

Study Title: Combination of Pioglitazone and Tyrosine Kinase Inhibitor (TKI) in Relapsed Chronic Myeloid Leukemia Following a First TKI Discontinuation

Study Phase: Phase 2

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PROTOCOL SYNOPSIS

Study Treatments	Pioglitazone
Study Title	Combination of Pioglitazone and Tyrosine Kinase Inhibitor (TKI) in Relapsed Chronic Myeloid Leukemia Following a TKI Discontinuation
Phase	II
Summary and Study Rationale	Approximately 40-60% of patients with chronic myeloid leukemia (CML) who discontinue imatinib (IM) or second-generation TKI after sustained deep molecular remission will experience molecular relapse, but regain molecular response after restarting TKI. The success rate of a second TKI discontinuation trial is low (25%), and continuous TKI is recommended for these patients. Pioglitazone (PIO) is a well-tolerated orally bioavailable Peroxisome Proliferator-Activated Receptors, (PPARγ) agonist with <i>in vitro</i> <i>BCR-ABL1</i> tyrosine kinase inhibition activity that is synergistic with TKI. In this study, we will assess safety and efficacy of PIO combined with TKI in CML patients with molecular relapse following a first TKI discontinuation trial. Success of a second TKI discontinuation trial, following 6 months of sustained deep molecular response while on PIO and TKI combination will be assessed.
Study Design	This is a single-center, prospective, phase II trial. For the purposes of this protocol and all analyses, a cycle of treatment is defined as 28 days. Eligible subjects will start PIO in addition to TKI, at 30mg/d for 2 cycles (56 days) and, in the absence of grade 2 or higher toxicities, increase to 45mg/d. Subjects will receive PIO in addition to TKI for 6 months from the first documented \leq molecular response (MR) ^{4,5} and if subjects maintain \leq MR ^{4,5} for 6 months with less or equal than 2 <i>BCR/ABL1</i> transcript fluctuation $<$ major molecular response (MMR) will be eligible for a second TKI discontinuation. Subjects who fail to achieve MMR after 4 months, or \leq MR ^{4,5} after 6 months of the combination treatment of PIO and TKI will be taken off treatment. Subjects who lose MMR while on the combination will be taken off study and managed at the discretion of the treating physician. While the combination of PIO and TKI, blood quantitative real time Polymerase Chain Reaction (qRT-PCR) for <i>BCR-ABL1</i> will be collected on a monthly basis, and after discontinuation of PIO and TKI, disease be monitored by blood qRT-PCR for <i>BCR-ABL1</i> obtained monthly for the first 3 months, quarterly for 1 year, and then bi-annually for 1 year.
Study Objectives	<ul style="list-style-type: none"> To assess safety of the combination of PIO and TKI in CML subjects who experience a loss of MMR following a first TKI discontinuation To assess survival without loss of MMR following a second TKI discontinuation trial in subjects who achieve or maintain \leqMR^{4,5} for at least 6 months with the combination PIO and TKI
Study Endpoints	<ul style="list-style-type: none"> <i>Safety:</i> To describe adverse events (AE) in subjects receiving the combination of PIO and TKI. AEs will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 assessed through scheduled assessment and subject reported diary To determine the proportion of subjects who maintain MMR following discontinuation of PIO and TKI using blood qRT-PCR for <i>BCR-ABL1</i> obtained monthly for the first 3 months, quarterly following 1 year and bi-annually thereafter.
Diagnosis and Main Inclusion Criteria	Age \geq 16 diagnosed with CML in any phase Molecular relapse (loss of MMR) post-first TKI discontinuation trial Complete cytogenetic and complete hematologic response

Number of Subjects	26 subjects
Duration of Subject Participation	2 years
Approximate Duration of Study	5years
Dosage and Administration	PIO 30mg/d, 45mg/d, oral tablets
Concomitant Medications/treatment	TKI
Safety Evaluations	Monthly assessments as needed with CTCAE v. 4.0
Statistical Analysis	<p>Under Simon's optimal two stage design with a 5% significance level, 80% power, and 8% drop out rate assuming a lower boundary of interest in the response rate of 20% and an upper boundary of interest in the response rate of 50%, a total of 26 evaluable subjects are required for the evaluation of the primary endpoint; 9 in Stage 1, additional 15 in Stage 2 and 2 for drop out.</p> <p>If less than 2 of 9 evaluable subjects in Stage 1 maintain MMR after second-TKI discontinuation or > 2 subject develop > grade 3 cardiac toxicity then the study will terminate, otherwise the study shall continue as planned and enrollment of an additional 15 subjects shall continue into Stage 2. If, at the final analysis, less than 9 of 24 evaluable subjects in both Stage 1 and 2 maintain MMR then it will be concluded that PIO and TKI does not deserve further investigation.</p> <p>Fisher exact test and the χ^2 test were used to compare categorical variables, and the Mann-Whitney U test was used for continuous variables. The probability of molecular relapse free survival will be estimated using the Kaplan-Meier method, and be calculated as the time from the first day of TKIs discontinuation to the date of the first documentation of molecular relapse or the last follow up day for patients who did not relapse. $P < 0.05$ will be considered statistically significant.</p>

ABBREVIATIONS

2G	Second-Generation
AE	Adverse Event
AP	Accelerated Phase
AUC	area under the curve
BC	Blast crisis
BM	Bone Marrow
BOS	Bosutinib
BP	Blood Pressure
CBC	Complete Blood Count
CCyR	Complete Cytogenetic Response
CHR	Complete Hematologic Response
CLcr	Creatinine Clearance
Cmax	Peak Serum Concentration
Cmin	Trough Serum Concentrations
CML	Chronic Myeloid Leukemia
CMR	Complete molecular remission
CP	Chronic Phase
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAS	Dasatinib
DSMC	the Data and Safety Monitoring Committee
ELN	European Leukemia Net
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
ICH	International Conference on Harmonization
IRB	Institutional Review Board
IS	International Scale
IM	Imatinib Mesylate
IND	Investigational New Drug
LSC	Leukemia Stem Cell
mCyR	minor Cytogenetic Response
minCyR	minimal Cytogenetic Response
MMAS	Morisky Medication Adherence Scales
MMR	Major Molecular Response
MR	Molecular Response
NCCN	National Comprehensive Cancer Network
NCI	The National Cancer Institute
NIL	Nilotinib
noCyR	no Cytogenetic response
NYHA	New York Heart Association
PCyR	Partial Cytogenetic Response
Ph +	Philadelphia chromosome
PI	Principal Investigator
PIO	Pioglitazone
PON	Ponatinib
PPAR	Peroxisome Proliferator-Activated Receptors
PPRE	Peroxisome Proliferator Response Elements
qRT-PCR	quantitative Real-Time Polymerase Chain Reaction
RFS	Relapse-Free Survival
RXR	Retinoid X Receptor
SAE	Serious Adverse Events
TKI	Tyrosine Kinase Inhibitor

TZD	Thiazolidinedione
UA	Urine Analysis
UP	Unanticipated Problem posing risks to subjects or others
USD	United States dollar

1. Background

1.1 Chronic Myeloid Leukemia

Chronic myeloid leukemia (**CML**) is a clonal myeloproliferative stem cell disorder characterized by the presence of a signature hybrid oncogene, the *BCR-ABL1* [1], and a unique triphasic clinical course. The majority of patients (~80%) are diagnosed during the early phase, or chronic phase (**CP**), however, without effective treatment, CML invariably progresses to the advanced phases of the disease: accelerated phase (**AP**) or blast crisis (**BC**). The Philadelphia chromosome (**Ph+**), the driving leukemogenic mechanism in CML, is a reciprocal translocation between chromosome 9 and 22 that juxtaposes two genes involved in cell signaling, signal transduction, and cell proliferation: *BCR* and *ABL1*. The *ABL1* gene encodes non-receptor tyrosine kinases that become deregulated and constitutively active by the juxtaposition of *BCR*. BCR-ABL is central in controlling downstream pathways involved in cell proliferation, regulation of cellular adhesion and apoptosis [1]. The understandings of the pathophysiology of CML led to development of drugs that specifically target the tyrosine kinase activity in BCR-ABL. These tyrosine kinase inhibitors (**TKI**) became the mainstay of modern therapy in CML and have revolutionized patients' outcomes. Currently, there are 5 TKIs approved for the treatment CML: Imatinib (**IM**), nilotinib (**NIL**), and dasatinib (**DAS**) for both first- and second-line therapy, bosutinib (**BOS**) for second- or third-line therapy and ponatinib (**PON**) for patients with the T315I mutation or for whom no other TKI is indicated. Indeed, treatment of patients with CML with TKIs is ranked as one of the great medical success stories of the past 30 years, which translates into life spans indistinguishable from those of similar persons without leukemia [2].

1.2 Definitions of Response in CML

Several organizations, such as the European Leukemia Net (**ELN**) and the National Comprehensive Cancer Network (**NCCN**), provide extensive evidence based recommendations that define response and provide milestones or algorithms to monitor response on TKIs (<http://www.nccn.org/clinical.asp>) [3]. Response criteria for hematologic, cytogenetic (based on metaphase cytogenetic preparations), and molecular responses (**MR**) are shown in **Table 1**. In the absence of universal standards or reagents, the International Scale (**IS**) was developed to harmonize MRs across laboratories by applying a laboratory specific conversion factor [4]. All molecular response criteria and recommendations for intervention in the NCCN or ELN guidelines are based on IS molecular responses. Major molecular response (**MMR**) is defined by a level of *BCR-ABL1* transcript by quantitative Real-Time Polymerase Chain Reaction (**qRT-PCR**) of

Table 1. Definitions of Response in CML

Response	Definition
Hematologic	
Complete (CHR)	WBC $< 10 \times 10^9/L$ Basophils $< 5\%$ No myelocytes, promyelocytes, myeloblasts in the differential Platelet count $< 450 \times 10^9/L$ Spleen nonpalpable
Cytogenetic[*]	
Complete (CCyR)	No Ph+ metaphases
Partial (PCyR)	1% to 35% Ph+ metaphases
Minor (mCyR)	36% to 65% Ph+ metaphases
Minima (minCyR)	66% to 95% Ph+ metaphases
None (noCyR)	$> 95\%$ Ph+ metaphases
Molecular[†]	
Major (MMR)	Ratio of <i>BCR-ABL1</i> to <i>ABL</i> (or other housekeeping genes) $\leq 0.1\%$ on the IS
MR ^{4.0}	Ratio of <i>BCR-ABL1</i> to <i>ABL</i> $< 0.01\%$
MR ^{4.5}	Ratio of <i>BCR-ABL1</i> to <i>ABL</i> $< 0.032\%$
MR ^{5.0}	Ratio of <i>BCR-ABL1</i> to <i>ABL</i> $< 0.001\%$

<0.1%, MR⁴ by a *BCR-ABL1*< 0.01%, MR⁴ by a *BCR-ABL1*< 0.0032%, and MR^{5.0} by a *BCR-ABL1*< 0.001%. Complete molecular remission (CMR) is defined by a *BCR-ABL1* level < MR^{4.5}.

1.3 Tyrosine Kinase Inhibitor Discontinuation Trials

Although TKIs dramatically improved CML patient outcomes and improved quality of life compared to previous treatments, therapy with a TKI is currently life-long therapy, requiring patients to take medication daily and to make frequent visits for monitoring. Patients often exposed to chronic, frequently low grade adverse events (AE), and negative emotional consequences can manifest even in the absence of overt disease symptoms [5]. Thus, daily adherence to TKI therapy might be challenging for a significant proportion of patients. However, TKI may not appear to be needed as a lifelong therapy. Recent data suggests that in patients in CP and AP treated with IM for ≥ 3 years and who have maintained CMR for at least 2 years, IM can be safely discontinued without reappearance of the *BCR-ABL1* in approximately 40~60% of patients, and these remissions are durable. (Figure 1. A~D) [6-9]. Although *BCR-ABL1* becomes detectable in the other 40~60% within a year from IM discontinuation, reinstitution of IM is associated with a second molecular remission in all cases. No patients transformed to BC on any of these discontinuation trials. Similar to IM, discontinuation of the second-generation (2G) DAS or NIL used in front-line or for IM intolerance or resistance is associated with a 60% treatment-free survival without loss of MMR [10].

However, for CP-CML patients who lose MMR after first IM discontinuation and regain CMR following a restart of IM, the current recommendations are to keep these patients on IM lifelong given the low success rates (25%) of a second TKI discontinuation trial [11].

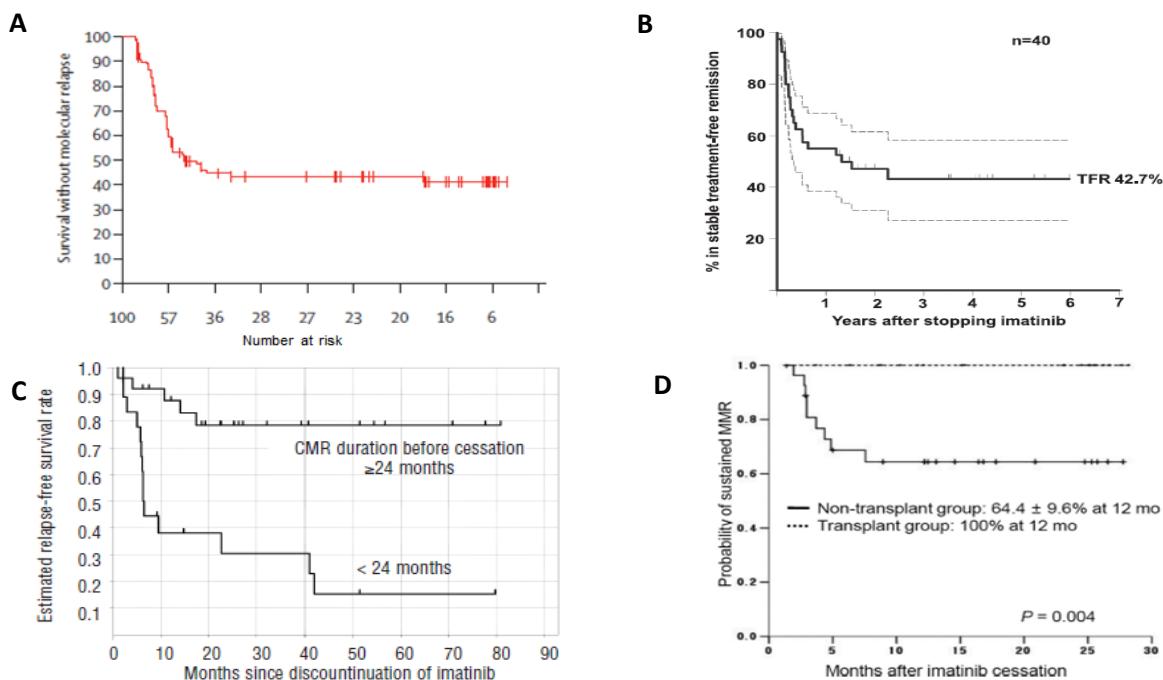


Figure 1. Molecular relapse free survival following imatinib discontinuation: A. data on 100 patients; B. data on 40 patients, C. data on 43 patients; D. data on 48 patients

Institutional Experience with TKIs discontinuation. From Jan 2010 and Jul 2015, 24 subjects with CML in CP (n=21), AP (n=2) and lymphoid BC (n=1) discontinued IM (n=17), DAS (n=4), or BOS (n=3), (the case of CML BC was previously published [12].). Subjects were treated with TKI for a median of 78.1(38.4-168.1) months, and had sustained CMR for median 54.2 (10.4-156.5) months. Subjects were monitored by qRT-PCR for *BCR-ABL1* monthly for the first 3 months, quarterly for the following 2 years and then bi-annually. Reason for TKI discontinuation was predominantly driven by patients' request and TKI intolerance. Seven subjects discontinued 2G TKIs for resistance (n=3) or intolerance (n=4) to previous TKIs. With a median follow-up of 38.3 months (range, 2.3-69.7), probability of survival without loss of MMR (molecular relapse free survival) at 1 year was 68%, as depicted in **Figure 2**. Eight (33 %) subjects lost MMR a median of 4 months (range, 1-14) after IM was stopped. Among seven evaluable subjects, 5 regained MMR, of which 4 <MR^{4.5} following restart of IM. Median time to regain MMR after restart of IM was 2.2 (1.5-28) months, and <MR^{4.5} was 2.2 (1.5-2.9) months.

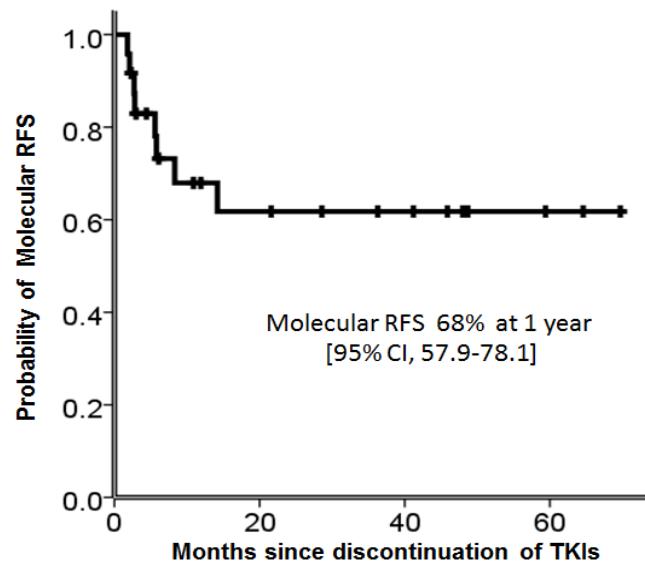


Figure 2. Kaplan-Meier curve of probability of molecular Relapse Free Survival (RFS); TKI, Tyrosine Kinase Inhibitor

1.4 CML stem cell and Peroxisome Proliferator-Activated Receptors γ

1.4.1 The role of leukemia stem cell in CML

Targeting the leukemic stem cell (LSC) is currently one of the major therapeutic focuses in leukemia research. The definition of a stem cell is functional rather than morphological, and these cells are characterized by three distinct properties: self-renewal, differentiation, and proliferative capacity [13]. The LSC is responsible for disease initiation, drug resistance and relapse [14, 15]. The precise molecular mechanism of LSC mediated resistance to current therapies has not been fully elucidated, but one critical factor might be the quiescence of these cells that allows this population to evade therapies that target rapidly dividing cells. These LSC are thought to be the reservoir for disease progression [16-22]. **Given that CML is a stem cell disease, and given the known resistance of CML LSC to TKI [22], novel approaches targeting the CML LSC are needed.**

1.4.2 The role of STAT5 in CML

BCR-ABL activates many signaling pathways in leukemic cells, such as STAT5, RAS, PI-3K and NF- κ B [23]. STAT5 was one of the first pathways to be described as being constitutively activated by BCR-ABL [24-26]. BCR/ABL activates STAT5 not only directly [27], but also indirectly via phosphorylation of JAK2 (**Figure 3**) [28, 29]. Phosphorylated STAT5 forms STAT5 dimers that translocate to nucleus, bind to DNA binding domains, and promote activation of downstream target genes. These target genes include the anti-apoptosis Bcl-XL6 [30], the drug resistance phenotype Rad51 [31] and the Peroxisome Proliferator-Activated Receptor γ (PPAR γ).

Role of STAT5 in preclinical CML models. In murine transplant models, bone marrow (**BM**) CD34+ cells collected from STAT5 knockout mice retrovirally transduced with *BCR-ABL1*, co-cultured with growth factors and then transplanted into lethally irradiated syngeneic mouse, did not lead to a leukemic phenotype in these syngeneic transplant recipients [32-34]. Furthermore, high levels of STAT5 were protective for *BCR-ABL1* cells co-cultured with TKI [35] and specific targeting of STAT5 activity increased eradication of *BCR-ABL1* cells, including primary CML CD34+ cells and CML cells resistant to TKI [36]. **This data highlights the importance of STAT5 in the pathogenesis of CML.**

1.4.3 Peroxisome Proliferator-Activated Receptors

The PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes [37]. PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, protein), and tumorigenesis of higher organisms. Three types of PPARs have been identified: α , γ , and δ (Figure 4). PPAR α is expressed predominantly in liver, kidney, heart, muscle, and adipose tissue. PPAR δ is expressed in many tissues but markedly in brain, adipose tissue, and skin. PPAR γ is expressed at high levels in adipose tissue and macrophage, but also in normal marrow and blood CD34+ progenitor cells [25-27], normal neutrophils and lymphocytes, as well as circulating leukemic cells from patients with AML, ALL, CML [38]. PPAR γ activation occurs predominantly through interactions with ligands. Natural ligands of PPAR γ include some long-chain polyunsaturated fatty acids, arachidonic acid metabolites and fatty acid derived components. The anti-diabetic thiazolidinedione class of drugs (TZD), certain non-steroidal anti-inflammatory drugs and some non-thiazolidinedione tyrosine are the synthetic ligands of PPAR γ . After ligand activation, PPAR γ forms a heterodimer with the retinoid X receptor (**RXR**) and then binds to the peroxisome proliferator response elements (PPREs) in the target gene, and regulates gene transcription (Figure 4).

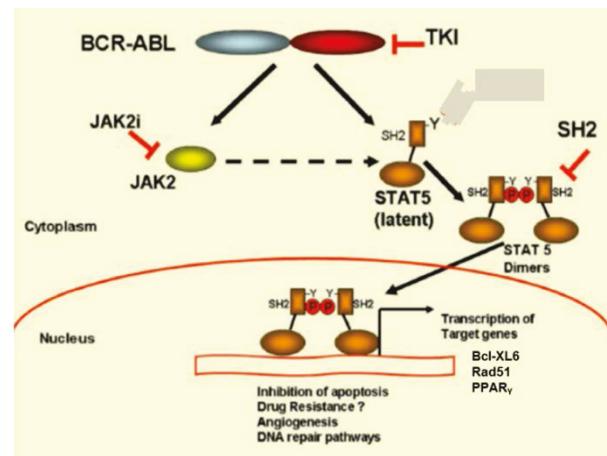


Figure 3. Schematic representation of STAT5 activation in CML and strategies of STAT5 inhibition. BCR-ABL phosphorylates STAT5 at a critical Tyrosine (Y) residue close to SH2 domain, with subsequent dimerization and nuclear translocation of STAT5: STAT5 dimers, which bind to DNA at consensus sequences of the target gene promoters.

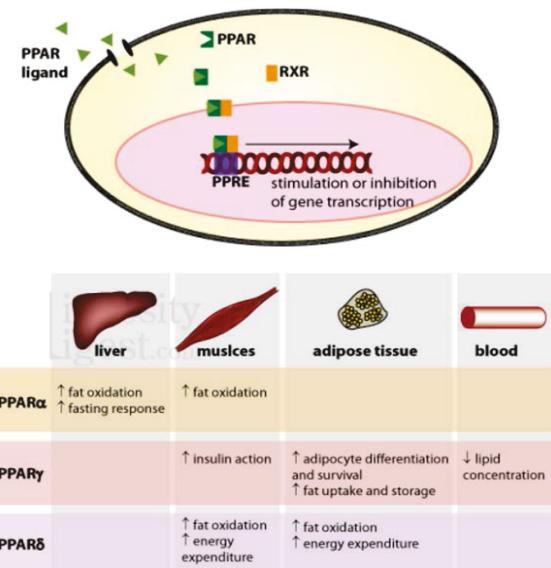


Figure 4. PPAR which is a subfamily of nuclear receptors. PPAR proteins bind DNA in combination with retinoid X receptors (RXRs), and these two proteins pair up to regulate transcription of various genes. There are three subtypes of PPARs: PPAR-alpha, PPAR-delta, and PPAR-gamma.

1.4.4 Interactions between PPAR γ and STAT5 in CML

PPAR γ is an important regulator of STAT 5 activity. Indeed in hematopoietic stem cells, a bidirectional crosstalk between STAT5 and PPAR γ signaling occurs. Phosphorylated STAT5 dimers bind in the nucleus to promotor of PPAR γ to initiate transcription. **Increased PPAR γ , in return, downregulates STAT5 and decreases cell proliferation (Figure 5)** [39, 40].

Synthetic ligands of PPAR γ such as the commercially available TZD, pioglitazone (**PIO**) was shown to decrease expression of STAT5 as well as proliferation of CD34+ cells collected from CML-CP patients as evidenced by a decrease the number of colony forming cells in vitro. In addition, **PIO appeared to induce non dividing cells into cycling by decreasing key guardians of the quiescence and stemness of CML LSCs (HIF2a and CITED2)** [41].

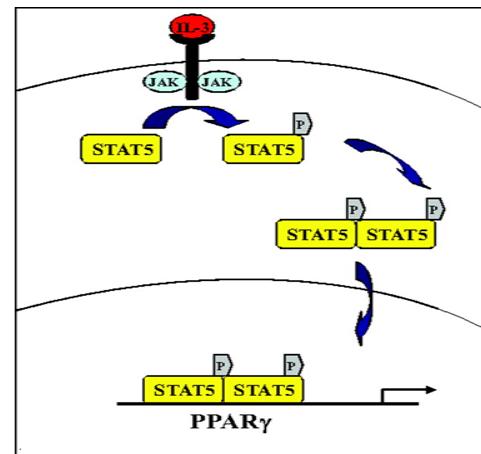


Figure 5. The phosphorylated STAT5s translocate to the nucleus where they bind to specific DNA binding sites on PPAR γ promoter and initiate transcription.

1.4.5 Clinical activity of a combination of PPAR γ ligands and TKI in CML

The combination of PIO and IM was administered to 3 (2 diabetics and 1 non-diabetic) CML patients who never achieved CMR while on IM (**Figure. 6**). **Patient 1** received 2 courses of PIO: one for 10 months and the second for 8 months, after a 28 months gap. Patient attained CMR 10 months after the first exposure to the combination of PIO and IM, and maintained CMR despite transient discontinuation of PIO. This patient remains in CMR on continuous IM 56 months after the initiation of PIO. **Patient 2** achieved CMR within 1 year of initiating PIO, and maintained CMR after sequential discontinuation of PIO and IM. **Patient 3** achieved CMR 6 months after starting PIO and also maintained CMR for 38 months after sequential discontinuation of PIO and IM. Of note all 3 patients had a single fluctuation in the PCR below the MMR level after initial achievement of CMR. Of interest, the level of STAT5 mRNA in the BM CD34+ cells collected from patient 3 decreased by 11.9 fold from baseline following initiation of PIO [41]. **These observations suggest that the combination of PIO and TKI can lead to BCR-ABL1 eradication in patients unable to achieve CMR with TKI alone**, and served as basis for an ongoing multi-center phase 2 clinical trial assessing the incidence of CMR conversion following the addition of PIO to IM in patients who never reached CMR with IM alone (<https://www.clinicaltrialsregister.eu/ctr-search/trial/2009-011675-79/FR#E>).

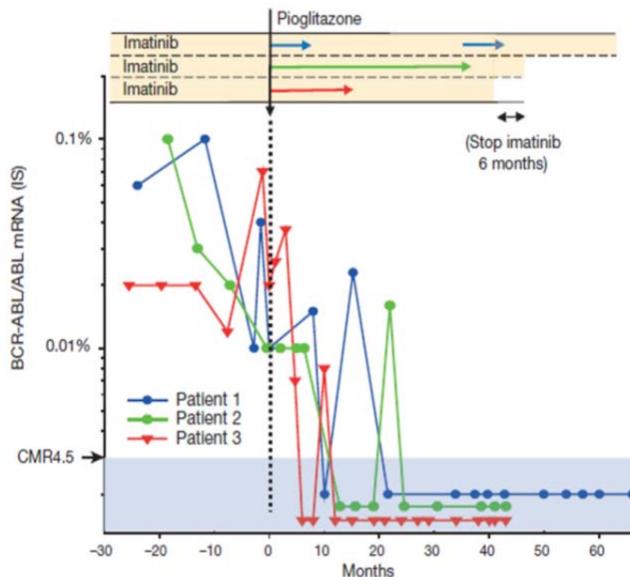


Figure 6. Pioglitazone induces complete and sustained molecular response (CMR) in CML patients.

1.5 *Pioglitazone*

PIO and Rosiglitazone belong to the TZD class of synthetic PPAR γ ligands, and are approved as anti-diabetic medication by the Food and Drug Administration (**FDA**) in the US. PIO is approved for noninsulin dependent type 2 diabetes mellitus as monotherapy or combination therapy in adjunct to diet and exercise, to improve glycemic control. PIO controls blood glucose by increasing insulin sensitivity [42]. Via activation of the PPAR γ nuclear receptor, PIO modulates gene expression, trigger adipocyte differentiation, and induce remodeling of adipose tissue, which is associated with changes in adipocyte signal output. Those signals include free fatty acids and peptide hormones (adiponectin, resistin, leptin, and tumor necrosis factor- α), and mediate the PIO-induced improvement of skeletal muscle glucose disposal [43-45].

1.5.1 *Pharmacokinetics and Pharmacodynamics*

Following once-daily administration of PIO, steady-state serum concentrations of both PIO and its major active metabolites, M-III (keto derivative of PIO) and M-IV (hydroxyl derivative of PIO), are achieved within seven days. At steady-state, M-III and M-IV reach serum concentrations equal to or greater than that of PIO. At steady-state, in both healthy volunteers and patients with type 2 diabetes, PIO comprises approximately 30% to 50% of the peak total PIO serum concentrations (PIO plus active metabolites) and 20% to 25% of the total area under the curve (AUC). Peak serum concentration (**C_{max}**), AUC, and trough serum concentrations (**C_{min}**) for PIO and M-III and M-IV, increased proportionally with doses of 15 mg and 30 mg per day. The mean serum half-life (t_{1/2}) of PIO and its metabolites (M-III and M-IV) range from three to seven hours and 16 to 24 hours, respectively. PIO has an apparent clearance, CL/F, calculated to be five to seven L/hr.

Bioavailability and Food Effects. Following oral administration of PIO, Tmax of PIO was reached within two hours. Food delays the Tmax to three to four hours but does not alter the extent of absorption (AUC).

Distribution; The mean apparent volume of distribution (Vd/F) of PIO following single-dose administration is 0.63 ± 0.41 (mean \pm SD) L/kg of body weight. PIO is extensively protein bound (>99%) in human serum, principally to serum albumin. PIO also binds to other serum proteins, but with lower affinity. M-III and M-IV are also extensively bound (>98%) to serum albumin.

Metabolism; PIO is extensively metabolized in the liver by hydroxylation and oxidation; the metabolites also partly convert to glucuronide or sulfate conjugates. In vitro data demonstrate that multiple CYP isoforms are involved in the metabolism of PIO, which include CYP2C8 and, to a lesser degree, CYP3A4 with additional contributions from a variety of other isoforms including the mainly extrahepatic CYP1A1. In vivo study of PIO in combination with gemfibrozil, a strong CYP2C8 inhibitor, showed that PIO is a CYP2C8 substrate. Urinary 6 β -hydroxycortisol/cortisol ratios measured in patients treated with PIO showed that PIO is not a strong CYP3A4 enzyme inducer.

Excretion and Elimination; Following oral administration, approximately 15% to 30% of the PIO dose is recovered in the urine. Renal elimination of PIO is negligible, and the drug is excreted primarily as metabolites and their conjugates in the feces.

Special population:

Renal Impairment; The serum elimination half-life of PIO, M-III, and M-IV remains unchanged in patients with moderate (creatinine clearance [CLcr] 30 to 50 mL/min) and severe (CLcr <30 mL/min) renal impairment when compared to subjects with normal renal function. Therefore, no dose adjustment in patients with renal impairment is required.

Hepatic Impairment; Compared with healthy controls, subjects with impaired hepatic function (Child-Turcotte-Pugh Grade B/C) have an approximate 45% reduction in PIO and total PIO (PIO, M-III, and M-IV) mean Cmax but no change in the mean AUC values. Therefore, no dose adjustment in patients with hepatic impairment is required.

Geriatric Patients; in healthy elderly subjects, Cmax of PIO was not significantly different, but AUC values were approximately 21% higher than those achieved in younger subjects. The mean t1/2 of PIO was also prolonged in elderly subjects (about ten hours) as compared to younger subjects (about seven hours). These changes were not of a magnitude that would be considered clinically relevant.

Ethnicity; Pharmacokinetic data among various ethnic groups are not available.

1.5.2 Side effects associated with the use of PIO

A summary of the overall incidence and types of common AEs reported in the PROactive trial (the Prospective Pioglitazone Clinical Trial in Macrovascular Events) is provided in **Table 2**. PROactive is a randomized, double-blind outcome study in 5238 patients with type 2 diabetes managed with diet and/or oral blood glucose-lowering drugs \pm insulin [46]. Patients were randomized to PIO (forced titration from 15 to 30 to 45 mg, depending on tolerability) or placebo in addition to existing therapy. **Table 2** summarizes AEs that occurred in >5% of subjects.

Table 2. PROactive Trial: Incidence and Types of Adverse Events Reported in >5% of Patients Treated with PIO and More Commonly than Placebo

	% of Patients	
	Placebo N=2633	PIO N=2605
Hypoglycemia	18.8	27.3
Edema	15.3	26.7
Cardiac Failure	6.1	8.1
Pain in Extremity	5.7	6.4
Back Pain	5.1	5.5
Chest Pain	5.0	5.1

1.5.3 Warnings and Precautions associated with the use of PIO (Package insert)

Congestive Heart Failure; PIO, like other TZD, can cause dose-related fluid retention when used alone or in combination with other antidiabetic medications and is most common when PIO is used in combination with insulin. Fluid retention may lead to or exacerbate congestive heart failure. Patients should be observed for signs and symptoms of congestive heart failure.

Hypoglycemia; Patients receiving PIO in combination with insulin or other antidiabetic medications (particularly insulin secretagogues such as sulfonylureas) may be at risk for hypoglycemia. A reduction in the dose of the concomitant antidiabetic medication may be necessary to reduce the risk of hypoglycemia.

Hepatic Effects; There have been post-marketing reports of fatal and non-fatal hepatic failure in patients taking PIO, although the reports contain insufficient information necessary to establish the probable cause. There has been no evidence of drug-induced hepatotoxicity in the PIO controlled clinical trial database to date. Patients with type 2 diabetes may have fatty liver disease or cardiac disease with episodic congestive heart failure, both of which may cause liver test abnormalities, and they may also have other forms of liver disease, many of which can be treated or managed. Patient who develop abnormal liver tests (ALT greater than 3 times the upper limit of the reference range); PIO treatment should be interrupted.

Urinary Bladder Tumors; Tumors were observed in the urinary bladder of male rats in the two-year carcinogenicity study. In two 3-year trials in which PIO was compared to placebo or glyburide, there were 16/3656 (0.44%) reports of bladder cancer in patients taking PIO compared to 5/3679 (0.14%) in patients not taking PIO. After excluding patients in whom exposure to study drug was less than one year at the time of diagnosis of bladder cancer, there were six (0.16%) cases on PIO and two (0.05%) cases on placebo. A five-year interim report of an ongoing 10-year observational cohort study found a no significant increase in the risk for bladder cancer in subjects ever exposed to PIO, compared to subjects never exposed to PIO (HR 1.2 [95% CI 0.9 –1.5]). Compared to never exposure, a duration of PIO therapy longer than 12 months was associated with an increase in risk (HR 1.4 [95% CI 0.9 –2.1]), which reached statistical significance after more than 24 months of PIO use (HR 1.4 [95% CI 1.03 –2.0]). Interim results from this study suggested that taking PIO longer than 12 months increased the relative risk of developing bladder cancer in any given year by 40% which equates to an absolute increase of three cases in 10,000 (from approximately seven in 10,000 [without PIO] to approximately 10 in 10,000 [with PIO]). There are insufficient data to determine whether PIO is a tumor promoter for urinary bladder tumors. Consequently, PIO should not be used in patients with active bladder cancer and the benefits of glycemic control versus unknown risks for cancer recurrence with PIO should be considered in patients with a prior history of bladder cancer.

Edema; In controlled clinical trials, edema was reported more frequently in patients treated with PIO than in placebo-treated patients and is dose-related. In post marketing experience, reports of new onset or worsening edema have been received. PIO should be used with caution in patients with edema. Because TZD, including PIO, can cause fluid retention, which can exacerbate or lead to congestive heart failure, PIO should be used with caution in patients at risk for congestive heart failure. Patients treated with PIO should be monitored for signs and symptoms of congestive heart failure.

Fractures; In PROactive (the Prospective Pioglitazone Clinical Trial in Macrovascular Events), 5238 patients with type 2 diabetes and a history of macrovascular disease were randomized to PIO (N=2605), and were force-titrated up to 45 mg daily or placebo (N=2633) in addition to standard of care. During a mean follow-up of 34.5 months, the incidence of bone fracture in females was 5.1% (44/870) for PIO versus 2.5% (23/905) for placebo. This difference was noted after the first year of treatment and persisted during the course of the study. The majority of fractures observed in female patients were non-vertebral

fractures including lower limb and distal upper limb fractures. No increase in the incidence of fracture was observed in men treated with PIO (1.7%) versus placebo (2.1%).

Macular Edema; Macular edema has been reported in post marketing experience in diabetic patients who were taking PIO or another TZD. Some patients presented with blurred vision or decreased visual acuity, but others were diagnosed on routine ophthalmologic examination. Most patients had peripheral edema at the time macular edema was diagnosed. Some patients had improvement in their macular edema after discontinuation of the TZD.

Ovulation; Therapy with PIO, like other TZD, may result in ovulation in some premenopausal anovulatory women. As a result, these patients may be at an increased risk for pregnancy while taking PIO. This effect has not been investigated in clinical trials, so the frequency of this occurrence is not known. Adequate contraception in all premenopausal women treated with PIO is recommended.

Macrovascular Outcomes; There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with PIO or any other antidiabetic drug.

1.5.4 Contraindications for the use of PIO (Package insert):

PIO is contraindicated in the following settings:

- Hypersensitivity to PIO or any component of the formulation;
- New York Heart Association (NYHA) Class III/IV heart failure
- pregnancy
- serious hepatic impairment (Canadian labeling)
- Active bladder cancer; history of bladder cancer; uninvestigated macroscopic hematuria (Canadian labeling)

1.6 Rationale for the Study

New approaches are needed for the management of the 50% of CML patients who experience a molecular relapse following a first trial of TKI discontinuation. Given the potential risks of long-term TKI exposure, the contra-indication for pregnancies/child conception while on TKI, and the expenses associated with the use of these agents (92,000 USD/year for IM, > 120,000 USD/year for DAS or NIL), many patients express interests in TKI discontinuation trials. However, with the low success rate of a second discontinuation trial (20%), patients who fail their first TKI discontinuation have currently no other option but to continue TKI therapy.

Given the interactions between PPAR γ and STAT5 in CML LSCs, the synergy between PIO and IM [41], and the safety profile of PIO, a trial testing the combination of PIO and TKIs is a reasonable novel approach to eradicate minimal residual disease in CML .

2. Study Objectives and Hypothesis

2.1 *Objectives*

- To assess safety of the combination of PIO and TKI in CML subjects who experience a loss of MMR following a first TKI discontinuation
- To assess survival without loss of MMR following a second TKI discontinuation in subjects who achieve or maintain \leq MR^{4,5} with the combination PIO and TKI administered for at least 6 months

2.2 *Hypothesis*

Subjects who lose MMR after a first TKI discontinuation trial, but are still in CCyR and CHR regardless of their molecular status, will be offered enrollment on this trial. We hypothesize that PIO in combination with TKI will be well tolerated and that subjects will achieve or maintain \leq MR^{4,5} with the combination of TKI and PIO. Subjects who achieve \leq MR^{4,5} will be offered a second TKI discontinuation trial. We also hypothesize that MMR will be sustained following a second TKI discontinuation in at least 50% of subjects.

3. Investigational Plan

3.1 *Overall Study Design and Plan*

This is an open-label phase 2 study in which eligible subjects will receive PIO in combination with TKI. Safety of PIO in combination with TKI will be assessed continuously. Subjects who lose MMR while on the combination will be taken off study and managed at the discretion of the treating physician. Loss of MMR is defined by a detectable *BCR-ABL1* level $>0.1\%$ and confirmed within 4 weeks. For the purposes of this protocol and all analyses, a cycle of treatment is defined as 28 days. Study schema is depicted in Figure 7.

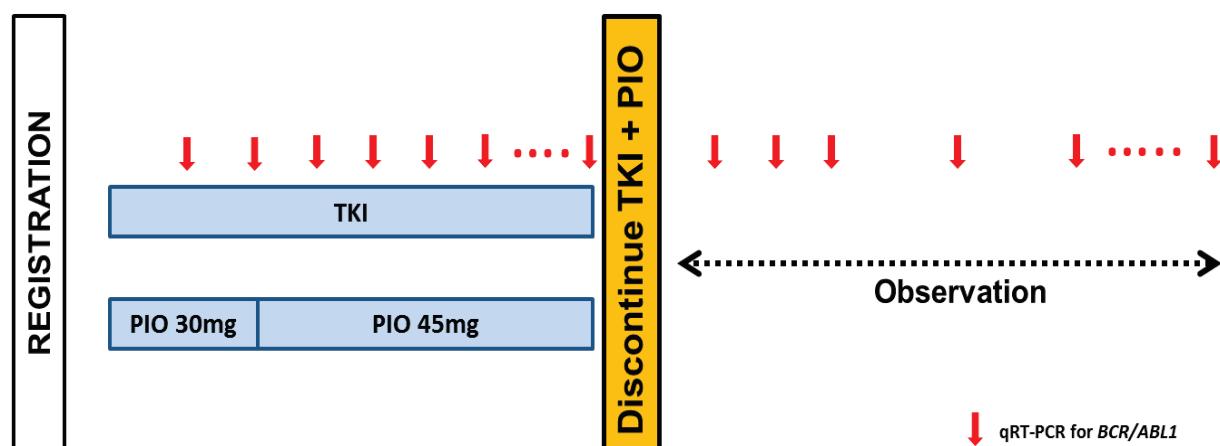


Figure 7. Study schema

Subjects may enter the study before or after restarting the TKI that was previously discontinued. Disease status can therefore be $>$ MMR but still in CCyR and CHR or \leq MMR. Subject will start PIO in addition to TKI, at 30mg/d for 2 cycles (56 days) and, in the absence of grade 2 or higher toxicities, PIO doses will be increased to 45mg/d. If grade 2 or higher toxicities occur on PIO 45 mg/d, PIO dose will be held until toxicities are \leq grade 1, and PIO doses will be decreased to 30 mg/d (**Dose modification was described section 7.1**). Subjects will receive PIO in addition to TKI for 6 months from the first documented \leq MR^{4,5}. During the combination of PIO and TKI, subjects will be monitored by blood qRT-PCR for *BCR-ABL1* obtained monthly.

Subjects entering the study in $>$ MMR but still in CCyR and CHR who do not achieve MMR within 4 months of the combination of PIO and TKI will be taken off study. Subjects entering the study in \leq MMR or who achieve MMR within 4 months of combination of PIO and TKI, will receive PIO for 6 months from the first documented \leq MR^{4,5}. Subjects who lose MMR at any time on the combination of PIO and TKI will be taken off treatment. Subjects who fail to achieve \leq MR^{4,5} after 6 months of the combination will also be taken off treatment. Fluctuations of PCR from MR^{4,5} at \leq MMR levels are expected and are acceptable during the combination of PIO and TKI.

Subjects who maintain \leq MR^{4,5} for 6 months with less or equal than 2 *BCR/ABL1* transcript fluctuation $<$ MMR will be eligible for a second TKI discontinuation trial. Subjects who discontinue TKI will be monitored by blood qRT-PCR for *BCR-ABL1* obtained monthly for the first 3 months, quarterly for 1 year, and then bi-annually for 1 year. After 2 years of follow-up off TKI, or if MMR is lost off TKI, subjects will have completed their participation on the trial and will enter the Long-Term Follow-up which includes once a year update on disease status (qRT-PCRs) for 5 years.

3.2 Endpoints

- *Safety*: To describe AEs in subjects receiving the combination of PIO and TKI. AEs will be graded according to the Common Terminology Criteria for Adverse Events (**CTCAE**) v.4.03 assessed through scheduled assessment and subject reported diary
- *Efficacy*: To determine the proportion of subjects who maintain MMR following a second discontinuation of TKI.

3.3 Description of Treatment

Trial will be discussed with subjects in the out-patient clinic. Interested subjects will be offered to participate in the study. The primary physician (co-investigator) will discuss the study and counsel both subject and family. The objectives of the study, procedures, alternative therapies and potential risks and benefits of participation will be explained. Subject will be given the time to read the consent and the opportunity to ask questions before signing the consent form approved by Institutional Review Board (**IRB**). Protocol registration procedures will be completed within 48 hours of enrollment. Eligibility checklist form will be completed prior to enrollment.

Eligible subjects will start PIO 30 mg/d orally for 2 cycles (56 days) and then increase to 45 mg/d. Subjects will either start or continue the same TKI at the pre-discontinuation doses. After initiation of PIO, blood qRT-PCR for *BCR-ABL1* will be collected on a monthly basis. Disease status will be monitored by blood qRT-PCR for *BCR-ABL1* at study specific time points (**Figure 7**).

4. Selection of Study Population

4.1 *Inclusion Criteria*

1. CML in any phase
2. Philadelphia chromosome positive acute lymphoblastic leukemia
3. Loss of MMR following a first TKI discontinuation trial
4. Age \geq 16 years
5. Serum bilirubin <1.5 x upper limit of normal values
6. AST (SGOT)/ALT (SGPT) <2.5x upper limit of normal values
7. Females of child bearing potential must agree to abstain from sexual activity or to use a medically approved contraceptive measure/regimen during and for 3 months after the treatment period. Women of child bearing potential must have a negative urine pregnancy test at the time of enrollment. Acceptable methods of birth control include oral contraceptive, intrauterine device, transdermal/implanted or injected contraceptives and abstinence.
8. Patient requiring anti-diabetic medications to manage hyperglycemia are eligible. Adjustments of other anti-diabetic agents will be made with close monitoring of blood glucose
9. Males must agree to abstain from sexual activity or agree to utilize a medically-approved contraception method during and for 3 months after the treatment period.
10. Informed consent
11. Be able and willing to comply with study visits and procedures

4.2 *Exclusion Criteria*

1. Known loss of CCyR by marrow cytogenetic or blood FISH for *BCR-ABL1*
2. Loss of CHR
3. Participation in another clinical trial with any investigative drug within 30 days prior to study enrolment
4. Chronic graft-versus-host disease requiring systemic immunosuppression post-allogeneic hematopoietic stem cell transplantation
5. Cardiovascular disease: history of congestive heart failure, myocardial infarction within the 6 months of study entry, symptomatic cardiac arrhythmia requiring treatment
6. History of bladder cancer
7. Gross (visible) hematuria
8. Known history of osteoporosis
9. Known history of macular edema
10. Known history of ABL1-domain mutation that predicts resistance to the discontinued TKI
11. Significant medical or psychiatric disorder that would interfere with consent, study participation, or follow-up
12. Known allergy to PIO
13. Pregnant or breastfeeding
14. Use of TZD within 28 days prior to enrollment

15. Significant gastrointestinal condition that could potentially impair the absorption or disposition of the drug
16. Uncontrolled peripheral edema (2+ or more) of any etiology.
17. Active cancer that requires therapy in the form of chemotherapy or radiation

5. Study Procedures

5.1 Schedule of Events

The following describes the procedures/tests required for this study:

Screening, on study, and end of study procedures are shown in **Table 3**. Screening tests and procedures to establish eligibility of the subject for the trial will be done within 14 days prior to initiation of study drug. Evaluation, visit samples or activities should occur within range defined below.

Table 3. Schedule of Events

	Screening ± 14days	Day1	On PIO and TKI		Off PIO and TKI		End of Treatment	12 Long-Term Follow up
			Evaluations on day 14 ± 2days	Evaluations on days 28 ± 2days then monthly ± 2days	Monthly Evaluations ± 2days for first 3 months	Quarterly Evaluations ± 7days for one year		
¹ Informed Consent and Registration	X							
² History and Demographics	X							
³ CML history, prior therapy, and response	X							
⁴ Vital Signs, Physical Examination	X		X	X			X	
⁵ Concomitant medications	X		X	X			X	
⁶ CBC and differential, and Serum Chemistries	X		X	X			X	
⁷ Urine HCG	X							
⁸ Blood qRT-PCR for <i>BCR-ABL1</i>	X			X	X	X	X	X
⁹ Diary card			X	X				
¹⁰ MMAS-8			X	X				
¹¹ Adverse Events Assessments		X						→
PIO administration		X		X				

¹**Informed Consent:** All subjects must take part in the informed consent process prior to any study related activity. During the consent process, the person obtaining consent must inform the subject of all elements of informed consent. Adequate time must be allowed for questions and for the subject to make a voluntary decision. No protocol-specific procedures are to be performed until the subject has signed and dated the

IRB approved informed consent form. Each subject's participation in the trial begins with the signing and dating of the informed consent form.

²*Medical/Surgical History and Demographics:* Medical/surgical history that includes diagnoses and therapies as well as demographic information that consists of the subject's age, gender, race, and ethnicity will be recorded.

³*CML History, Prior Therapy, and Response:* The initial presentation history and pathology including the date of onset of CML must be recorded. TKI therapy including date of initiation and stop, doses, dose reductions or interruptions will be recorded. Sequential monitoring response to therapy and date of first TKI discontinuation, as well as post-discontinuation monitoring data will be collected. If TKI was restarted prior to enrollment on study, date of re-initiation, and dosing information, as well as response monitoring data will be collected.

⁴*Vital signs and Physical Examination:* Vital signs including temperature, pulse, and blood pressure, and complete physical examination, including weight, must be performed at screening, day 14 and 28 as part of study. After that, this will be done every two to three months as part of standard of care unless new symptoms occur.

⁵*Concomitant medications:* All prescribed, over the counter, and supplemental/herbal medications, including route of administration (oral, topical, intravenous, rectal, subcutaneous, etc.), frequency, and duration will be recorded.

⁶*Complete Blood Count (CBC), differential and Serum Chemistries:* CBC with automated differential and serum chemistries will be obtained at screening as part of standard of care, and day 14 and 28 as part of study. After that, this will be done every two to three months as part of standard of care unless new symptoms occur.

⁷*Urine human chorionic gonadotropin (HCG):* HCG will only be performed in pre-menopausal women of childbearing age with no history of hysterectomy or bilateral tubal ligation.

⁸*Blood qRT-PCR for BCR-ABL1:* Blood qRT-PCR for *BCR-ABL1* will be obtained monthly while on PIO and TKI. Following discontinuation of TKI, monthly blood qRT-PCR for *BCR-ABL1* for the first 3 months, quarterly for a year and bi-annually thereafter will be done as part of standard of care.

⁹*Diary card:* A diary card will be completed daily to indicate the date and approximate time of administration of each dose of PIO and TKI, including any deviations from schedule such as missed or repeated doses. (**Appendix 1**)

¹⁰*Morisky Medication Adherence Scales – 8 (MMAS-8):* A MMAS-8 will be completed at each clinic visits while on PIO and TKI. (**Appendix 2**)

¹¹*Adverse event assessments:* All AEs will be assessed at each clinic visit as part of standard of care regardless the event is considered related to the treatment or not.

End of Treatment or Early Termination Procedures: The End-of-Treatment (or early termination) Visit should be performed within 2 weeks (14 days) of the subject's last dose of study drug or the subject/investigator decision to end treatment, whichever is later. Subjects will also return to the clinic or

will be contacted for a safety follow-up assessment 30 days \pm 3 days after the last dose of study drug was taken.

¹²*Long Term Follow-up:*

- **Safety:** All AEs ongoing or starting within 30 days after the End-of-Treatment must be recorded on the case report form (CRF). After this time, ongoing AEs thought to be at least possibly study-drug related and all ongoing serious adverse events (SAE) should be followed at least every 4 weeks until they resolve to baseline (or CTCAE grade ≤ 1), stabilize, or are considered to be chronic or irreversible.
- **Relapse-free Survival:** After 2 years of follow-up off TKI, if MMR is lost at any time, or if subjects withdraw from study for toxicity or any reasons, information on disease status (qRT-PCRs) will be collected once a year for 5 years as part of the Long-Term Follow-up. This information does not need to be obtained during a visit; review of local hematologist's records is acceptable.

5.2 **Description of Study Visits**

5.2.1 **Visit 1 (Day -14 to -1): Screening**

- Obtain written informed consent
- Record demographic data including age, gender, and race
- Review medical, surgical, and CML history described above.
- Record prior and concomitant medications including start/stop dates, indication, dose and frequency taken within 30 days of Day 1.
- Collect blood samples for CBC with differential count and serum chemistries.
-
- Collect urine for pregnancy test for female subjects who are not postmenopausal or surgically sterile. If positive, repeat and confirm results within a week and prior to start of study drug. A second positive test will result in exclusion of the subject from the study.
- Unless done within 72 hours, measure and record height in cm and weight in kg, record vital signs. Measure body temperature, systolic / diastolic blood pressure (BP) and pulse rate. Perform and record physical examination.
- Collect blood samples for qRT-PCR of *BCR/ABL1* unless done it within 30 days

5.2.2 **Visit 2 (Day 1): Baseline/Drug Dispensing/Initiation of Treatment**

- Review and document all inclusion and exclusion criteria to ensure that subjects meet all inclusion criteria and none of the exclusion criteria.
- Record AEs as part of standard of care.
- Dispense PIO at 30 mg/d and instruct subjects how to take study drug, daily every 24 hours and how to fill the diary card.

5.2.3 **Visits 3 (Days 14 \pm 2): Early Follow-up**

- Measure and record height in cm and weight in kg. Record vital signs. Measure body temperature, systolic/diastolic BP and pulse rate. Perform and record physical examination.

- Record medications including start/stop dates, indication, dose and frequency for any changes since previous visit.
- Collect blood samples for CBC with differential count and serum chemistries.
- Assess study drug compliance by pill count, review of the diary card and MMAS-8.
- Assess and record AEs and SAEs

5.2.4 Visits 4+ (Day 28 ±2, then q28+/-2 days during combination of PIO and TKI)

- On day 28, measure and record height in cm and weight in kg. Record vital signs. Measure body temperature, systolic / diastolic BP and pulse rate. Perform and record physical examination. Unless new symptoms occur, these will be performed every other month as part of standard of care.
- On day 28, record medications including start/stop dates, indication, dose and frequency for any changes since previous visit. Unless new symptoms occur, these will be performed every other month as part of standard of care.
- On day 28, collect blood samples for CBC with differential count and serum chemistries. Unless new symptoms occur, these will be performed every other month as part of standard of care.
- On day 56, in the absence of grade 2 or higher toxicities, PIO doses will be increased to 45 mg/d
- Dispense drug supply for self-dosing, remind subjects of dosing instructions, and obtain all unused study drug
- Assess study drug compliance by pill count, review of the diary card and MMAS-8.
- Collect blood samples for qRT-PCR of *BCR/ABL1* every month.
- Assess and record AEs and SAEs continuously

5.2.5 Post-discontinuation of PIO and TKI Visits (Monthly for 3 months ± 2 days and quarterly ±7 days for 1 year and biannually thereafter)

- Collect blood samples for qRT-PCR of *BCR/ABL1* as part of standard of care
- Assess and record AEs and SAEs continuously

5.2.6 Unscheduled Visits

Unscheduled visits may occur at any time at the investigator's discretion, and appropriate clinical and laboratory measurements performed based on AEs or other findings.

5.2.7 End of Treatment Visit (±14 days)

At the time of last study monitoring, loss of MMR, failure to achieve <MMR after 4 months or < MR^{4,5} after 6 months of combination treatment of PIO and TKI, the End of Treatment Visit should be completed for all subjects as soon as possible, and every effort should be made to perform the procedures required. Subjects will also return to the clinic or will be contacted for a safety follow-up assessment 30 days ± 3 days after the last dose of study drug was taken. Only information about AEs and SAEs will be collected at this visit.

The following procedures will be performed at End of Treatment Visit:

- Measure and record height in cm and weight in kg. Record vital signs. Measure body temperature, systolic / diastolic BP and pulse rate. Perform and record physical examination which is part of standard of care
- Record medications including start/stop dates, indication, dose and frequency for any changes since previous visit.
- Collect blood samples for CBC with differential count, blood chemistries, and qRT-PCR of *BCR/ABL1* as part of standard of care
- Collect blood samples for ABL1 domain mutation analysis as part of standard of care in case of loss of MMR
- Obtain all unused study drug if subjects drop out before discontinuation of PIO and TKI.
- Assess study drug compliance by pill count and review of the diary card
- Assess and record AEs and SAEs

5.3 *Subject Registration and Identification*

Demographic information on all subjects who sign the Informed Consent Form will be recorded on the master subject log. Those subjects who complete screening procedures and meet all eligibility criteria may be enrolled. At the time of registration, the subject will be assigned a unique identification code (number), consisting of a study site number and a unique consecutive number.

5.3.1 *Screen Failure*

Subjects who have signed informed consent and subsequently fail to meet the inclusion and/or exclusion criteria are defined as screen failures. For all screen failures, the Investigator is to maintain a screening log that documents the subject initials and reason(s) for screen failure. A copy of the log will be retained in the Investigator's study files. Any subject who is rescreened after screen failure must, in addition to the failed procedure, repeat only those screening procedures outlined in the Schedule of Events that have fallen outside the specified screening period.

5.3.2 *Early Discontinuation*

In the event that a subject is withdrawn from the study, every effort will be made by the Investigator to document and report the reasons for withdrawal as thoroughly as possible. The reason(s) for termination must be clearly reported in the subject's CRF. An End-of-Treatment reason for discontinuation must be recorded for any subject who is registered. If a subject is discontinued from the trial for any reason, every effort must be made to perform all clinical and laboratory procedures as scheduled for the End-of-Treatment Visit. In the event that the subject fails to return for the necessary visit(s), an effort must be made to contact the subject to determine the reason, and this information should be recorded in the appropriate source record and the end-of-treatment CRF.

5.4 *Study Duration*

The total estimated duration of the study is 5 years, including 2 years to accrue subjects, and 3 years of follow up for the last subject enrolled. Subjects will be supplied the study drug PIO until they discontinue treatment.

5.5 *Withdrawal Criteria*

Subjects will be discontinued from further study drug administration if any of the following occur:

- Loss of MMR at any time
- Subjects entering the study in > MMR but still in CCyR and CHR who do not achieve MMR within 4 months of the combination of PIO and TKI
- Subjects who fail to achieve < MR^{4,5} after 6 months of the combination of PIO and TKI
- Intolerable toxicity as determined by the Investigator or the subject
- Any grade 3 or higher cardiac toxicity
- Any grade 4 toxicity possibly associated with PIO
- A treatment interruption for study-drug related non-hematologic toxicities lasting > 28 days
- Intolerance to PIO
- Significant deviation from the protocol or eligibility criteria
- Noncompliance with study or follow-up procedures
- Pregnancy
- Subject withdrawal of consent and decision to discontinue participation
- Termination of the trial
- Any other reason that, in the opinion of the Investigator, would justify removal of the subject from the study

5.6 *Study Termination*

If the sponsor-investigator, Data Monitoring Committee, or regulatory agencies discover conditions during the study that indicate that the study should be terminated, this action may be taken after appropriate consultation between the sponsor-investigator, and Data Monitoring Committee. Conditions that may warrant termination of the study include, but are not limited to:

- The discovery of a serious, unexpected, or unacceptable risk to subjects enrolled in the study
- Submission of knowingly false information from the research facility to the regulatory authorities
- Insufficient adherence to protocol requirements

Study termination and follow-up will be performed in compliance with the conditions set forth in the guidelines for Good Clinical Practice (**GCP**), International Conference on Harmonization (**ICH**) of Technical Requirements for Registration of Pharmaceuticals for Human Use.

6. Safety Assessments

Safety Assessments: Safety will be assessed by routine physical and laboratory evaluations according to the schedule (**Table 3**) and as medically indicated. AEs will be recorded and the severity will be graded according to the CTCAE v.4.03.

Adverse Events: Type, incidence, severity (graded in accordance with the CTCAE v.4.03), timing, seriousness and relatedness, outcome, action taken with study drug, and treatment will be assessed and documented by the investigator continuously throughout the study. Baseline malignancy-related signs and

symptoms will be recorded as AEs during the study if they worsen in severity or increase in frequency. Procedures for following AEs at the end of treatment and beyond are outlined in Section 5.1.

7. Study Treatment

Subjects will be provided with the study drug PIO to take in addition to TKI. The TKI that was previously discontinued will be restarted at doses administered prior to the first discontinuation. PIO will be self-administered on a once daily schedule.

7.1 Dose Selection, Treatment Administration, Compliance, and Dose Modifications

The starting dose of PIO will be 30 mg and will be taken orally once daily. After 56 days of PIO 30 mg/d, the dose will be increased to 45 mg/d in the absence of grade >1 related AE. The dose of PIO was selected based on previously published pilot report [47].

Subjects will take the prescribed tablets at approximately the same time each day, regardless of meals. Subjects will be provided a diary card where the date and time of administration will be recorded (**Appendix 1**). Subjects who forget to take their dose can make up the missed dose as long as this is done within the 12 hours of the due time. Any missing dose should be recorded. Drug compliance will be measured using the Morisky Medication Adherence Scales-8 (MMAS8) at every visit while on PIO and TKI (**Appendix 2**). Dose modification will be done as follows:

Table 4. Dose Modification

Toxicity		Immediate action	Later action
Hematologic toxicity	≥ Grade 3 Neutropenia or Thrombocytopenia	1. Hold PIO and TKI until ≤ Grade 1	1. First episode: resume TKI and PIO without dose modifications 2. Second episode: resume at 1 lower dose level (previous dose -15 mg) 3. Third episode: permanent discontinuation of PIO, resume TKI alone
	≤ Grade 3 anemia	1. Continue PIO 2. RBC transfusion if indicated	1. First episode: resume TKI and PIO without dose modifications 2. Second episode: resume PIO at 1 lower dose level (previous dose -15 mg) 3. Third episode: permanent discontinuation of PIO, resume TKI alone
	Grade 4 anemia	1. Hold PIO and TKI 2. RBC transfusion if indicated	1. First episode: resume PIO at 1 lower dose level (previous dose -15 mg) 2. Second episode: permanent discontinuation of PIO, resume TKI alone
Non-hematologic toxicity	Grade 2	1. Hold PIO until ≤ Grade 1	1. First episode: resume TKI and PIO without dose modifications 2. Second episode: resume at 1 lower dose level (previous dose -15 mg)
	≥ Grade 3*	1. Hold PIO and TKI until ≤ Grade 1	1. First episode: resume PIO at 1 lower dose level (previous dose -15 mg) 2. Second episode: permanent discontinuation of PIO, resume TKI alone

*In case of cardiac toxicity, subject will be taken off the study

7.2 Allocation of Treatment

The results of screening assessments for each subject will be reviewed by the study principal investigator (PI), who will approve each subject. Upon this approval, subjects will receive a 28 days (1 cycle) + 2 days supply of 30 tablets of PIO.

7.3 Concomitant Medications

All concomitant medications administered from the time of informed consent signature through 30 days after the end of treatment (last dose or investigator/subject decision to discontinue, whichever is later) are to be reported on the CRF for each subject. No medications will be prohibited, but caution will be used for medications that interact with PIO according to the package insert.

7.4 Treatment Supply:

7.4.1 Formulation, Packaging and Labeling:

PIO will be purchased by the Investigational Pharmacy at Emory for use in this trial. PIO is available as a tablets for oral administration containing 15, 30, and 45 mg of PIO (as the base) formulated with the following excipients: lactose monohydrate NF, hydroxypropylcellulose. Tablet will be supplied as follows: 30 counts in Rexam's 1-ClicR vials (Rexam PLC, London, UK) that comes with induction-sealed child resistant caps. Bottle labels will bear the appropriate label text as required by governing regulatory agencies, to include product name, product strength, number of capsules, and lot number.

7.4.2 Treatment Storage, Dispensing, and Accountability

The recommended storage condition for PIO is room temperature (15-30°C), and should be kept in the original container to protect from light and from humidity. The study pharmacist or designee at the site will be responsible for handling, dispensing study drug, completing associated documentary paperwork, handling dispensing log/accountability form. Each time study medication is dispensed for a subject, the following information will be recorded: the subject's initials, the subject's study number, tablet strength, the number of tablets dispensed with the corresponding lot number, and the initials of the person dispensing the drug. These logs are to be maintained by the study pharmacist in the pharmacy throughout the duration of the study. The Investigator is responsible for ensuring that the subject diary card(s) and study drug provided to the subject and returned from the subject are accounted for and noted in source documentation.

7.4.3 Disposition of used and unused supplies

All used bottles or packs of study drug must be destroyed according to the standard practice. Destruction of such supplies will be documented. During the trial and at termination, subjects must return all unused study drug supplies and the return of these unused study drug supplies must be recorded. Returned supplies must not be re-dispensed. No other utilization of PIO intended for use in this study will be authorized. The investigator or his/her designee will be responsible for the appropriate handling and disposition of residual study drug.

8. Adverse Event Reporting

8.1 Adverse Events

8.1.1 Adverse Event Definition

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product which does not necessarily have to have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product whether or not considered related to the medicinal product. Any worsening of a preexisting condition, which is temporally associated with the use of the study drug (i.e., occurs after the first dose of study drug), is also an AE. AEs include:

- Abnormal test findings
- Changes in physical exam findings
- Other untoward medical events, regardless of their relationship to the study drug, such as injury, events that require surgery, accidents, or apparently unrelated illnesses, and hypersensitivity
- AEs may include signs or symptoms resulting from: drug overdose, drug withdrawal, drug abuse, drug misuse, drug interactions, drug dependency, and, in utero drug exposure

8.1.2 Abnormal Laboratory Tests

The criteria for determining whether an abnormal objective test finding should be reported as an AE are as follows:

- Test result is associated with accompanying symptoms that are considered clinically significant in the opinion of the investigator
- Test result requires additional diagnostic testing (other than merely repeating an abnormal test) or medical/surgical intervention
- Test result leads to a change in study drug dosing or discontinuation from the study, significant additional concomitant drug treatment, or other therapy
- Test result is considered to be an AE by the investigator

8.1.3 Performing Adverse Event Assessment

All observed or volunteered AEs, regardless of suspected causal relationship to the investigational product, will be reported as described in the following sections. For all AEs, the investigator must pursue and obtain information adequate to determine the outcome of the AE and to assess whether it meets the criteria for classification as a serious AE requiring immediate notification to the appropriate regulatory agencies.

8.1.4 Reporting Period

Serious AEs require notification of the appropriate regulatory agencies beginning from the time the subject starts receiving the investigational product, through and including 30 days after the last

administration of the assigned study treatment or the investigator/subject decision to discontinue treatment, whichever occurs later. Any SAE occurring any time after the reporting period must be promptly reported if a causal relationship to the investigational product is suspected. Any SAE ongoing at the end of the reporting period should be followed as described below. For all enrolled subjects, AEs (serious and non-serious) should be recorded on the CRF beginning with the signing of the informed consent form and concluding 30 days following the last dose of the assigned study treatment or the investigator/subject decision to discontinue treatment, whichever occurs later. AEs ongoing after the reporting period: Any ongoing AEs thought to be at least possibly study-drug related and all ongoing SAEs after this time should be followed at least every 4 weeks until they resolve to baseline (or CTCAE grade ≤ 1), stabilize, or are considered to be chronic/irreversible.

8.1.5 Adverse Event Severity

The severity of AEs will be assessed according to the CTCAE, v.4.03 If the AE is not defined in the CTCAE, the investigator will determine the severity of the AE based on the following definitions:

- Mild (grade 1): The AE is noticeable to the subject but does not interfere with routine activity. The AE does not require discontinuing administration or reducing the dose of the study drug
- Moderate (grade 2): The AE interferes with routine activity but responds to symptomatic therapy or rest. The AE may require reducing the dose, but not discontinuing administration of the study drug
- Severe (grade 3): The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy. In addition, the AE leads to discontinuing administration or reducing the dose of the study drug
- Life-Threatening (grade 4): The AE requires discontinuing administration of the study drug. The subject is at immediate risk of death
- Death (grade 5): The subject dies as a direct result of the complication or condition induced by administration of the study drug

8.1.6 Causality

The investigator's assessment of causality must be provided for all AEs (serious and non-serious). An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to the AE. In addition, if the investigator determines a SAE is associated with study procedures, the investigator must record this causal relationship in the source documents and on the SAE form and report such an assessment in accordance with the SAE reporting requirements. The investigator will use medical consideration and use the following categories of causality to determine the relatedness of an AE with the study drug based on the following definitions. Not all criteria in each category of relatedness must be present.

- Definitely Not Related (not drug related): The subject did not receive study drug, OR the temporal sequence of the AE onset relative to the administration of the study drug is not reasonable, OR there is another obvious cause of the AE
- Probably Not Related (not drug related): there is evidence of exposure to study drug, there is another more likely cause of the AE, dechallenge (if performed) is negative or ambiguous, rechallenge (if performed) is negative or ambiguous

- Possibly Related (drug related): there is evidence of exposure to study drug, the temporal sequence of the AE onset relative to administration of the study drug is reasonable, the AE could have been due to another equally likely cause, dechallenge (if performed) is positive
- Probably Related (drug related): there is evidence of exposure to study drug, the temporal sequence of the AE onset relative to administration of the study drug is reasonable, the AE is more likely explained by the study drug than by another cause
- Definitely Related (drug related): there is evidence of exposure to study drug, the temporal sequence of the AE onset relative to administration of the study drug is reasonable, dechallenge is positive, rechallenge (if feasible) is positive, the AE shows a pattern consistent with previous knowledge of the test drug or a test drug class

8.1.7 Expectedness

The expectedness of an AE is assessed by the Investigator in the overall classification of AEs and SAEs for regulatory reportability.

8.2 Serious Adverse Events

The definitions and reporting requirements of FDA and ICH Guidelines for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2A, will be adhered to.

8.2.1 Serious Adverse Event Definition

An AE is considered an SAE if at least one of the following conditions applies:

- Death: An AE that results in death is any subject death within 30 days of the last dose of study drug administration. The cause of death or AE that resulted in a fatal outcome is the SAE
- Life-threatening AE: An AE that places the subject, in the view of the investigator or the sponsor, at immediate risk of death from the event as it occurred (i.e., this does not include an event that had it occurred in a more severe form might have caused death)
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions: Any substantial disruption of a person's ability to conduct normal life functions
- Inpatient hospitalization or prolongation of existing hospitalization: Hospitalization refers to admission of a subject into a hospital for any length of time
- A congenital anomaly/birth defect: A fixed, permanent impairment established at or before birth
- Cancer: Occurrence or diagnosis of a new cancer during the study is considered an SAE. A new cancer is a cancer that is histopathologically different than the cancer under study in the trial (i.e., does not include metastatic or progressive disease)
- Overdose: Occurrences of overdose must be reported as an SAE
- Important medical event: Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not result in death, be life-threatening, or require hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other outcomes listed in the definition above, the important medical events should be reported as serious. Examples of such events are intensive treatment in an emergency room or at home for allergic

bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization; or the development of drug dependency or drug abuse.

AEs (reported from clinical studies) that require hospitalization or prolongation of hospitalization are considered serious. Any initial admission (even if less than 24 hours) to a health care facility meets these criteria. AEs that require emergency room care that do not result in hospital admission are not SAEs unless assessed by the investigator to be an important medical event. Hospitalization does not include the following: Hospice facilities, Respite care, skilled nursing facilities, Nursing home, Routine emergency room admissions, and same day surgeries (as outpatient/same day/ambulatory procedure)

Hospitalization or prolongation of hospitalization in the absence of a precipitating AE is not in itself an SAE. Examples include: social admission (e.g., subject has no place to sleep), optional admission not associated with a precipitating AE (e.g., for elective cosmetic surgery that was planned prior to study enrollment [appropriate documentation is required for these cases]), hospitalization or prolongation of hospitalization for scheduled therapy of the target malignancy of the study is not considered an SAE

8.2.2 Reporting Adverse Events

Written Investigational New Drug (IND) safety reports will be submitted to the FDA by the IND sponsor, for serious, unexpected suspected adverse reactions within 15 calendar days of the sponsor determining that the information requires reporting. If the event is fatal or is deemed to be life threatening, the report will be made within 7 calendar days. The IND sponsor will also make an assessment of whether the event constitutes an unanticipated problem posing risks to subjects or others (UP). This assessment will be provided to the Emory University IRB, which, in turn will make a final determination. If the Emory IRB determines an event is a UP it will notify the appropriate regulatory agencies and institutional officials. The Sponsor- Investigator is responsible for reviewing all IND Action Letters, Safety Reports and any other safety related Investigator Notification and determining the action to be taken (i.e. prompt reporting to the IRB, consent change/modification, routine reporting, etc).

The following information about the subject and the event will need to be collected and available:

- Investigator identification
- Subject identification code (e.g., sex, age, or date of birth)
- Information on study drug (e.g., start/stop date, dose and frequency of study drug administered)
- Description of event

In addition to the above information, the sponsor will require the investigator's assessment of the following:

- Severity of the SAE
- Relationship of the SAE to the study drug
- Outcome of the SAE

8.2.3 Follow-up Information of Serious Adverse Events

Appropriate diagnostic tests should be performed and therapeutic measures, as medically indicated, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the

event has resolved or is otherwise explained by the investigator. For all SAEs, the investigator is obligated to pursue and provide information to the appropriate regulatory agencies. There should be routine follow-up through and including 30 days after the last administration of assigned study treatment or the investigator/ subject decision to discontinue treatment, whichever occurs later, in all subjects in order to monitor for the occurrence of SAEs. If an SAE continues after the 30-day evaluation period, then the subject must be followed until the event resolves or returns to baseline. The medical monitor may specify a longer period of time if required to assure the safety of the subject.

8.3 *Other Safety Issues*

8.3.1 Pregnancy

Females of childbearing potential and fertile males will be informed as to the potential risk of conception while participating in this study and will be advised that they must use effective contraception from beginning study drug through at least 30 days after the end of treatment. A pregnancy test will be performed on each pre-menopausal female of childbearing potential immediately prior to beginning study drug. A negative pregnancy test must be documented prior to administration of study drug. If a subject is confirmed pregnant during the trial, study drug administration must be discontinued immediately. The investigator must immediately notify the appropriate regulatory agencies of this event and record the pregnancy on a Pregnancy Form. The investigator must also report follow-up information to the appropriate regulatory agencies regarding the course of the pregnancy, including perinatal and neonatal outcome, regardless of whether the subject has discontinued participation in the study. If the pregnancy results in the birth of a child, additional follow-up information may be requested. If the pregnancy results in spontaneous abortion or stillbirth, the event should be reported as an SAE. Pregnancy outcomes must be collected for the female partners of any males who took study drug in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the female partner.

8.3.2 Overdose

An overdose is defined as the accidental or intentional ingestion or infusing of any dose of a product that is considered BOTH excessive AND medically important. All occurrences of overdose must be reported as an SAE. These AEs will be monitored as instructed in the schedule of events and as clinically indicated.

9. Statistical Considerations

9.1 *General Considerations*

For the purposes of this protocol and all analyses, a cycle of treatment is defined as 28 days.

9.2 *Analysis Population*

Intention to treat (ITT) population: The ITT population includes all subjects who are enrolled in the study.

Safety Population: The safety population includes all subjects who have received at least 1 dose of PIO.

Per-protocol Population: The per-protocol population includes all subjects who are enrolled, receive at least one dose of study drug, and have no major protocol violations that could be expected to impact response data, such as: failure to satisfy one or more eligibility criteria, administration of other anti-cancer therapy concurrent with study drug, and administration of incorrect study drug dose. Major protocol violations will be finalized and documented prior to final data analysis.

9.3 Study Endpoints

The primary endpoint of this study is to determine safety and efficacy of PIO administered in combination with TKI. Therefore, description of AEs in subjects receiving the combination of PIO and TKI will be collected. AEs will be graded according to the CTCAE v.4.03 assessed through scheduled assessment and subject reported diary. Efficacy is determined by the proportion of subjects who achieve and maintain < MR^{4,5} using blood qRT-PCR for *BCR-ABL1* obtained monthly while on PIO and TKI.

Secondary endpoint of the study is to determine the proportion of subjects who lose MMR following discontinuation of PIO and TKI using blood qRT-PCR for *BCR-ABL1* obtained monthly for the first 3 months then quarterly.

9.4 Determination of Sample Size

Previous reports suggest that PIO is well tolerated at the doses of 30 and 45 mg [47]. Under Simon's optimal two stage design with a 5% significance level and 80% power, assuming a lower boundary of interest in the response rate of 20% and an upper boundary of interest in the response rate of 50%, a total of 24 evaluable subjects are required for the evaluation of the primary endpoint; 9 in Stage 1 and an additional 15 in Stage 2. If $\leq 2/9$ evaluable subjects in Stage 1 maintain MMR after second-TKI discontinuation or ≥ 2 subject develop \geq grade 3 cardiac toxicity then the study will terminate, otherwise the study shall continue as planned and enrollment of an additional 15 subjects shall continue into Stage 2. If, at the final analysis, less than 9 of 24 evaluable subjects in both Stage 1 and 2 maintain MMR then it will be concluded that the combination of PIO and TKI does not deserve further investigation. After adjusting for 8% drop out rate, the actual required sample size will be 26 subjects. Therefore, the total sample size of the study will be 26 subjects.

9.5 Safety and Efficacy Analysis

All subjects receiving at least 1 dose of study drug will be considered evaluable for safety. Listings of laboratory test results will also be generated, and descriptive statistics summarizing the changes in laboratory tests over time will be presented. Exposure to study drug over time will also be summarized. The AE incidence rates, as well as the frequency of occurrence of overall toxicity, categorized by toxicity grades (severity) will be described. The number of subjects that has reappearance of *BCR-ABL1* will be described. Descriptive statistics that will summarize the changes in *BCR-ABL1* testing over time will be presented. Loss of MMR is defined as a *BCR-ABL1* $>0.1\%$ by qRT-PCR confirmed within a week and associated with a rise in the titer on a confirmatory test obtained 4 weeks later (ELN definition). For the primary and secondary efficacy endpoints, subgroup analyses will be performed by baseline potential prognostic factors. Subgroups will include: age, gender, race, underlying diagnosis, and other disease-related prognostic factors (disease status).

9.6 *Interim Analysis*

An interim analysis is planned after 9 subjects have been enrolled and their results have been obtained. This interim analysis will focus on efficacy as discussed in section 9.4 and safety. If grade 3-4 toxicity is observed at any moment in 2 subjects, the trial will be put on hold and no further subjects will be enrolled until analyses of these cases is complete and reviewed by the data safety monitoring board (**DSMB**).

9.7 *Protocol Deviations and Violations*

To be protocol-compliant, a subject must not have any major protocol deviations during the study period. Protocol deviations will be identified prior to final analysis and will be listed by treatment group in the clinical study report.

10. Quality Control and Quality Assurance and Study Monitoring

The investigator is responsible for performing quality control and assurance checks. Before enrolling any subjects into this study, the investigator will review the protocol, the CRFs and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs with all personnel listed on the delegation of authority and FDA1572. The investigator will evaluate the conduct of the trial at least once monthly and perform the following:

- Review information recorded in the CRFs and verify against source documents
- Review data for safety information and to identify missing data
- Review protocol violations, out-of-range data, and other data inconsistencies

This study will be monitored by the DSMB Winship Committee.

11. Ethical Conduct of the Study

This study will be conducted in accordance with the ethical standards that have their origin in the Declaration of Helsinki and that are consistent with GCP guidelines and the applicable regulatory requirements.

11.1 *Institutional Review Boards Approval*

The protocol and the informed consent document must have the initial and at continuing approvals of the IRB. The signed IRB approval letter must identify the documents approved (i.e., list the investigator's name, the protocol number and title, the date of the protocol and informed consent document, and the date of approval of the protocol and the informed consent document). Any advertisements used to recruit subjects should also be reviewed by the IRB.

11.2 *Subject Information and Consent*

The study informed consent form will follow regulations that provide protection for human subjects in clinical investigations and describe the general requirements for informed consent. The informed consent

document will contain all of the elements of the informed consent specified in the regulations. Nothing in this protocol or the regulations is intended to limit the authority of a physician to provide emergency medical care under applicable regulations. In addition, regulatory agencies will be granted permission to conduct inspections and review records pertaining to this clinical investigation.

11.3 *Subject Confidentiality*

Throughout the course of the study, all private information will be kept securely using password protected computer terminals. Access to the data will be on a need to know basis, therefore, only those investigators listed in the protocol will have access to the data. A high premium will be placed on ensuring that identifying information is not made available to staff engaged in routine care of the subject unless necessary for the safe performance of their duties. For this reason, all research-related samples and clinical data will be de-identified using assigned study-specific registration numbers for such non-standard of care procedures as much as possible.

The investigator agrees to keep all information in strict confidence and to request similar confidentiality from his/her staff and the IRB. Study documents will be stored appropriately to ensure their confidentiality. The information provided to the investigator may not be disclosed to others without direct written authorization, except to the extent necessary to obtain informed consent from subjects who wish to participate in the trial.

11.4 *Data Safety Monitoring Committee*

Subject safety, study efficacy and compliance will be reviewed at the weekly Hematology working group meetings. The Data and Safety Monitoring Committee (**DSMC**) of the Winship Cancer Institute will also oversee the conduct of this study. This committee will review pertinent aspects of study conduct including subject safety, compliance with protocol, data collection and efficacy. The committee will review the charts of 10% of subjects enrolled to the study and two of the first 5 subjects entered to the study. The Committee reserves the right to conduct additional audits if necessary. The PI or designee is responsible for notifying the DSMC about the accrual of subjects when the first 5 have been entered to the study. The PI or designee will also notify the DSMC of study status within 2 months before the next scheduled review is due.

12. Data Handling and Record Keeping

12.1 *Case Report Forms and Study Records*

Study-specific CRFs will be made available for the conduct of this study. Study data, contained in source documentation, will be entered into the CRFs for all subjects enrolled in the study. All pertinent data records will be maintained as required after completion or termination of the study.

12.2 *Source Documents*

The investigator agrees that qualified representatives of the sponsor and regulatory agencies will have the right, both during and after this study, to conduct inspections and to audit and review medical records pertinent to the clinical study as permitted by the regulations. Subjects will not be identified by name in

any reports stemming from the study, and confidentiality of information in medical records will be preserved. The confidentiality of the subject will be maintained unless disclosure is required by regulations.

12.3 Retention of Data

Trial documents (including correspondence related to this clinical study, subject records, source documents, CRFs, study drug inventory records and IRB and sponsor correspondence pertaining to the study, original subject, laboratory, and study drug inventory records relating to the study) will be retained as specified in FDA regulations and institutional policy.

12.4 Study Termination

The study will be terminated at any time for any of the following reasons:

- Less than 2 of 9 evaluable subjects maintain MMR after second-TKI discontinuation
- More than 2 subjects develop \geq grade 3 cardiac toxicities
- More than 2 subjects develop grade 4 toxicity possibly associated with PIO
- Failure to enroll subjects
- Protocol violations
- Inaccurate or incomplete data
- Unsafe or unethical practices
- Questionable safety of the study drug
- Administrative decision

In the event of the termination of the Study, a written statement describing why the study was terminated prematurely will be made available by the investigator.

13. References

1. Faderl, S., et al., *The biology of chronic myeloid leukemia*. N Engl J Med, 1999. **341**(3): p. 164-72.
2. O'Brien, S.G., et al., *Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia*. N Engl J Med, 2003. **348**(11): p. 994-1004.
3. Baccarani, M., et al., *Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet*. J Clin Oncol, 2009. **27**(35): p. 6041-51.
4. Branford, S., et al., *Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials*. Blood, 2008. **112**(8): p. 3330-8.
5. Cella, D., C.J. Nowinski, and O. Frankfurt, *The impact of symptom burden on patient quality of life in chronic myeloid leukemia*. Oncology, 2014. **87**(3): p. 133-47.
6. Mahon, F.X., et al., *Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial*. Lancet Oncol, 2010. **11**(11): p. 1029-35.
7. Ross, D.M., et al., *Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study*. Blood, 2013. **122**(4): p. 515-22.
8. Takahashi, N., et al., *Discontinuation of imatinib in Japanese patients with chronic myeloid leukemia*. Haematologica, 2012. **97**(6): p. 903-6.
9. Lee, S.E., et al., *Predictive factors for successful imatinib cessation in chronic myeloid leukemia patients treated with imatinib*. Am J Hematol, 2013. **88**(6): p. 449-54.
10. Rea, D., et al., *Dasatinib or Nilotinib Discontinuation in Chronic Phase (CP)-Chronic Myeloid Leukemia (CML) Patients (pts) with Durably Undetectable BCR-ABL Transcripts: Interim Analysis of the STOP 2G-TKI Study with a Minimum Follow-up of 12 Months – on Behalf of the F....* Blood, 2014. **124**(21): p. 811-811.
11. Legros, L., et al., *Second attempt to discontinue imatinib in CP-CML patients with a second sustained complete molecular response*. Blood, 2012. **120**(9): p. 1959-60.
12. Hill, B.G., et al., *Sustained Complete Molecular Remission After Discontinuation of Tyrosine Kinase Inhibitors in Blast-Phase Chronic Myeloid Leukemia*. J Clin Oncol, 2014.
13. Molofsky, A.V., et al., *Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16Ink4a and p19Arf senescence pathways*. Genes Dev, 2005. **19**(12): p. 1432-7.
14. Morrison, S.J., N. Uchida, and I.L. Weissman, *The biology of hematopoietic stem cells*. Annu Rev Cell Dev Biol, 1995. **11**: p. 35-71.
15. Weissman, I.L., *Stem cells: units of development, units of regeneration, and units in evolution*. Cell, 2000. **100**(1): p. 157-68.
16. Holyoake, T., et al., *Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia*. Blood, 1999. **94**(6): p. 2056-64.
17. Chu, S., et al., *Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leukemia patients in complete cytogenetic remission on imatinib mesylate treatment*. Blood, 2005. **105**(5): p. 2093-8.
18. Barnes, D.J. and J.V. Melo, *Primitive, quiescent and difficult to kill: the role of non-proliferating stem cells in chronic myeloid leukemia*. Cell Cycle, 2006. **5**(24): p. 2862-6.

19. Copland, M., et al., *Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction*. Blood, 2006. **107**(11): p. 4532-9.
20. Jorgensen, H.G., et al., *Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells*. Blood, 2007. **109**(9): p. 4016-9.
21. Konig, H., et al., *Enhanced BCR-ABL kinase inhibition does not result in increased inhibition of downstream signaling pathways or increased growth suppression in CML progenitors*. Leukemia, 2008. **22**(4): p. 748-55.
22. Zhou, H. and R. Xu, *Leukemia stem cells: the root of chronic myeloid leukemia*. Protein Cell, 2015. **6**(6): p. 403-12.
23. Turhan, A.G., *STAT5 as a CML target: STATinib therapies?* Blood, 2011. **117**(12): p. 3252-3253.
24. Carlesso, N., D.A. Frank, and J.D. Griffin, *Tyrosyl phosphorylation and DNA binding activity of signal transducers and activators of transcription (STAT) proteins in hematopoietic cell lines transformed by Bcr/Abl*. J Exp Med, 1996. **183**(3): p. 811-20.
25. Ilaria, R.L., Jr. and R.A. Van Etten, *P210 and P190(BCR/ABL) induce the tyrosine phosphorylation and DNA binding activity of multiple specific STAT family members*. J Biol Chem, 1996. **271**(49): p. 31704-10.
26. Shuai, K., et al., *Constitutive activation of STAT5 by the BCR-ABL oncogene in chronic myelogenous leukemia*. Oncogene, 1996. **13**(2): p. 247-54.
27. Hantschel, O., et al., *BCR-ABL uncouples canonical JAK2-STAT5 signaling in chronic myeloid leukemia*. Nat Chem Biol, 2012. **8**(3): p. 285-93.
28. Xie, S., et al., *Involvement of Jak2 tyrosine phosphorylation in Bcr-Abl transformation*. Oncogene, 2001. **20**(43): p. 6188-95.
29. Samanta, A.K., et al., *Janus kinase 2: a critical target in chronic myelogenous leukemia*. Cancer Res, 2006. **66**(13): p. 6468-72.
30. Gesbert, F. and J.D. Griffin, *Bcr/Abl activates transcription of the Bcl-X gene through STAT5*. Blood, 2000. **96**(6): p. 2269-76.
31. Slupianek, A., et al., *BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance*. Mol Cell, 2001. **8**(4): p. 795-806.
32. Hoelbl, A., et al., *Stat5 is indispensable for the maintenance of bcr/abl-positive leukaemia*. EMBO Mol Med, 2010. **2**(3): p. 98-110.
33. Walz, C., et al., *Essential role for Stat5a/b in myeloproliferative neoplasms induced by BCR-ABL1 and JAK2(V617F) in mice*. Blood, 2012. **119**(15): p. 3550-60.
34. Ye, D., et al., *STAT5 signaling is required for the efficient induction and maintenance of CML in mice*. Blood, 2006. **107**(12): p. 4917-25.
35. Warsch, W., et al., *High STAT5 levels mediate imatinib resistance and indicate disease progression in chronic myeloid leukemia*. Blood, 2011. **117**(12): p. 3409-20.
36. Nelson, E.A., et al., *The STAT5 inhibitor pimozide decreases survival of chronic myelogenous leukemia cells resistant to kinase inhibitors*. Blood, 2011. **117**(12): p. 3421-9.
37. Evans, R.M., G.D. Barish, and Y.X. Wang, *PPARs and the complex journey to obesity*. Nat Med, 2004. **10**(4): p. 355-61.
38. Greene, M.E., et al., *Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping*. Gene Expr, 1995. **4**(4-5): p. 281-99.
39. Shipley, J.M. and D.J. Waxman, *Down-regulation of STAT5b transcriptional activity by ligand-activated peroxisome proliferator-activated receptor (PPAR) alpha and PPARgamma*. Mol Pharmacol, 2003. **64**(2): p. 355-64.

40. Zhou, Y.C. and D.J. Waxman, *Cross-talk between janus kinase-signal transducer and activator of transcription (JAK-STAT) and peroxisome proliferator-activated receptor-alpha (PPAR α) signaling pathways. Growth hormone inhibition of ppar α transcriptional activity mediated by stat5b*. J Biol Chem, 1999. **274**(5): p. 2672-81.
41. Prost, S., et al., *Erosion of the chronic myeloid leukaemia stem cell pool by PPAR[ggr] agonists*. Nature, 2015. **525**(7569): p. 380-383.
42. Kahn, S.E., et al., *Glycemic Durability of Rosiglitazone, Metformin, or Glyburide Monotherapy*. New England Journal of Medicine, 2006. **355**(23): p. 2427-2443.
43. Farnsworth, C. and W. Waldhausl, *Thiazolidinediones: metabolic actions in vitro*. Diabetologia, 2002. **45**(9): p. 1211-23.
44. Rosen, E.D. and B.M. Spiegelman, *PPAR γ : a nuclear regulator of metabolism, differentiation, and cell growth*. J Biol Chem, 2001. **276**(41): p. 37731-4.
45. Yamauchi, T., et al., *The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPAR γ) deficiency and PPAR γ agonist improve insulin resistance*. J Biol Chem, 2001. **276**(44): p. 41245-54.
46. Charbonnel, B., et al., *The prospective pioglitazone clinical trial in macrovascular events (PROactive): can pioglitazone reduce cardiovascular events in diabetes? Study design and baseline characteristics of 5238 patients*. Diabetes Care, 2004. **27**(7): p. 1647-53.
47. Rousselot, P., et al., *Targeting STAT5 Expression Resulted in Molecular Response Improvement in Patients with Chronic Phase CML Treated with Imatinib*. Blood 2012. **120**(21).

PLEASE BRING EMPTY BOTTLE AND
REMAINING PILLS AT EACH CLINIC
VISIT

Appendix 1: Diary Card

Medication Diary

Today's date _____

Page 1

Subject's Initials _____ Subject Study ID _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle.
2. You will take your dose PIO each day at approximately the same time. PIO tablets should be swallowed whole, and should not be broken, chewed or opened. You will take ____ mg tablets each day.
3. Record the date, the number of PIO tablets of each size you took, and when you took them.
4. Record the number of TKI tablets of each size you took.
5. **Please bring your pill bottle and this form to your physician when you go for your next appointment.**

Date	Day	Time of daily dose	PIO			TKI _____ _____ mg	Comments
			15 mg	30 mg	45 mg		
	1						
	2						
	3						
	4						
	5						
	6						
	7						
	8						
	9						
	10						
	11						
	12						
	13						
	14						

Subject's Initials _____ Subject Study ID _____

		INSTRUCTIONS TO THE PATIENT:					
		<ol style="list-style-type: none"> 1. Complete one form for each cycle. 2. You will take your dose PIO each day at approximately the same time. PIO tablets should be swallowed whole, and should not be broken, chewed or opened. You will take ____ mg tablets each day. 3. Record the date, the number of PIO tablets of each size you took, and when you took them. 4. Record the number of TKI tablets of each size you took. 5. If you have any comments or notice any side effects, please record them in the Comments column. 5. Please bring your pill bottle and this form to your physician when you go for your next appointment. 					

Date	Day	Time of daily dose	PIO			TKI _____ _____ mg	Comments
			15 mg	30 mg	45 mg		
	15						
	16						
	17						
	18						
	19						
	20						
	21						
	22						
	23						
	24						
	25						
	26						
	27						
	28						

	Patient's Signature: _____	Date: _____
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	Physician's Office will complete this section:	
	1. Patient's planned daily dose _____ Total number of pills taken this month _____	
	Physician/Nurse/Data Manager's Signature _____ Date : _____	

Appendix 2: Morisky Medication Adherence Scales – 8 (MMAS-8)

1) Do you sometimes forget to take your pills?	YES=0 <input type="checkbox"/> NO=1 <input checked="" type="checkbox"/>
2) People sometimes miss taking their medications for reasons other than forgetting. Thinking over the past two weeks, were there any days when you did not take your medicine?	YES=0 <input type="checkbox"/> NO=1 <input checked="" type="checkbox"/>
3) Have you ever cut back or stopped taking your medicine without telling your doctor because you felt worse when you took it?	YES=0 <input type="checkbox"/> NO=1 <input checked="" type="checkbox"/>
4) When you travel or leave home, do you sometimes forget to bring along your medicine?	YES=0 <input type="checkbox"/> NO=1 <input checked="" type="checkbox"/>
5) Did you take all your medicine yesterday?	YES=1 <input type="checkbox"/> NO=0 <input type="checkbox"/>
6) When you feel like your symptoms are under control, do you sometimes stop taking your medicine?	YES=0 <input type="checkbox"/> NO=1 <input checked="" type="checkbox"/>
7) Taking medicine every day is a real inconvenience for some people. Do you ever feel hassled about sticking to your treatment plan?	YES=0 <input type="checkbox"/> NO=1 <input checked="" type="checkbox"/>
8) How often do you have difficulty remembering to take all your medicine? ___ A. Never/rarely ___ B. Once in a while ___ C. Sometimes ___ D. Usually ___ E. All the time	A=4 <input type="checkbox"/> B=3 <input type="checkbox"/> C=2 <input type="checkbox"/> D=1 <input type="checkbox"/> E=0 <input type="checkbox"/> Divide score by 4

Adherence	MMAS-8 Score
High Adherence	8
Medium Adherence	6-8
Low Adherence	<6