed in a quantitative way. These results will provide fundamental insights into the roles of PI3Ks in cell biology and help understand the underlying mechanisms by which different PI3K inhibitors act upon B cell leukemias. Furthermore, these results could provide evidence for specific PI3K inhibitor treatments of CLL patient in the future.