Cancer-Specific Stress and Absolute Lymphocyte Count Trajectories in Patients with Chronic Lymphocytic Leukemia

Master’s Thesis

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Abstract

Chronic stress has been commonly observed in cancer patients and is associated with immune system down regulation. The effect of stress on immunity in hematologic cancers such as chronic lymphocytic leukemia (CLL) has not been studied despite the role of immune system dysfunction in CLL’s pathogenesis. In a phase II clinical trial, 154 patients with relapsed/refractory CLL received ibrutinib, provided blood samples, and completed a self-report measure of psychological stress specific to cancer over an 18-month treatment period (nine assessments). Targeted treatments like ibrutinib have been effective in reducing disease progression in CLL, despite the occurrence of lymphocytosis, which is an increase in absolute lymphocyte counts (ALC) and marker of progressive disease. Controlling for demographic, health status, number of prior treatments, and CLL genetic risk (del17p) factors, random changepoint models were estimated to evaluate the impact of stress on ALC trajectories. Stress was associated with pretreatment ALC ($\beta_0 = 0.13; 95\% \text{ CI } = 0.02, 0.25$) but did not impact the timing of lymphocytosis ($\varphi_{x1} = 0.03, \text{ CI } = -0.15, 0.22$), or the treatment response before ($\beta_{x1} = -0.11, \text{ CI } = -0.23, 0.01$) and after ($\delta_{x1} = 0.10, -0.01, 0.22$) lymphocytosis. Stress affects pre-treatment ALC but has little impact on ALC trajectories after beginning drug therapy. Additional analysis showed that lymphocytosis occurs later in the treatment trajectory for individuals classified as drug non-responders by 18-months compared to those regarded as drug responders ($\varphi_{x2} = -0.95; \text{ CI } = -1.50, -0.41$), though it is unclear whether clinical or
demographic risk factors can predict these groups. Results suggest patients exhibit similar ALC trajectories after ibrutinib initiation. Distress screening and management should be initiated prior to cancer treatments.
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Chapter 1: Introduction

Receiving a cancer diagnosis can be distressing for many individuals. Rates of moderate to high psychological distress (30% - 43%) have been observed across many cancer sites, including leukemia (33%) (Zabora, Brintzenhofeszoc, Curbow, Hooker, & Piantadosi, 2001). This distress is not solely limited to the time of cancer diagnosis but has been observed at 12 months post-diagnosis in samples of patients with varying sites of disease (Carlson, Waller, Groff, Giese-Davis, & Bultz, 2013). A cross-sectional study of leukemia and lymphoma patients has shown approximately 51% met criteria for moderate levels of anxiety and depressive symptoms (Montgomery, Pocock, Titley, & Lloyd, 2003).

Examining psychological stress in cancer patients is important as higher levels of stress have been associated with higher rates of recurrence and cancer death (Groenvold et al., 2007; Pinquart & Duberstein, 2010). Accounting for why this may be, stress hormones may impair immune functioning, which may impact disease progression (Repasky, Eng, & Hylander, 2015). Andersen and colleagues (1994) have proposed a biobehavioral model to illustrate how psychological and behavioral responses to cancer diagnosis/treatment may influence biological/immune processes that impact health outcomes. Therefore, establishing that psychological stress as a common feature associated with cancer is important as meta-analyses have shown that chronic stress is linked to immunosuppression (Segerstrom & Miller, 2004).
Effects of stress may be particularly important for hematologic cancers such as chronic lymphocytic leukemia (CLL). The most common form of adult leukemia (Siegel, Miller, & Jemal, 2015), CLL arises from dysfunctions in the immune system (Riches & Gribben, 2013). Additionally, individuals with CLL may be more vulnerable for chronic psychological stress as there is no cure and while treatment may bring remission, relapses are likely to occur. Whether chronic stress affects the course of CLL or hinders cancer treatment in CLL patients is unknown. What is known is that psychological stress can limit cancer patients’ ability to cope which may further complicate the heterogeneous disease course seen in CLL.

The proposed study examines the role of cancer-specific psychological stress on a disease marker (absolute lymphocyte counts; ALC) in patients with relapsed/refractory CLL who are undergoing treatment. This introduction provides a brief description to orient the reader. First, a review of the construct of stress will provide a rationale for how stress is conceptualized in the proposed study. Second, a brief description of the immune system will allow for better understanding of the relationship between stress and the immune system with a specific focus on cancer populations. Next, a detailed account of CLL, ibrutinib (cancer treatment), and the phase-II trial from which the patient data is taken will be provided. Taken together, these sections provide context for the final section: aims and hypotheses.

**Stress**

Stress can conceptualized as an individual’s response to environmental challenges, frequently called stressors, over time (Monroe, 2008). Modern conceptualizations of stress are often derived from Selye’s (1946; 1950) model in which
individuals were viewed as having both short-term and long-term reactions to environmental challenges. Selye’s model provides an account of the body’s physiological response to stress. Demonstrating a prescient sense of the model’s applicability, Selye suggested in 1950 that his model could inform leukemia treatments, due to the involvement of the endocrine system in leukemia pathology.

While Selye’s model emphasized the individual’s physiological response to environmental stressors, subsequent models have emphasized individuals’ cognitive appraisals and coping responses to stressors in what has been termed the transactional stress model (Folkman, Lazarus, Dunkel-Schetter, Delongis, & Gruen, 1986). Combining this model with Selye’s model, if an individual perceives a stressor as more challenging or lacks the ability to cope, the physiological response to that stressor becomes amplified.

While the aforementioned models define stress by the individual’s response, others define stress by the characteristics of the stressor (Elliot & Eisdorfer, 1982). Elliot and Eisdorfer (1982) identified five stress categories that may affect the magnitude or the duration of the stress response. These five categories include: 1) acute or time-limited stressors (e.g., giving a speech), 2) brief naturalistic stressors (e.g., student examinations), 3) stressful event sequences (e.g., death of a spouse), 4) chronic stressors (e.g., caregiving), and 5) distal events (e.g., trauma).

For the proposed study, all patients have relapsed/refractory CLL, indicating that they have received a cancer diagnosis and have received prior treatment. Chronic lymphocytic leukemia is non-curative with a cycling of remission and relapse. According to Elliot and Eisdorfer (1982), a chronic stressor is a challenge where the individual does not know if or when the challenge will end. Additionally, using both Selye’s model and
the transactional model, an individual’s perceptions or cognitive appraisals of CLL may vary and this variation may cause differences in the body’s physiological responses. Provided with a background on how stress has been theoretically defined, the next step is to determine how the proposed study can operationalize the CLL stress.

Approaches to measuring stress include physiological assessments of stress (e.g. salivary cortisol or heart rate variability), objective quantification of stressors (e.g. life event counts), and assessment of an individual’s appraisal of stress (Monroe, 2008). Each type of assessment measures different components of stress (i.e., physiological, psychological) and ideally, multiple approaches are utilized. Considering that an individual’s appraisal of a stressor may affect the physiological (and perhaps immunological) response to the stressor, patient perceptions of stress specific to cancer will be measured in this study by the Impact of Event Scale (IES; Horowitz, Wilner, & Alvarez, 1979). As this study aims to assess the impact of stress on an immune marker, a basic understanding of the immune system will be provided in the next section.

**Immune System**

The immune system is designed to protect the body from disease-causing microorganisms or foreign materials, also called antigens (Herbert & Cohen, 2003). To aid in understanding the immune system, Segerstrom and Miller (2004) distinguish the natural and specific arms of immunity; others refer to these separate arms as innate and adaptive (Coico, Sunshine, & Benjamin, 2003). Each arm has specific cells that have specialized functions to protect the host (Figure 1). Immune cells in the innate arm of immunity attack and destroy cells it does not recognize as belonging to the host (Segerstrom & Miller, 2004). Immune cells in the adaptive arm produce antibodies
against specific antigens or help augment the immune response. The proposed study focuses on adaptive immunity, which in contrast to innate immunity, is characterized by greater specificity and requires more time for an immune response. This is a crucial distinction when considering the role of psychological stress in a chronic disease such as CLL.

Specific or adaptive immunity can be assessed via lymphocytes. Lymphocytes are the cells that respond to an antigen, and when an antigen-specific lymphocyte is activated, it proliferates in order to create a population of cells to destroy the antigen (Segerstrom & Miller, 2004). There are three different types of lymphocytes: T-helper cells (CD4), cytotoxic T cells (CD8), and B cells (Figure 2). B cells produce antibodies that enhance the immune response (Segerstrom & Miller, 2004). Antibodies will bind to specific antigen which will release a series of enzymes that culminate in the destruction of the antigen (Coico, Sunshine, & Benjamin, 2003). T-helper cells produce cytokines that aid in inflammatory responses in the natural arm of immunity (Segerstrom & Miller, 2004). Additionally, T-helper cells produce cytokines that aid in signaling B-cells to produce antibodies or produce cytokines to suppress the immune response (Coico, Sunshine, & Benjamin, 2003). Finally, the third type of lymphocyte cell, cytotoxic T cells, destroys antigens (Segerstrom & Miller, 2004).

When examining the role of the immune system in cancer, one must consider that the immune system can protect against mutated cells (i.e., cancer cells) but can also support cancer cell survival (Narendra, Reddy, Shantikumar, & Ramakrishna, 2013). Lymphocytes can recognize cancer cells by the unique antigens the cancer cell generates and destroy these cells in a process called immune surveillance (Narendra et al., 2013;
Pardoll, 2015). In other instances, tumor cells have resistance mechanisms that can block immune responses initiated by the lymphocytes (Pardoll, 2015). Furthermore, through tumor cell signaling, the immune system can be reorganized in a way that protects cancer cells from immune responses and supports and promotes tumor growth. This interplay between the immune system and cancer is further complicated in hematologic cancers like CLL, which is thought to originate from mutations during B-cell development (Zhang & Kipps, 2014).

Assessment of the immune system in CLL is critical as CLL cells develop in the same environment as normal immune cells (Dhodapkar & Dhodapkar, 2015). Measurement of the immune system can be conducted with enumerative indicators or functional assays (Segerstrom & Miller, 2004). Counts of the number of each type of immune cell provide an indication of whether counts are within normal range. Functional assays assess how well an immune cell can perform its function (e.g., T-cell proliferation) and may be more useful in determining dysfunctions in the immune system when cell counts are within normal range. In, CLL an enumerative indicator like absolute lymphocyte counts (ALCs) represents a key indicator of disease status in CLL and was used as the outcome variable in the current study.

**Stress and Immunity**

As the proposed study aims to examine the impact of cancer-specific stress on ALCs, it is necessary to review the effects of stress on the immunity. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) produce adrenocortico-tropic hormone and glucocorticoids during times of stress which are thought to inhibit the immune response (Antoni et al., 2006; Chrousos & Gold, 1992).
Meta-analyses show stress to be negatively related to the number of T-helper cells, T-cytotoxic cells, and B cells (Herbert & Cohen, 1993). Other meta-analyses have demonstrated that chronic stress results in negative effects on functional measures (e.g., the ability of lymphocytes to proliferate) in healthy individuals (Segerstrom & Miller, 2004). However, recent research has shown that stress can activate hematopoietic stem cells to increase the number of lymphocytes in a sample of medical residents (Heidt et al., 2014).

Research using animal models has demonstrated that chronic stress suppresses immunity as observed by increases to regulatory/suppressor T cells (thought to protect cancer cells), increases in inflammatory cytokines, and decreases in NK cells (Ben-Eliyahu, Yirmiya, Liebeskind, Taylor, & Gale, 1991; Saul et al., 2005; Zhao, Xu, Liang, Li Zhang, & Sun, 2015). These immunosuppressive effects have concomitantly been shown to result in cancer progression as measured by tumor burden metastases (Ben-Eliyahu et al., 1991; Zhao et al., 2015). Furthermore, research examining the effect of stress on cancer treatment have shown chronically stressed mice developed larger tumors compared to non-stressed mice suggesting that stress can hinder cancer treatment (Liu et al., 2015).

Human studies have shown that elevated nocturnal cortisol levels and reduced cortisol variability are associated with higher levels of inflammatory cytokines in patients with ovarian cancer (Schrepf et al., 2015). Additionally, long-term follow-ups from the same study showed that ovarian cancer patients with high nocturnal cortisol levels had shorter survival intervals (3.8 years) compared to patients with low nocturnal cortisol levels (10.8 years). While these studies have shown associations between physiological
markers of stress (i.e., cortisol) and markers of immunity, similar results can be seen with psychological measures of stress. Greater preoperative cancer-related stress was associated with higher post-operative levels of cytokines associated with tumor growth in colorectal cancer patients (Sharma, Greenman, Sharp, Walker, & Monson, 2008). Higher levels of baseline distress have been associated with lower rates of survival at 2 years in women with ovarian cancer (Price et al., 2016). Finally, a meta-analysis has demonstrated that stress is associated with higher incidence of cancer and poorer survival in cancer patients (hazard ratio=1.29; 95% CI 1.16-1.44) (Chida, Hamer, Wardle, & Steptoe, 2008).

The majority of research, as discussed above, examines the effect of stress on measures of innate immunity measures; fewer studies assess indicators of adaptive immunity. Chronic stress was observed to result in lower counts of T lymphocytes, which resulted in down regulation of T-cell mediated immunity and increases in tumor proliferation in mice with lymphoma (Frick et al., 2009). One past study has found fatigue to be associated with lower counts of T lymphocytes in breast cancer survivors (Collado-Hidalgo, Bower, Ganz, Colw, & Irwin, 2006). Additionally, prior research has found psychological stress specific to cancer (i.e., higher IES scores) is associated with decreased proliferation of T cells in post-operative breast cancer patients (Andersen et al., 1998). Taken together, these findings indicate stress reduces adaptive immune responses. There are limited findings in hematologic cancer and it is unknown whether stress-induced immune dysfunctions occur in CLL. Specifically, the current study will address this question by examining whether stress impacts ALCs in patients with CLL.
**Chronic Lymphocytic Leukemia**

Chronic lymphocytic leukemia is the most common form of adult leukemia in the U.S. with an estimated 15,000 newly diagnosed cases and 5,000 deaths expected to occur in 2015 (Siegel et al., 2015). The disease is typically diagnosed in older adults (Coico, Sunshine, & Benjamin, 2003) and can be described as a B-cell malignancy (Chiorazzi, Rai, & Ferrarini, 2005). In examining how CLL can affect the immune system, malignant B cells proliferate and accumulate which hinders the ability of healthy, mature B cells from functioning, leaving individuals at higher risk of infections (Nabhan & Rosen, 2014). For example, research has shown that infections are the leading cause of death in CLL, occurring in approximately 60% of patients (Forconi & Moss, 2015). Higher rates of infections are not only caused by immune dysfunction due to the proliferation of CLL cells but also by down regulation of T-cell mediated immunity (Bosch & Abrisqueta, 2015). Furthermore, deficits in T lymphocytes result in the secretion of cytokines that aid in the proliferation of CLL cells and protect CLL cells from cell death (apoptosis) (Riches & Gribben, 2013). Finally, research has indicated that B-cell receptor (BCR) signaling results in proliferation of malignant B cells in CLL and may also account for the immune system’s inability to destroy malignant CLL cells (Burger & Chiorazzi, 2013).

Though it is clear that CLL can have detrimental effects on immunity, one must account for the heterogeneity of the disease as seen in patients. Patients may be asymptomatic and may not require treatment for years while others have active or aggressive disease (Chiorazzi et al., 2014). Markers of active disease include rapid increases of lymphocyte counts (lymphocytosis), severe constitutional symptoms (e.g.,
night sweats, fever, fatigue), bone marrow failure, or enlargement of the lymph nodes (Ghia & Hallek, 2014). Demographic, clinical, and genomic factors influence the course or severity of the disease (Nabhan & Rosen, 2014). Older age, male sex, and presence of multiple comorbidities are predictive of more aggressive disease and/or poorer outcomes (Baumann et al., 2014; Catovsky, Wade, & Else, 2014; Goede et al., 2014; Houldsworth et al., 2013). Furthermore, the severity of the disease may vary due to the presence of genetic abnormalities (Nabhan, Raca, & Wang, 2015; O’Gorman & Donneberg, 2008). Specifically, individuals with genetic deletions 17p and 11q have poorer responses to cancer treatments (Nabhan et al., 2015). Accounting for this clinical complexity, treatment strategies vary based on these demographic and clinical factors and are only initiated when the disease is active (Ghia & Hallek, 2014).

Chemoimmunotherapies are the standard first-line treatments with different agents being indicated depending on the aforementioned factors (Jain & O’Brien, 2015). For example, fludarabine, cyclophosphamide, and rituximab (FCR) is standard for younger patients while chlorambucil and obinutuzumab may be recommended for older patients with comorbidities. Second-line treatments may be a repetition of the first-line treatment if the remission was longer than 24 months or new chemotherapeutic agents (Ghia & Hallek, 2014). Treatment options are fewer and typically more toxic with each relapse. When relapse occurs, patients are characterized as having relapsed or refractory CLL; these patients are the focus of the current study.

Current research on relapsed/refractory CLL patients has emphasized treatment efficacy with duration of progression free survival as the primary outcome. In general, there is limited research on psychological responses as a secondary outcome as existing
studies focus on global quality of life (QoL) (Holzner, Kemmler, Kopp, Holzner, & Kopp, 2004; Levin, Li, Riskind, & Rai, 2007; Pashos et al., 2013; Shanafelt et al., 2007). For example, existing research has demonstrated that individuals who have received two or more treatments have lower QoL than individuals who were beginning a first-line treatment (Pashos et al., 2013). Additionally, two trials that compared untreated patients and patients undergoing treatment show lower quality of life scores for patients being treated for CLL (Levin et al., 2007; van den Broek et al., 2014). These results suggest that someone with relapsed/refractory CLL may experience greater levels of distress compared to a patient who is initiating a first-line treatment. In analogous research on solid tumor cancers, patients with recurrent cancer have been observed to have higher levels of cancer-specific distress compared to disease free cancer patients (Andersen, Shapiro, Farrar, Crespin, & Wells-Digregorio, 2005). These findings suggest that patients with relapsed/refractory disease who are undergoing a novel treatment, ibrutinib, may be at risk of high levels of cancer specific stress and stress-induced immune impairments.

**Ibrutinib**

Ibrutinib is a novel, targeted therapy for patients with refractory/relapsed CLL (Byrd et al., 2013; Maddocks et al., 2015). Ibrutinib is an orally administered drug that inhibits a kinase (Bruton tyrosine kinase; BTK) crucial in B-cell receptor (BCR) signaling and has been observed to have no negative effects on T cells (Jain & O’Brien, 2013). In one trial with 36 patients with CLL (13 were treatment naïve), ibrutinib was shown to be effective in inhibiting BCR in CLL cells and decreased CLL proliferation and cell survival (Herman et al., 2014). Early studies have not only shown ibrutinib to be mechanistically effective but have also shown improvements in psychosocial functioning.
over time. A single arm, phase II trial of ibrutinib demonstrated increases in overall QoL ratings after 6 and 12 months of treatment (Burger et al., 2014).

While these findings indicate ibrutinib may be an effective treatment for patients with CLL, it has been observed to cause lymphocytosis (Wodarz et al., 2014). A phase I trial demonstrated that lymphocytosis was reversed in ibrutinib-treated patients during a non-treatment period indicating lymphocytosis is caused by ibrutinib and not the disease itself (Advani et al., 2012). A phase II trial has reported that lymphocytosis can occur rapidly, within 7 days of treatment (median = 4 weeks) (Byrd et al., 2013). Researchers believe that BTK inhibition causes CLL cells to be released from the lymph nodes into the bloodstream (Schwarzbich & Witzens-Harig, 2014; Wodarz et al., 2014).

The occurrence of lymphocytosis may seem problematic as it is typically regarded as a negative prognostic marker and a symptom of active or progressive disease (Ghia & Hallek, 2014). However, a new category of cancer response, partial response with lymphocytosis, is used as a clinical marker and does not represent progressive disease (Rai & Jain, 2015). This is supported by data showing ibrutinib-treated patients with persistent lymphocytosis have similar overall survival rates to patients without persistent lymphocytosis (Figure 3; Byrd et al., 2015; Woyach et al., 2014). Another phase II trial has shown treatment-induced lymphocytosis to be associated with other positive prognostic signs such as concurrent reductions in lymph node size (Byrd et al., 2013). Additionally, molecular research has shown that BTK may remain inhibited but CLL cells exhibit downstream BCR signaling in cases of prolonged lymphocytosis (Woyach et al., 2014). This indicates that though these CLL cells cannot proliferate in the bloodstream they may continue to survive. Given prior research, while ibrutinib may be
effective in releasing CLL cells from the tumor producing niche and inhibiting CLL proliferation, chronic stress, through its effects on T-cell immunity, may promote CLL cell survival. Additionally, T-regulatory cell counts have been observed to be higher in CLL patients compared to healthy controls and is correlated with progressive disease (D’Arena et al., 2013). No research has evaluated whether cancer-specific stress impacts ALCs or lymphocytosis in patients treated with ibrutinib. The next section will detail how the present investigation will answer these key questions.

**Focus of the Present Investigation**

There is little research on the impact of psychological stress on adaptive immunity for individuals with hematologic cancers like CLL. Understanding the potential relationship between psychological stress and immunity becomes even more vital when patients undergo novel cancer treatments that impact immunity. Ibrutinib has been observed to cause lymphocytosis, an elevation in ALCs, that may persist over the course of treatment. While lymphocytosis due to ibrutinib is not associated with adverse outcomes (Woyach et al., 2014), it is unknown whether stress specific to cancer impacts treatment via the occurrence or timing of lymphocytosis. For example, stress hormones have been observed to mediate the desensitization of cancer cells to drug treatment in animal models (Liu et al., 2015). Additionally, stress has been shown to depress T-cell mediated immunity which may result in an environment that supports the proliferation and protection of malignant CLL cells (Frick et al., 2009; Saul et al., 2005). Specifically, past research has shown that chronic stress likely results in an increase in regulatory/suppressor T-cells, a decrease in T-helper cells, and a concomitant increase in tumor volume in cancer mouse models (Dhabhar 2013; Partecke et al., 2016; Saul et al.,
Research indicates disease progression in CLL is associated with increases in T-regulatory cells and decreases in T-helper cells (Yousefi et al., 2015). These findings suggest that stress, via changes in T-cell mediated immunity, fosters an environment conducive to the proliferation and survival of CLL cells.

The primary outcome for the current study is the trajectory of ALCs over an 18-month period in relation to stress. Absolute lymphocyte counts will be used as a measure of immunity. Prior research involving CLL have utilized ALCs for staging purposes (Eichhorst et al., 2015) and for observing treatment responses for therapeutic agents like ibrutinib (Byrd et al., 2013). Psychological stress will be assessed with the Impact of Event Scale - Revised (Horowitz, Wilner, & Alvarez, 1979). If chronic stress increases regulatory/suppressor T-cells, decreases T-helper cells (Dhabhar 2013; Partecke et al., 2016) and supports tumor proliferation in mouse models (Frick et al., 2009), higher levels of stress likely support CLL cell survival and proliferation (Riches & Gribben, 2013).

Though cancer treatments, like ibrutinib, have been shown to be effective inhibiting CLL proliferation and survival (Herman et al., 2009), chronic stress has been shown to hinder cancer treatment in animal models (Liu et al., 2015). In applying past research to the present investigation, stress should result in higher ALCs throughout the trajectory and delay the occurrence of lymphocytosis, a consequence of ibrutinib’s mechanism of action (Schwarzbich & Witzens-Harig, 2014). Detailed hypotheses of how stress impacts specific trajectory components can be found in the section below.

**Specific Aims**

In a single arm trial of ibrutinib for patients with relapsed/refractory CLL (N=154), the effect of cancer-specific stress on trajectories of ALCs will be studied. For clarity, Figure
4 provides an illustration of the linear components of a projected ALC trajectory and how each relates to the hypotheses.

**Hypotheses**

1. Pre-treatment cancer-specific stress will be positively correlated with pre-treatment ALCs as reflected by the location of the intercept (Figure 4). Patients with higher levels of stress will have higher intercepts.

2. Higher levels of cancer-specific stress will delay the occurrence of treatment-induced lymphocytosis as reflected by differences in slope 1 and the location of the knot. Patients with higher levels of stress will exhibit a flatter slope 1 and the knot will be shifted to the right, indicating a delay in lymphocytosis.

3. Higher levels of cancer-specific stress will be associated with a slower rate of decline from the knot as reflected by slope 2 (i.e., patients with higher levels of stress will have flatter slopes compared to patients with lower levels of stress).

4. As an exploratory hypothesis, ALC trajectories will be compared based on treatment response classifications at 18 months. Category of treatment response will be introduced as a covariate in the model generated for the previous hypotheses. Individuals categorized as treatment non-responders will exhibit higher levels of stress, greater ALCs throughout the trajectory, and delayed occurrences of lymphocytosis relative to treatment responders.
Chapter 2: Methods

Design

Data were collected from a phase II, open-label, non-randomized monotherapy study of ibrutinib in 154 relapsed and refractory CLL patients (see Table 1). The primary aim of the study was to compare progression free survival rates in patients with or without the genetic deletion (del17p). Secondary endpoints for the study included 6-month and best overall response rates, patient-reported emotional distress.

Subjects

The majority were males (71%), Caucasian (97%), and with a mean age of 64.1 years (SD = 10.80 years; range = 26-91). The majority of patients had greater than a high school degree (69%), had an income greater than $50,000 (55%), and were in a relationship with a significant other (86%). Additionally, patients received an average of 3.5 (SD = 2.6; range = 1-16) prior treatments and 51% had disease with del17p.

Procedures

Study procedures were approved by an institutional review board at the Ohio State University. All patients were enrolled after providing informed consent from May 2012 to April 2014 at the Ohio State University Comprehensive Cancer Center – Arthur G. James Cancer Hospital and Richard J. Solove Research Institute. The study was conducted according to the principles of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. Inclusion criteria were
as follows: 1) at least 18 years of age with a confirmed diagnosis of relapsed and refractory CLL, 2) failure of at least one prior therapy, 3) Eastern Cooperative Oncology Group scores (i.e. performance status) greater than 2, 4) life expectancy greater than 2 months, 5) normal organ function, and 6) ability to understand and willingness to sign a written informed consent document.

Exclusion criteria were as follows: 1) receipt of chemotherapy, radiotherapy, or immunotherapy within 4 weeks prior to the first dose of ibrutinib, 2) occurrence of adverse events from agents administered more than 4 weeks ago, 3) receipt of other investigational drugs, 4) secondary malignancies that limited survival to less than two years, 5) malabsorption syndrome or a disease affecting gastrointestinal functioning, 6) life-threatening illness, 7) uncontrolled or active infection requiring antibiotic therapy, 8) significant cardiovascular disease (e.g., symptomatic arrhythmias, congestive heart failure, 9) active central nervous system involvement by lymphoma, 10) pregnant or breastfeeding.

After eligibility was determined, ibrutinib was administered orally at a dose of 420 mg once daily in 28-day cycles. Treatment continued until disease progression or unacceptable toxicity. Patient self-reports of stress and other psychosocial variables were collected at the screening visit and day 1 on cycles 1, 2, 3, 6, 9, 12, 15, 18. Hospital staff collected 6-mL potassium EDTA [lavender-top vacutainer] from peripheral blood in order to provide complete blood cell counts. These blood draws were collected at screening, and day 1 of all subsequent cycles. All patients also had results from cytogenetic analyses available to determine the presence of the genetic deletion, 17p13.
Measures

Impact of Event Scale-Revised (IES-R) – The IES is a 15-item self-report questionnaire that is widely used as a measure of subjective stress (Sundin & Horowitz, 2002). Based on Horowitz’ theory about the stress response, the scale was initially conceived to assess two common responses to stress: intrusion (repetitive thoughts after stressful events) and avoidance (Horowitz, 1975). However, additional items were added in order to assess hyperarousal symptoms in a 22-item revised version of the scale (IES-R; Weiss & Marmar, 1997). Respondents are asked about the frequency they have been experiencing symptoms related to the stressful event in the last seven days. Factor analytic studies indicate that the measure examines three factors: intrusive thoughts (e.g., “I had dreams about being a cancer patient”), avoidant behaviors/thoughts (e.g., “I tried not to talk about it”), and hyperarousal (e.g., “I was jumpy and easily startled”). Participants rate the frequency of these feelings or events in the past week, using a five-point Likert scale ranging from 0 = not at all to 4 = extremely. Past research has demonstrated high internal consistency (Cronbach’s alpha = 0.85-0.91) in cancer patients (Mystakidou, Tsilika, Parpa, Galanos, & Vlahos, 2007; Thewes et al., 2001). Baseline scores from the current study also showed high internal consistency (Cronbach’s alpha = 0.88). For the current study items are summed for a total score that can range from 0 to 88. The IES has been utilized as a predictor of immunity in several cancer studies (Ah, Kang, & Carpenter, 2007; Andersen et al., 1998; Mundy-Bosse, Thornton, Yang, Andersen, & Carson, 2011).

Absolute Lymphocyte Count (ALC) – Absolute lymphocyte counts are used to diagnose new cases of CLL and is regarded as an essential assessment in pretreatment
evaluations (Hallek et al., 2008). Additionally, previous studies have utilized ALCs to track treatment responses to ibrutinib (Byrd et al., 2013; Smith et al., 2015). Lab reports from the James Cancer Hospital provided number of neutrophils, lymphocytes, basophils, monocytes, eosinophils, blast cells, hemoglobin, red blood cells, and platelets.

_Treatment Response_ – Treatment responses at 18 months (or the last-known response) will be used for the exploratory hypothesis. Physician coded treatment responses are defined by the International Workshop on Chronic Lymphocytic Leukemia guidelines and include the following (Hallek et al., 2008):

1) **Complete response**: Patients have normal complete blood counts (ALC < 5,000 µ/L, platelets > 100,000 µ/L, hemoglobin > 11.0 g/dl), absence of constitutional symptoms, and no enlargement of the lymph nodes, spleen, or liver.

2) **Partial response**: Patients have a > 50% decrease in ALC from pre-treatment value, a > 50% decrease in lymph node, spleen, or liver enlargement, and meet at least one of the three normal complete blood count criteria listed for complete responders.

3) **Partial response with lymphocytosis**: Patients meet partial response criteria but have elevated ALCs indicative of lymphocytosis.

4) **Progressive disease**: Patients have a > 50% increase in the products of at least two lymph nodes, a > 50% increase in liver and/or spleen size, or transformation to a more aggressive histology (e.g., Richter’s syndrome).

5) **Stable disease**: Patients did not fulfill criteria for the aforementioned treatment responses.
**Sample Size**

The Phase II trial was powered to evaluate progression free survival. For this outcome the 2-year progression free survival for patients with the genetic deletion was estimated to be 55%. Progression free survival for patients without the genetic deletion was projected to be 70%. A priori power analysis indicated that a sample size of 146 would be sufficient to detect a significant difference given the estimations of progression free survival for patients with and without the genetic deletion. Furthermore, a comparable study (N=116) found that treatment arm (treatment naïve vs. high risk and relapsed/refractory CLL) predicts ALC values in a phase II open label ibrutinib study (Smith et al., 2015). Patients were followed over a median time of 8 months (range = 1-12) and provided a median of 13 blood samples (range= 2–20) for ALC.

The primary outcome of the proposed study is ALC. This study was not powered to evaluate secondary outcomes (i.e. stress and ALC values) with the aforementioned hypotheses. Previously cited data, however, suggests that a sample size of N=146 is adequately powered to examine all hypotheses.

**Analysis Plan**

Descriptive statistics on demographic variables as well as ALCs and IES scores for each timepoint (9) were using SPSS 22 (IBM, 2014). Trajectories of ALCs in patients treated with ibrutinib will be modeled using a mixed model with a random changepoint as implemented in R ‘segmented’ package (Muggeo 2008, 2014). This type of model allows for the identification of a knot, or changepoint (Cudeck & Klebe, 2002). Additionally, using random and fixed effects (i.e. mixed effects) allows for correlated observations between timepoints (9) for each individual (Fitzmaurice, Laird, & Ware, 2011). This
permits the analysis of between-subject and within-subject variance in longitudinal observations. Thus a random changepoint model with varying intercepts, knots, and slopes and can be represented by the equation:

\[ Y_{ij} = \beta_{0i} + \beta_1 t_{ij} + \delta_i (t_{ij} - \varphi_i) + \epsilon_{ij}, \quad j = 1, \ldots, n_i, \]

where \( i \) represents the individual, \( j \) represents the time point of the observation. For each subject \( i \), the parameter \( \beta_0 \) is the mean ALC at baseline and \( \varphi \) is the changepoint in the trajectory. Given this, \( \beta_1 \) is Slope 1 (Figure 4) when \( t < \varphi \), and \( \delta \) is the difference in slopes. Therefore, \( \beta_1 + \delta \) is Slope 2, when \( t \geq \varphi \). Each parameter is specified as a sum of fixed and random effects.

In applying this to the proposed study, changepoint models will provide a trajectory for the longitudinal observations of ALCs. Specifically, changepoint models will provide pretreatment ALCs (intercept), the time when lymphocytosis occurs (changepoint), and the rate of change in ALCs before and after lymphocytosis. In order to evaluate hypotheses, cancer specific stress will be tested as a predictor for each parameter. Treatment response categories (e.g. treatment responders vs. treatment non-responders) will be tested as a predictor for the exploratory hypothesis. Additional covariates included age, gender, prognostic variables, and treatment history. Models were fitted with the ‘nlme’ and ‘segmented’ packages in R, which use a likelihood framework. In order to escape possible local solutions, estimates were refined via a bootstrap restarting approach (Muggeo 2016). For all changepoint models, parameter estimates were calculated using 100 bootstrap samples.
Chapter 3: Results

Preliminary Analyses

All 152 participants completed the screening and baseline assessments. At the 2\textsuperscript{nd} month assessment, 147 participants (96.7\%) completed psychosocial questionnaires and provided blood for immunologic assessment. Steady attrition continued throughout the study; 110 participants (72.4\%) completed the 18-month follow-up assessment (see Figure 5 for CONSORT diagram). Of the 152 enrolled participants, 16 (10.5\%) died, 16 (10.5\%) were taken off the study drug for progressive disease by 18 months, and 10 (6.6\%) were lost to follow up for other reasons (e.g., taken off drug due to adverse events or withdrew consent).

Means and standard deviations for the IES scores and absolute lymphocyte counts can be found in Table 2. Screening stress scores were significantly correlated with ALC values at screening ($\rho = .20$; 95\% CI = 0.01, .39) and treatment initiation stress scores were correlated with treatment initiation ALC values ($\rho = .24$; CI = 0.8, .38). These correlational analyses would confirm hypothesis 1 that pre-treatment stress scores will be positively correlated with absolute lymphocyte counts, indicating that individuals with higher levels of stress begin treatment with higher lymphocyte counts.

Distributions of stress and lymphocyte counts were positively skewed and were log transformed for model estimations. For the exploratory hypothesis, treatment responses were recoded to be dichotomous (0 = treatment non-responder; 1 = treatment
Patients who were coded as having a complete response (n=1), partial response (n=87), and partial response with lymphocytosis (n=10) at the 18-month follow-up were recoded as treatment responders (n=98). Patients who were coded as stable disease (n=18), progressive disease (n=16), or who died during the study (n=16) by the 18-month follow-up were recoded as treatment non-responders (n=52).

**Missing Data**

For participants who missed no more than 25% of all items on a scale, scores were calculated by averaging across the number of items completed and multiplying that by the possible number of items. If a participant did not complete more than 75% of a scale and/or subscale their score on that scale was regarded as missing. Examining the missing data pattern revealed there are 1,150 instances (84.1%) out of a total of 1,368 observations of complete data for participants. There 53 instances (3.8%) of a participant missing a total score on the IES, 6 instances (< .01%) of a participant only missing a value for absolute lymphocyte count, and 159 instances (11.6%) of a participant missing both a total score on the IES and an absolute lymphocyte count measurement for a total of 218 (15.9%) instances of missing data.

The 159 instances of missing stress scores and lymphocyte counts are due to reasons of attrition and cannot be classified as missing at random. Scores that were missing at random (i.e., not attributable to death, progressive disease or other reasons of attrition) were imputed using the MICE (multiple imputation by chained equations) in R (van Buuren & Groothuis-Oudshoorn, 2011). This package can handle data that is missing at random (e.g., participant not completing items at a particular timepoint). Missing values for IES total scores and absolute lymphocyte counts were imputed using
predictive mean matching by using other variables as predictors. Predictor variables include variables included in the data models (IES total scores and absolute lymphocyte counts) as well as variables that are not included in the model but may be correlated with imputed scores (e.g. demographic and disease characteristics).

Five sets of imputed scores were calculated using the MICE package and checked to ensure values were within the possible range. Each of the five sets of imputed scores were evaluated in the final selected model to ensure results were not dependent on which imputed values were used. All parameter estimates for each set of imputed data were within the confidence interval for the first iteration of imputed data. Therefore, only model results from the first iteration of imputed data are presented for simplicity.

**Changepoint Models**

An initial mixed model with a random changepoint was fitted to examine the trajectory of absolute lymphocyte counts over time without the predictor variable and covariates. A scatterplot and spaghetti plot of absolute lymphocyte count trajectories can be found in Figures 6 and 7. The first model does not include the predictor variable and covariates. Parameter estimates for model 1 can be found in Table 3. Parameter estimates indicate that the changepoint occurs between the first and second cycles of treatment ($\phi = 1.40$, 95% CI = 0.98, 1.84) and is preceded by a steep increase ($\beta_1=1.19$, CI = 0.94, 1.44) and followed by a gradual decrease ($\delta=-1.37$, CI = -1.64, -1.10). Variances of the random effects suggest higher heterogeneity among the intercept ($\sigma_{\beta_0}=1.43$) and changepoint $\phi=0.54$) compared to the first ($\sigma_{\beta_1}>.001$) or second slope ($\sigma_{\delta}=.06$). The model residual standard deviation is estimated at 0.91. Given the small variance in the first slope, it was not included as a random effect in subsequent models.
Covariates and stress scores were fitted for two subsequent models such that effects of stress on components of a changepoint trajectory (Figure 4) could be evaluated. Model 2 was fitted with a block diagonal covariance matrix with no additional correlation structure while an autoregressive covariance structure was included for model 3. An autoregressive covariance structure is recommended for longitudinal, repeated measures designs (Little, Pendergast, & Natarajan, 2000). An autoregressive covariance structure specifies that measurements from the same subject taken at adjacent timepoints are highly correlated and decrease toward zero as the time between measurements increases.

Parameter estimates for models 2 and 3 can be found in Tables 4 and 5, respectively. A loglikelihood ratio test indicates an autoregressive covariance structure (model 3) results in a better fitting model ($\chi^2(1) = 101.84$, $p < .001$).

Parameter estimates for model 3 (Table 5) indicate that the changepoint is occurring between cycles 1 and 2 of treatment ($\phi = 1.69$, CI = 1.20, 2.18) and is preceded by a steep increase in absolute lymphocyte counts ($\beta_1 = 0.99$, CI = 0.63, 1.35) and followed by a gradual decrease ($\delta = -1.15$, CI = -1.52, -0.79). Stress parameter estimates suggest stress increases result in absolute lymphocyte count elevations at baseline ($\chi_I = 0.13$, CI = 0.02, 0.25). However, stress did not affect the location of the changepoint ($\phi_{x_1} = 0.03$, CI = -0.15, 0.22), or the steepness of slope 1 ($\beta_{x_1} = -0.11$, CI = -0.23, 0.01) or slope 2 ($\delta_{x_1} = 0.10$, -0.01, 0.22). While parameter estimates were not significant, there is a trend consistent with the hypotheses such that stress results in flatter slopes; higher levels of stress result in a more gradual increase to the changepoint and a more gradual decrease after the changepoint.
Sociodemographic and disease variable covariates were not significant. Variances of the random effects suggest higher heterogeneity among the intercept (\(\sigma_{\beta_0}=1.01\)) with less heterogeneity at the changepoint (\(\sigma_\phi=0.23\)) and with stress (\(\sigma_\chi_1=0.10\)). Variance of the difference slope indicates little to no heterogeneity (\(\sigma_\delta<.001\)). The model residual standard deviation is estimated at 1.18. Figure 8 provides a visualization of the mixed random changepoint model for a subset of individuals along with a comparison of the population estimate, which is based on fixed effects only. As indicated from random effects estimates, much of the variation is accounted for by the intercept (e.g. subject 30 or subject 37), while little there appears to be little variation of the slopes or changepoints. Finally, the autocorrelation function (\(\phi=0.53\)) indicates that measurements between timepoints were moderately correlated.

**Exploratory Changepoint Model**

Treatment response was added as an additional covariate to model 3. Figure 9 provides scatterplots of absolute lymphocyte counts for treatment responders and treatment non-responders along with a line representing the population average. Parameter estimates indicate that treatment response is not significantly associated with absolute lymphocyte count trajectories at baseline (\(x_2=0.15, \text{CI}=-0.35, 0.64\)). However, parameter estimates indicate that treatment non-responders had a delay of when the changepoint occurs (\(x_2\phi=-0.95; \text{CI}=-1.50, -0.41\)). That is, lymphocytosis occurs approximately one month prior for treatment responders compared to treatment non-responders. Fixed effects of stress are not significant in model (\(x_1 = -0.002; \text{CI}=-0.06, 0.06\)).
Chapter 4: Discussion

The present study examined the impact of stress on trajectories of ALCs in relapsed/refractory CLL patients treated with ibrutinib. Higher levels of psychological stress have been associated with higher mortality rates in individuals with 2 or more long-term health conditions (Prior et al., 2016) and with individuals with a history of solid tumor cancers (Hamer, Chida, & Molloy, 2009). There are too few studies that examine stress’s impact on disease trajectories in cancer; and the majority of studies are limited to animal models. No prior study has evaluated psychological stress and immunity longitudinally in hematologic cancer patients. Addressing this lack of knowledge becomes even more vital when patients undergo novel treatments. While past researchers often assess whether treatment type predicts stress (Admiraal, Reyners, Hoekstra-Weebers, 2013), few studies assess whether stress impacts treatment responses. The current study provides a novel way to address these research gaps by accounting for how psychological stress impacts disease trajectories in patients undergoing a new cancer therapy.

Results indicate that higher levels of stress result in higher pretreatment ALCs. These results may reflect that chronic stress, via impaired T-cell mediated immunity, promotes an environment conducive to CLL cell proliferation and survival (Riches & Gribben, 2013). Assessment of baseline/pretreatment psychosocial variables such as stress is important, as previous research has found that higher levels of baseline
hopelessness predicted shorter survival times at two years in women with ovarian cancer (Price et al., 2016). Stress was not associated with the ALC trajectory between treatment initiation and lymphocytosis (slope 1), the time period after lymphocytosis (slope 2), or the occurrence of lymphocytosis. However, there was a trend such that higher levels of stress resulted in a more gradual increase in ALC values before lymphocytosis (slope 1) and a more gradual decline in ALC values after lymphocytosis (slope 2). Stress does not appear to hinder ibrutinib’s mechanism of action (i.e., inhibition of BCR signaling and redistribution of CLL cells from the tissue to the bloodstream), which is reflected by the occurrence of lymphocytosis. However, Woyach and colleagues (2014) have described that in cases of prolonged lymphocytosis, CLL cells in the bloodstream may exhibit BCR signaling. While this signaling does not allow CLL cells to proliferate in the blood stream it does help these cells survive. This combined with prior research demonstrating chronic stress increases T-regulatory cells and decreases T-helper cells (Dhabhar 2013; Partecke et al., 2016), suggests stress may create an environment advantageous to CLL cell survival after lymphocytosis. This may explain why individuals with higher levels of stress exhibit greater ALCs after lymphocytosis.

While changes in T-cell mediated immunity may help explain the effect of stress post-lymphocytosis it is more difficult to explain why higher stress results in a more gradual slope in the time period prior to lymphocytosis. Prior research has observed an inverse correlation between T-regulatory cells and lymphocyte doubling time, indicating that the higher the T-regulatory cell count the shorter time it takes for ALC values to double (Lad et al., 2013). Short lymphocyte doubling times (i.e., less than 6-months) are a marker of active disease and strong indicator to begin treatment (Hallek 2015). Given
this, some patients may have been exhibiting elevations in ALCs before the start of treatment. While there are no established ALC maximums, individuals who initiate ibrutinib with higher ALCs may have a shorter “distance” to reach lymphocytosis. Since individuals with higher levels of stress were likely to initiate treatment with higher ALC values, ALC increases to lymphocytosis (which did not vary by stress) are likely to be more gradual. Past studies have utilized change in ALC values from baseline as a primary outcome (Byrd et al., 2015); future studies may want to utilize ALC change from baseline as a way to standardize outcome variables.

Stress did not impact the occurrence or timing of the changepoint. One factor that may account for this null finding is the low observed rate of stress across the trajectory. Median values of stress peaked before treatment (median = 11), continued to decline at treatment initiation (median = 9), and remained constant throughout the rest of the trajectory (median = 4). These decreases in stress mirror the improvements in overall quality of life that occur during ibrutinib treatment for relapsed/refractory CLL patients (Burger et al., 2014). However, given the range of scores for the IES (0-88), results indicate that the majority of patients were not endorsing high levels of stress throughout the trajectory. In this sample, patients with relapsed/refractory CLL had received an average of 3.5 prior therapies. Effects of stress on ALCs may be magnified in the treatment naïve or newly diagnosed, as these populations have been observed to exhibit more stress (Carlson et al., 2013) and higher lymphocyte increases (Smith et al., 2015). Future studies may want to consider utilizing multiple methods of measuring stress (i.e. physiological measurements such as salivary cortisol) or employing other self-reported assessments. Additionally, analyses involved examination of concurrent measurements of
stress and ALC and did not examine time-lagged associations. Prior research has found lagged associations between acute stress and markers of inflammation (Slavish, Graham-Engeland, Smyth, & Engeland, 2015) as well as depression and disease progression six months later in rheumatoid arthritis patients (Overman et al., 2012).

The linear changepoint models for the current study were estimated using a likelihood based framework and have been used to estimate longitudinal changes in depressive symptoms among three treatment groups (Mueggo et al., 2014). Other researchers have used Bayesian estimation as well as non-linear estimators to estimate the changepoint of cognitive decline (Yu et al., 2012) and T-cell counts in immunocompromised populations (Chu et al., 2005). The likelihood-based framework used for the current study is computationally less burdensome compared to Bayesian alternatives. However, it is unclear whether alternative methods may result in a better-fitting, albeit more computationally burdensome, model.

One limitation is that the present study does not allow for causal inferences to be made on the association between stress and immunity; it is an observational study. Furthermore, all patients in the current sample received the same treatment, meaning that there are no comparison groups. Similar statistical methods can be employed in future comparative studies that aim to assess immunological or psychological changes based on treatment group. For example, adding a treatment naïve group to the current study would allow for examination of stress and ALC trajectories and compare those trajectories to those receiving ibrutinib. Results from the present study indicate that much of the individual variability occurs at the intercept (pretreatment) where higher levels of stress were associated with higher ALCs. Examining changepoint models in a treatment naïve
group could help answer whether the relationship between stress and ALC persists over time.

One strength of this study is its relatively large sample size. Additionally, this is one of the few studies that has examined the impact of psychological stress on immunity in a sample of patients with a hematological cancer. Previous research, specific to CLL, has been limited to longitudinal changes in quality of life (Holzner et al., 2004; Levin et al., 2007, Pashos et al., 2013; Shanafelt et al., 2007). The current sample only included patients with relapsed/refractory CLL. Future studies should examine the impact of stress on immunity in the newly diagnosed or treatment naïve CLL patients.

In summary, this study’s results should encourage clinicians who are considering using ibrutinib for relapsed/refractory CLL patients as results indicate that individual ALC trajectories tended to follow a similar pattern. While higher levels of stress are associated with higher ALC values prior to treatment, stress does not impact ALC trajectories after treatment initiation. Furthermore, while model estimates indicate there is some individual variability about the changepoint and with stress levels, much of the variability occurs at the intercept (i.e. treatment initiation) when median stress levels were at their highest. These results suggest that assessment and management of psychological stress should occur prior to initiation of cancer treatments. Though stress did not impact treatment ALC trajectories, exploratory analysis revealed differing ALC trajectories for treatment responders compared to non-responders. However, it is unclear whether these groups can be differentiated based on clinical or demographic factors.
References


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Wodarz, D., Garg, N., Komarova, N. L., Benjamini, O., Keating, M. J., Wierda, W. G.,


Appendix A: Tables

Table 1:
Sociodemographic and disease variables

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=152)</td>
</tr>
<tr>
<td><strong>Sociodemographics</strong></td>
<td></td>
</tr>
<tr>
<td>Age (in years), M (SD)</td>
<td>64.1 (10.80)</td>
</tr>
<tr>
<td>Gender (Male)</td>
<td>107 (71%)</td>
</tr>
<tr>
<td>Married/Partnered (Yes)</td>
<td>131 (86%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>147 (97%)</td>
</tr>
<tr>
<td>African-American</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
</tr>
<tr>
<td>High School/Technical School or Below</td>
<td>44 (29%)</td>
</tr>
<tr>
<td>Some College/College Graduate</td>
<td>58 (38%)</td>
</tr>
<tr>
<td>Some Graduate School/Graduate Degree</td>
<td>46 (30%)</td>
</tr>
<tr>
<td>Missing</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>Household income (K)</td>
<td></td>
</tr>
<tr>
<td>≤ 50</td>
<td>41 (27%)</td>
</tr>
<tr>
<td>51-100</td>
<td>38 (25%)</td>
</tr>
<tr>
<td>&gt;100</td>
<td>46 (30%)</td>
</tr>
<tr>
<td>Prefers Not to Answer</td>
<td>24 (16%)</td>
</tr>
<tr>
<td>Unknown to Participant</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (1%)</td>
</tr>
<tr>
<td><strong>Disease Variables</strong></td>
<td></td>
</tr>
<tr>
<td>Number of prior therapies, M (SD)</td>
<td>3.5 (2.6)</td>
</tr>
<tr>
<td>Deletion of 17p (Yes)</td>
<td>78 (51%)</td>
</tr>
<tr>
<td>Unadjusted Charlson Comorbidity Index(a), M(SD)</td>
<td>2.5 (1.0)</td>
</tr>
<tr>
<td><strong>Treatment Response</strong></td>
<td></td>
</tr>
<tr>
<td>Complete Response</td>
<td>1 (.7%)</td>
</tr>
<tr>
<td>Partial Response</td>
<td>87 (57%)</td>
</tr>
<tr>
<td>Partial Response with Persistant</td>
<td>10 (7%)</td>
</tr>
<tr>
<td>Lymphocytosis</td>
<td></td>
</tr>
<tr>
<td>Stable Disease</td>
<td>18 (12%)</td>
</tr>
<tr>
<td>Progressive Disease</td>
<td>16 (11%)</td>
</tr>
<tr>
<td>Death</td>
<td>16 (11%)</td>
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</table>
Table 2:

Descriptive statistics by timepoint

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean(SD)</td>
<td>Mean(SD)</td>
<td>Mean(SD)</td>
<td>Mean(SD)</td>
<td>Mean(SD)</td>
</tr>
<tr>
<td>IES Total</td>
<td>12.33(10.23)</td>
<td>11.81(10.65)</td>
<td>7.07(8.02)</td>
<td>6.74(7.79)</td>
<td>6.72(7.31)</td>
<td>6.38(7.28)</td>
<td>6.40(8.08)</td>
<td>6.79(8.17)</td>
<td>6.85(8.21)</td>
</tr>
<tr>
<td>ALC</td>
<td>50.61(75.94)</td>
<td>58.37(58.26)</td>
<td>83.75(83.15)</td>
<td>82.79(87.03)</td>
<td>48.40(48.23)</td>
<td>30.42(36.84)</td>
<td>18.80(28.33)</td>
<td>12.48(25.55)</td>
<td>7.77(19.39)</td>
</tr>
</tbody>
</table>
Table 3:

Parameter estimates from model 1 with a diagonal covariance matrix for the random effects.

<table>
<thead>
<tr>
<th>Fixed Effects</th>
<th>Random Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Est</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.17</td>
</tr>
<tr>
<td>Left Slope</td>
<td>1.19</td>
</tr>
<tr>
<td>Difference</td>
<td>-1.37</td>
</tr>
<tr>
<td>Slope Changepoint</td>
<td>1.41</td>
</tr>
</tbody>
</table>
Table 4:

Parameter estimates from model 2 with a diagonal covariance matrix

<table>
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<tr>
<th></th>
<th>Fixed Effects</th>
<th>Random Effects</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Est</td>
<td>SE</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.15</td>
<td>0.78</td>
</tr>
<tr>
<td>Left Slope</td>
<td>1.60</td>
<td>0.13</td>
</tr>
<tr>
<td>Difference Slope</td>
<td>-1.77</td>
<td>0.13</td>
</tr>
<tr>
<td>Changepoint</td>
<td>1.35</td>
<td>0.15</td>
</tr>
<tr>
<td>Stress</td>
<td>0.19</td>
<td>0.06</td>
</tr>
<tr>
<td>Stress*Left Slope</td>
<td>-0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Stress*Change</td>
<td>-0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Stress*Diff Slope</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Age</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.12</td>
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<td>0.05</td>
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<tr>
<td>Del17p</td>
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Table 5:

Parameter estimates from model 3 with an autoregressive covariance structure.

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<th>Random Effects</th>
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<tr>
<td>Stress*Diff Slope</td>
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<tr>
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<tr>
<td>Gender</td>
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<td>-</td>
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<tr>
<td>Prior Therapies</td>
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<tr>
<td>Del17p</td>
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<td>-</td>
</tr>
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</table>

Note: Autocorrelation function: 0.53
Appendix B: Figures

Figure 1: The immune system and its components
Figure 2: Types of lymphocytes with their specific function.
Figure 3: Kaplan Meyer survival plots comparing complete responders/partial responders and partial responders with lymphocytosis
Figure 4: Components of a linear trajectory of absolute lymphocyte counts.
Figure 5: Study enrollment flow chart
Figure 6: Scatterplot of lymphocyte counts by timepoint with fixed effects changepoint line
Figure 7: Spaghetti plot of absolute lymphocyte count trajectories for first 25 patients
Figure 8: Trellis plots for individual subjects
Note: Solid lines indicate model 3 fit, while dashed lines indicate population average fit.
Figure 9: ALC trajectories for drug-responders and drug non-responders