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It is entitled:
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The Relationship of 6-Mercaptopurine Medication Adherence to Clinical Outcomes in Pediatric Cancer

A dissertation submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Psychology of the College of Arts and Sciences

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ABSTRACT

Objective. To investigate a multidimensional framework for investigating oral 6-mercaptopurine (6MP) medication adherence using indirect (i.e., behavioral: electronic monitoring) and direct measures (i.e., pharmacological: metabolites) of adherence to predict clinical outcomes over time in a multisite cohort of pediatric patients diagnosed with ALL or LBL. Methods. Adherence to 6MP and its relationship to clinical outcomes were examined for 139 patients ages 7-19 years diagnosed with ALL or LBL across six centers. Medication adherence was measured using two objective methods: behavioral adherence (i.e., electronic monitoring of 6MP) and pharmacological adherence (i.e., metabolite profiles of 6MP). Behavioral measures of adherence were collected daily and downloaded at three-month intervals. Pharmacological measures of adherence were obtained at three-month intervals via serum assay. Health outcomes were measured at quarterly intervals through medical chart reviews. Results. Unconditional growth curve modeling indicated that the mean percentage of behavioral adherence was 84.4% at baseline and declined over 15 months of follow-up to 75.2%. Three trajectories of 6MP behavioral adherence were identified: 1) optimal adherence (67.1% of patients): averaging 95% behavioral adherence across 15 months of follow-up; 2) moderate adherence (20%): relatively stable poor adherence (67%); and, 3) chronically nonadherent (12.9%): behavioral adherence decreased from 62.7% to 30%. Three metabolite profiles were identified: high levels of one metabolite and low levels of the second metabolite were considered adherent: 1) high TGN-low MMP (18.9%); and, 2) low TGN-high MMP (46.2%). Low levels of both metabolites were considered nonadherent: low TGN-low MMP (34.9%). Patients presenting with the nonadherent metabolite profile (low TGN-low MMP) had the lowest adherence rates over time (70% to 76%) relative to those presenting with adherent metabolite profiles (85 to
90%). With respect to patterns of medication adherence and relationship to clinically-relevant health outcomes, there were no significant differences observed between patients in the adherent versus nonadherent behavioral adherence trajectories with respect to mean absolute neutrophil counts (ANC), risk for infection as measured by ANC, healthcare utilization, or risk for relapse. On the other hand, there were significant differences observed among the three metabolite profiles and clinically-relevant health outcomes. Those patients in the low TGN-low MMP metabolite group had higher ANCs, had a decreased risk for infection, and were at a higher risk for relapse relative to the adherent metabolite profiles. There were no differences observed among the three metabolite clusters with respect to healthcare utilization. Conclusions. This study provided the first example of how behavioral adherence can be mapped on to pharmacological measures of medication adherence in a study of pediatric cancer. Although behavioral patterns of medication adherence deteriorated over time, pharmacological measures of medication adherence indicated relatively stable patterns of medication adherence across the 15 month period. Medication adherence predicted ANC and relapse rates. Adherence promotion interventions might be tailored to subgroups of patients who demonstrated problematic patterns of treatment adherence that could place them at risk for relapse. In addition, patients who demonstrate adequate levels of medication adherence could benefit from less intensive, preventative interventions to sustain and improve their adherence over time.
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Chapter 1. Introduction

Pediatric Cancer: Acute Lymphoblastic Leukemia (ALL) and Lymphoblastic Lymphoma (LBL): Prevalence, Survival Rates, and Medical Treatment

Pediatric cancer treatment has evolved in the past 20 years. Survival rates have increased from 58% in 1977 to 89% in 2005 (National Cancer Institute, 2008). Improvements in survival are largely related to the progress made in clinical trials that test the efficacy and side effect profiles of cancer treatment (Brennan, Federico, & Dyer, 2010; National Cancer Institute, 2008). Although survival rates for pediatric acute lymphoblastic leukemia (ALL) have improved, it was estimated that approximately 442 pediatric deaths (infancy to 14 years) in 2010 were attributed to leukemia and ALL continues to result in more deaths than any other form of cancer in patients’ ages 10 to 19 (American Cancer Society, 2010; National Cancer Institute, 2008).

Acute lymphoblastic leukemia (ALL) and cancers of the brain and central nervous system account for more than 50% of the current cancer diagnoses, with ALL being the most common form of pediatric cancer (American Cancer Society, 2010). Hodgkin and Non-Hodgkin Lymphomas are the third most common childhood malignancy. Lymphoblastic lymphoma (LBL) affects 30% of pediatric patients diagnosed with non-Hodgkin’s lymphoma (National Cancer Institute, 2008). Similarities in morphology, genetics, and immunophenotypes between LBL and ALL indicated that ALL and LBL be considered as part of a spectrum of malignant lymphoproliferative disorders (Reddy & Perkins, 2004). Furthermore, ALL and LBL require similar cancer treatment protocols (Weiss, Bindl, Picozzi, Link, & Warnke, 1986).

Children and adolescents with ALL and LBL undergo both an intensive phase of cancer treatment as well as a maintenance phase of treatment (Lau, Matsui, Greensberg, & Koren, 1998; Pai, Drotar, & Kodish, 2008). The intensive phase of cancer treatment can last several months up
to one year depending on diagnosis, gender, age, response to treatment, and clinical severity (Lau et al., 1998; Pai et al., 2008). The intensive phase of cancer treatment typically involves extensive inpatient stays, at home nursing care once discharged from hospital, and medical treatment that is directly administered by the medical team. Once the patient’s ALL and LBL is in remission, children and adolescents undergo maintenance therapy, which lasts approximately 2-3 years and involves multiple medications given on an outpatient basis. Maintenance therapy is considered vital for survival and long-term success for pediatric patients diagnosed with ALL and LBL, and contributes to a decreased likelihood for adverse clinical outcomes, such as relapse (Bhatia et al., 2012; Davies & Lilleyman, 1995; Kennard et al., 2004; Pritchard, Butow, Stevens, & Duley, 2006; Relling, Hancock, Boyett, Pui, & Evans, 1999). In fact, relapse prevention is the primary goal, which necessitates patient adherence to a lengthy and complex course of maintenance therapy that may be very difficult for many children, adolescents, and their parents to implement in accord with prescribed recommendations (Nachman et al., 2006).

During maintenance treatment, patients are required to take several oral medications as prescribed, including those that are prescribed daily (e.g., 6-mercaptopurine) and those with a variable administration (e.g., methotrexate is a medication that is taken for five consecutive days once per month); receive monthly injections; take other medications as needed (e.g., steroids, pain medication, etc.); and, attend monthly medical follow-ups (Kondryn, Edmondson, Hill, & Eden, 2010; Pai et al., 2008). A prescribed daily dose of 6-mercaptopurine (6MP), which is an oral chemotherapy medication is a cornerstone of ALL and LBL treatment across all centers in the U.S. and totals approximately 548 to 1125 doses over the course of maintenance treatment. Such multifaceted treatment is very burdensome for children, adolescents, and their families.
(Kennard et al., 2004; Malbasa, Kodish, & Santacroce, 2007; Pai et al., 2008; Pritchard et al.,
2006).

Scope, Prevalence, and Impact of Nonadherence to Medical Treatment for Pediatric
Chronic Illness, Including Pediatric Cancer

Nonadherence to medical treatments is defined as not completing a treatment regimen
that was prescribed or recommended by a health care provider (Drotar, 2000; Osterberg &
Blaschke, 2005; Rapoff, 2010). Nonadherence to prescribed treatment regimens in pediatric
chronic illness is a significant problem and may have an impact on child and adolescent long-
term health outcomes. As noted by the World Health Organization, treatment nonadherence is
prevalent with rates of 50% or greater across pediatric chronic illness populations (WHO, 2003).
Consequences of nonadherence to medical treatment regimens include: increased symptoms
(Osterberg & Blaschke, 2005), functional impairment (Drotar, 2000; Rapoff, 2010), medical
complications (Osterberg & Blaschke, 2005), increased health care utilization (WHO, 2003), and
mortality (Simpson et al., 2006). Moreover, the economic costs of nonadherence are very high in
the U.S. and worldwide (Cutler & Everett, 2010; Drotar, 2000; Lee, Balu, Cobden, Joshi, &
Pashos, 2006; Osterberg & Blaschke, 2005; Rapoff, 2010; Simpson et al., 2006; WHO, 2003).

The importance of treatment adherence in the outcomes of ALL and LBL necessitates a
methodologically sound study of adherence in current cohorts of children and adolescents
diagnosed with ALL and LBL. More than 20 published studies have reported data on treatment
adherence in ALL and related conditions such as lymphoma. Previous research has suggested
that nonadherence to prescribed medication, including 6MP, during the maintenance phase of
treatment is very high in children and adolescents diagnosed with cancer. Davies and Lillyman’s
(1995) review identified rates of nonadherence to prednisone and 6MP ranging from 10 to 42%. 

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Similarly, Lau et al. (1998) assessed 6MP adherence over a four to six week period in a sample of youth diagnosed with ALL (ages 2.6 to 17.2 years) and found a mean adherence rate of 89.4% (range 38-100%). In a randomized trial investigating the effectiveness of a videogame in improving adherence to chemotherapy medications in a sample of children, adolescents and young adults (ages 13-29 years), medication adherence was higher in the intervention group (62.3%) compared to the control group (52.5%) (Kato, Cole, Bradlyn, & Pollock, 2008). In a large Children’s Oncology Group (COG) study, adherence rates declined from 94.1% at the end of the first month to 90.2% at six months (Bhatia et al., 2012). Decreased adherence was associated with Hispanic ethnicity, being age 12 years or older, and living in a single-mother household. These researchers examined adherence cutoffs and found that adherence rates of less than 95% were associated with an increased risk of relapse. In fact, nonadherent patients were 2.5 times more likely to relapse compared to adherent patients (Bhatia et al., 2012). Furthermore, 44% of patients enrolled in the COG study over a period of six months were identified as nonadherent based on the cutoff of 95% adherence.

**Measurement of 6-Mercaptopurine Medication Adherence in Pediatric ALL and LBL**

It is well known that systematic and reliable measurement of treatment adherence in pediatric chronic illness is a necessity for determining the precise relationship between medication adherence and its relationship to health outcomes (Quittner, Modi, Lemanek, Ievers-Landis, & Rapoff, 2008). Kenna and colleagues (2005) suggested that adherence measures be considered as one of two categories: 1) *direct measures* such as biological assays or clinician observation of medication ingestion; and, 2) *indirect measures* of medication administration such as self-report/parent-report (*subjective*), medical chart review (*objective*), pill counts (*objective*), or electronic monitoring (*objective*). A large number of studies, including those investigating
adherence to medication regimens in pediatric ALL and LBL, have used physician-, parent-, or patient-reported adherence measures. However, physicians, parents, and patients tend to overestimate adherence levels (Kenna, Labbé, Barrett, & Pfister, 2005; Lau et al., 1998; Riekert & Rand, 2002) and hence these reports are not optimal for identifying and targeting nonadherence. Similarly, pill counts often yield higher adherence rates and do not provide objective information such as the date and time medication was administered (Kenna et al., 2005). On the other hand, electronic monitoring data provides information regarding daily medication taking, including the date/time the medication cap was opened (Kenna et al., 2005; Riekert & Rand, 2002; Vrijens et al., 2005). However electronic monitoring does not provide a direct measure of adherence. For example, patients’ might remove the medication from the bottle but might not actually ingest the medication (Ingerski, Hente, Modi, & Hommel, 2011; Kenna et al., 2005; Lau et al., 1998). For this reason, Kenna and colleagues (2005) suggested examining multiple adherence measures, including metabolite assays, in the context of a multivariate model to examine the influences of adherence on drug exposure, therapeutic efficacy, and clinical outcomes.

Integration of Pharmacological and Behavioral Measures of Treatment Adherence

Although measurement of medication adherence and its potential relationship to clinical outcomes and biomarkers is important in pediatric ALL and LBL, most studies investigating 6MP medication adherence in pediatric ALL and LBL have used a subjective measure of medication adherence (e.g., self, physician, or parent-reported adherence) or a single, objective measure of treatment adherence (e.g., pill counts; electronic monitoring; or, pharmacological methods). To my knowledge, previous research has not described adherence to 6MP medication in the context of multiple, objective, direct, and indirect adherence measures such as behavioral
measures (i.e., real-time assessment of daily medication adherence: electronic monitoring, which is an indirect measure of adherence) and pharmacological measures (i.e., metabolites of 6MP, a direct measure of adherence) of medication adherence. Behavioral and pharmacological measures of 6MP adherence provide useful and potentially complementary data regarding medication adherence across time.

Electronic monitoring, which is recognized as an important indirect objective method for monitoring treatment adherence, has been used in a number of studies and provides an estimate of patients' adherence behavior (e.g., medication taking). However, electronic monitoring does not provide information regarding whether the medication was actually ingested by the patient (Ingerski et al., 2011; Kenna et al., 2005; Lau et al., 1998). On the other hand, metabolite concentration profiles of 6MP provide information regarding 6MP medication adherence for the time period immediately prior to the blood draw (Lau et al., 1998; Pritchard et al., 2006). When 6MP is ingested, it is metabolized into two metabolites: thioguanine nucleotides (TGN) and methylated mercaptopurine (MMP) (Landier et al., 2004; Pentheroudakis & Pavlidis, 2007). Active intracellular metabolites have longer half-lives than the parent drug. The half-life of 6-thioguanine (TGN) and 6-methylated mercaptopurine (MMP) is approximately 5 days following the medication dose (Derijks et al., 2004; Fishman & Mrozek-Orlowsk, 1999).

Metabolite concentration profiles can provide direct objective information regarding medication adherence and can be mapped on to electronic monitoring data. Low concentrations of both metabolites: thioguanine nucleotides (TGN) and methylated mercaptopurine (MMP) are indicative of poor bioavailability of the drug (e.g., inadequate dosing or inability of the patient to absorb the medication), nonadherence, or both and are associated with poor disease prognosis in children with ALL and LBL, including higher risk for disease relapse (Bhatia, 2011;
Chrzanowska, Kolecki, Duczmal-Cichocka, & Fiet, 1999; Gaynon, 2006; Hawwa et al., 2009; Lilleyman & Lennard, 1994; Pai et al., 2008; Traore et al., 2006). However, research has suggested that if a patient is adherent and took 6MP as prescribed, then the expected metabolite profile would be a negative correlation between the two metabolites (i.e., high values of one metabolite would be associated with low values of the other, such as high TGN-low MMP or low TGN-high MMP) (Davies & Lilleyman, 1995; Pai et al., 2008; Traore et al., 2006).

Two previous research studies have used cluster analysis to identify subgroups of individuals who present with similar levels of 6MP metabolite concentrations (Hawwa et al., 2009; Traore et al., 2006). Traore and colleagues (2006) examined metabolite concentration levels of TGN and MMP metabolites for 48 patients (Mage = 15 years, SD = 2 years) and found four groups representing different levels of 6MP metabolite concentrations: 1) high TGN-low MMP metabolite levels, 2) low TGN-high MMP metabolite levels, 3) low TGN-low MMP metabolite levels, and 4) low TGN-very high MMP metabolite levels. Hawwa and colleagues (2009) conducted a similar analysis of 19 pediatric patients diagnosed with ALL ages 3-17 years (Mage = 10 years). These researchers identified three metabolite profiles in their sample: 1) high TGN-low MMP profile, 2) low TGN-high MMP profile, and 3) low TGN-low MMP profile.

**Methodological Problems in Previous Research**

Previous research describing medication adherence in pediatric ALL and LBL has been limited by significant methodological problems, which limits internal validity and generalizability of study findings. Significant limitations of previous research included collecting data at a single site and recruiting small sample sizes (N = 19 to 68) with the exception of three studies (Bhatia et al., 2012; Kato et al., 2008; Lennard, Welch, & Lilleyman, 1995). Previous research has typically used physician- or self-reported adherence measures, which typically
overestimate adherence levels (Lau et al., 1998; Riekert & Rand, 2002). Another methodological problem that is addressed by the proposed study is the lack of data on the combined use or integration of indirect measures of adherence such as behavioral adherence and direct measures of adherence such as pharmacological measures of 6MP adherence in one study. These two measures provide objective data regarding 6MP dosing patterns (i.e., daily adherence as measured by electronic monitoring) and drug intake as measured by metabolite levels of 6MP. Furthermore, no studies have examined the collective contribution or relationship between these two objective measures to clinically-relevant outcomes.

Additionally, research investigating medication adherence in pediatric ALL and LBL has typically been cross-sectional or time-limited (e.g., a few weeks to a month), which limits the clinical significance of research for understanding how adherence may impact clinical outcomes (Hawwa et al., 2009; Lau et al., 1998). Moreover, those studies that utilized electronic monitoring did not exploit the full potential of electronic monitoring and typically reported the average frequency of adherence as the sole metric in data analyses. Use of modern data analytic methods (e.g., growth curve analysis, group-based trajectory analysis, and cluster analysis) that examine critical patterns of treatment adherence across time was limited in prior research. Such analytic methods are important because they provide detailed information regarding group and individual differences in medication adherence across time.

**Relationship between Adherence in Pediatric ALL or LBL and Clinical Outcomes**

Research has documented that nonadherence to cancer treatment, including ALL and LBL treatment, occurs frequently and contributes to morbidity and mortality in pediatric patients (Bhatia et al., 2012; Festa, Tamaroff, Chasalow, & Lanzkowsky, 1992; Lau et al., 1998; Lennard et al., 1995; Tebbi, 2006). Treatment nonadherence may be related to clinical outcomes and
biomarkers, such as worse disease prognosis, disease relapse, adverse side effects, increased risk for late effects of chemotherapy treatment, and mortality (Bhatia et al., 2012; Davies & Lilleyman, 1995). It has been estimated that nonadherence to prescribed treatment regimens accounted for 33-69% of hospital admissions in the US, which translates to $100 billion healthcare related costs per year (Bhatia, 2011; Osterberg & Blaschke, 2005).

Research has suggested that clinical biomarkers (e.g., white blood cell counts and neutrophil counts) can be used to influence chemotherapy dosing decisions as well as to assess adherence to 6MP (Butow et al., 2010; De Oliveira, Viana, Zani, & Romanha, 2004; Landier, 2011; Malbasa et al., 2007). Both white blood cell counts and absolute neutrophil counts (ANC) were found to be related to the blood cell levels of the cytotoxic metabolites of methotrexate and 6-mercaptopurine, which are commonly prescribed chemotherapy agents for treatment of ALL and LBL (Lennard, Rees, Lilleyman, & Maddocks, 1983; Schmiegelow, 2006). Absolute neutrophil count (ANC) is also an important clinical biomarker to evaluate because it provides information regarding the child’s ability to fight infection. Absolute neutrophil count (ANC) is commonly used to determine hematological toxicity and risk for infection: ANC values of 1500 to 2000: no increased risk for infection; 1000 to 1500: mild risk for infection; 500 to 1000: moderate risk for infection; < 500: high risk for infection (Marrs, 2006; Segel & Halterman, 2008). Low ANC values (i.e., ≤ 500) are indicative of neutropenia (i.e., low numbers of neutrophils which are the most important type of white blood cells), and puts the child at an increased risk for infection (Bhatia, 2011; Hakim et al., 2010; Malbasa et al., 2007). ANC values ≤ 500 will likely result in the medical team putting the patient on a medication hold until the ANC is increased to 500 or greater (Lau et al., 1998; Malbasa et al., 2007; Relling et al., 1999; Segel & Halterman, 2008).
Secondary outcomes such as healthcare utilization and relapse risk are also potentially related to medication nonadherence. Bhatia and colleagues (2012) provided data documenting the association between 6MP medication nonadherence (i.e., adherence rates <95%) and relapse risk. Furthermore, it has been well documented that nonadherence is related to increased hospitalizations (Bhatia, 2011; Osterberg & Blaschke, 2005). However, the relationship of adherence to frequency of healthcare utilization for pediatric cancer patients during the maintenance phase of therapy has not been well examined. Research has documented the relationship between increased healthcare utilization and worse disease outcomes in cancer survivors (Castellino et al., 2005; Robison et al., 2005). However, there is limited data supporting the relationship between medication adherence and increased healthcare utilization during the maintenance phase of cancer treatment. To address these needs, healthcare utilization and relapse risk will be examined in the current study.

**Rationale and Significance of the Current Study**

The primary focus of the current study was adherence to 6MP medication because of its critical importance in the treatment regimen for ALL and LBL. It is an oral medication that is administered daily and can be monitored with an electronic monitor (e.g., MEMS: AARDEX Corporation, Palo Alto, CA), and the metabolites of 6MP (i.e., TGN and MMP metabolites) can be measured reliably and validly (Hawwa et al., 2009; Lennard et al., 1995; Traore et al., 2006). Behavioral and pharmacological measures of 6MP adherence provide complementary data regarding medication adherence across time. This study investigated behavioral and pharmacological measures of adherence and their relationship to clinical outcomes (e.g., healthcare utilization, relapse) and biomarkers (absolute neutrophil count: ANC) in a multisite cohort of pediatric ALL and LBL patients (7-19 years at baseline, \( N = 139 \)) across 15 months.
Prospective studies of adherence have generally noted deterioration in treatment adherence over time (Bhatia et al., 2012; Lau et al., 1998; Pai et al., 2008; Pritchard et al., 2006), but little is known about patterns and changes in 6MP medication adherence over time as measured by two objective methods of adherence during the maintenance phase of treatment for pediatric cancer. This study addressed several limitations of previous research by describing the relationship between an indirect and direct measure of medication adherence: electronic monitoring and pharmacological measures. In addition, the current study provided information regarding how well these measures predict clinical outcomes and biomarkers across 15 months.

**Specific Aims, Research Questions, and Hypotheses**

The current study had three aims. See Table 1 for a description of the relevant research aims, hypotheses, data analyses and purpose of each data analysis associated with aims 1-3 that are presented in the current study.

**Aim 1. Describe 6MP medication adherence patterns over 15 months based on behavioral and pharmacological measures of 6MP medication adherence.** The first aim was to describe 6MP medication adherence patterns over 15 months based on behavioral and pharmacological measures of 6MP medication adherence. Previous research in pediatric ALL and LBL has shown that behavioral adherence typically decreases over time (Bhatia et al., 2012; Lau et al., 1998; Pai et al., 2008; Pritchard et al., 2006). Thus, it was hypothesized that the overall rate of 6MP adherence as measured by behavioral measures would decrease over a 15 month period.

Furthermore, based on baseline (i.e., first month of study enrollment) behavioral adherence patterns of 6MP medication adherence that were observed in the current sample (Rohan, Drotar, Alderfer, et al., 2013), it was hypothesized that there would be group
differences in medication adherence patterns across 15 months. Three groups demonstrating different behavioral adherence patterns over time would be identified in the longitudinal study: 1) optimal adherence, 2) moderate or deteriorating adherence, and 3) chronic nonadherence.

Based on previous research investigating cross-sectional 6MP metabolite concentration levels (Hawwa et al., 2009; Traore et al., 2006), it was hypothesized that there would be three unique metabolite clusters demonstrating different metabolite profiles over 15 months: a group that demonstrated significant nonadherence (i.e., low levels of both metabolites, TGN/MMP), and two groups that demonstrated a negative correlation between the two metabolites, which typically reflects adherence to 6MP as indicated by (Lilleyman & Lennard, 1994): high TGN-low MMP and low TGN-high MMP.

**Aim 2. Document the longitudinal relationship between 6MP adherence measured by behavioral and pharmacological methods.** The second aim of this research was to document the longitudinal relationship between 6MP adherence as measured by behavioral (indirect measure) and pharmacological (direct measure) methods of medication adherence. Previous research has not examined the longitudinal relationship between these two measures. It was hypothesized that over the 15 month period, there would be a moderate relationship between pharmacological measures of adherence and behavioral adherence measures. Those individuals who were identified as nonadherent based on pharmacological methods (i.e., low-low metabolites) would also have the lowest behavioral adherence rates compared to the other metabolite clusters.

**Aim 3. Determine the relationship between behavioral and pharmacological measures of 6MP adherence to clinical outcomes and clinical biomarkers.** Finally, the third aim of this study was to determine the relationship between behavioral and pharmacological
measures of 6MP adherence to clinical outcomes (e.g., healthcare utilization, relapse) and a clinical biomarker (i.e., absolute neutrophil count: ANC). Previous research has suggested the importance of medication adherence in disease prognosis, including reduced risk for relapse and decreased healthcare utilization (Bhatia, 2011; Bhatia et al., 2012). However, no previous research has examined the relationship between behavioral and pharmacological measures of medication adherence and their respective relationship to clinical outcomes. It was hypothesized that consistently lower 6MP adherence rates as measured by behavioral and pharmacological adherence measures would predict worse clinical outcomes (e.g., higher frequencies of healthcare utilization and relapse) over a 15 month period.

In addition, previous research has examined the separate relationship between the two metabolites (TGN or MMP) and absolute neutrophil count (ANC) (Lancaster, Lennard, & Lilleyman, 1997; Lennard et al., 1983; Lennard et al., 1995; Lilleyman & Lennard, 1994), but previous research has not examined the relationship between behavioral and pharmacological (metabolite profiles) measures of medication adherence to a clinical biomarker (ANC). It was hypothesized that consistently lower 6MP adherence rates as measured by behavioral adherence and pharmacological measures of adherence would predict worse outcomes with respect to absolute neutrophil counts (ANC) over 15 months. Those patients identified as being nonadherent to 6MP would likely have an increased risk for infection as indicated by ANC values < 1500.

Chapter 2. Methods

Study Design

This research study was a secondary analysis of data that was collected as part of a prospective, multisite randomized controlled trial funded by the National Cancer Institute (PI:
Drotar; 1R01CA119162). The current study was conducted as part of an NRSA Fellowship Training Grant (F31) funded by the National Cancer Institute (1F31CA168307 to JMR). Adherence and medical data were collected as part of the 15-month longitudinal study of a family-centered problem-solving intervention to promote medication adherence for pediatric cancer. This research study focused on prospective measurement of adherence patterns based on an indirect measure of adherence: behavioral adherence (i.e., electronic monitoring) and a direct measure of adherence: a pharmacological adherence measure (6MP metabolite levels) during maintenance phase therapy and how adherence related to clinical outcomes and biomarkers. The research aims and data analyses presented in this study (see Table 1) were not proposed in the original grant submission. The research strategy and data are unique to this dissertation research.

Participants

Participants were 139 children and adolescents ages 7-19 years (at baseline) diagnosed with acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LBL) and their primary caregivers who were followed at six medical centers in the United States. The World Health Organization discussed similarities in morphology, genetics, and immunophenotypes between lymphoblastic lymphoma and acute lymphoblastic leukemia and suggested that ALL and LBL be considered as part of a spectrum of malignant lymphoproliferative disorders (Reddy & Perkins, 2004). Furthermore, ALL and LBL require similar treatment protocols and both require 6MP oral medication treatment, which provided support for including patients diagnosed with either ALL or LBL in the current research sample. Demographic and medical characteristics of the baseline sample are provided in Table 2. Ethnicity was largely representative of each clinic’s sample. Institutional Review Boards at each site approved the study.

Eligibility Criteria
To be eligible for study participation, participants were prescribed a daily dosage of 6-mercaptopurine (6MP) oral medication, were diagnosed with ALL or LBL in remission, and finished with at least one cycle (84 days) of the maintenance phase of therapy for ALL and LBL. Participants were excluded from study participation \( (n = 7) \): if they were involved in foster care or did not have a primary caregiver available to participate \( (n = 2) \), had known plans to relocate \( (n = 5) \), were diagnosed with a comorbid chronic condition requiring burdensome treatments (e.g., cystic fibrosis), or were diagnosed with an intellectual disability or psychiatric condition that made it difficult to complete study procedures.

In accord with HIPAA guidelines, families were first contacted by their medical provider to gain their permission for the study team to approach the family about the study. If families agreed to be contacted, they were approached by study coordinators at each site to obtain parental permission and consent and assent for children and adolescents ages 11 and older. Verbal assent was obtained for patients 10 years and younger. Of the 171 patients and families approached to participate, 18.7% \( (n = 32) \) refused to participate owing to the following reasons: being too busy \( (n = 12) \), not interested \( (n = 19) \), or having no transportation \( (n = 1) \). Comparisons of patients’ and families who participated in the study with those who did not participate indicated negligible associations with respect to patients’ age \( (d = -0.003) \) and gender \( (Φ = 0.09, p = 0.22) \). However, there was a moderate association in participation rates: non-Hispanic, Caucasian patients’ and families (9.4%) refused participation more compared to Hispanic (3.5%) and non-Hispanic, minority (5.8%) patients’ and families \( (V = 0.23; p = 0.01) \).

**Family-Centered Problem Solving Intervention**

Following the baseline study visit, children and adolescents and their primary caregivers were randomized in equal numbers to one of two groups using a stratified random permuted
blocks scheme design: *Family Problem Solving Training Intervention (FPST) (n = 69)* or *Current Psychosocial Care (CPC) (n = 70).* The primary aim of the RCT involved testing the efficacy of an FPST intervention that addressed barriers to treatment adherence, including: enhancing adolescent and family problem-solving strategies; facilitating parent-adolescent communication and collaboration; and, using behavioral reinforcement to enhance problem-solving skills. The FPST intervention model was a family based approach designed to address specific barriers to medication adherence that were commonly experienced by children with cancer and their families. The FPST included five in-person visits and two phone visits that were designed to enhance the durability of intervention effects. The essential features of the intervention model involved the promotion of parent-adolescent problem-solving and team work in developing solutions to specific barriers to medication adherence that were identified during the intervention sessions; improving child/adolescent-parent communication around cancer treatment and promoting development of collaborative strategies to improve medication adherence; promoting parental support and monitoring of medication adherence; and, utilizing behavioral strategies to reinforce adherence to treatment, including engagement and enhancement of motivation and problem solving methods (Nezu, Nezu, & Perri, 1989).

**Measures**

**Medical Characteristics and Prescribed Medical Treatment.** Medical charts were reviewed at quarterly intervals (baseline, 3, 6, 9, 12, and 15 months) using standardized forms to obtain information regarding: a) prescribed treatment regimens: medication type, dose, and timing of administration; b) healthcare utilization (i.e., number of clinic visits, ER visits, and hospitalizations); c) clinical biomarkers (absolute neutrophil count: ANC); d) and, disease relapse. Prescribed medical treatment was standardized across all sites based on treatment
protocols for ALL and LBL implemented by the Children’s Oncology Group (COG), which facilitated data collection across each site. Information regarding the prescribed treatment regimen was used to operationalize nonadherence (e.g., discrepancy between the daily dosage of 6MP versus what had been taken by the patient as measured by electronic monitoring and pharmacological data). Similar procedures have been used in previous research studies of adherence (Quittner, Espelage, Iever-Landis, & Drotar, 2000; Rohan et al., 2010).

**Medication Adherence.** 6MP medication adherence was measured from baseline to 15 months using two methods: 1) objective, indirect behavioral measures of medication adherence; and, 2) Direct, objective measure of medication adherence: pharmacological measure (Hawwa et al., 2009; Traore et al., 2006).

**Behavioral Adherence Measures: Electronic Monitoring of 6MP.** An electronic monitoring device (i.e., the Medication Event Monitoring System (MEMS®) from the AARDEX Corporation, Palo Alto, CA) was used to monitor adherence to 6MP oral medication therapy across 15 months. The MEMS® system is similar to a prescription bottle, but contains a micro-electronic chip in the cap that registers dates and times when the bottle is opened and closed. Time-stamped medication events were stored in the MEMS® and transferred to a program (i.e., PowerView) that records the daily history of medication taking. This information can be exported to statistical programs for analysis. Patients and families were aware of adherence monitoring, but were not given feedback regarding their medication adherence. Patients and families were instructed to take their 6MP only from the MEMS® bottle for the duration of the study, not to open the bottle unless they are taking a dose of medication at that time, and to close the bottle immediately after removing the prescribed dose. A standardized form was used during each download to capture information regarding extra openings, refills, and periods of nonuse during
the previous three-month period. Adherence was defined as the number of times that oral 
medication was taken as prescribed (Lau et al., 1998). Electronic monitoring of oral medication 
usage has been used by a number of investigators to study medication adherence in a range of 
pediatric chronic illnesses (Rapoff, 2010), including ALL and LBL (Kondryn et al., 2010; Lau et 
al., 1998). Specific methods of quality control of these data are included in Appendix 1.

During the quarterly MEMS downloads, research assistants collected information from 
patients and families about difficulties with using the MEMS cap, including any difficulties that 
were experienced when using the MEMS cap as a proxy to an already established medication 
administration system such as a pill box. Families who used the MEMS Cap with an already 
established medication administration system were asked to open the MEMS cap each time that 
medication was removed from the other medication administration system. Behavioral adherence 
rates for families in either group who indicated difficulties with using the MEMS cap were coded as 
a “non-monitored period” (i.e., missing data). Families in either group who indicated that they 
opened the MEMS device each time that a pill was ingested or removed from a medication bottle or 
pill box were included in all data analyses. Behavioral adherence data for patients and families who 
had periods of nonadherence but did not indicate difficulties with using the MEMS cap were 
included in the analyses and data was captured as true nonadherence. A mixed ANOVA indicated 
that there was a significant difference in five-day adherence rates between those patients and 
families who used a pill box to assist with medication adherence ($M = 70.19\%, SE = 3.91$) and those 
patients and families who used the MEMS bottles normally ($M = 86.95\%, SE = 2.57$) ($F (1,127) = 
6.16, p = 0.01, \eta^2_p = 0.09$). On the other hand, the mean five-day adherence rates did not 
significantly change over time ($p = 0.99, \eta^2_p = 0.00$). Similarly, there was not a significant 
interaction between 5-day behavioral adherence and time ($p = 0.33, \eta^2_p = 0.01$). See Appendix 2
for a graph describing adherence rates over time for normal MEMS users versus proxy users.

Many patients and families used the pill box to limit the difficulties they were having with taking medication as prescribed and the medical team often corroborated this report. Given the information regarding established patterns of nonadherence for the proxy users and the level of quality control in place for behavioral adherence data, the behavioral adherence data reflected here is likely an accurate representation of adherence rates over time and MEMS user type will not be included as a covariate in data analyses.

**Pharmacological Measures of Medication Adherence: 6MP Metabolite**

**Concentrations.** Blood samples, which provide metabolite concentrations of 6MP were obtained at six time points collected at quarterly intervals (baseline, 3, 6, 9, 12, and 15 months) using a standardized bioassay (Traore et al., 2006). High performance liquid chromatography (HPLC) with ultraviolet detection was employed to measure 6MP activity in RBC. The use of red blood cells (RBC), TGN, and MMP concentrations was based on an extensive review of the extant literature on 6MP pharmacology and utility to detect 6MP (Davies & Lilleyman, 1995; Traore et al., 2006). Pharmacology data were entered into a secure database. Metabolite levels at each time point, the last dosage amount, and the date and time of the last dose were mapped on and verified with electronic monitoring data. Specific methods of the serum assay and quality control procedures of these data are included in Appendix 1.

**Thiopurine Methyltransferase (TPMT) Activity.** Thiopurine methyltransferase (TPMT) activity was measured at the first and last blood draw using the same blood samples that were collected for the standardized bioassay described above. TPMT analyses were conducted by the pharmacology lab. Patients and their parents consented separately for genotyping. The genetic data for patients who consented for this analysis was entered into the
same secure database that provided the metabolite levels for each time point. Thiopurine methyltransferase (TPMT) is an enzyme that metabolizes 6MP into two active metabolites: methylated mercaptopurine (MMP) and thioguanine (TGN) (Relling, 2003; Relling et al., 2011). TPMT activity is a genetic trait that is inherited by both biological parents. One allele is inherited from the mother and the other allele is inherited from the father. TPMT activity can be described as the absolute values of TPMT activity; or, as a genotype (e.g., homozygous deficient, heterozygous, or homozygous wild type). TPMT activity is an important measure in the present study because it can affect how a patient metabolizes 6MP, which could potentially influence the metabolite profile group membership for that patient. Thus, TPMT absolute values and TPMT genotypes were obtained for all patients who consented to genetic testing. Absolute values were calculated using the average TPMT value for the first and last blood draw (baseline and 15 month values for most patients).

In the general population, one in 300 hundred patients are homozygous deficient, which means that the patient carries two inactive TPMT alleles and will present with low/deficient TPMT activity. If patients are adherent to their 6MP medication, patients with this genotype will often present with extremely high concentrations of TGN metabolites and little to no MMP metabolites. Those patients who are heterozygous (10% of population) carry both a variant copy of the gene and a normal/wild type copy and will present with intermediate levels of TPMT activity. If patients are taking their 6MP as prescribed, these patients will often present with high concentrations of TGN metabolites and low levels of MMP metabolites. Finally, 90% of patients are homozygous/wild type and will present with normal or high TPMT activity and not carry any genetic variations. Patients who present with this genotype will express low levels of TGN and high levels of MMP if taking their medication as prescribed (Relling, 2003; Relling et al., 2011).
Differences in TPMT activity and genotype will be examined across the metabolite clusters to determine if TPMT activity should be used as a covariate in analyses.

**Clinical Outcomes: Absolute Neutrophil Count (ANC).** Information regarding absolute neutrophil count (ANC) was collected via medical chart reviews at quarterly intervals (baseline, 3, 6, 9, 12, and 15 months). ANC is an important clinical biomarker to evaluate because it provides information regarding the child’s ability to fight infection. Values ≥ 1500 indicate no increased risk for infection, values between 1000-1500 indicate mild risk for infection, values 500-1000 suggest moderate risk, and values ≤ 500 indicate high risk for infection. Some clinicians’ have postulated that values ≥ 500 and ≤ 1500 are considered normal, whereas, low values (i.e., ≤ 500) are indicative of severe neutropenia (i.e., low numbers of neutrophils, which are the most important type of white blood cells), and puts the child at an increased risk for infection (Bhatia, 2011; Hakim et al., 2010; Malbasa et al., 2007; Marrs, 2006).

**Clinical Outcomes: Disease Relapse.** Information regarding disease remission or disease relapse during the research study or shortly following completion of the research study was documented using a standardized data collection form.

**Clinical Outcomes: Healthcare Utilization.** Information regarding healthcare utilization related to pediatric cancer treatment was collected at quarterly intervals (baseline, 3, 6, 9, 12, and 15 months). Frequency of healthcare utilization was the total number of clinic visits for pediatric cancer, and the number of ER visits and hospitalizations related to pediatric cancer.

**Data Analytic Plan**

See Table 1, which provides a summary of the analyses conducted for Aims 1-3, including the aims/hypotheses, data analytic method, and purpose of each analysis. Normality distributions and homogeneity of variance was examined (as relevant) for all continuous
variables that were included in this study. Generalized linear models were used for categorical outcomes, which were appropriate for both normal and non-normal distributions. Baseline demographic and medical factors were examined to determine which variables, if any, should be included as covariates in analyses of Aims 2-3 that examined group differences among adherence subgroups. Based on prior research (Bhatia, 2011; Bhatia et al., 2012; Davies & Lilleyman, 1995), the following covariates were examined: patient gender, patient age, patient ethnicity/race, single parent versus two parent households, and TPMT activity (genotype).

Results of the randomized controlled trial indicated that there were no significant differences in behavioral medication adherence and metabolite profiles between those patients who participated in the family-centered problem-solving intervention compared to those who received clinical care as usual ($p = 0.12, d = 0.21$). However, given the potential importance of group membership on relevant health outcomes for individual patients, RCT group was used as a covariate in Aim 3 analyses.

**Quality Control of Data Used in the Proposed Study.** All of the study data was sent to CCHMC (the Central Coordinating Site) and was cleaned by research coordinators in Cincinnati under the supervision of J. Rohan. Electronic monitoring data was reviewed for quality and data integrity and issues were addressed with specific site personnel. Electronic monitoring data was stored in a secured database and double-checked for accuracy. Blood samples were sent to a Central Lab at CCHMC (The Laboratory of Applied Pharmacokinetics and TDM in the Division of Clinical Pharmacology) and the senior research assistant in that lab completed all analyses and entered the metabolites of 6MP in a secured database. Results were double-checked for accuracy. See Appendix 1 for detailed information about quality control procedures for adherence data.

**Description of Adherence Patterns over 15 months Using Behavioral and**
Pharmacological Adherence Measures: Aim 1. 6MP medication adherence patterns over time were separately examined for behavioral adherence measures (e.g., electronic monitoring; indirect adherence measure) and pharmacological adherence measures (e.g., metabolite concentrations for 6MP: direct adherence measures). Behavioral measures of adherence were modeled using two different approaches to describe adherence over time: 1) an *unconditional growth curve model* was used to model the overall, population-based behavioral adherence patterns for the current sample; and, 2) *group-based trajectory modeling* was used to identify distinct, specific behavioral adherence subgroups that followed similar patterns over time. Pharmacological measures of medication adherence were analyzed using *two-step cluster analysis*, which was used to identify metabolite profiles representing different patterns of metabolite levels for 6-mercaptopurine. See *Table 1*, which provides a summary of the analyses conducted for Aim 1.

*Changes in Behavioral Measures of Adherence: Individual Growth Curve Modeling.* In order to test the hypothesis that behavioral adherence declined over time, an unconditional growth curve model was used to measure changes in daily adherence rates over time (Singer & Willet, 2003). Unconditional growth curve modeling was performed using SAS 9.3. The unconditional growth model yielded a fitted intercept (i.e., baseline adherence percentage) and fitted slope (i.e., change across time) for the population included in the current sample (Singer & Willet, 2003). A unique feature of growth curve analysis was that it accounted for missing data such that it used whatever data was available for an individual subject (Singer & Willet, 2003). Restricted maximum likelihood estimations were used to avoid biased estimates of the variance components. Unstructured covariance matrices were used to allow variances and covariances to vary across time rather than to conform to a priori constraints (Singer & Willet, 2003).

Growth curve modeling described change over time for the population as a whole and has been used in previous studies of pediatric cancer (Bhatia et al., 2012). However, growth curve modeling did not provide information regarding distinct, specific subgroups that followed similar adherence patterns over time (Nagin, 2005). Group-based trajectory analyses identified clusters of individuals that followed similar patterns of change over time and identified the optimal number of groups (or trajectories) within a data set over time (Jones & Nagin, 2007). Group-based trajectory modeling was used in the current study to identify adherence subgroups for behavioral measures of 6MP medication adherence from baseline to 15 months. It was hypothesized that three subgroups would be identified representing different levels of behavioral adherence from baseline to 15 months: optimal adherence, deteriorating adherence, and chronic nonadherence. This is based on previous research investigating adherence in other chronic illness populations (Modi, Rausch, & Glauser, 2011) as well as adherence rates over the first month of monitoring in this sample of pediatric cancer patients, which identified optimal adherence, deteriorating adherence, and chronic nonadherence subgroups (Rohan, Drotar, Alderfer, et al., 2013).

SAS 9.3 Proc Traj was used to identify trajectories of behavioral 6MP medication adherence over time based on latent group-based trajectory modeling (LGTM) (Jones & Nagin, 2007; Nagin, 2005). When using LGTM, each person is assumed to belong to a single subgroup for the duration of the observational period. Solutions with two, three, four, five, and six subgroups were examined and modeling allowed for linear, cubic, and quadratic trajectories within each subgroup to determine the final model with the best fit. The adequacy of the final model was evaluated using statistical diagnostics recommended by...
Nagin (2005): Bayesian Information Criterion (BIC) and subgroup proportions, model estimates of group probability, average posterior probability, and odds correct classification. Nagin (2005) recommended BIC and subgroup proportions of at least .10, the latter to ensure the group size is large enough to be of practical utility. It also was recommended that if the average posterior probability was greater than 0.7 and the odds correct classification was greater than five for all of the groups in the model, the trajectory model represented a high level of accuracy in classifying individuals into their specific trajectory assignment (Nagin, 2005).

_A Priori Power Analysis._ Based on a Monte Carlo simulation in M-plus, a sample size of 130 patients was sufficient to detect the three hypothesized trajectories. The Monte Carlo simulation illustrated that a three group solution provided substantially better fit based on the average BIC values compared to a two or four group solution. Specifically, when using the BIC log Bayes factor approximation illustrated in Jones et. al. (2007), the average BIC provided “very strong” evidence against both the two or four group solutions as compared to the three group solution.

_6-Mercaptopurine Metabolite Profiles from Baseline to 15 months: Two-Step Hierarchical Cluster Analysis._ Longitudinal cluster analysis was used to identify metabolite profiles for the two metabolites (TGN and MMP) of 6-mercaptopurine (6MP) based on results of a pharmacological bioassay. The purpose of cluster analysis was to define mutually exclusive groups of individuals who had similar patterns of metabolite levels over time (e.g., low levels of both metabolites or high levels of one metabolite and low levels of another metabolite) (Aldenderfer & Blashfield, 1984; Davies & Lilleyman, 1995; Hawwa et al., 2009; Lancaster et al., 1997; Lennard et al., 1995; Traore et al., 2006). Standardized z-scores for TGN and MMP
were used as the unit of analysis rather than absolute scores because cluster analysis required commensurability (i.e., equal scale units). Performing a z score transformation prior to the two-step analysis ensured that commensurability was obtained (Aldenderfer & Blashfield, 1984).

IBM SPSS 20 was used to run the longitudinal cluster analyses. Hierarchical two-step cluster analysis was used to identify robust and clinically relevant cluster patterns (Aldenderfer & Blashfield, 1984; Garson, 2010; Steele & Aylward, 2007). The two-step cluster analysis first identified ‘pre-clusters’ and then treated these ‘pre-clusters’ as single cases in hierarchical cluster analysis (Garson, 2010). Cluster membership was determined by the cluster distances approach, in that between groups differences were maximized and within group differences minimized to generate similar groups or patterns of individuals (Aldenderfer & Blashfield, 1984; Garson, 2010). The Bayesian Information Criterion (BIC) was used to determine the appropriate number of clusters, which was based on the lowest BIC and the largest BIC change between the number of clusters (Aldenderfer & Blashfield, 1984; Garson, 2010).

**Relationship between Behavioral and Pharmacological Measures of Adherence: Aim 2.**

Aim 2 examined the relationship between indirect behavioral measures and direct pharmacological measures of medication adherence. It was hypothesized that there would be a moderate relationship between 6MP metabolites and behavioral adherence rates over time. The half-life of 6-thioguanine (TGN) and 6-methylated mercaptopurine (MMP) is approximately 5 days following the medication dose (Derijks et al., 2004; Fishman & Mrozek-Orlowsk, 1999). Thus, behavioral adherence rates were mapped on to the assay data and behavioral adherence rates were examined at five days prior to the date of the blood draw. It was hypothesized that those individuals who presented with low levels of both TGN and MMP metabolites would also have the lowest behavioral adherence rates across time compared to the other metabolite groups. See
Table 1, which provides a summary of the analyses conducted for Aim 2.

**Description of Adherence Patterns and Relationship to Clinical Outcomes and a Clinical Biomarker: Aim 3.** Aim 3 examined whether indirect behavioral adherence measures and direct pharmacological measures of medication adherence predicted clinical outcomes and clinical biomarkers over time. See Figure 1. The primary analysis utilized longitudinal mixed effects models to examine whether 6MP medication adherence as measured by behavioral and pharmacological measures predicted clinically-relevant health outcomes or a clinical biomarker. General linear mixed models were used for normal/continuous outcomes and generalized linear mixed effects models for non-normal/categorical outcomes (mixed effects longitudinal logistic regression). Working correlation structures were examined for all models and the “best” model was chosen using the appropriate model fit statistics (e.g., AIC or QIC values, which was dependent on the model type used for the analysis) (Cui, 2007; Vaida & Blanchard, 2005). All analyses were conducted with SAS 9.3. See Table 1, which provides a summary of the analyses conducted for Aim 3.

**A Priori Power Analysis.** Based on previous literature that examined the relationship between pharmacological measures of medication adherence and clinical outcomes/biomarkers in cancer ($r = -0.30$) (Lennard et al., 1983), a sample size of 85 individuals was needed for the analyses involving a single measure of medication adherence. Using data from previous studies investigating the relationship between clinical outcomes and multiple adherence measures in pediatric type 1 diabetes (Hood, Peterson, Rohan, & Drotar, 2009), a sample size of 138 patients was needed to yield at least 80% power for the analyses that examined whether medication adherence measures (e.g., behavioral and pharmacological) predicted relevant clinical outcomes.

**Chapter 3. Results**
A description of the data analytic methods for Aims 1-3 and associated hypotheses are presented in Table 1. Normality and homogeneity of variance criteria was met as relevant. Review of relevant covariates (assessed one at a time) based on previous research (Bhatia, 2011; Bhatia et al., 2012; Davies & Lilleyman, 1995) indicated that there were no significant differences ($p > 0.05$) between the behavioral adherence trajectories or the metabolite profiles on relevant baseline demographic and medical characteristics (see Tables 3 and 4) or health outcomes. Thus, demographic and medical characteristics were not included as covariates in the statistical models discussed in Aims 2-3. On the other hand, RCT group was used as a covariate in the models discussed in Aim 3.

Aim 1: Description of Adherence Patterns over 15 months Using Behavioral and Pharmacological Adherence Measures.

Changes in Behavioral Measures of Adherence: Individual Growth Curve Modeling. Research has shown that 6MP medication adherence typically decreased over time in pediatric samples of patients diagnosed with ALL and LBL (Bhatia et al., 2012; Butow et al., 2010; Kato et al., 2008; Kondryn et al., 2010; Lau et al., 1998; Pritchard et al., 2006). It was hypothesized that the overall sample of patients in the current sample would show decreases in 6MP behavioral adherence from baseline to 15 months. Unconditional growth curve modeling ($n = 131$) indicated that average behavioral medication adherence was 84.4% at baseline (intercept; SE = 2.03, 95% CI: 80.38-88.41) and significantly decreased at a rate of -0.02% units per day (slope; SE = 0.01, 95% CI: -0.002 to -0.03) from baseline to 15 months [$F (1, 92.6) = 4.87, p = .03$]; such that at 15 months behavioral adherence was 75.2%.

Group Differences in Behavioral Adherence: Group-based Trajectory Modeling. Consistent with hypotheses based on behavioral adherence patterns of 6MP medication
adherence across the first month of study enrollment (Rohan, Drotar, Alderfer, et al., 2013), three behavioral adherence trajectories were identified using LGTM: optimal adherence, moderate adherence, and chronic nonadherence. Three trajectories had the best model fit relative to 2 and 4 trajectories using recommendations provided by Nagin (2005). Table 5 provides LGTM model estimates for each trajectory group. As shown in Figure 2, the majority of patients \(n = 88, 67.1\%\) demonstrated exemplary adherence rates across 15 months: starting at 96.3\% (week 1) and decreasing at a rate of -0.10\% per week; such that at 15 months behavioral adherence was 94.8\%. A second, much smaller group \(n = 26, 20\%\) demonstrated poor behavioral adherence at the start of monitoring (67.6\% at week 1), which remained relatively stable over time, decreasing at a rate of -0.01\% to an average of 67.5\% at 15 months. The third and smallest group \(n = 17, 12.9\%\) never established an adequate pattern of behavioral adherence with adherence levels of only 62.69\% at baseline and decreasing at a rate of -2.8\% per week; such that at 15 months behavioral adherence was approximately 30\%.

Baseline demographic and medical characteristics for the three behavioral adherence groups are shown in Table 3. Given the relatively small sample sizes for the chronic nonadherence \(n = 17\) and moderate adherence \(n = 36\) groups relative to the optimal adherence group, for statistical analyses discussed in Aims 2-3, the chronic nonadherence and the moderate adherence groups were collapsed into a single group referred to as the “nonadherent group.” The optimal adherence group was referred to as the “adherent group.” Demographic and medical characteristics were compared across the two groups using independent t-tests (continuous variables) or chi-squares (categorical variables) to determine which demographic and medical variables, if any, should be included as covariates in statistical models described in Aims 2-3. Significant differences are denoted in Table 3.

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6-Mercaptopurine Metabolite Profiles from Baseline to 15 months: Two-Step Hierarchical Cluster Analysis. Based on the findings from previous research examining cross-sectional metabolite concentrations for 6MP using hierarchical cluster analytic techniques and their relationship to medication adherence (Davies & Lilleyman, 1995; Hawwa et al., 2009; Lennard et al., 1983; Lennard et al., 1995; Traore et al., 2006), it was hypothesized that three metabolite group profiles would be identified in the present sample: 1) a group demonstrating low levels of both metabolites of 6MP, which is indicative of nonadherence to 6MP; and two groups representing better adherence to 6MP as evidenced by high levels of one metabolite and low levels of the second metabolite: 2) high levels of MMP and low levels of TGN, and 3) high levels of TGN and low levels of MMP (Davies & Lilleyman, 1995; Hawwa et al., 2009; Lennard, Lilleyman, Van Loon, & Weinshilboum, 1990; Lennard et al., 1995; Traore et al., 2006).

Consistent with hypotheses, a two-step hierarchical cluster analysis indicted that a 3 group model had the best fit based on BIC criterion: high TGN-low MMP metabolite profile (n = 146, 18.9%), low TGN-high MMP metabolite profile (n = 356, 46.2%), and a low TGN-low MMP metabolite profile (n = 269, 34.9%).

*Figure 3* provides the average z scores for TGN and MMP for each of the three metabolite profiles from baseline to 15 months. As shown, there is a clear separation between the three metabolite profiles with respect to the mean standardized z score of TGN and MMP. *Table 6* provides the absolute TGN and MMP metabolite values from baseline to 15 months and overall means for the three metabolite profiles. As shown in *Figures 3* and *Table 5*, TGN and MMP metabolite levels were relatively stable over time. However, consistent with previous research (Davies & Lilleyman, 1995; Lennard et al., 1995), the TGN metabolites that were observed in the present study appear to be more stable across the three metabolite profiles.
compared to the MMP metabolites, as evidenced by significant variability in MMP metabolite values over time relative to TGN metabolite values.

Baseline demographic and medical characteristics for the three metabolite profiles are shown in Table 4. Multivariate mixed models (e.g., general linear models and generalized linear models) were used to determine whether baseline demographic and medical characteristics should be included as covariates in the statistical models described in Aims 2-3. Results of these analyses indicated that there were no significant differences between the metabolite profiles.

**Aim 2: Relationship between Behavioral and Pharmacological Measures of Adherence**

Since the half-life of the 6MP metabolites: 6-thioguanine (TGN) and 6-methylated mercaptopurine (MMP) is approximately 5 days following the medication dose (Derijks et al., 2004; Fishman & Mrozek-Orlowsk, 1999); behavioral adherence rates were mapped on to the assay data and adherence rates were examined at five days prior to the date of the blood draw.

**Relationship between Five-Day Behavioral Adherence Rates and Metabolite Profiles.**

Five day behavioral adherence rates for the three metabolite profiles are presented in Figure 4. Table 7 provides the descriptive statistics (M ± SD, range) for behavioral adherence rates across the three metabolite profiles from three to 15 months. It was hypothesized that there would be a significant relationship between behavioral adherence rates and metabolite profiles, such that behavioral adherence rates would be higher for the low TGN-high MMP and high TGN-low MMP groups relative to the low TGN-low MMP group. There was a significant association over time between metabolite profile membership and five day behavioral adherence rates (r’s from baseline to 15 months ranged between -0.16 to -0.26).

**Examination of Mean Differences in Five-Day Adherence Rates for Metabolite Profiles from Three to 15 months.** It was hypothesized that those individuals who expressed low levels
of both TGN and MMP metabolites were most likely to be nonadherent to 6MP and hence would have the lowest behavioral adherence rates across time compared to the low TGN-high MMP and high TGN-low MMP groups. Between subjects ANOVAs were used to examine cross-sectional mean differences in 5 day adherence rates between the three metabolite profiles across 3 to 15 months. Post hoc comparisons were conducted using Tukey’s HSD.

Five day behavioral adherence rates for the three metabolite profiles are provided in Figure 4 and Table 7. There was a significant difference in mean 5-day adherence rates between the three metabolite profiles at 6 [$F (2,115) = 4.19, p = 0.02, \eta^2_p = 0.07$] and 12 months [$F (2, 97) = 3.60, p = 0.03, \eta^2_p = 0.07$]. Those in the low TGN-high MMP metabolite profile, which is the group commonly associated with adherence to 6MP as suggested by previous research (Davies & Lilleyman, 1995; Hawwa et al., 2009; Lennard et al., 1995; Traore et al., 2006) demonstrated higher behavioral adherence rates compared to the low TGN-low MMP group, which is the group that is referred to as nonadherent in previous research (Davies & Lilleyman, 1995; Hawwa et al., 2009; Lennard et al., 1995; Traore et al., 2006). Significant differences are denoted in Figure 4.

Previous research suggested that patients who were identified as having a high TGN-low MMP metabolite profile were likely TPMT deficient and have trouble metabolizing 6MP (Relling et al., 2011). In the current sample, 46.2% of patients in the high TGN-low MMP profile were identified as heterozygote and hence TPMT deficient (see Table 4). In fact, the overwhelming majority of TPMT deficient patients (67%) in the present sample were identified as having a high TGN-low MMP metabolite profile. Thus, patients with high TGN-low MMP profiles ($n = 32$) were omitted from behavioral adherence-pharmacological adherence comparisons. Cross-sectional differences in behavioral adherence rates from three to 15 months
were examined for the low TGN-low MMP profile \((n = 48)\) and the low TGN-high MMP profile \((n = 59)\). Independent sample t-tests indicated significant differences in behavioral adherence rates between these two metabolite profiles at 6 \([t (94) = 2.84, p = 0.01, d = 0.59]\), 9 \([t (86) = 2.17, p = 0.03, d = 0.25]\), 12 \([t (81) = 2.44, p = 0.02, d = 0.56]\), and 15 months \([t (73) = 2.07, p = 0.04, d = 0.52]\). Significant differences are denoted in Figure 4.

**Aim 3: Description of Adherence Patterns and Relationship to Clinical Outcomes and a Clinical Biomarker**

Mixed effects ANOVAs were used to determine whether baseline demographic and medical characteristics should be included as covariates in models examining the influence of direct (metabolites of 6MP) and indirect (behavioral adherence: electronic monitoring of 6MP) measures of medication adherence on clinical outcomes/biomarkers (e.g., healthcare utilization, disease relapse, absolute neutrophil count: ANC). Generalized linear models (Proc Genmod) were used for categorical outcomes, which was appropriate for both normal and non-normal distributions. Based on prior research (Bhatia, 2011; Bhatia et al., 2012; Davies & Lilleyman, 1995), the following baseline medical and demographic characteristics were examined: child age, duration of cancer diagnosis, absolute TPMT values, patient gender, ethnicity, one versus two parent households, and TPMT genotype. None of the baseline demographic and medical characteristics significantly predicted mean differences in clinical outcomes from baseline to 15 months \((p’s > 0.05)\) and hence were not included in models as covariates unless otherwise noted.

**Behavioral Adherence Trajectories and Absolute Neutrophil Count (ANC).**

**Absolute Neutrophil Count (ANC): Absolute Values.** It was hypothesized that patients who were “nonadherent” (chronic nonadherence, moderate adherence) would have higher ANC absolute values from baseline to 15 months compared to those patients who were “adherent”
(optimal adherence). The mean absolute neutrophil count (ANC) values from baseline to 15 months were compared across the adherent (optimal adherence) versus nonadherent (moderate adherence, chronic nonadherence) behavioral adherence trajectories using a Mixed ANOVA. Descriptive statistics for ANC values from baseline to 15 months for the behavioral adherence trajectories are provided in Table 8. All continuous variables were normal and homogeneity of variance criteria were met as relevant. RCT group was entered as a covariate and was not significant in the model ($p = 0.33$).

Contrary to hypotheses, the between groups test indicated that there was not a significant difference in mean ANC values between the optimal adherence versus nonadherent behavioral adherence trajectories ($p = 0.20, \eta^2_p = 0.01$). On the other hand, the mean ANC values for the adherent versus nonadherent behavioral trajectories significantly changed over time: $F(5,138) = 2.54, p = 0.03, \eta^2_p = 0.02$. See Table 8. ANC increased across behavioral adherence groups. However, there was not a significant interaction between behavioral adherence and time ($p = 0.50, \eta^2_p = 0.01$).

**Absolute Neutrophil Count (ANC): Risk for Infection.** It was hypothesized that those with lower behavioral adherence rates (i.e., chronic nonadherence or moderate behavioral adherence) would also have an increased risk for disease severity and infection as indicated by absolute neutrophil counts less than 1500. A longitudinal binomial logistic regression was used to examine whether infection risk as measured by absolute neutrophil count (ANC) could be predicted by adherent (optimal adherence) versus nonadherent behavioral adherence trajectories (chronic nonadherence, moderate adherence). Risk for infection was based on ANC values and was categorized as no risk for infection (i.e., ANC values 1500 and above) and a mild to high risk for infection (ANC values 0 to 1499).
When controlling for RCT group ($\chi^2 = 4.59$, $df = 1$, $p = 0.03$) and contrary to hypotheses, having no risk for infection was not predicted by membership in adherent versus nonadherent behavioral trajectories ($p = 0.62$). As shown in Figure 5, the majority of patients in both the adherent and nonadherent trajectories had a decreased risk for infection over time. However, the odds of having no risk for infection were 0.86 times higher in the nonadherent group compared to the adherent group (95% CI: 0.47, 1.57). On the other hand, the proportion of patients who did not have an increased risk for infection significantly changed over time ($\chi^2 = 10.06$, $df = 1$, $p = 0.002$). Having no risk for infection increased by 6% per month over time (OR = 1.06, 95% CI: 1.02, 1.09). However, there were not significant differences observed between membership in a behavioral adherence trajectory and prediction of infection risk from baseline to 15 months ($p = 0.06$). In other words, the adherent and nonadherent behavioral adherence trajectories typically had similar proportions of patients in the no increased risk and increased risk groups from baseline to 15 months and prediction of infection risk did not differ between the two behavioral adherence trajectories.

**Metabolite Profiles and Absolute Neutrophil Count (ANC).**

**Absolute Neutrophil Count (ANC): Absolute Values.** It was hypothesized that patients who were identified as having low levels of both TGN and MMP, which could be indicative of nonadherence (Davies & Lilleyman, 1995; Hawwa et al., 2009; Lancaster et al., 1997; Lennard et al., 1983; Lennard et al., 1995; Traore et al., 2006), would also have higher ANC absolute values from baseline to 15 months compared to patients in the other metabolite profile groups. The mean absolute neutrophil count (ANC) values from baseline to 15 months were compared across the three metabolite profiles using a mixed ANOVA. Post hoc comparisons were conducted using sequential Bonferroni corrections. All continuous variables were normal and
homogeneity of variance criteria were met as relevant. RCT group was entered as a covariate and was not significant in the model \((p = 0.82)\). *Figure 6* provides the least square mean estimates of ANC values for the three metabolite profile groups.

Consistent with the hypothesis, the between groups test indicated that there was a significant difference in mean ANC between the three metabolite profiles \([F(2,138) = 3.01, p = 0.05, \eta^2_p = 0.04]\). The mean absolute ANC value \((M = 2296, SE = 95)\) for the low TGN-low MMP metabolite profile was significantly higher than the mean absolute ANC value for the low TGN-high MMP metabolite profile \((M = 2039, SE = 88)\) \((p < 0.02)\). On the other hand, ANC values across the three metabolite profiles did not significantly change over time \((p = 0.07, \eta^2_p = 0.01)\). Finally, there was not a significant interaction between metabolite profiles and time \((p = 0.88, \eta^2_p = 0.09)\).

**Absolute Neutrophil Count (ANC): Risk for Infection.** It was hypothesized that those with low levels of both TGN and MMP would also have an increased risk for disease severity and infection as indicated by an absolute neutrophil count (ANC) less than 1500. A longitudinal binomial logistic regression was used to examine whether infection risk as measured by absolute neutrophil count (ANC) could be predicted by metabolite profiles. Risk for infection was based on dichotomized ANC values and was categorized as no risk for infection: ANC values 1500 and above; and, a mild to high risk for infection: ANC values 0 to 1499. RCT group was entered as a covariate and was not significant in the model \((p = 0.14)\).

As hypothesized, having no risk for infection as indicated by ANC\(_S\) 1500 and greater was significantly predicted by metabolite profile group membership \((\chi^2 = 6.37, df = 2, p = 0.04)\). As shown in *Figure 7*, patients in the low TGN-low MMP group had the highest odds for not having an increased risk for infection as evidenced by ANC\(_S\) 1500 or greater \((\text{OR: 2.96, 95\% CI: 2.14 to 3.68})\).
4.08). For the high TGN-low MMP metabolite profile, the odds of not having an increased risk for infection was 2.37 (95% CI: 1.59 to 3.52); and, for the low TGN-high MMP metabolite profile, the odds of not having an increased risk of infection was 1.65 (95% CI: 1.28 to 2.13). The odds of not having an increased risk for infection was 1.90 times higher for the low TGN-low MMP metabolite profile compared to the high TGN-low MMP metabolite profile (95% CI = 9.95, 3.79, \( p = 0.06 \)). Similarly, the odds of not having an increased risk for infection was 1.98 times higher for the low TGN-low MMP metabolite profile compared to the low TGN-high MMP metabolite profile (95% CI = 1.12, 3.48; \( p = 0.02 \)). The proportion of patients who did not have an increased risk for infection significantly changed over time (\( \chi^2 = 10.45, df = 1, p = 0.001 \)). No risk for infection increased by 5% per month over time (OR = 1.05, 95% CI = 1.02, 1.09; \( p = 0.0009 \)). As shown in Figure 7, although there was some variability within the metabolite profiles, for the most part, infection risk decreased over time across the three metabolite profiles. However, there were not significant differences between metabolite profile membership and time with respect to prediction of infection risk from baseline to 15 months (\( p = 0.34 \)). In other words, prediction of infection risk over time did not differ between the three metabolite profiles.

**Behavioral Adherence Trajectories (Adherent versus Non-Adherent) and Healthcare Utilization.** It was hypothesized that those in the “nonadherent” behavioral adherence trajectories (chronic nonadherence, moderate adherence) would also have more healthcare utilization visits compared to those with optimal behavioral adherence. A mixed ANOVA was conducted to examine whether there were differences in quarterly rates of healthcare utilization across adherent (optimal adherence) versus nonadherent (chronic nonadherence, moderate adherence) behavioral adherence trajectories. Post hoc comparisons
were conducted using sequential Bonferroni corrections. All continuous variables were normal and homogeneity of variance criteria were met as relevant. Descriptive statistics for healthcare utilization visits from baseline to 15 months across the behavioral adherence trajectories are provided in Table 9. RCT group was entered as a covariate and was not significant in the model ($p = 0.55$).

Contrary to hypotheses, the between groups test indicated that there was no significant difference between the adherent (optimal adherence) and nonadherent (chronic nonadherence, moderate adherence) behavioral adherence trajectories with respect to mean healthcare utilization ($p = 0.10, \eta^2_p = 0.02$). On the other hand, frequency of healthcare utilization across the adherent (optimal adherence) and nonadherent (chronic nonadherence, moderate adherence) behavioral adherence trajectories significantly changed over time ($F(5, 609) = 58.29, p = 0.0001, \eta^2_p = 0.31$). Finally, there was no significant interaction between behavioral adherence trajectories and time ($p = 0.28, \eta^2_p = 0.01$).

**Metabolite Profiles and Healthcare Utilization.** It was hypothesized that those who demonstrated poor adherence (i.e., low levels of both TGN and MMP) as measured by pharmacological measures of adherence would also have increased healthcare utilization compared to those with metabolite levels consistent with medication adherence (i.e., high TGN-low MMP, low TGN-high MMP). A mixed ANOVA was conducted to examine whether there were differences in quarterly rates of healthcare utilization across the three metabolite profiles. Post hoc comparisons were conducted using sequential Bonferroni corrections. All continuous variables were normal and homogeneity of variance criteria were met as relevant. Descriptive statistics for quarterly rates of healthcare utilization (i.e., the total number of clinic visits, hospitalizations, and emergency room visits for the preceding three months prior to each visit)
are provided in Table 10. RCT group was entered as a covariate and was not significant in the model \( (p = 0.87) \).

Consistent with hypotheses, the between groups test indicated that there was a significant difference among the three metabolite profiles on frequency of healthcare utilization \( (F(2, 118) = 3.30, p = 0.04, \eta^2_p = 0.05) \). Patients in the low-low metabolite profile had more healthcare utilization visits \( (M = 4.04, SE = 0.14) \) relative to the low TGN-high MMP \( (M = 3.60, SE = 0.12) \) metabolite profile. Similarly, frequency of healthcare utilization for each profile significantly changed over time \( (F(5, 606) = 63.09, p = 0.0001, \eta^2_p = 0.32) \). Finally, there was not a significant interaction between metabolite profiles and time \( (p = 0.55, \eta^2_p = 0.01) \).

**Behavioral Adherence Trajectories and Risk for Relapse.** It was hypothesized that those in the “nonadherent” behavioral adherence trajectories (chronic nonadherence, moderate adherence) would have an increased risk for relapse compared to those with optimal behavioral adherence. Relapse rates for the adherent versus nonadherent behavioral adherence groups are shown in Table 3. A binomial logistic regression was used to examine whether disease relapse (remission versus disease relapse) could be predicted by adherent (optimal adherence) versus nonadherent (chronic nonadherence or moderate adherence) behavioral adherence trajectories. RCT group was entered as a covariate and was not significant in the model \( (p = 0.39) \). Contrary to hypotheses, cancer remission was not predicted by membership in adherent versus nonadherent behavioral adherence trajectories \( (p = 0.92) \). The odds of cancer remission were higher for those patients in the adherent trajectory compared to the nonadherent trajectory \( (OR = 1.05, 95\% CI: 0.36, 3.09, p = 0.92) \).

**Metabolite Profiles and Risk for Relapse.** It was hypothesized that those who demonstrated poor adherence (i.e., low levels of both TGN and MMP) as measured by
pharmacological measures of adherence would have an increased risk for relapse compared to those with metabolite levels consistent with medication adherence (i.e., high TGN-low MMP, low TGN-high MMP). Total relapse rates and mortality for the three metabolite profiles are shown in Table 11. Relapse was a dichotomous, static variable and coded as whether or not the patient relapsed at any point during the study or shortly following completion of the study. Metabolite profiles were dynamic variables and patients’ metabolite profiles could change over time. A longitudinal binomial logistic regression was used to examine whether the probability for cancer remission versus disease relapse could be predicted by metabolite profile membership. RCT group was entered as a covariate and was not significant in the model ($p = 0.32$).

Contrary to hypotheses, remission was not significantly predicted by metabolite profile membership ($p = 0.39$). The odds of cancer remission were 1.01 times higher in the low TGN-high MMP metabolite profile relative to the low-low metabolite profile (95% CI: 0.94, 1.10). However, the proportion of patients who remained in remission significantly changed over time ($\chi^2 = 8.05, df = 1, p = 0.005$). Similarly, there was a significant difference observed between metabolite profile membership and prediction of staying in remission from baseline to 15 months ($\chi^2 = 5.99, df = 2, p = 0.05$). In other words, there were significant differences over time between the three metabolite profiles with respect to predicting the number of patients who remained in remission versus those whose disease relapsed from baseline to 15 months. As shown in Table 11, those patients with low levels of both metabolites (i.e., low TGN-low MMP group) from baseline to 15 months typically had higher rates of disease relapse during the study or following study completion (8.6 to 16.7%) compared to patients in the high TGN-low MMP (5.0 to 11.5%) and the low TGN-high MMP groups (1.9 to 14.0%) with the exception of 6 and 15 months. At 6 months, there were more patients who relapsed during or after the study in both the high TGN-
low MMP (24%) and the low TGN-high MMP groups (9.8%) relative to the low TGN-low MMP group (7.0%). Similarly, at 15 months there were more patients in the high TGN-low MMP group who had relapsed during or following study completion (17.6%) compared to the low TGN-high MMP group (1.9%) and the low TGN-low MMP group (5.3%).

**Chapter 4. Discussion**

**Conclusions**

To my knowledge, this study provided the first comprehensive description of multiple objective measures of 6-mercaptopurine (6MP) medication adherence and their relationship to clinically-relevant outcomes during the maintenance phase of cancer treatment for pediatric ALL and LBL. Medication adherence to 6MP was monitored using a behavioral adherence measure (i.e., real-time measures of daily 6MP adherence using electronic monitors) and a pharmacological adherence measure (i.e., metabolites of 6-mercaptopurine) over the course of 15 months. This comprehensive measurement approach addressed two important scientific and clinically-relevant questions: 1) What is the course of treatment adherence and nonadherence in ALL and LBL based on two objective measures of adherence?; and, 2) How does adherence to ALL and LBL treatment relate to clinically-relevant outcomes (e.g., relapse rates, healthcare utilization) and a clinical biomarker (e.g., absolute neutrophil count: ANC)?

Similar to adherence results documented in previous longitudinal research studies in pediatric cancer (Bhatia et al., 2012; Kato et al., 2008; Lau et al., 1998), behavioral 6MP adherence rates for the present sample demonstrated a significant level of deterioration across the fifteen months of monitoring (84.4% to 75.2% at the 15 month visit). This finding is consistent with previous research that used electronic monitoring data to describe longitudinal patterns of medication adherence across a number of different prescribed medications for pediatric cancer.
treatment, which documented mean adherence rates of 50% to 94% over time (Bhatia et al., 2012; Kato et al., 2008; Lau et al., 1998). It is also notable that the present study had a longer follow-up period of behavioral adherence (i.e., 15 months) compared with previous research. Lau and colleagues (1998) monitored adherence rates of 6MP across a period of four to six weeks (mean follow-up of 44 days; \( N = 24 \) patients). Kato et al. (2008) monitored behavioral adherence of trimethoprim-sulfamethoxole across three months (\( N = 200 \)). Bhatia et al. (2012) examined behavioral adherence of 6MP across 6 months (\( N = 327 \)).

Examination of pharmacological measures of medication adherence (i.e., metabolites of 6-mercaptopurine: 6MP) using hierarchical cluster analysis indicated three distinct metabolite profiles across 15 months that were consistent with previous research (Hawwa et al., 2009; Traore et al., 2006). In the present sample, two of the three metabolite profiles were consistent with better adherence to 6MP as indicated by high levels of one metabolite and low levels of the second metabolite (e.g., high TGN-low MMP and low TGN-high MMP metabolite profiles) (Davies & Lilleyman, 1995; Lennard et al., 1995). On the other hand, the third metabolite profile was consistent with lower adherence to 6MP as indicated by low levels of both TGN and MMP metabolites (Davies & Lilleyman, 1995; Lennard et al., 1995).

A new contribution of this study was the measurement of TPMT activity in analyses of metabolite levels of 6MP. It is well known that pediatric cancer patients with TPMT deficiencies (low or absent TPMT) will have difficulty metabolizing 6MP, which could influence metabolite levels and outcomes (Davies & Lilleyman, 1995; Relling et al., 2011). If patients are adherent to 6MP medication, those patients with low or absent TPMT activity (heterozygous) will present with high levels of TGN and low levels of MMP, whereas patients with high TPMT activity (wild type) will present with low levels of TGN and high levels of MMP. In the present study,
the majority of patients with the heterozygous TPMT genotype were in the high TGN-low MMP metabolite group (67%). On the other hand, low levels of both metabolites (TGN-MMP) cannot be explained by metabolic differences and are likely indicative of nonadherence or poor bioavailability of the medication (Davies & Lilleyman, 1995).

To my knowledge, the current study is the first to report data concerning the relationship between an indirect measure of 6MP behavioral adherence and a direct measure of pharmacological adherence. The differences in the behavioral adherence rates between the adherent (high TGN-low MMP and low TGN-high MMP groups) versus the nonadherent metabolite profiles (low TGN-low MMP) suggests that adherence behaviors influence metabolite levels and hence exposure to medication (Kenna et al., 2005). Furthermore, the majority of patients who were adherent to 6MP based on electronic monitoring were likely in one of the two groups with metabolic profiles representative of adherence.

The significant intra-sample and intra-patient variability in the measures of medication adherence in the current study is consistent with previous research investigating behavioral and pharmacological measures of adherence (Bhatia et al., 2012; Davies, Lennard, & Lilleyman, 1993; Hawwa et al., 2009; Kato et al., 2008; Lau et al., 1998; Pai et al., 2008; Traore et al., 2006). The present study utilized group-based trajectory analyses to identify subgroups or clusters of patients representing similar levels of behavioral adherence patterns across 15 months of monitoring. Group-based analyses investigating behavioral adherence rates over time documented that 32.8% of patients in the current sample presented with moderate or chronic nonadherence patterns across 15 months. In fact, those patients who were identified as having moderate adherence or chronic nonadherence patterns presented with behavioral adherence rates far below the 95% cutoff that Bhatia et al. (2012) indicated was a risk factor for disease relapse.
In addition, these patients never established an adequate level of adherence across the 15 month period with adherence rates ranging from 30% to 68%. On the other hand, there was a subsample of patients whose adherence remained relatively high across the 15 month period. It is notable that patients with optimal adherence presented with behavioral adherence levels above 95% at week 1, but demonstrated slight declines in adherence over time such that at 15 months adherence rates were 94.8%. Furthermore, previous studies that investigated prospective behavioral adherence patterns did not exploit the full potential of electronic monitoring data to describe individual variation. These studies generally described the average frequency of adherence (Kato et al., 2008; Lau et al., 1998) or described changes in adherence over change over time for the population as a whole for a relatively short period (Bhatia et al., 2012).

Previous research documented that higher ANC levels were associated with nonadherence to 6MP medication as measured by behavioral adherence (Bhatia et al., 2012). On the other hand, previous research has not investigated the relationship between nonadherence and infection risk as measured by ANC. Contrary to hypotheses, adherent versus nonadherent behavioral adherence trajectories across time did not predict significant changes in absolute values of ANC over 15 months, nor were there differences in prediction of infection risk (as measured by ANC) across the nonadherent and adherent behavioral adherence trajectories. It is possible that these non-significant findings were influenced by the small samples of patients in each of the subgroups. In addition, it is possible that the cumulative 15 month adherence rates for the behavioral adherence trajectories are not sensitive enough to predict mean ANC or infection risk (as measured by ANC). A more sensitive measure of the relationship between behavioral adherence rates and prediction of ANC could be adherence rates that are collected immediately prior to the ANC data. Lennard and colleagues (1983) found a significant relationship between
TGN levels at baseline and ANC collected 14-days post-TGN data collection, but did not find a relationship between cross-sectional TGN values and ANC or ANC that was collected seven days post-TGN blood draw.

Previous research also documented that nonadherence to 6MP medication as measured by low metabolites of 6MP was associated with higher ANC levels (Innocenti et al., 2000; Lennard et al., 1983; Traore et al., 2006). This finding was replicated in the current study such that there was a significant difference in absolute values of ANC between the three metabolite profiles. Those patients’ with low TGN-low MMP metabolite levels had significantly higher mean ANC values compared to the low TGN-high MMP metabolite profile.

Previous research has documented the importance of optimal medication adherence in decreasing risk for disease relapse, as well as, decreasing healthcare utilization (Bhatia, 2011; Bhatia et al., 2012). Although there is a lack of research in pediatric cancer that examined the relationship between medication adherence and healthcare utilization, one might expect that nonadherence to prescribed treatment regimens would be associated with increased healthcare utilization or less optimal clinical status. However, in the present study there were no differences in total healthcare utilization observed for the two behavioral adherence trajectories. Healthcare utilization remained relatively stable across the 15 months for all three behavioral adherence trajectories. The nonadherent behavioral adherence groups generally had higher (though non-significant) rates of average healthcare utilization (4.2 to 4.9) relative to the adherent group (3.8 to 4.5). On the other hand, there were significant differences in healthcare utilization across the three metabolite profiles. The nonadherent metabolite group (low TGN-low MMP) generally had higher (though non-significant) average healthcare utilization (4.1 to 5.0) compared to the adherent metabolite groups (high TGN-low MMP: 3.6 to 4.9; low TGN-high MMP: 3.9-4.4). It
is quite possible that these findings were influenced by the small samples of patients in the adherence subgroups.

Bhatia and colleagues (2012) provided evidence for decreased adherence (less than 95%) being associated with an increased risk for disease relapse over time in a large sample ($N = 327$). Contrary to hypotheses, there was not a significant difference in risk for disease relapse between the adherent and nonadherent behavioral adherence trajectories. There was a greater (though non-significant) percentage of patients who relapsed in the nonadherent group (14%; chronic nonadherence, moderate adherence) compared to the optimal adherence group (12.8%). It is possible that these findings were influenced by the small samples of patients in the two groups. On the other hand, there was a significant difference between the three metabolite profiles and risk for disease relapse. Those in the nonadherent metabolite profile (low TGN-low MMP) generally had higher disease relapse rates across the 15 months (8.6 to 16.7%) relative to the adherent profiles: high TGN-low MMP (5.0 to 11.5%) and the low TGN-high MMP groups (1.9 to 14.0%).

Clinical Implications

Clinical Implications for Targeted and Tailored Adherence Promotion Intervention. Data concerning the prospective course and individual patterns of treatment adherence could allow for more effective preventive intervention by enhancing more precise targeting of adherence promotion interventions to those who need them most (e.g., the most nonadherent patients) at the time that they need it (e.g., when adherence deteriorates or nonadherence becomes chronic). In the current study, there were relatively large numbers of patients in the current sample who demonstrated rates of nonadherence that could place them at an increased risk of disease relapse based on recommendations discussed in Bhatia et al. (2012). In addition,
nonadherence to 6MP may place patients at risk for elevated ANC values, which could impact therapeutic efficacy or treatment outcomes. Furthermore, nonadherence to 6MP in the current sample was associated with an increased risk for disease relapse.

Although some patients demonstrated gains in behavioral 6MP adherence over time or demonstrated optimal adherence rates from baseline to 15 months, Bhatia et al.’s findings suggest that all of the patients in the present sample could benefit from some type of adherence promotion intervention (Cortina, Somers, Rohan, & Drotar, 2013; Kahana, Drotar, & Frazier, 2008; Wu et al., 2013). In the present study, even those subgroups that were identified as adherent to prescribed treatment regimens, demonstrated adherence rates below the 95% cutoff at some point across the 15 month period. This deterioration in medication adherence could place many patients in the current sample at an increased risk for disease relapse. Patients in the optimal adherence group or those patients demonstrating sufficient levels of metabolite levels could benefit from preventative interventions, which are less intensive in nature but assist patients with maintaining or improving adherence. Patients in the chronic nonadherence and moderate adherence subgroups would be prime candidates for more intensive, empirically-supported adherence promotion interventions (e.g., behavioral and problem-solving approaches) (Cortina et al., 2013; Kahana et al., 2008; Kondryn et al., 2010; Wu et al., 2013).

Adherence data based on MEMS, especially coupled with data on metabolite profiles provided an objective means of identifying patients who are most in need of interventions to promote adherence. For example, patients with patterns of chronic nonadherence based on behavioral adherence (MEMS) or specific metabolite profiles (low levels of both 6MP metabolites) or those patients who show deteriorating patterns of adherence are most at risk. Interventions such as that described in Kato et al. (2008) in which patients played a videogame to
promote adherence to 6MP and other medications have been shown to be effective. Feedback concerning adherence has been effective in other conditions and might be in cancer, especially if coupled with targeted problem-solving and feedback from electronic monitoring to increase the power of effects. Sharing results of electronic monitoring with patients and families is a unique and innovative method for improving or maintaining optimal adherence levels (Cortina et al., 2013; Rohan, Drotar, Perry, et al., 2013; Spaulding, Devine, Duncan, Wilson, & Hogan, 2012; Wu et al., 2013). Furthermore, a multidisciplinary team of healthcare providers can utilize principles of anticipatory guidance during routine medical follow-up (e.g., education concerning importance of adherence, identification of potential barriers to adherence, and follow-up on the success of adherence promotion efforts) in an effort to prevent adherence problems from occurring during the maintenance phase of treatment (Pai & Drotar, 2009).

**Clinical Implications for Medical Follow-Up.** Ongoing measurement of behavioral and pharmacological adherence measures of 6MP over the course of cancer treatment could be important for optimal clinical management of dosing recommendations and promotion of positive health outcomes. Although implementing multiple measures of medication adherence in routine clinical care could be costly and likely not practical for all patients who are treated for pediatric cancer, these findings suggested that using direct and indirect measures of medication adherence provided an effective method for identifying patients who are in need of intensive adherence promotion intervention. For example, behavioral and pharmacological measures of medication adherence could assist with identification of those children and adolescents who are suspected to have moderate adherence or chronic nonadherence patterns early in treatment (e.g., chronically problematic trajectories of adherence or low levels of 6MP metabolites across the first several months of treatment). Intensive adherence promotion interventions could significantly improve
the health outcomes of these patients, and hence decrease their risk for disease relapse.

Assessment of behavioral and pharmacological measures of adherence could assist clinicians with making dose recommendations for 6MP in order to reduce the risk for adverse events associated with drug toxicity. Undetected nonadherence to ALL and LBL treatment may contribute to potential medication toxicity. Nonadherence to prescribed medications could be misinterpreted by a treating physician as poor absorption of the medication, and the physician may prescribe a higher medication dose, which could be too much for the patient and result in significant drug toxicity if patients resume taking the medication at the newly prescribed dose (Davies & Lilleyman, 1995; Hawwa et al., 2009; Lau et al., 1998; Partridge, Kato, & DeMichele, 2009; Traore et al., 2006).

Lau and colleagues (1998) proposed an algorithm for monitoring response to maintenance therapy for pediatric ALL, which included examining both behavioral adherence rates, as well as, metabolites of 6MP using ANC as a clinical biomarker of treatment success. It was proposed that behavioral adherence rates be used as a first line of adherence measurement and be examined during the entirety of maintenance treatment. Lau and colleagues also proposed using metabolite levels as a second line of adherence measurement if behavioral adherence rates were below 90% or ANC levels were above the target range (i.e., 1500 or greater).

Limitations and Future Directions

Several limitations should be considered when interpreting these findings. The limitations of MEMS data as a measure of behavioral adherence is well documented (Ingerski et al., 2011; Kenna et al., 2005; Riekert & Rand, 2002; Rohan, Drotar, Alderfer, et al., 2013). It would have been ideal if all patients used the MEMS cap. However, it was not clinically appropriate, nor ethically desirable to request that patients start using the MEMS cap for the purposes of research.
if they were already using a pill box to limit the difficulties they were having with taking medication as prescribed, which was corroborated by the medical team. Future research should consider methods other than MEMS, including using pill boxes with electronic monitors. The present study only included patients’ ages 7-19 years. Future studies should examine a heterogeneous age range in a much larger sample of pediatric cancer patients from infancy to young adulthood. Furthermore, it is unknown whether 6MP adherence generalizes to other chemotherapy medications. Thus, adherence across multiple medications should be examined to determine if adherence differs across medications or regimens. Finally, 6MP medication adherence in the current study was examined for 15 months of maintenance treatment. It will be important to assess medication adherence across the entire duration of maintenance to assess for treatment burden and its relationship to adherence and health outcomes.

Several directions for future research are needed to address the above limitations. First, future research should attempt to replicate the findings presented here in a larger sample of pediatric cancer patients. Future research should utilize the full breadth of medical data that is collected in routine medical care for pediatric patients (e.g., white blood cell count, erythrocyte count, and platelet counts). Collecting metabolite levels and clinical outcomes at monthly intervals during a patient’s routine medical visit would provide a more powerful measure of the relationship between behavioral and pharmacological measures of medication adherence and how adherence relates to these clinical outcomes. Moreover, the statistical methods presented here can be readily applied to other chronic illness populations that have capacities for indirect and direct measures of longitudinal medication adherence. It will be important to determine if metabolite profiles can be used to measure adherence in other chronic illness populations. In addition, with some exceptions (Cortina et al., 2013), objective measures of medication
adherence are not typically used in ongoing clinical management of medication adherence. Nevertheless, implementing multiple, objective direct and indirect measures of treatment adherence in the management of pediatric cancer and other chronic illness populations will provide real-time information regarding adherence patterns over time, including average adherence percentages, metabolite levels, therapeutic efficacy, etc. Future research investigating the specific relationship between adherence and health outcomes in pediatric cancer is imperative for ensuring that patients receive the most optimal treatment that promotes positive health outcomes. Furthermore, research investigating optimal adherence levels and the therapeutic levels of TGN and MMP metabolites and their relationship to clinical outcomes will be important for clinical decision-making involving dose recommendations. Finally, it will important to assess the feasibility of using objective adherence measures in routine clinical care to improve treatment and health outcomes for pediatric cancer.
REFERENCES


Chrzanowska, M., Kolecki, P., Duczmal-Cichocka, B., & Fiet, J. (1999). Metabolites of mercaptopurine in red blood cells: a relationship between 6-thioguanine nucleotides and


Pediatrics, 159(4), 528-534.


Kondryn, H. J., Edmondson, C. L., Hill, J. W., & Eden, T. O. B. (2010). Treatment non-adherence in teenage and young adult patients with cancer. Lancet Oncology, 12, 100-


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<table>
<thead>
<tr>
<th>Aims</th>
<th>Hypotheses</th>
<th>Data Analytic Method</th>
<th>Purpose of Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Describe 6MP medication adherence patterns over 15 months based on behavioral measures of 6MP medication adherence</td>
<td>• The overall rate of 6MP adherence for the entire sample as measured by behavioral measures (e.g., electronic monitoring) would decrease over a 15 month period&lt;br&gt;• There would be group differences in behavioral adherence patterns over time representing optimal adherence, moderate or deteriorating adherence, or chronic nonadherence patterns from baseline to 15 months</td>
<td>• Unconditional Growth Curve Modeling&lt;br&gt;• Group-Based Trajectory Modeling</td>
<td>• Describe population change in behavioral adherence rates from baseline to 15 months&lt;br&gt;• Describe groups or clusters representing similar behavioral adherence trajectories over time</td>
</tr>
<tr>
<td>1. Describe 6MP medication adherence patterns over 15 months based on pharmacological measures of 6MP medication adherence</td>
<td>• There would be three unique clusters demonstrating different metabolite profiles over 15 months: a group that demonstrated potential nonadherence or inadequate dosing: low levels of both metabolites, TGN-MMP; and, two groups that demonstrated a negative correlation between the two metabolites, which typically reflects adherence to 6MP: high TGN-low MMP and low TGN-high MMP</td>
<td>• Two-Step Hierarchical Cluster Analysis</td>
<td>• Identify groups or clusters representing different levels of 6MP metabolites (e.g., high levels of MMP, low levels of TGN; low levels of TGN, high levels of MMP; low levels of TGN, low levels of MMP)</td>
</tr>
<tr>
<td>2. Document the longitudinal relationship between 6MP adherence measured by behavioral and pharmacological methods</td>
<td>• Over a 15 month period, there would be a relationship between 6MP metabolites (TGN and MMP) and behavioral adherence as measured by electronic monitoring: Those individuals who are identified as nonadherent based on pharmacological methods (i.e., low-low metabolites) would also have the lowest behavioral adherence rates, on average, compared to the other metabolite clusters</td>
<td>• Correlations&lt;br&gt;• Descriptive Statistics&lt;br&gt;• Mixed ANOVA (continuous outcomes)</td>
<td>• Determine whether behavioral adherence rates as measured by electronic monitoring are associated with pharmacological measures of adherence as documented by metabolite profiles</td>
</tr>
<tr>
<td>3. Determine the</td>
<td>• Lower 6MP behavioral adherence rates as</td>
<td>• Mixed ANOVA</td>
<td>• Assess the relationship between</td>
</tr>
<tr>
<td>relationship between behavioral and pharmacological measures of 6MP adherence to clinical outcomes (e.g., healthcare utilization, relapse)</td>
<td>evidenced by group-based trajectory modeling would predict worse clinical outcomes (e.g., higher frequencies of health care utilization and increased risk for relapse) over a 15 month period</td>
<td>(continuous outcomes)</td>
<td>behavioral adherence and frequency of health care utilization and risk for disease relapse</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>• Lower 6MP adherence rates as measured by pharmacological adherence measures would predict worse clinical outcomes (e.g., higher frequencies of health care utilization and relapse) over a 15 month period</td>
<td>• Mixed ANOVA (continuous outcomes)</td>
<td>• Assess the relationship between pharmacological measures of adherence and risk for frequency of health care utilization and risk for disease relapse</td>
<td></td>
</tr>
<tr>
<td>3. Determine the relationship between behavioral and pharmacological measures of 6MP adherence to a clinical biomarker (i.e., absolute neutrophil count: ANC): Risk for infection as defined by ANC</td>
<td>• Patients who were identified as nonadherent to 6MP using behavioral adherence measures would likely have an increased risk for infection as indicated by ANC values &lt; 1500</td>
<td>• Mixed ANOVA (continuous outcomes)</td>
<td>• Assess the relationship between behavioral adherence and risk for infection as indicated by ANC</td>
</tr>
<tr>
<td></td>
<td>• Those patients identified as being nonadherent to 6MP using pharmacological measures of adherence would likely have an increased risk for infection as indicated by ANC values &lt; 1500</td>
<td>• Mixed ANOVA (continuous outcomes)</td>
<td>• Assess the relationship between pharmacological adherence and risk for infection as indicated by ANC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Binomial logistic regression (dichotomous outcomes)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. *Demographic and Medical Characteristics of Baseline Sample (N = 139).*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child’s/Adolescent’s age at baseline (years), M ± SD (Range)</td>
<td>12.29 years ± 3.44 (7-19.1 years)</td>
</tr>
<tr>
<td>Type of Cancer Diagnosis</td>
<td></td>
</tr>
<tr>
<td>ALL, n (%)</td>
<td>133 (95.7)</td>
</tr>
<tr>
<td>LBL, n (%)</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td>Duration of Cancer Diagnosis at Baseline (Years), M ± SD (Range)</td>
<td>1.29 years ± 0.35 (0.68-2.27 years)</td>
</tr>
<tr>
<td>Child’s Gender</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>94 (67.6)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>45 (32.4)</td>
</tr>
<tr>
<td>Child’s Ethnicity/Race</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic, Caucasian, n (%)</td>
<td>75 (54.0)</td>
</tr>
<tr>
<td>Non-Hispanic, Other, n (%)</td>
<td>15 (10.9)</td>
</tr>
<tr>
<td>Hispanic, n (%)</td>
<td>49 (33.9)</td>
</tr>
<tr>
<td>Ethnicity Unknown/Not Reported, n (%)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Primary Caregiver Age (years), M ± SD (Range)</td>
<td>40.66 years ± 7.31 (24-59 years)</td>
</tr>
<tr>
<td>Caregiver Relationship who Participated in Baseline Visit</td>
<td></td>
</tr>
<tr>
<td>Biological Mother</td>
<td>126 (90.6)</td>
</tr>
<tr>
<td>Biological Father</td>
<td>12 (8.6)</td>
</tr>
<tr>
<td>Step-Father</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Primary Caregiver’s Marital Status</td>
<td></td>
</tr>
<tr>
<td>Married, n (%)</td>
<td>96 (69.1)</td>
</tr>
<tr>
<td>Not Married, n (%)</td>
<td>43 (30.9)</td>
</tr>
<tr>
<td>Highest Level of Education Completed by Primary Caregiver</td>
<td></td>
</tr>
<tr>
<td>No High School Diploma, n (%)</td>
<td>26 (18.7)</td>
</tr>
<tr>
<td>High School Diploma or G.E.D., n (%)</td>
<td>32 (23.0)</td>
</tr>
<tr>
<td>College Courses/Vocational/Trade School/Associate's Degree, n (%)</td>
<td>45 (32.4)</td>
</tr>
<tr>
<td>Bachelor’s/Master’s/Professional Degree (MD, PhD, JD), n (%)</td>
<td>36 (25.9)</td>
</tr>
<tr>
<td>Household Composition</td>
<td></td>
</tr>
<tr>
<td>One caregiver household</td>
<td>45 (32.4)</td>
</tr>
<tr>
<td>Two caregiver household</td>
<td>94 (67.6)</td>
</tr>
<tr>
<td>Total Annual Household Income Before Taxes, Median</td>
<td></td>
</tr>
<tr>
<td>Less than $18,745</td>
<td>36 (25.9)</td>
</tr>
<tr>
<td>$18,745 to $32,874</td>
<td>18 (12.9)</td>
</tr>
<tr>
<td>$32,875 to $48,999</td>
<td>13 (9.4)</td>
</tr>
<tr>
<td>$49,000 to $72,999</td>
<td>20 (14.4)</td>
</tr>
<tr>
<td>$73,000 to $126,500</td>
<td>31 (22.3)</td>
</tr>
<tr>
<td>More than $126, 500</td>
<td>17 (12.2)</td>
</tr>
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</table>
Table 3. Baseline Demographic and Medical Characteristics of Behavioral Adherence Trajectory Groups.

<table>
<thead>
<tr>
<th></th>
<th>Model Derived Group-Based Trajectories</th>
<th>Adherent versus Nonadherent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chronic Nonadherence (n=17)</td>
<td>Moderate Adherence (n=26)</td>
</tr>
<tr>
<td>Patient's age (years), M ± SD</td>
<td>13.34 ± 3.40</td>
<td>13.57 ± 3.36</td>
</tr>
<tr>
<td>Child's Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (58.8)</td>
<td>18 (69.2)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>7 (41.2)</td>
<td>8 (30.8)</td>
</tr>
<tr>
<td>Child's Ethnicity/Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic, Caucasian, n (%)</td>
<td>10 (58.8)</td>
<td>14 (53.8)</td>
</tr>
<tr>
<td>Non-Hispanic, Other, n (%)</td>
<td>2 (11.8)</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>Hispanic, n (%)</td>
<td>5 (29.4)</td>
<td>9 (34.6)</td>
</tr>
<tr>
<td>Household Composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One caregiver household</td>
<td>6 (35.3)</td>
<td>13 (50.0)</td>
</tr>
<tr>
<td>Two caregiver household</td>
<td>11 (64.7)</td>
<td>13 (50.0)</td>
</tr>
<tr>
<td>TPMT Absolute Value, M ± SD</td>
<td>12.57 ± 3.40</td>
<td>12.79 ± 3.50</td>
</tr>
<tr>
<td>TPMT Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygote, n (%)</td>
<td>5 (62.5)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Wild type, n (%)</td>
<td>14 (53.8)</td>
<td>21 (87.5)</td>
</tr>
<tr>
<td>Disease Relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients Relapsed, n (%)</td>
<td>1 (5.9)</td>
<td>5 (19.2)</td>
</tr>
<tr>
<td>Patients Deceased, n (%)</td>
<td>1 (5.9)</td>
<td>1 (3.8)</td>
</tr>
<tr>
<td>Duration of Relapse from Baseline (Years), M±SD</td>
<td>1.39</td>
<td>1.98 ± 1.06</td>
</tr>
<tr>
<td>Duration of Death from Baseline (Years), M±SD</td>
<td>1.39</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Note: Significant differences between behavioral adherence trajectories denoted by **p < 0.01; *p < 0.05
Table 4. *Baseline Demographic and Medical Characteristics of Metabolite Profiles.*

<table>
<thead>
<tr>
<th></th>
<th>High TGN-Low MMP (n=32)</th>
<th>Low TGN-High MMP (n=59)</th>
<th>Low TGN-Low MMP (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's age (years), M ± SD</td>
<td>11.82 ± 3.36</td>
<td>11.97 ± 3.44</td>
<td>12.99 ± 3.46</td>
</tr>
<tr>
<td>Child’s Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>24 (75.0)</td>
<td>39 (66.1)</td>
<td>31 (64.6)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>8 (25.0)</td>
<td>20 (33.9)</td>
<td>17 (35.4)</td>
</tr>
<tr>
<td>Child’s Ethnicity/Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic, Caucasian, n (%)</td>
<td>23 (71.9)</td>
<td>30 (50.8)</td>
<td>23 (47.9)</td>
</tr>
<tr>
<td>Non-Hispanic, Other, n (%)</td>
<td>3 (9.4)</td>
<td>4 (6.8)</td>
<td>9 (18.8)</td>
</tr>
<tr>
<td>Hispanic, n (%)</td>
<td>6 (18.8)</td>
<td>25 (42.4)</td>
<td>16 (33.3)</td>
</tr>
<tr>
<td>Household Composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One caregiver household</td>
<td>7 (21.9)</td>
<td>19 (32.2)</td>
<td>19 (39.6)</td>
</tr>
<tr>
<td>Two caregiver household</td>
<td>25 (78.1)</td>
<td>40 (67.8)</td>
<td>29 (60.4)</td>
</tr>
<tr>
<td>TPMT Absolute Value, M ± SD</td>
<td>10.96 ± 4.02</td>
<td>13.76 ± 3.57</td>
<td>12.63 ± 2.93</td>
</tr>
<tr>
<td>TPMT Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygote, n (%)</td>
<td>12 (46.2)</td>
<td>3 (5.6)</td>
<td>3 (6.7)</td>
</tr>
<tr>
<td>Wild type, n (%)</td>
<td>14 (53.8)</td>
<td>51 (94.4)</td>
<td>42 (93.3)</td>
</tr>
</tbody>
</table>

*Note: No significant differences between metabolite profiles on relevant demographic and medical characteristics.*
Table 5. *Group-Based Trajectory Modeling: Weekly Behavioral Adherence (N = 131).*

<table>
<thead>
<tr>
<th>Behavioral Adherence Group Trajectory</th>
<th>Parameter</th>
<th>Estimate (Std. Err.)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Adherence</td>
<td>Intercept</td>
<td>96.32 (0.83)</td>
<td>115.67</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>-0.10 (0.06)</td>
<td>-1.67</td>
<td>0.09</td>
</tr>
<tr>
<td>Moderate Adherence</td>
<td>Intercept</td>
<td>67.58 (1.59)</td>
<td>42.61</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>-0.006 (0.002)</td>
<td>-3.14</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Chronic Nonadherence</td>
<td>Intercept</td>
<td>62.69 (1.93)</td>
<td>32.50</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>-2.82 (0.15)</td>
<td>-18.67</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>
Table 6. Metabolite Profiles: Absolute TGN and MMP Values from Baseline to 15 months (M ± SD; Range).

<table>
<thead>
<tr>
<th></th>
<th>High TGN - Low MMP</th>
<th>Low TGN - High MMP</th>
<th>Low TGN - Low MMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-TGN 6-MMP</td>
<td>6-TGN 6-MMP</td>
<td>6-TGN 6-MMP</td>
</tr>
<tr>
<td>Baseline</td>
<td>1269 ± 541 (720-3233)</td>
<td>6855 ± 5117 (0-18609)</td>
<td>583 ± 226 (232-1482)</td>
</tr>
<tr>
<td>3 months</td>
<td>1196 ± 524 (743-2647)</td>
<td>5384 ± 4226 (122-14734)</td>
<td>581 ± 211 (249-1141)</td>
</tr>
<tr>
<td>6 months</td>
<td>1166 ± 368 (715-2248)</td>
<td>6738 ± 5610 (116-23707)</td>
<td>537 ± 185 (253-1102)</td>
</tr>
<tr>
<td>9 months</td>
<td>1096 ± 366 (733-2178)</td>
<td>7017 ± 4900 (226-15526)</td>
<td>577 ± 228 (198-1123)</td>
</tr>
<tr>
<td>12 months</td>
<td>1111 ± 370 (706-1957)</td>
<td>8679 ± 8356 (230-30263)</td>
<td>560 ± 211 (209-1008)</td>
</tr>
<tr>
<td>15 months</td>
<td>1184 ± 483 (711-2551)</td>
<td>4482 ± 5213 (156-17299)</td>
<td>580 ± 173 (266-1006)</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>1176 ± 449 (706-3233)</td>
<td>6600 ± 5645 (0-30263)</td>
<td>569 ± 206 (198-1482)</td>
</tr>
</tbody>
</table>
Table 7. Behavioral Adherence Rates for Metabolite Profiles: Descriptive Statistics (M ± SD, Range).

<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
<th>15 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High TGN-Low MMP</strong></td>
<td>90.0 ± 26.7</td>
<td>85.5 ± 29.7</td>
<td>84.3 ± 28.3</td>
<td>89.4 ± 27.5</td>
<td>75.1 ± 37.2</td>
</tr>
<tr>
<td></td>
<td>(0-100)</td>
<td>(0-120)</td>
<td>(20-100)</td>
<td>(0-100)</td>
<td>(0-100)</td>
</tr>
<tr>
<td><strong>Low TGN-High MMP</strong></td>
<td>85.0 ± 29.6</td>
<td>88.6 ± 25.2</td>
<td>88.3 ± 22.9</td>
<td>87.2 ± 25.7</td>
<td>90.4 ± 23.8</td>
</tr>
<tr>
<td></td>
<td>(0-100)</td>
<td>(0-120)</td>
<td>(0 - 100)</td>
<td>(0-100)</td>
<td>(0-120)</td>
</tr>
<tr>
<td><strong>Low TGN-Low MMP</strong></td>
<td>74.8 ± 37.2</td>
<td>70.9 ± 35.9</td>
<td>74.9 ± 34.2</td>
<td>70.5 ± 36.3</td>
<td>76.4 ± 35.1</td>
</tr>
<tr>
<td></td>
<td>(0-120)</td>
<td>(0-120)</td>
<td>(0 - 100)</td>
<td>(0-100)</td>
<td>(0-120)</td>
</tr>
</tbody>
</table>
Table 8. *Descriptive Statistics for Behavioral Adherence Trajectories and ANC (Absolute Values) (M ± SD)*.

<table>
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<tr>
<th></th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic Nonadherence (n=17)</strong></td>
<td>1865 ± 1377</td>
<td>1931 ± 916</td>
<td>2067 ± 1046</td>
<td>2070 ± 1036</td>
<td>2021 ± 1107</td>
<td>2384 ± 985</td>
</tr>
<tr>
<td><strong>Moderate Adherence (n=26)</strong></td>
<td>2135 ± 1134</td>
<td>2222 ± 926</td>
<td>2188 ± 23928</td>
<td>2667 ± 1022</td>
<td>2415 ± 880</td>
<td>2898 ± 1582</td>
</tr>
<tr>
<td><strong>Optimal Adherence (n=88)</strong></td>
<td>1863 ± 1018</td>
<td>2168 ± 1418</td>
<td>2063 ± 1242</td>
<td>2054 ± 1108</td>
<td>2194 ± 1535</td>
<td>2321 ± 1806</td>
</tr>
</tbody>
</table>
Table 9. Descriptive Statistics for Behavioral Adherence Trajectories and Healthcare Utilization (Total Clinic Visits, Hospitalizations and Emergency Room Visits for Preceding Three Months) (M ± SD, Range).

<table>
<thead>
<tr>
<th></th>
<th>Baseline to 3 Months</th>
<th>3 months to 6 months</th>
<th>6 months to 9 months</th>
<th>9 months to 12 months</th>
<th>12 months to 15 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chronic Nonadherence (n=17)</strong></td>
<td>5.12 ± 3.06 (3-14)</td>
<td>4.41 ± 2.09 (3-10)</td>
<td>4.76 ± 2.31 (2-10)</td>
<td>5.29 ± 4.12 (3-19)</td>
<td>4.50 ± 2.92 (1-10)</td>
</tr>
<tr>
<td><strong>Moderate Adherence (n=26)</strong></td>
<td>4.69 ± 2.41 (2-13)</td>
<td>5.16 ± 3.17 (2-18)</td>
<td>3.72 ± 0.98 (2-6)</td>
<td>4.39 ± 1.75 (3-9)</td>
<td>4.09 ± 1.88 (2-10)</td>
</tr>
<tr>
<td><strong>Optimal Adherence (n=88)</strong></td>
<td>4.43 ± 2.04 (0-9)</td>
<td>4.53 ± 2.35 (2-13)</td>
<td>4.28 ± 1.86 (1-10)</td>
<td>3.78 ± 2.00 (0-13)</td>
<td>3.95 ± 2.31 (0-13)</td>
</tr>
</tbody>
</table>
Table 10. Descriptive Statistics for Metabolite Profiles and Healthcare Utilization (Total Clinic Visits, Hospitalizations and Emergency Room Visits for Preceding Three Months) (M ± SD, Range).

<table>
<thead>
<tr>
<th></th>
<th>Baseline to 3 Months</th>
<th>3 months to 6 months</th>
<th>6 months to 9 months</th>
<th>9 months to 12 months</th>
<th>12 months to 15 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>High TGN-Low MMP</td>
<td>4.91 ± 2.92</td>
<td>4.36 ± 2.33</td>
<td>4.27 ± 1.76</td>
<td>3.60 ± 1.31</td>
<td>4.24 ± 2.54</td>
</tr>
<tr>
<td></td>
<td>(2-14)</td>
<td>(2-13)</td>
<td>(3-9)</td>
<td>(1-7)</td>
<td>(2-10)</td>
</tr>
<tr>
<td>Low TGN-High MMP</td>
<td>4.15 ± 1.96</td>
<td>4.43 ± 2.24</td>
<td>4.29 ± 1.89</td>
<td>4.11 ± 1.98</td>
<td>3.85 ± 1.85</td>
</tr>
<tr>
<td></td>
<td>(0-9)</td>
<td>(2-13)</td>
<td>(1-10)</td>
<td>(1-11)</td>
<td>(2-10)</td>
</tr>
<tr>
<td>Low TGN-Low MMP</td>
<td>4.86 ± 2.31</td>
<td>5.00 ± 2.86</td>
<td>4.12 ± 1.79</td>
<td>4.54 ± 3.34</td>
<td>4.36 ± 2.72</td>
</tr>
<tr>
<td></td>
<td>(0-13)</td>
<td>(2-18)</td>
<td>(2-10)</td>
<td>(2-19)</td>
<td>(1-13)</td>
</tr>
</tbody>
</table>
Table 11. Metabolite Profiles: Total Relapse Rates and Mortality from Baseline to 15 Months.

<table>
<thead>
<tr>
<th>Metabolite Profile</th>
<th>Baseline</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
<th>12 months</th>
<th>15 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Patients Relapsed by Metabolite Cluster from Baseline to 15 months, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High TGN-Low MMP</td>
<td>3 (9.4)</td>
<td>2 (8.7)</td>
<td>6 (24.0)</td>
<td>3 (11.5)</td>
<td>1 (5.0)</td>
<td>3 (17.6)</td>
</tr>
<tr>
<td>Low TGN-High MMP</td>
<td>7 (12.7)</td>
<td>8 (14.0)</td>
<td>6 (9.8)</td>
<td>3 (5.7)</td>
<td>4 (6.5)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>Low TGN-Low MMP</td>
<td>8 (16.7)</td>
<td>8 (16.0)</td>
<td>3 (7.0)</td>
<td>6 (12.2)</td>
<td>3 (8.6)</td>
<td>2 (5.3)</td>
</tr>
<tr>
<td><strong>Total Patients Deceased by Metabolite Cluster from Baseline to 15 months, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High TGN-Low MMP</td>
<td>1 (3.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Low TGN-High MMP</td>
<td>4 (6.8)</td>
<td>5 (8.8)</td>
<td>2 (3.3)</td>
<td>1 (1.9)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Low TGN-Low MMP</td>
<td>1 (2.1)</td>
<td>1 (2.0)</td>
<td>2 (4.7)</td>
<td>3 (6.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Duration of Relapse from Baseline (Years) by Metabolite Cluster from Baseline to 15 months, M±SD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High TGN-Low MMP</td>
<td>2.03 ± 1.67</td>
<td>2.62 ± 1.86</td>
<td>2.62 ± 0.98</td>
<td>2.31 ± 0.97</td>
<td>3.94 ± NA</td>
<td>2.55 ± 1.32</td>
</tr>
<tr>
<td>Low TGN-High MMP</td>
<td>1.14 ± 1.14</td>
<td>0.88 ± 0.93</td>
<td>1.36 ± 1.11</td>
<td>2.57 ± 1.68</td>
<td>2.47 ± 0.85</td>
<td>3.12 ± NA</td>
</tr>
<tr>
<td>Low TGN-Low MMP</td>
<td>1.70 ± 1.12</td>
<td>1.91 ± 1.05</td>
<td>0.91 ± 0.43</td>
<td>1.71 ± 0.91</td>
<td>2.48 ± 0.72</td>
<td>3.15 ± 0.12</td>
</tr>
<tr>
<td><strong>Duration of Death from Baseline (Years) by Metabolite Cluster from Baseline to 15 months, M±SD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High TGN-Low MMP</td>
<td>1.36 ± NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Low TGN-High MMP</td>
<td>1.57 ± 0.29</td>
<td>1.53 ± 0.27</td>
<td>1.39 ± 0.04</td>
<td>1.42 ± NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Low TGN-Low MMP</td>
<td>1.39 ± NA</td>
<td>1.39 ± NA</td>
<td>1.57 ± 0.24</td>
<td>1.50 ± 0.21</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Note:* Relapse and death are static variables indicating whether or not the patient relapsed/passed away at any time during or shortly after the study. Metabolite profile is a dynamic variable: patients’ can be in different metabolite profiles across time.
Figure 1. The proposed relationship between multiple objective measures of 6MP medication adherence and clinical outcomes (absolute neutrophil count, healthcare utilization, and risk for disease relapse).
Figure 2. Group-based trajectories for behavioral adherence from baseline to 15 months (entire sample; $N = 131$).
Figure 3. Metabolite profiles for TGN and MMP: Mean z scores by metabolite profile from baseline to 15 months ($N = 139$).
Figure 4. 5-Day Adherence rates from 3 to 15 months for 6MP metabolite profiles.

Note: Significant differences in cross-sectional comparisons between the three group metabolite profiles: * denotes significant differences in 6MP behavioral adherence at that time point; Significant differences in cross-sectional comparisons between the two group metabolite profiles: † denotes significant differences in 6MP behavioral adherence at that time point.
Figure 5. Behavioral Adherence Trajectories and Absolute Neutrophil Count: Risk for Infection as Measured by Absolute Neutrophil Count.
Figure 6. Metabolite Profiles and Absolute Neutrophil Count (Absolute Values)

Note: * indicates between groups significance between the low TGN-low MMP metabolite profile and the low TGN-high MMP metabolite profile.
Figure 7. Metabolite Profiles and Absolute Neutrophil Count: Risk for Infection as Measured by Absolute Neutrophil Count.
Appendix I. Quality Control Procedures for Pharmacological and Behavioral Data

**Electronic Monitoring of 6MP:** An electronic monitoring device (i.e., the Medication Event Monitoring System (MEMS®) from the AARDEX Corporation, Palo Alto, CA) was used to monitor adherence to 6MP oral medication therapy across 15 months. Time-stamped medication events are stored in the MEMS® and transferred to a program (i.e., PowerView) that records the daily history of medication taking. This information will then be exported to a secure Excel database and cleaned for analysis.

**Serum Assay (6MP Metabolite Concentrations):** Blood samples, which provide metabolite concentrations of 6MP were collected at 6 points in time (baseline, 3, 6, 9, 12, and 15 months) using a standardized bioassay (Traore et al., 2006). The use of red blood cells (RBC), TGN, and MMP concentrations was based on an extensive review of the extant literature on 6MP pharmacology and utility to detect 6MP (Davies & Lilleyman, 1995; Traore et al., 2006). Pharmacology data is entered into a secure database that provides the metabolite levels for each time point, the last dosage, and date/time of the last dose. This data will be mapped on and verified with the electronic monitoring data. If there is a discrepancy between the electronic monitoring data and the information regarding the date/time of the last dose captured on the 6MP metabolite concentration form, the information captured from the electronic monitoring data will be used because this is an objective measure and the information captured on the form is based on patient/parent self-report.

High performance liquid chromatography (HPLC) with ultraviolet detection will be employed to measure the 6MP activity in RBC. Samples for metabolite determination are first treated with acetonitrile and perchloric acid to precipitate protein and acidify the mixture. Hydrolysis at 95-100°C converts the thioguanine nucleotides to 6-thioguanine (6-TG) and the
methylated thiopurine nucleotides to a derivative of 6-methylmercaptopurine (6-MMP). Thioxanthine is the internal standard. The 6-TG has a quantitation range of 0.15-10 µ mol l⁻¹. In this assay, the coefficient of variation is less than 5%. The 6-MMP has a quantitation range of 0.015-10 µ mol l⁻¹ and the coefficient of variation is less than 3% (Dervieux & Boulieu, 1998; Medard, Nafa, & Jacqz-Aigrain, 1997).

Analysts are separated using the Synergi Hydro RP 3mm x 250mm column and a mobile phase of 6% Acetonitrile and 94% 20mM phosphate buffer at pH 3.0. The 6-TG has a quantitation range of 25 – 1600 pmol/8 x 10^8 RBC and the 6MMPd range is 188 – 12000 pmol/8 x 10^8 RBCs. 6TG intraday controls of 75, 250 and 1200 pmol have CVs of 5.1, 5.2 and 5.2 respectively. 6MMPd intraday controls of 563, 2250 and 9000 pmol have CV’s of 5.1, 5.0 and 4.6 respectively.
Appendix II. Five-Day Adherence Rates for MEMS Users versus Proxy MEMS Users