IBRUTINIB FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): IMPACT OF THE YOU&I™ PATIENT SUPPORT PROGRAM ON TREATMENT ADHERENCE

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Background: Oral therapies for cancer can present several advantages over intravenous drugs, including greater flexibility and convenience for the patient and reduced healthcare costs. However, for treatment to be effective, a high degree of adherence must be maintained. Prior studies have shown a range of non-adherence to long-term oral anticancer therapy, with adherence rates varying considerably with patient characteristics. Consequences of low adherence can include increased healthcare costs and inferior patient outcomes, including decreased time to relapse and decreased survival. The YOU&i™ Patient Support Program (PSP) supports patients with reimbursement services and provides patients and their physicians with a comprehensive nurse-coaching program in an effort to encourage treatment adherence.

Aim: To examine patient satisfaction and adherence to ibrutinib within the YOU&i™ PSP.

Methods: Using evidence-based literature reviews and global/local market research, barriers to treatment adherence were identified. These included a lack of disease/treatment education, high financial burden, negative psychosocial/motivational factors, poor medication routine-building, and suboptimal patient:physician communication. Each patient’s risk for non-adherence was calculated using the Morisky Medication Adherence Scale© score and the total number of barriers identified at initial assessment, and the frequency of nurse-coaching calls was adjusted accordingly. Patients were categorized as “adherent” if they had ≥85% compliance (obtained refills within 4 days of expected refill date) and as “discontinued” if ibrutinib was not dispensed for ≥3 months, if the patient was confirmed as discontinued prior to ibrutinib not being dispensed for ≥3 months, or if the patient was confirmed as deceased. All other patients were categorized as “partially adherent”. Patient questionnaires were used to gauge satisfaction with the YOU&i™ PSP.

Results: As of January 19, 2016, a total of 903 CLL patients were enrolled in the PSP; 87% of patients opted in for nurse coaching. Most patients (58%) had received 1-2 prior lines of therapy. At 9 months from treatment initiation, 81% of patients who received nurse coaching vs 59% of those who did not were categorized as “adherent” (excludes patients who discontinued due to disease progression or death). Similarly, only 7% of those who received nurse coaching vs 23% of those who did not were discontinued from ibrutinib for reasons other than disease progression or death. In the overall PSP population, 17% discontinued the program; among the patients discontinuing, the most common reason was death (49%). Overall, 91% of patients reported that they were satisfied/very satisfied with the PSP, with 87% likely to recommend the program. Furthermore, 78% reported that the PSP was very helpful in supporting their adherence to ibrutinib.

Conclusions: The results from the YOU&i™ PSP suggest that nurse coaching may contribute to improved patient adherence to oral anticancer treatment. In the YOU&i™ PSP, nurse coaching appeared to also be associated with decreased treatment discontinuation. This program, or similar supportive programs, may be helpful in assisting patients in maintaining adherence to treatment with ibrutinib.
THE ROLE OF SRC-FAMILY KINASE FELINE-GARDNER RASHEED IN CLL

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The Src-family kinase Feline-Gardner Rasheed (Fgr) is not expressed in peripheral B-cells, but is ectopically expressed in circulating CLL cells and Epstein-Barr Virus immortalized B-cells. Its known functions in myeloid lineage and FCRL4+ memory B-cells include regulation of migration, B-cell antigen receptor (BCR) signaling, metabolism, and cell-survival. Fgr’s function in CLL is still unknown. Because these processes are altered in CLL, we hypothesize that Fgr is contributing to aggressive disease by playing a role in the regulation of migration, BCR signaling, metabolism, and cell survival. Preliminary results with the BJAB and RAJI cell lines show that Fgr may be contributing to survival signaling downstream of BCR activation and regulation of mitochondrial metabolism. Work with primary patient samples suggest that Fgr may be more highly expressed in Zeta-Associated Protein 70 Kinase (ZAP-70) positive CLL patient samples at both the transcript and protein level. Whether Fgr expression is a predictor of or contributes to more aggressive disease is still unknown.
UGT2B17 OVEREXPRESSING CLL CELLS DISPLAY A PROLIFERATIVE ADVANTAGE AND ARE CHARACTERIZED BY DISTINCTIVE GENE AND PROTEIN EXPRESSION PROFILES.

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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: The study was undertaken to explore gene and protein expression profiles characterizing leukemic cells overexpression UGT2B17. 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. RNA sequencing and stable isotope labeling by amino acids in cell culture (SILAC) coupled to mass spectrometry (MS) were performed to identify pathways associated with UGT2B17 overexpression.

Results: Compared to MEC1-KD, cells overexpressing UGT2B17 displayed a significant proliferative advantage (30% vs. KD, \( p = 0.03 \)). Differential gene expression followed by pathway and network analysis revealed that high UGT2B17 levels predominantly affects genes involved in regulation of proliferation, adhesion, signaling and immune function. mRNA levels of other important CLL biomarkers correlated with UGT2B17 expression in our cell model, suggesting an UGT2B17-dependent mechanism for promotion of CLL progression. In line, SILAC identifies numerous differentially expressed proteins (1.5 fold change, FDR<0.01), critical for hematopoietic development, viral response and cell motility. Validation of top candidates as well as assessment of the metabolic functions of CLL cell models is currently under investigation.

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.*

Eligible for George H Smith Award
FK866 AND GMX-1778 INDUCED CELL DEATH THROUGH NAD DEPLETION IS RESCUED BY EXOGENOUS NAD AND OLAPARIB

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Background: Chronic Lymphocytic Leukemia (CLL) is primarily a disease of the elderly. Chemotherapy remains the standard of care treatment for CLL, but patients are often elderly and frail and thus poorly tolerate treatment. Recently a novel strategy for treatment has been selectively targeting the altered metabolism of CLL cells. Nicotinamide Phosphoribosyl Transferase (NAMPT), the rate limiting enzyme in the Nicotinamide Adenine Dinucleotide (NAD) salvage pathway, is upregulated in CLL. NAMPT inhibition by FK866 and GMX-1778 leads to loss of NAD, ATP and cell viability.

In this study, we evaluated the effects of exogenous NAD or the PARP inhibitor olaparib on NAD content, ATP content and cell viability in CLL cells treated with FK866 or GMX-1778 to determine if they could rescue the effects of NAMPT inhibition.

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 pretreated with either exogenous NAD, olaparib or vehicle control overnight. The following day, cells were treated with either NAMPT inhibition induced by FK866 or GMX-1778, or vehicle control for three days. NAD and ATP content were then assessed using NAD/NADH GLO™ and CellTiter-Glo® (Promega) assays respectively. Cell viability was assessed by flow cytometry using annexin V-FITC/7AAD staining.

Results: Exogenous NAD significantly rescued NAD depletion, ATP depletion and loss of cell viability in CLL cells treated with FK866 or GMX-1778. ATP depletion, cell viability and to a lesser extent NAD content were also significantly rescued by olaparib.

Conclusions: FK866 and GMX-1778 induced loss of NAD content, ATP content and cell viability was rescued by an increased NAD pool, suggesting cell death due to an on-target effect of these NAMPT inhibitors. This also suggests a similar mechanism of action for both inhibitors.

Eligible for George H Smith Award
DEL11Q IS ASSOCIATED WITH INCREASED SENSITIVITY TO INHIBITORS OF GLUCOSE METABOLISM, MITOCHONDRIAL METABOLISM, OR IBRUTINIB

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Background and rationale. Chronic Lymphocytic Leukemia (CLL) is one of the most common leukemia’s in the western world. CLL is biologically and clinically heterogeneous. For example, patients with del11q or del17p are associated with faster disease progression and poor response to traditional chemotherapy. Among the leading therapeutic interventions in CLL is the use of ibrutinib, which is a highly specific and irreversible BTK (Bruton’s tyrosine kinase) inhibitor. Despite showing impressive clinical results, ibrutinib treatment is not curative and resistant patients display an aggressive disease. Thus, new therapeutic strategies are needed. Metabolic reprogramming is an emerging therapeutic target. For instance, a clinical trial involving Ritonavir (GLUT4 inhibitor) and Metformin (OxPhos inhibitor) for Multiple myeloma is currently ongoing. Additionally, our group has shown that the inhibition of BCR signaling can modify the metabolic reprogramming of CLL primary cells.

Objective: To evaluate for targetable metabolic differences associated with del11q. Specifically our aim is to identify metabolic adaptations to ibrutinib treatment in a panel of primary CLL lymphocytes. In this work, we used media containing a physiological concentration of glucose, ibrutinib, inhibitors of glucose metabolism, and inhibitors of mitochondrial metabolism.

Results: We have found that del11q positive cells were more sensitive to glucose and glutamine pathway inhibitors, which does not seem to be associated with decreased ATM activity, as an inhibitor of this kinase did not affect survival or ROS levels in primary CLL lymphocytes in vitro. In addition, del11q positive samples were more sensitive to an inhibitor of mitochondrial respiration, oligomycin A. Interestingly, glucose uptake inhibition by using Ritonavir, caused an increase in glutamine uptake, suggesting a compensation effect.

Ibrutinib induced a general AMPK activation regardless the subset analyzed, and was preferentially cytotoxic to del11q positive samples. Ibrutinib induced glutamine uptake in del11q positive and negative samples, but induced glucose uptake only in del11q positive samples without affecting the expression of glucose transporters. Suggesting that del11q positive and negative samples face ibrutinib inhibition in a different manner. In accordance to this, we observed decreased basal levels in p-PLCγ2 in del11q positive samples, which could contribute to the increased sensitivity to ibrutinib in these cells.

Previously, the increment in oxidative stress has been associated to cancer cell death; however, it has also been observed that cells can take advantage of ROS to maintain the activation of signaling networks, using ROS for phosphatases inhibition. Sensitivity to glucose and glutamine pathways, or ibrutinib, was accompanied by ROS levels increase. However, the use of a ROS scavenger did not rescue the decrease in viability observed; excluding it as the responsible for the observed cell death. Finally, we explored the effect of metabolic inhibitors on ibrutinib-mediated cytotoxicity in primary CLL lymphocytes both positive and negative for del11q.

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Eligible for George H Smith Award
ZAP70 ALTERS CHRONIC LYMPHOCYTIC LEUKEMIC CELL METABOLISM

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Chronic lymphocytic leukemia (CLL) is the leukemia with the highest incidence among adults in the Western countries. Prognosis of CLL is poor but one of the biomarker zeta-chain-associated protein 70 (ZAP-70) over expression is associate with aggressive disease with time to treatment being 2.6 yrs for ZAP70 expressing (ZAP70+) patients and 8 yrs for ZAP70 deficient (ZAP70-) patients. Thus ZAP-70 may play a role in CLL progression. Metabolic reprograming plays a central role in cancer progression and is altered in CLL cells. However, the role of ZAP70 in metabolism in CLL cells is remain unexplored. Our study demonstrated that ZAP70 expression alters metabolism through association with pyruvate kinase M2 (PKM2). PKM2 is key isoform of Pyruvate kinase which is rate limiting enzyme and its provide metabolic advantages to tumour cells by their growth promotion. Our results indicated the PKM2 is expressed CLL cells and binds to ZAP70. In addition, we found ZAP70+ cells have high susceptible rate of oxygen consumption with extracellular acidification rate compare with CLL cells lacking ZAP-70 as determined by Seahorse analysis. This indicates ZAP70+ CLL cells have increased mitochondrial respiratory capacity with high glycolytic rate. Collectively, our data reveals a new function for ZAP70, which is to regulate the Warburg effect and may play a role in CLL progression.

Eligible for George H Smith Award
ALTERATION OF PI3Kγ SIGNALING ENHANCES MICROENVIRONMENTAL INTERACTION AND SURVIVAL OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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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-1α, 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.

Improper activation of the phosphoinositide-3 kinase (PI3K) survival pathway has been implicated in tumorigenesis in several tissue types. PI3K enzymes are phospholipid kinases that phosphorylate the 3’ hydroxyl group of the inositol ring of phosphoinositide lipids and are subdivided into 3 classes. 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 p110γ has not been extensively studied in B cells, despite their well-established functions in chemokine receptor signaling in other cell types; however, p110δ 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). Current data suggest that levels of class 1B PI3K subunits may be a critical factor controlling malignant cell survival and invasiveness.

Hypothesis: Altered expression of class 1B subunits enhances CLL survival and disease progression by modulating interactions with lymphoid tissue stromal cells.

Results: Expression of p110γ was significantly increased in CLL cells, which was positively correlated with increases in p101 protein in response to a simulated microenvironment (anti-CD40 + IL-4 stimulation). p84 mRNA was minimally expressed in CLL cells and its protein was not detectable. Moreover, p110γ inhibition significantly reduced CLL cell migration in response to the chemokine SDF1-α and adhesion to bone-marrow derived stromal cells, while the overexpression of either p110γ or p101 significantly enhanced CLL-like cells’ migration.

Eligible for George H Smith Award
TARGETING THE METABOLIC CASCADE TO TREAT CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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CLL is the most common leukemia diagnosed and managed by practicing hematologists. CLL is characterized by abnormal proliferation and accumulation of mature CD5 positive B-lymphocytes in blood, bone marrow, spleen and lymph nodes. Therapies for CLL have evolved over the last two decades, however newer treatment have yet to improve overall survival. Altered mitochondrial metabolism serve as a novel target to advance treatment options for CLL.

SIRT(silent mating type information regulation 2 homolog), a histone deactylation protein, plays a ubiquitous role in the homeostatic regulation of cellular metabolism in cytoplasmic, mitochondrial and some time nuclear compartments that senses changes in intracellular NAD⁺ and cellular caloric levels. We reported that the reduction in NAD production by NAMPT inhibition induced apoptosis in CLL cells compared to control B cells. The present study focuses on the impact of SIRT1 on NAD regulation in CLL cells. The baseline protein expression of NAMPT and SIRT1 was altered in CLL cells versus control B cells. Thus, we will explore the co-relation of NAMPT and SIRT1 protein expression with their respective inhibitors FK866 and EX527 in CLL cells. We will measure the impact of SIRT1 inhibition on reactive oxygen species and mitochondrial membrane potential, release of cyto C and mitochondrial related proteins level including BAX, BCL2. Thus, targeting SIRT1 and altering mitochondrial function may be proven as effective therapeutic option for CLL.
CROSS-RESISTANCE AND SYNERGY WITH BENDAMUSTINE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Bendamustine (BEN) has structural similarities to an alkylating agent and a nucleoside analog, and is effective against tumor cells that are resistant to standard therapy. The activities of BEN was compared against that of the alkylating agent, chlorambucil (CLB), and the nucleoside analogs, fludarabine (FLU) and deoxyadenosine/pentostatin (dADO/PEN), in primary chronic lymphocytic leukemia (CLL) cells in vitro. Cross-resistance was observed between BEN, CLB and FLU, with previously treated patients or those with a deletion 17p being most resistant. In contrast, some resistant CLL cells retained moderate sensitivity to dADO/PEN. Like FLU and CLB, BEN induced apoptosis through both the mitochondrial and death receptor pathways. There was a greater increase in DNA double-strand breaks (DSB) following FLU, as compared to BEN and CLB. Synergistic cytotoxicity was seen on combining BEN or CLB with FLU or dADO/PEN, but not when combining BEN with CLB. These results demonstrate that BEN acts as an alkylating agent, demonstrates cross-resistance to CLB and FLU and resistance to cells with a deletion 17p. DNA repair may play a role in the mechanism of CLL cell resistance to chemotherapy. DNA repair and DNA damage signaling pathway genes were evaluated for differential expression before and after in vivo treatment of the patient and in patients with a deletion 17p or 11q. Synergistic antitumor activity was seen between BEN and dADO/PEN suggesting that the combination of BEN and PEN should be evaluated in the clinic.
A UNIQUE SPLICED ISOFORM OF TIN2, MISSING EXON 2, IS EXPRESSED IN CHRONIC LYMPHOCYTIC LEUKEMIA AND HAS PROGNOSTIC SIGNIFICANCE.

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Telomeres are distinctive complex structure of short repetitive DNA (TTAGGG) and associated protein complexes that cap the ends of chromosomes. In human nature, telomeres shorten in each cell cycle and become dysfunctional as at this stage cells go through senescence. Telomeres are important in maintaining the integrity of the chromosomes. A series of shelterin proteins (TRF1, TRF2, RAP1, POT1a and b, TIPP1 and TIN2), and their dynamic interactions with the telomeric DNA regulate telomere maintenance. TIN2 is a key player in telomere chromatin complex, because of its higher affinity to bind both TRF1 and TRF2. There are two isoforms of TIN2, TIN2S and TIN2L, the former being found in human fibroblasts and mammary epithelial cells, but have not been investigated in B cells. In chronic lymphocytic leukemia (CLL), the telomeres are shorter than in normal lymphocytes, with very short telomeres being associated with poor survival. In the present study, we demonstrate that the leukemia cell telomere length shortens over time, indicating that telomere shortening is an ongoing process. When CLL cell metaphases were examined by Q-FISH, telomere shortening affected all chromosomes equally, but mechanism and effect of shelterins in this process is not well known. Therefore, we next investigated the transcriptional and translational status of TIN2 in B cells from B-CLL patients. Apart from TIN2, the all other shelterin proteins were increased in CLL cells as compared to normal lymphocytes. In contrast, TIN2S levels were dramatically reduced in CLL and an alternative spliced isoform of TIN2S was observed, both in IgVH mutated and unmuted cases. Targeted PCR for TIN2 followed by sequencing revealed a deletion of the entire exon 2, totaling 105 bp, in this spliced form of TIN2. When measured by RT-PCR, expression of spliced TIN2 and TIN2S (full length), were inversely correlated (r=-0.34, p= 0.038), estimated by correlation analysis. In 50 patients, a high level of spliced TIN2 correlated significantly with older age (P=0.032) and higher CIRS score with shorter survival (P=0.019), estimated by Fisher’s Exact test. In addition, in three out of eight patients this spliced form of TIN2 increased over time confirming survival advantage of this alteration.

To assess the functional role of TIN2, we also measured the binding ability of TIN2 and TRF2, since the exon2 of TIN2 is required for complex formation between TIN2 and TRF2. Immunoprecipitation of CLL cells and normal lymphocytes with TRF2 antibody and subsequent analysis with TIN2 antibody showed only the presence of TIN2 in normal but not in CLL. This suggests that the binding ability between TIN2 and TRF2 were lost in CLL. Moreover, immunofluorescence and western blot studies in CLL cells showed TRF2 was localized in both the nuclei and cytoplasm in CLL cells compared to localization only to the nucleus in normal lymphocytes. Despite these functional alterations, spliced TIN2 messenger levels did not correlated with telomere length. This is the first description of the splice variant of TIN2 detected in primary CLL cells. Although we also detected basal RNA levels of the spliced form of TIN2 in normal lymphocytes, mainly in B cells, we didn't detect it at the protein level. In CLL the expression of this spliced form of TIN2 could be interfering with the TIN2-TRF2 complex formation, therefore affecting chromosome instability and disease progression.
MICROENVIRONMENTAL EFFECT OF EXOSOME AND MICROVESICLES RELEASED FROM CLL

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The tumor microenvironment is a complex structure consisting of numerous components, including inflammatory cytokines, microvesicles (MVs) and exosomes secreted from the cancer cell as well as their surrounding cell types. MVs are 100 to 1000 nm diameter in size and generated by shedding from the plasma membrane whereas exosomes are small vesicles (50-150 nm) generated via an endocytic pathway, contain proteins, noncoding RNAs, mRNAs and tumor derived double-stranded DNA (dsDNA). These released MVs and exosomes play major role in cell-cell communication to deliver specific signals by fusing to nearby cells within their circulatory range. Compared to normal B cells, CLL cells release large numbers of MVs, exosomes and inflammatory cytokines and these may affect adjacent cells, such as lymphocytes and marrow stromal cells. In this study, we have begun evaluating the role of conditioned media from CLL cells on normal squamus cells and squamus cell carcinoma cells in vitro. Preliminary data suggest that MV released by CLL cells stimulate the growth of normal skin cells but not squamus cell carcinoma cells. In contrast, squamous carcinoma cells growth more rapidly in the presence of inflammatory cytokines released by CLL cells. Ongoing studies are confirming these results and determining whether the effects are related to the type of CLL cells producing the conditioned media.
A RETROSPECTIVE REVIEW OF IMMUNOGLOBULIN LEVELS IN A COHORT OF CLL PATIENTS AT A LARGE PROVINCIAL CANCER CENTER

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Hypogammaglobulinemia is the most commonly observed immune deficiency in patients with chronic lymphocytic leukemia (CLL) yet little is known about its prognostic significance. Patients are susceptible to persistent, recurrent infections which are a leading cause of death in this patient population. Disease duration and prior chemotherapy treatment are 2 recognized factors which affect an individual’s degree of hypogammaglobulinemia.

At diagnosis, one quarter of patients present with low IgG levels, and of those with normal levels, 25% subsequently develop hypogammaglobulinemia during longterm follow up. Once clinically indicated (IgG <4g/L and at least 2 courses of antibiotics in a 12 month period; or 1 severe bacterial infection requiring hospitalization or treatment with IV antibiotics) patients are treated with replacement IgG (IVIG or SCIG) but there is no evidence to predict which patients will develop hypogammaglobulinemia or which patients will become symptomatic.

Laboratory Reference Values: IgG 6.9-16.2 g/L, IgM 0.7-3.8 g/L, IgA 0.7-3.8 g/L

After obtaining ethical approval, we conducted a retrospective chart review of 293 patients newly diagnosed with CLL (SLL and MBL) referred to CLL clinic at CancerCare Manitoba over a 5 year period (January 1, 2007 - December 31, 2011). Immunoglobulin (Ig) levels (G, M, A) were measured at the time of referral/diagnosis (baseline) and annually thereafter. Ig levels were correlated with patient characteristics (age at diagnosis, gender, co-morbidities), CLL prognostic indicators (Rai stage, lymphocyte doubling time, creatinine, LDH, β2-microglobulin, IGHV mutational status, ZAP-70, CD38) and time to first treatment (TTFT). A subset analysis of patients treated with replacement gammaglobulin was also conducted. All clinical data was imported into the CCMB Clinical CLL database (Caisis) for analysis.

Results: N=293 with a median age at diagnosis of 68 years; 62.5% were male, 38% had received treatment for their CLL & 24% died during the follow up period (median follow up of 71 months). 70% had CLL (85% diagnosed with Rai 0/1), 15% monoclonal B-cell lymphocytosis (MBL) & 15% small lymphocytic lymphoma (SLL). Over a median follow-up of 4 years, one-third of patients with normal baseline IgG developed a low IgG and this was unrelated to receiving chemotherapy.

This study demonstrates that baseline Ig levels in CLL are informative of the underlying biology of this disease, and could provide valuable information regarding disease progression, survival and need for subsequent IgG replacement therapy or CLL treatment. Importantly, Ig levels may be elevated in CLL and this is associated with increased CIRS and a high β2-microglobulin level, suggesting that the increase is related to underlying comorbidities. Further studies are required to examine the cause for the progressive decline in Ig levels over time, despite an otherwise stable disease, and to determine whether this decline can be eliminated by earlier treatment with novel new therapies.
IMPLEMENTATION OF S SUBCUTANEOUS IMMUNOGLOBULIN (SCIG) CLINIC AT A LARGE PROVINCIAL CANCER CENTER: A NEW APPROACH TO AN OLD PROBLEM

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Immune dysfunction is a key feature of CLL with abnormalities in both B and T cell function characterized by infectious complications, reduction in immunoglobulin (Ig) levels, and an increase in circulating T cells. Hypogammaglobulinemia is the most notable immune deficiency, present in 24% of all patients at the time of diagnosis and progressing over time regardless if they have received prior chemotherapy treatment (Beiggi, 2013). Patients with hypogammaglobulinemia are more susceptible to frequent/recurrent infections (usually respiratory caused by S.pneumoniae and H.influenzae) causing significant patient morbidity and infections are a leading cause of death. Although not considered cost effective, IVIG every 3-4 weeks (weight-based dosing) has been the standard of care in CLL and has been shown to reduce the incidence of bacterial infections by 50% (no impact on the total number of severe bacterial infections and nonbacterial infections) but does not prolonging overall survival.

Methods: Based on similar programs for patients with primary immune deficiency (PID), in September 2014 we developed and implemented an advanced practice nurse-led SCIG program for oncology patients with secondary immune deficiency (SID) at our provincial cancer center. All patients with hematologic malignancies (lymphoma, multiple myeloma, chronic lymphocytic leukemia, and post stem cell transplant) receiving IVIG were screened for eligibility and transitioned to the new home-based program and all newly initiated replacement patients were screened and offered the choice of SCIG in addition to IVIG. Dosing: We adopted a low-dose approach (12 grams/month) with dose escalation based on the presence of infections rather than IgG trough levels.

Results:

Antibiotic Use: 3:1 reduction in antibiotic prescriptions (6 months pre:post SCIG initiation) 42% infection free, 22% required 1 prescription

Treatment Satisfaction (at 6 months): 82% somewhat/extremely satisfied with SCIG 79% somewhat/extremely satisfied with SCIG’s ability to prevent infections 9% reported side effects (100% mild and not requiring medical intervention)

QoL: 64% rate health the same or worse than 1 year prior to SCIG (79% at baseline), 11% report health/treatment interfering with their normal social activities (vs. 25% at baseline), 43% report they get sicker easier than people they know (vs. 66% at baseline)

Conclusion: SCIG has become the standard of care for all patients with hematologic malignancies (lymphoma, multiple myeloma, chronic lymphocytic leukemia) who require IgG replacement for SID. This new approach has demonstrated marked savings and benefits including significant labor (nursing), and resources savings (availability of infusion chairs), better regulation, monitoring and use of donor immunoglobulins; fewer prescribed antibiotics, and improved quality of life and treatment satisfaction for our patients and their caregivers.
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 dasatinib. 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. \textit{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. One mechanism to explain the resistance is acquisition of mutations in downstream regulatory genes. To explore this, we performed whole genome sequencing using HiSeq2500 analyzer (Macrogen Corporation) comparing genomic DNA isolated from the patient’s CLL and salivary cells. We identified 4 candidate genes which are mutated in the CLL cells but not the saliva, and have been reported in the literature as strong regulators of ERK / AKT / PI3K pathways. Current studies are underway to understand and validate these candidate genes as novel mechanisms of TKI resistance in CLL.

\textbf{Eligible for George H Smith Award}
FK866 is a specific inhibitor of NAMPT (nicotinamide phosphoribosyltransferase), a rate limiting enzyme involved in NAD generation. Much interest has been generated in the use of this drug for cancer treatment. It has been previously demonstrated in the lab that FK866 (10nM) induces cell death in CLL patient derived B-cells by an early depletion of NAD (1 day after treatment) followed by a subsequent decrease in cellular ATP levels (2 days after treatment).

In this abstract we report structural changes in primary CLL cells that were treated with FK866 for 6,18-20 hours. Electron microscopy has shown the presence of bound round/oval/square structures as well as nuclear invaginations. These structures are only observed in cells that were treated with FK866 and not with any other drugs used (fludarabine, bendamustine, chlorambucil) nor with the DMSO or untreated controls. To rule out the boxlike structures as artifacts, FK866 treated and untreated primary CLL cells had f-actin (a cytoskeletal protein) stained with FITC labelled phalloidin then viewed with a confocal microscope. This was done in the same time frame as the EM work (6,18-20 hours) and holes were observed only in the f-actin framework of the FK866 treated cells. These holes go through the entire cell and in some instances, go through the nuclei as well.

This phenomenon appears to occur only in CLL. Two Ewing’s sarcoma cell lines (A-673 and TC-32) as well as two small cell lung cancer cell lines (H209 and H69) that are known to be similarly sensitive to FK866 did not exhibit these structural differences.

Due to the FK866 mediated loss of NAD, CLL cells may be undergoing macropinocytosis an MTOR mediated phenomenon that is used by cells to extract nutrients from the environment. We have shown that FK866 causes increase in 4EBP1 phosphorylation compared to controls.

In conclusion FK866 may mediate macropinocytosis in CLL cells versus other FK866 sensitive tumour cells in a MTOR dependent manner.