Weill Cornell Medical College

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Weill Cornell Medical College

TITLE:	Detection, monitoring, and molecular characterization of leukemic stem cells from patients with chronic myeloid leukemia (CML) undergoing therapy with nilotinib
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INDICATION:	Chronic Myeloid Leukemia (CML)
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List of Abbreviations

AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
bid	bis in diem/twice a day
CHR	complete hematologic response
CML	chronic myelogenous leukemia
CML-AP	chronic myelogenous leukemia – accelerated phase
CML-BC	chronic myelogenous leukemia – blast crisis
CML-CP	chronic myelogenous leukemia – chronic phase
CR	complete response
CRF	case report/record form
CS&E	Clinical Safety and Epidemiology
CT	computerized tomography
CTC	common terminology criteria
CyR	cytogenetic response
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
GIST	gastrointestinal stromal tumor
HR	hematologic response
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	intention-to-treat
IULN	Institutional Upper Limit of Normal
iv	intravenous(ly)
LVEF	left ventricular ejection fraction
MCyR	major cytogenetic response
MTD	maximum tolerated dose
NCI	National Cancer Institute
NEL	no evidence of leukemia
NIH	National Institutes for Health
PD	progression of disease
PFS	progression free survival
Ph +	Philadelphia chromosome positive
po	per os/by mouth/orally
PR	partial response
qd	quaque die/every day
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	serious adverse event
SD	stable disease
ULN	upper limit of normal
WBC	White Blood Cell Count

1 Objectives

The overall objective of the study is to describe the quantitative and qualitative characteristics of the leukemic stem cell population in a cohort of patients with untreated chronic phase Ph+ CML during therapy with nilotinib.

Specific Objectives:

1. To measure the quantitative changes using multi-parameter flow cytometry, transcritptional profiling, and real-time RT-PCR in the leukemic stem cell population (CD45dimCD34+CD38-) in CML patients during therapy with nilotinib
2. To assess the molecular characteristics of the leukemic stem cell population before and during therapy with nilotinib. Immunophenotypic changes, transcriptional profiling, and changes in BCR-ABL expression using real-time RT-PCR will be evaluated at diagnosis, after 4 weeks, 12 weeks and 48 weeks after therapy.
3. To correlate the quantitative and qualitative changes in the CML stem cell population with clinical outcomes, including rapidity and depth of reduction of BCR/ABL transcript levels during therapy with nilotinib. MMR and CMR rates will be evaluated at 18 cycles and 24 cycles on study.

2 Study design

The study is a phase 4 clinical and translational trial designed to evaluate the effects of nilotinib on the leukemic stem cell population in patients with chronic phase Ph+ CML. Patients on study will be monitored according to accepted NCCN clinical guidelines for 24 cycles. Before therapy, and at specific time- points during therapy, (described in section 5.4.1.) peripheral blood and bone marrow samples will be obtained for cytogenetic and molecular evaluations. In addition, samples will be collected to analyze the quantitative and qualitative changes in the leukemic stem cell population before and during therapy with nilotinib. The study is intended as a hypothesis finding analysis in order to establish whether in response to nilotinib therapy, defined differences in the baseline or therapy-induced changes in the characteristics of the stem cell population will be predictive of the ability to successfully discontinue therapy in patients with CML.

3 Overview of chronic myelogenous leukemia

Chronic myeloid leukemia (CML) is a hematological stem cell disorder associated with a specific chromosomal translocation known as the Philadelphia (Ph) chromosome detected in 95% of patients (Nowell and Hungerford 1960; Rowley 1973). The molecular consequence of the translocation is the fusion of the ABL proto-oncogene to the BCR gene resulting in the production of an activated form of the ABL protein-tyrosine kinase (Bartram et al 1983; Heisterkamp et al 1983). Expression of the BCR-ABL protein is capable of inducing leukemia in mice, implicating the protein as the cause of these diseases (Daley et al 1990; Kelliher et al 1990).

Clinically, CML progresses through three distinct phases of increasing refractoriness to therapy: chronic phase (CP) (median duration 3-4 years; median survival up to 10 years with allogeneic

bone marrow transplant, 5-6 years with interferon (IFN) and beyond 10 years with imatinib), accelerated phase (AP) (median duration 3-9 months; median survival 8-18 months), and blast crisis (BC) (median survival 3-6 months) (Enright and McGlave 2000). Most patients, however, present in the CP, characterized by splenomegaly and leukocytosis with generally few symptoms.

National Comprehensive Cancer Network (NCCN) guideline on CML (NCCN guideline v 2.2012) and European Leukemia Net (ELN) (Baccarani et al 2009) recommend continuing tyrosine kinase inhibitor (TKI) treatment indefinitely in all responding patients. There are currently no recommendations or provisions for temporary or permanent stopping of TKI treatment.

4 Introduction to investigational treatment

4.2.1 Overview of Nilotinib

Nilotinib is a aminopyrimidine, available as an oral formulation that is an Adenosine triphosphate (ATP)-competitive inhibitor of the protein tyrosine kinase activity of BCR-ABL, which prevents the activation of BCR-ABL dependent mitogenic and anti-apoptotic pathways (e.g. Phosphatidylinositol 3-kinases (PI-3 kinase) and signal transducer and activator of transcription 5 (STAT5)), leading to the death of the BCR-ABL phenotype. Following oral administration to animals, nilotinib is moderately absorbed with approximately 30% bioavailability, and is well tolerated.

4.2.1.1 Non-clinical experience

Data from preclinical studies demonstrate that nilotinib achieves higher intracellular concentrations than imatinib, and that nilotinib inhibits BCR-ABL tyrosine kinase activity and induces apoptosis at lower concentrations than imatinib (Le Coutre et al 2004, White et al 2005). Therefore, based on the preclinical data, and observed efficacy of nilotinib in imatinib-resistant and intolerant patients, nilotinib was predicted to have significant efficacy in newly diagnosed CML-CP patients. For more details on non-clinical experience, please refer to the latest nilotinib [Investigator's Brochure].

4.2.1.2 Clinical experience

Clinical safety and tolerability

Overall, nilotinib has been found to be effective and well tolerated in patients with Philadelphia chromosome positive chronic myeloid leukemia (Ph+ CML)-CP, and AP who were resistant to imatinib or intolerant of imatinib as well as in patients with newly diagnosed Ph+ CML-CP.

For detailed nilotinib clinical safety and tolerability, please refer to the latest nilotinib [Investigator's Brochure]. Safety data in newly diagnosed Ph+ CML-CP have been acquired from the Phase III ENESTnd (Evaluating Nilotinib Efficacy and Safety in Clinical Trials - newly diagnosed CML-CP), [CAMN107A2303] study. Additional safety data have been acquired from a Phase I/II open-label study [CAMN107A2101] in CML patients, and a Phase III study in gastrointestinal stromal tumor (GIST) patients, as well as from further clinical studies and post-marketing experience. The most commonly reported (>5%) all grade nonhematologic adverse

reactions in patients with CML were rash, headache, nausea, pruritus, alopecia, myalgia, fatigue, dry skin, arthralgia, vomiting, abdominal pain upper, muscle spasms, diarrhea, constipation, peripheral edema, dyspepsia, erythema, abdominal pain, and asthenia. Hematologic adverse drug reactions included myelosuppression (thrombocytopenia, neutropenia and anemia). Clinically relevant biochemical abnormalities included hyperglycemia, hyperbilirubinemia, hypophosphatemia, and increases in lipase, ALT, and AST. There are insufficient safety data for using nilotinib during pregnancy. In very rare cases peripheral arterial occlusive disease (PAOD) occurred. In the majority of these reported cases, patients had associated risks (e.g. hypertension, hyperlipidemia, hypercholesterolemia, smoking, or diabetes mellitus) and/or had a pre-existing peripheral vascular disease. A causal relationship of the reported events with nilotinib has not been established but could not be excluded.

Clinical Pharmacokinetics

Nilotinib is metabolized by the liver, primarily via CYP3A4. Thus strong inhibitors or inducers of CYP3A4 can significantly alter the pharmacokinetics and systemic exposure of nilotinib in humans. Unchanged nilotinib represents the predominant systemic circulating component (approximately 88% of the total drug-related serum exposure). The terminal elimination half-life of nilotinib was estimated to be approximately 17 hours. Race was assessed as a covariate on the bioavailability or clearance of nilotinib in the population pharmacokinetic analysis which included a total of 550 patients. No significant differences were observed in nilotinib pharmacokinetics across various race groups, e.g. Caucasian (n=348 patients), Black (n=23 patients), Asian (n=138 patients) and other races (n=41 patients) [Modeling report CAMN107A2303]. These findings are consistent with the previous observations in patients with imatinib resistant or intolerant CML [Study CAMN107A2101 PopPK] and the ethnic insensitivity analysis conducted for the original Tasigna® submission. Population pharmacokinetics (PK) analysis also suggested that male patients had an approximately 10% lower bioavailability or 10% lower systemic exposure than female Patients. However, since the observed extent of the difference is relatively small, such a sex effect is unlikely to be clinically meaningful for nilotinib therapy. Other demographic variables, such as age and body weight did not significantly affect nilotinib PK. Thus, patient demographics are not a clinically important factor contributing to interpatient variability in nilotinib PK and exposure.

For additional nilotinib clinical pharmacokinetics information, please refer to the latest nilotinib Investigator's Brochure.

Clinical efficacy

Nilotinib is approved by US Food and Drug Administration (FDA) and EuropeanCommission (EC) to treat newly diagnosed adult patients with Ph+ CML in CP, and to treat CP and AP Ph+ CML in adult patients resistant to or intolerant to prior therapy that included imatinib. The recommended adult dosage of nilotinib is 300 mg orally twice daily for newly diagnosed Ph+ CML-CP and 400 mg orally twice daily for resistant or intolerant Ph+ CMLCP and CML-AP.

The results of study ENESTnd [CAMN107A2303] demonstrated superiority of nilotinib vs. imatinib in the CML-CP frontline setting (Saglio et al 2010, Larson et al 2012). This study was designed to compare the efficacy and safety of nilotinib 400 mg twice daily (BID) and nilotinib

300 mg BID with imatinib 400 mg once daily (QD) treatment. The primary efficacy endpoint is the rate of major molecular response (MMR) at 12 cycles after the start of first study medication. The data cut-off for this first analysis of study [CAMN107A2303] was 02- Sep-2009, at which time the last patient had completed 12 cycles of treatment. More patients treated with nilotinib achieved deeper molecular responses and the differences were significantly superior at each of the analyzed time points to date. The rate of major molecular response (MMR) at 12 cycles was 43% in the nilotinib 400 mg BID treatment arm, 44% in the nilotinib 300 mg BID treatment arm, and 22% in the imatinib treatment arm $p<0.001$ (primary endpoint). The cumulative incidence of molecular response 4.5 log reduction from standardized baseline (MR4.5) was 7%, 19% and 28% by 12, 24 and 36 cycles in the nilotinib 400 mg BID arm, and was 11%, 25% and 32% in the nilotinib 300 mg BID arm. In the imatinib arm the rates were 1%, 9% and 15% at the same time points. The differences between the rates of MR4.5 in nilotinib and imatinib arms increased over time (6%, 10% and 13% difference by cycle 12, 24 and 36 for the 400 mg BID arm and 10%, 16% and 17% difference for the 300 mg BID arm by the same time points respectively). These results demonstrated that more patients starting CML-CP therapy with nilotinib vs. imatinib can achieve levels of molecular response (MR) necessary to allow for a future treatment discontinuation. For additional nilotinib clinical efficacy information, please refer to the latest nilotinib [Investigator's Brochure].

Study Rationale

The use of specific BCR/ABL tyrosine kinase inhibitors has clearly and dramatically improved the outcomes for patients with CML. Current data suggests that close to 90% of patients treated in the chronic phase of CML will be long-term survivors with survival rates equivalent to age-matched cohorts (Saglio et al 2010). In addition to prevention of the progression of the disease to the acute phases of the illness, a high percentage of patients have achieved deep molecular responses in response to TKI therapy with some patients achieving undetectable levels of BCR/ABL protein transcript levels using highly sensitive PCR-based assays. These responses have been durable with continuous daily TKI therapy, which has remained the continued recommended therapy for patients with CML.

Despite the tremendous success of TKI therapy in CML there has been growing concern about the effects on the health and well-being of patients who are receiving continued administration of these agents. These concerns have centered around the detrimental effects on quality of life, risks of organ impairment, and financial hardships associated with long term TKI therapy. In this regard, a critical question has emerged as to whether patients with CML achieving a deep molecular response can be safely discontinued from TKI therapy.

To date the efforts to discontinue TKI therapy have been essentially empirical, that is, patients in durable deep molecular response have been discontinued from therapy and observed. The largest reported experience comes from the STIM (stop imatinib mesylate) trial conducted in France (Mahon et al 2010). The data from this trial, updated in December 2013, has demonstrated that only 40% of patients in deep molecular response, defined as a 4.5 log reduction of BCR/ABL levels from baseline were able to successfully stay off TKI therapy without recurrence of molecular positivity. The only variables predictive for lack of recurrence of disease were clinical and included a history of prior treatment with interferon-alpha and the length of time on imatinib therapy prior to discontinuation. Fortunately, all of the patients with recurrent molecular positivity achieved a complete or major molecular response with retreatment and so far only 1 patient has reportedly progressed to a more advanced stage of CML. This initial clinical trial, however, clearly indicates the need for better predictive markers for successful discontinuation of TKI therapy.

Emerging data from several laboratories has demonstrated that CML stem cells persist in all patients with CML on TKI therapy including patients achieving deep and durable molecular responses (Bhatia et al 2003, Graham et al 2002) Limited, but consistent data has shown that these BCR/ABL positive stem cells are not suppressed by current TKI therapy and remain capable of self-renewal (Bolton-Gillepsie et al 2013, Hamilton et al 2012) An important question therefore is why therapy can be successfully discontinued in some patients while others will have rapid repopulation of the marrow and blood with CML. In addition, these preliminary studies have suggested that these cells may be supported by non-BCR/ABL signaling pathways, which allow for survival despite prolonged exposure to BCR/ABL inhibitors. To date studies conducted on CML stem cells have utilized samples from patients who have already been exposed to TKI therapy for a long period of time and have not clearly and uniformly described the character and kinetics of the change in the stem cell population before and during therapy.

Accordingly, a prospective trial in a previously untreated cohort of chronic phase CML patients, uniformly treated with nilotinib, the current standard of care for these patients, will allow the opportunity to fully describe the character and changes of the stem cell population. The data obtained from these patients may identify stem cell variables that can more accurately predict the success of discontinuation of TKI therapy.

4 Population

Patients with newly diagnosed Ph+ CML in chronic phase will be eligible for enrollment in this trial. Prior treatment with nilotinib for less than 2 weeks and hydroxyurea is allowed.

Inclusion/exclusion criteria

4.1 Inclusion criteria

1. Patients ≥ 18 years of age.
2. ECOG Performance status 0,1, or 2
3. Documented diagnosis of Ph+ Chronic phase CML(Cortes et al 2012)

   Chronic phase: None of the criteria for accelerated or blastic phase

   Accelerated phase

- Blasts ≥ 15% in blood or BM
- Basophilia ≥ 20% in blood or BM
- Platelets < 100 × 109/L unrelated to therapy

Blast phase

- ≥ 30% blasts in blood or BM
- Extramedullary disease with localized immature blasts

4. Adequate end organ function, defined as the following:
   - Creatinine < 1.5 x ULN
   - ANC > 1.5 x 10^9/L
   - Platelets > 100 x 10^9/L
   - Total bilirubin < 1.5 x ULN
     - Does not apply to patients with isolated hyperbilirubinemia (e.g., Gilbert's disease) grade <3.
   - AST (SGOT) and ALT (SGPT) < 3 x ULN
   - Serum amylase and lipase ≤ 2 x ULN
   - Alkaline phosphatase ≤ 2.5 x ULN
   - Patients must have the following laboratory values (WNL = within normal limits at the local institution lab) or corrected to within normal limits with supplements prior to the first dose of study medication:
     - Potassium (WNL)
     - Magnesium (WNL)
     - Phosphorus (WNL)
     - Corrected Calcium (WNL)

4.2 Exclusion criteria

1. Previous treatment with any other tyrosine kinase inhibitor except for up to 2 weeks of nilotinib
2. Impaired cardiac function including any one of the following:
   - Inability to monitor the QT interval on ECG
   - Congential long QT syndrome or a known family history of long QT syndrome.
   - Clinically significant resting brachycardia (<50 beats per minute)
   - QTcF > 450 msec on baseline ECG. If QTcF >450 msec and electrolytes are not within normal ranges, electrolytes should be corrected and then the patient re-screened for QTcF
   - Myocardial infarction within 12 months prior to starting study

- Other clinically significant uncontrolled heart disease (e.g. unstable angina, congestive heart failure or uncontrolled hypertension)
- History of or presence of clinically significant ventricular or atrial tachyarrhythmias
- Complete left bundle branch block
- Right bundle branch block plus left anterior/posterior hemiblock
- Use of ventricular-paced pacemaker
- History of unstable angina within 1 year of study entry

3. Patients currently receiving treatment with strong CYP3A4 inhibitors and treatment cannot be either discontinued or switched to a different medication prior to starting study drug. (http://medicine.iupui.edu/clinpharm/ddis/)).
4. Patients currently receiving treatment with any medications that have the potential to prolong the QT interval and the treatment cannot be either discontinued or switched to a different medication prior to starting study drug (http://crediblemeds.org/)
5. Impaired gastrointestinal (GI) function or GI disease that may significantly alter the absorption of study drug (e.g., ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, small bowel resection or gastric bypass surgery).
6. History of acute pancreatitis within 1 year of study entry or past medical history of chronic pancreatitis
7. Severe and/or uncontrolled concurrent medical disease that in the opinion of the investigator could cause unacceptable safety risks or compromise compliance with the protocol (e.g. uncontrolled diabetes, uncontrolled infection)
8. History of another active malignancy within 5 years prior to study entry with the exception of previous or concomitant basal cell skin cancer and previous carcinoma in situ treated curatively
9. Known presence of significant congenital or acquired bleeding disorder unrelated to cancer
10. Major surgery within 4 weeks prior to Day 1 of the study or who have not recovered from prior surgery
11. Treatment with other investigational agents within 30 days of Day 1.
12. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during the study and for 14 days after the final dose of nilotinib. Highly effective contraception is defined as either:
    - Total abstinence (when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
    - Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment.
    - Male sterilization (at least 6 months prior to enrolling). For female patients on the study the vasectomized male partner should be the sole partner for that patient.

- Use of a combination of any two of the following:
  a. Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception.
  b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
  c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository In case of use of oral contraception women should have been stable on the same pill for a minimum of 3 months before taking study treatment

Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks prior to enrolling. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

If a study patient becomes pregnant or suspects being pregnant during the study or within 30 days after the final dose of nilotinib, the Study Doctor needs to be informed immediately and ongoing study treatment with nilotinib has to be stopped immediately.

5 Treatment

5.1 Investigational therapy and reference therapy

All patients enrolled on the study will receive nilotinib at a dose of 300 mg P.O. BID daily. Therapy will be continued until progressive disease or unacceptable toxicity is documented

5.1.1 How supplied

Novartis will supply nilotinib as 150 and/or 200 mg capsules packaged in cards for an exposure period of up to 24 cycles or so long as the patient remains on study provided the patient shows continuous benefit from treatment with nilotinib and there are no safety concerns. Medication labels will comply with the legal requirements of the US and will be printed in English. The storage conditions for nilotinib will be described on the medication label. Bottles must be stored in a safe, secure location.

5.2 Study drug administration

Patients should be instructed to take their doses every 12 hours. After a two hour fast the patient should take their morning dose of nilotinib. Patients should continue to fast for 1 hour after the administration of the morning nilotinib dose. In the evening, the patient should have their evening meal, fast for 2 hours, and then take their evening dose. Patients should continue to fast for 1 hour after the administration of the evening nilotinib dose.

If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose. If the morning or evening dose is delayed (i.e., missed by a few hours), patients should take the dose and wait a minimum of 8 hours before the next dose; if that is not possible, the patient should skip the affected dose and take the next scheduled dose.

Water is allowed during the fasting period on all days. Patients should be instructed to take their doses at approximately the same time each day. Each dose of nilotinib should be taken with a glass of water and consumed over as short a time as possible. Patients should be instructed to swallow the capsules whole and not chew them. Patients must avoid grapefruit, grapefruit juice, star fruit, and Seville oranges during the entire study.

5.3 Concomitant therapy

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed, provided their use is documented in the patient records and on the appropriate case report form. Nilotinib may be given in combination with hematopoietic growth factors such as erythropoietin or G-CSF if clinically indicated. Nilotinib may be given with hydroxyurea or anagrelide if clinically indicated. The administration of any other anti-cancer agents including, chemotherapy and biologic agents is not permitted. Similarly, the use of other concurrent investigational drugs is not allowed, unless it is part of the study design.

5.3.1 CYP 3A4 inhibitors

Every effort should be made NOT to administer strong CYP3A4 inhibitors CYP3A4 inhibitors may decrease the metabolism of nilotinib and thereby increase serum concentrations and increase exposure. If administration of a strong CYP3A4 inhibitor cannot be avoided during the study and cannot be switched to an alternative therapy, study treatment must be STOPPED. Furthermore, increased awareness should be exercised when administering moderate inhibitors and/or multiple weak inhibitors. A list of these medications and inhibitor classifications can be found at the following website: http://medicine.iupui.edu/clinpharm/ddis/, however this list may not be comprehensive.CYP 3A4 inducers

Every effort should be made NOT to administer strong CYP3A4 inducers **however, if** administration of a CYP3A4 inducer cannot be avoided during the study, temporary discontinuation of study treatment is NOT required. A list of these medications and inducer classifications can be found at the following website: http://medicine.iupui.edu/clinpharm/ddis/, however this list may not be comprehensive.

5.3.2 QT Prolonging Drugs

Every effort should be made NOT to administer a QT prolonging agent when a patient is on nilotinib treatment. If when a patient is on nilotinib treatment, concomitant administration of an agent known to prolong the QT interval is required and cannot be switched to an alternative therapy, nilotinib must be STOPPED. Please see http://crediblemeds.org/ for a list of agents that prolong the QT interval (this list may not be comprehensive).

5.3.3 Drug metabolizing enzymes and drug transporter systems

Nilotinib is a competitive inhibitor of CYP3A4, CYP2C8, CYP2C9, CYP2D6 and UGT1A in vitro, potentially increasing the concentration of drugs eliminated by these enzymes. In addition, single-dose administration of nilotinib with midazolam (a CYP3A4 substrate) to healthy subjects increased midazolam exposure by 30%. Caution should be exercised when co-administering nilotinib with substrates for these enzymes that have a narrow therapeutic index. Since warfarin is metabolized by CYP2C9 and CYP3A4 it should be avoided if possible.

In vitro studies also suggest that nilotinib may induce CYP2B6, CYP2C8 and CYP2C9, and thereby has the potential to decrease the concentrations of drugs which are eliminated by these enzymes.

Nilotinib is a substrate of the efflux transporter P-glycoprotein (Pgp, ABCB1). If nilotinib is administered with drugs that inhibit Pgp, increased concentrations of nilotinib are likely and caution should be exercised.

5.3.4 Nilotinib dose reduction

Dose Adjustments or Modifications

If clinically appropriate, dose adjustments may be made at the discretion of the Primary Investigator.

QT Interval Prolongation:

Table 1: Dose Adjustments for QT Prolongation

ECGs with a QTc >480 msec	1. Withhold Tasigna, and perform an analysis of serum potassium and magnesium, and if below lower limit of normal, correct with supplements to within normal limits. Concomitant medication usage must be reviewed. 2. Resume within 2 weeks at prior dose if QTcF returns to <450 msec and to within 20 msec of baseline. 3. If QTcF is between 450 msec and 480 msec after 2 weeks, reduce the dose to 400 mg once daily. 4. If, following dose-reduction to 400 mg once daily, QTcF returns to >480 msec, Tasigna should be discontinued. 5. An ECG should be repeated approximately 7 days after any dose adjustment. If clinically appropriate escalation of the dose back to 300 mg twice daily should be attempted.

Myelosuppression

Withhold or dose reduce Tasigna for hematological toxicities (neutropenia, thrombocytopenia) that are not related to underlying leukemia (Table 2).

Table 2: Dose Adjustments for Neutropenia and Thrombocytopenia

Newly diagnosed Ph+ CML in chronic phase at 300 mg twice daily Resistant or intolerant Ph+ CML in chronic phase at 400 mg twice daily	ANC* <1.0 x 109/L and/or platelet counts <50 x 109/L	1. Stop Tasigna, and monitor blood counts 2. Resume within 2 weeks at prior dose if ANC >1.0 x 109/L and platelets >50 x 109/L 3. If blood counts remain low for >2 weeks, reduce the dose to 400 mg once daily 4. If dose is adjusted for low counts and values recover to grade zero, patients may be dose escalated back to 300mg PO, BID.

*ANC=absolute neutrophil count

Table 3: Dose Adjustments for Selected Non-hematologic Laboratory Abnormalities

Elevated serum lipase or amylase ≥Grade 3	1. Withhold Tasigna, and monitor serum lipase or amylase 2. Resume treatment at 400 mg once daily if serum lipase or amylase returns to ≤Grade 1
Elevated bilirubin ≥Grade 3	1. Withhold Tasigna, and monitor bilirubin 2. Resume treatment at 400 mg once daily if bilirubin returns to ≤Grade 1
Elevated hepatic transaminases ≥Grade 3	1. Withhold Tasigna, and monitor hepatic transaminases 2. Resume treatment at 400 mg once daily if hepatic transaminases returns to ≤Grade 1

*If clinically appropriate escalation of the dose back to 300 mg twice daily should be attempted

Other Non-hematologic Toxicities

If other clinically significant moderate or severe non-hematologic toxicity develops, withhold dosing, and resume at 400 mg once daily when the toxicity has resolved. If clinically appropriate, escalation of the dose back to 300 mg (newly diagnosed Ph+ CML-CP) or 400 mg (resistant or intolerant Ph+ CML-CP) twice daily should be attempted. For Grade 3 to 4 lipase elevations, dosing should be withheld, and may be resumed at 400 mg once daily once lipase has normalized to grade ≤ 1. Test serum lipase levels monthly or as clinically indicated. For Grade 3 to 4 bilirubin or hepatic transaminase elevations, dosing should be withheld, and may be resumed at 400 mg once daily once the abnormalities have normalized to grade ≤ 1. Test bilirubin and hepatic transaminases levels monthly or as clinically indicated.

5.4 Interruption or discontinuation of treatment

Reasons that a patient may have an interruption or discontinuation of treatment in a clinical study are considered to constitute one of the following:

1. Adverse event(s)
2. Abnormal laboratory value(s)
3. Drug Toxicity
4. Unsatisfactory therapeutic effect (including disease progression/relapse)
5. Subject's condition no longer requires study treatment
6. Intercurrent illness
7. Investigator’s decision
8. Protocol violation
9. Subject withdrew consent
10. Lost to follow-up
11. Administrative problems
12. Death

5.4.1 Study Visit and Assessment Schedule:

Patients enrolled in the study will be cared for according to established clinical guidelines for the use of nilotinib according to the FDA approved prescribing information and according to established NCCN guidelines for CML patients on TKI therapy. Specific study assessments and visits are outlined below:

Baseline Studies (can be performed up to 28 days prior to enrollment):

1. Bone marrow aspirate and biopsy
2. Peripheral blood for Correlative Research
3. Peripheral blood for BCR/ABL, FISH and RT-PCR
4. Informed consent/inclusion and exclusion evaluation

5. History and physical examination
6. Pregnancy test (if applicable)
7. Calculation of EUTOS score
8. ECOG performance status
9. Hematology (Complete CBC w/ Platelets and Differential)Blood Chemistry (Complete Metabolic Panel, Direct Bilirubin, Phosphorous, Amylase, Lipase, Uric Acid, LDH and Magnesium)
10. Hepatitis B surface antigen (HBsAG)
11. 12 lead electrocardiogram
12. Fasting lipid panel (total cholesterol, LDL, HDL, triglycerides)
13. Hemoglobin A1C level

On study visits:

Patients will be treated with Nilotinib for to 2 years, or so long as the patient remains on study provided they show continuous benefit from treatment with nilotinib and there are no safety concerns. Patient's will return to clinic for evaluation after 4 weeks of cumulative treatment (+/- 7 days), after 12 weeks of cumulative treatment (+/- 7 days) and once every 12 weeks thereafter (+/- 14 days) until they are removed from protocol treatment. The following assessments will be performed at stated time points based off of cumulative treatment:

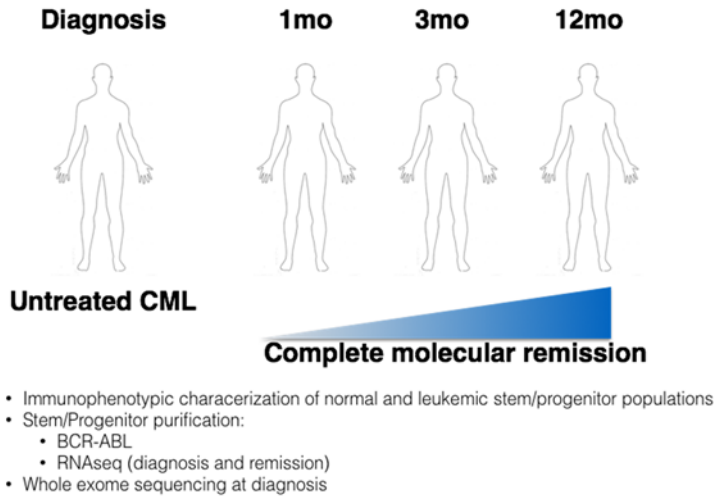
1. Bone marrow aspiration and biopsy will be performed at 4, 12, and 48 weeks of cumulative dose
2. Peripheral blood studies for quantitative BCR/ABL levels will at 4, 12, and 48 weeks thereafter while on study up to Week 96.
3. Hematology (Complete CBC w/ Platelets and Differential) every 24 weeks
4. Blood Chemistry (Complete Metabolic Panel, Direct Bilirubin, Phosphorous, Amylase, Lipase, Uric Acid, LDH and Magnesium) every 24 weeks
5. Peripheral blood for RT-PCR every 12 weeks (if bone marrow has not already been submitted)
6. ECG at 4 weeks, 48 weeks and every 24 cycles thereafter or in the event of a dose modification.

Safety Follow Up:

Patients will be contacted 30 days after cessation of treatment with nilotinib to assess any safety issues that may have arisen after stopping the study drug. This follow-up can be performed in person or over the phone.

Correlative Science/Experimental design.

In order to determine the effect of nilotinib in stem and progenitor populations we will evaluate 40 newly diagnosed CML patients undergoing treatment with nilotinib at different time points. We will evaluate the levels of expression of BCR-ABL in purified stem cell populations during the course of treatment. In addition, we will compare the stem and progenitor populations present during the course of treatment in peripheral blood and bone marrow. We will perform transcriptional profiling of such populations to determine changes in signaling pathways driving survival, self-renewal or proliferation. Whole exome sequencing will be also performed in all diagnostic samples to determine whether there are novel cooperating mutations. Figure 1, shows a summary of the correlative studies.



Specifically we plan to:

I. To determine the effect of nilotinib in hematopoietic stem cells from chronic phase CML patients. (Guzman Lab)

Bone marrow and peripheral blood samples will be collected at diagnosis, 4, 12, and 48 weeks after exposure to nilotinib. Mononuclear cells obtained from at least 10cc of bone marrow aspirate will be isolated to perform the following assays:

(1) Complete immunophenotypic characterization of the progenitor and stem cell populations will be performed using the high throughput features of the BD-LSR-II flow cytometer. Multiparameter flow cytometry will be performed for the following markers: CD45, CD123, CD34, CD38,IL1-RAP, CD99 and CD90.

(2) qPCR in purified stem cell populations (CD45dim, CD34+CD38-) for BCR-ABL transcripts. Lymphocytes will be also isolated to evaluate the BCR-ABL trasncripts.

(3) RNAseq or open array taqman assays will be perform in purified stem cell populations (CD45dim, CD34+CD38-) to determine transcriptional changes.

II. *To investigate the mutation profile that may contribute to disease .(Hassane Lab)*

Whole exome sequencing will be conducted using the Nimblegen SeqCap exome capture platform, spanning 64 Mb of target. For each diagnosis sample, we compare leukemia cells alongside purified lymphocytes (CD19/CD3) as a germline control. Somatic variants will be assessed on Illumina sequencing performed to a depth of 400x to ensure detection of subclonal variants. Genes and pathways on which variants converge will be determined using gene set enrichment analysis. We will correlate gene and pathway-level alterations uncovered in the 40 patients with phenotypic features such as stem cell content.

III. To determine whether gene mutations in abl within the stem/progenitor pool contribute to the inability of nilotinib to eliminate the malignant clone.(Guzman and Hassane labs)

We will perform targeted re-sequencing in stem/progenitor population at the 48 week time point for patients that show detectable levels of BCR-ABL transcript. The targeted re-sequencing will involve ABL and related pathway genes.

5.4.2 Safety assessments

Safety assessments will consist of evaluating adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

5.4.2.1 Adverse events

An adverse event is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient's signed informed consent has been obtained.

Abnormal laboratory values or test results occurring after informed consent constitute adverse events only if the induce clinical signs or symptoms, are considered clinically significant, require therapy (e.g., hematologic abnormality that requires transfusion or hematological stem cell support), or require changes in study medication(s).

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events eCRF. This includes any adverse events that occur in the TFR Phase.

Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's eCRF. Adverse event monitoring should be continued for at least 30 days (or 5 half-lives, whichever is longer) following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate

Adverse Event. Adverse events will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, corresponding to Grades 1 - 4, will be used. CTCAE Grade 5 (death) will not be used in this study; rather, information about deaths will be collected though a Death form.

The occurrence of adverse events should be sought by non-directive questioning of the patient during the screening process after signing informed consent and at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during the screening process or between visits, or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:
1. The severity grade (CTCAE Grade 1-4)
2. Its duration (Start and end dates or Ongoing at End of Study)
3. Its relationship to the study treatment
4. Action taken with respect to study or investigational treatment
5. Whether medication or therapy was given
6. Whether it is serious, where a serious adverse event (SAE) is defined as in section 9

All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded on the Adverse Event eCRF.
Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

5.4.2.2 Serious Adverse Events

Serious adverse event (SAE) is defined as one of the following:
- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Note that hospitalizations for the following reasons should not be reported as serious adverse events:
- Routine treatment or monitoring of the studied indication (CML), not associated with any deterioration in condition
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's

general condition
- Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above

is not a serious adverse event

5.4.2.3 Reporting

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided the main informed consent and until at least 30 days after the patient has stopped study treatment will be reported to Novartis (using the SAE Report Form) within 24 hours of learning of its occurrence. SAEs will be followed until resolution or until clinically relevant improvement or stabilization. Any SAEs experienced after this 30 days period (or 5 half-lives, whichever is longer) should only be reported to Novartis if the investigator suspects a causal relationship to the study treatment. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. If the serious adverse event has not been previously documented (new occurrence) and it is thought to be related to study drug (or therapy), the Medical Safety Expert of the Clinical Safety & Epidemiology (CS&E) Department may contact the investigator to obtain further information. If warranted, an investigator alert may be issued, to inform all investigators involved in any study with the same drug (or therapy) that this serious adverse event has been reported.

Information about all SAEs is collected and recorded on the FDA Med Watch 3500a form and Novartis Serious Adverse Event Report Form; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and send the completed, signed form by fax to the oncology Novartis Drug Safety and Epidemiology (DS&E) department within 2 to 3 calendar days for deaths or life-threatening events and 5 calendar days for other serious adverse events.

The telephone and telefax number of the contact persons in the local department of DS&E, specific to the site, are listed in the investigator folder provided to each site. The original copy of the SAE Report Form and the fax confirmation sheet must be kept with the case report form documentation at the study site.

Follow-up information is sent to the same contact(s) to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the [Investigator's Brochure] or Package Insert (new occurrence) and is thought to be related to the Novartis study treatment, an oncology DS&E department associate may urgently require further information from the investigator

for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

5.5 Precautions and potential risks

Patients enrolled in any nilotinib clinical study and who are at risk for any of the medical conditions discussed in this section should be managed according to the guidelines in the study protocol.

5.5.1 Myelosuppression

Complete blood counts should be performed every protocol mandated visit, or as clinically indicated. Myelosuppression was generally reversible and usually managed by withholding nilotinib temporarily or reducing the dose.

5.5.2 QT prolongation

Significant prolongation of the QT interval may occur when nilotinib is inappropriately taken with food, and/or strong CYP3A4 inhibitors and/or medicinal products with a known potential to prolong QT. Therefore, co-administration with food must be avoided and concomitant use with strong CYP3A4 inhibitors and/or medicinal products with a known potential to prolong QT should be avoided. The presence of hypokalemia and hypomagnesaemia may further enhance this effect. Nilotinib should be used with caution in patients who have or who are at significant risk of developing prolongation of QTc, such as those: (1) with long QT syndrome, (2) with uncontrolled or significant cardiac disease including recent myocardial infarction, congestive heart failure, unstable angina or clinically significant bradycardia. Specific recommendations for instances of QTcF prolongations are outlined below:

Cardiac QTc prolongation

QTcF > 480 msec Hold nilotinib when an ECG with a QTcF > 480 msec.

In addition to the standard procedures, the investigator should follow their local standards of practice and treatment guidelines for treating prolonged QT intervals. For example in France, this would include consultation with a cardiologist and close cardiac monitoring.

In case of recurrent QTcF prolongation to > 480 msec despite dose reduction the patient must be discontinued unless the reason for QTcF prolongation can be corrected (such as discontinuing or replacing of QT-prolonging concomitant drugs).

5.5.3 Drug interactions

The administration of nilotinib with agents that are strong CYP3A4-inhibitors and drugs that may prolong the QT interval such as anti-arrhythmic medicines should be avoided. Should treatment with any of these agents be required, it is recommended that therapy with nilotinib be interrupted if possible. If transient interruption of treatment with nilotinib is not possible, close monitoring of the individual for prolongation of the QT interval is indicated.

Concomitant use of nilotinib with medicinal products that are potent inducers of CYP3A4 is likely to reduce exposure to nilotinib to a clinically relevant extent. Therefore, in patients receiving nilotinib, concomitant use of alternative therapeutic agents with less potential for CYP3A4 induction should be selected.
Nilotinib is a moderate CYP3A4 inhibitor. As a result, the systemic exposure of other drugs primarily metabolized by CYP3A4 (e.g. certain HMG-CoA reductase inhibitors) may be increased when co-administered with nilotinib. Appropriate monitoring and dose adjustment may be necessary for drugs that are CYP3A4 substrates and have a narrow therapeutic index.

5.5.4 Sudden death

Death in CML is most frequently due to infection and/or hemorrhage. Risk of death increases in accelerated and blast crisis phases of the disease. Case reports of sudden death or sudden cardiac death have been observed with nilotinib. All deaths occurring in patients receiving nilotinib have been assessed by Novartis for the possibility of sudden cardiac death based on the clinical presentation, not solely based on the reported verbatim of "sudden death" or "sudden cardiac death". Reports of sudden death are identified based on the following definition: Death with an abrupt loss of consciousness due to disruption of cerebral blood flow that occurs within one hour of the onset of acute symptoms in case the event is witnessed or within 24 hours from having been observed live and symptom-free in case of an unwitnessed fatal event. This definition excludes progression of the underlying disease or other non -cardiac chronic and terminal illness on the basis that such deaths are not considered as unexpected. Likewise excluded are all cases with an identifiable non-cardiac origin as well as death associated with trauma, violence, overdose, drowning or suicide. Additionally, the cases of sudden unexplained death are also included. A review of all cases received to date from all sources has identified a total of 27 cases that meet the conservatively modified definition of sudden cardiac death (SCD) established by the MAH. Fourteen cases of sudden death were identified from the post-marketing spontaneous reporting and the other thirteen cases were from clinical trials including those for compassionate use. A total of approximately 12,406 patients have received nilotinib in Novartis-sponsored clinical trials through 31 January 2016. Based on the cumulative patient exposure, the risk of sudden cardiac death in clinical trial patients is 0.104%. The estimated post-marketing exposure for nilotinib is 118,282 patient-years. Hence, the reporting rate of spontaneous reports of sudden death is 0.012 per 100 patient-years.
Sudden death was not observed or reported in the [CAMN107A2303] clinical study for the newly diagnosed CML-CP patients as of the 84-month analysis.

5.5.5 Ischemic vascular or ischemic cardiovascular events

Newly-diagnosed or worsened ischemic vascular or cardiovascular events have occurred in a relatively small number of CML-CP patients while on study medication. If a patient experiences such an adverse event the physician should ensure that the patient is assessed by a vascular or cardiovascular specialist. Further recommendations for the management of ischemic vascular or cardiovascular-related events are outlined below:

Ischemic vascular or cardiovascular events

Grade 2* Hold nilotinib and refer patient for assessment by a vascular or cardiovascular

specialist. Resume nilotinib next lower dose level after recovery to ≤ Grade 1 is seen
l→ 400 mg QD.
If another recurrence
l→ discontinue nilotinib treatment.
If recovery to ≤ Grade 1 is greater than 28 days, nilotinib treatment must be discontinued.
Grade 3* or Grade 4* Hold nilotinib and refer patient for assessment by a vascular or cardiovascular specialist. Consideration should be given for discontinuation of nilotinib treatment. The patient must be discontinued from nilotinib treatment if recovery to ≤ Grade 2 is greater* Patient should be assesstehdanfo2r8podtaeynsti.al risk factors for the event including causality secondary to CML therapy.

5.5.6 Serum total cholesterol

In the Phase III study [CAMN107A2303] comparing nilotinib and imatinib therapy in newly diagnosed CML patients, elevations in total cholesterol and low density lipoprotein cholesterol have been very commonly observed.
Blood lipid panel tests should be performed for all patients prior to initiating nilotinib therapy and monitored during treatment. If test results warrant intervention, physicians should follow their local standards of practice or treatment guidelines, which may recommend treatment even for grade 1 cholesterol elevation. Before prescribing a lipid lowering medication, the possibility of drug-drug interactions should be considered as some HMG-CoA reductase inhibitors are also metabolized via the CYP3A4 pathway.

5.5.7 Glucose

Increases in blood glucose levels have been reported with nilotinib. Blood glucose levels should be assessed for all patients prior to initiating nilotinib therapy and monitored during treatment. If blood glucose results warrant intervention, physicians should follow their local standards of practice and treatment guidelines in order to normalize blood glucose levels.

5.5.8 Other Cardiac risk factors

Patients should be assessed or monitored for any other cardiac risk factors such as family history, cardiovascular events in the past medical history, smoking, hypertension, and obesity. If the assessment for presence of any other cardiovascular risk factors warrants intervention, physicians should follow their local standards of practice or treatment guidelines.

5.5.9 Serum lipase

Elevation in serum lipase has been observed. Caution is recommended in patients with previous history of pancreatitis. In case lipase elevations are accompanied by abdominal symptoms, doses should be interrupted and appropriate diagnostics should be considered in order to exclude pancreatitis.

5.5.10 Total gastrectomy

The bioavailability of nilotinib might be reduced in patients with total gastrectomy. More frequent follow-up of these patients should be considered.

5.5.11 Lactose

Since the capsules contain lactose, nilotinib is not recommended for patients with rare hereditary problems of galactose intolerance, severe lactase deficiency or of glucose-galactose malabsorption.

5.5.12 Hepatitis B reactivation

Reactivation of hepatitis B can occur in patients who are chronic carriers of this virus and are receiving a BCR-ABL tyrosine kinase inhibitor (TKI), such as nilotinib.. Some cases involving drugs of the BCR-ABL TKI class resulted in acute hepatic failure or fulminant hepatitis leading to liver transplantation or a fatal outcome. Patients should be tested for hepatitis B infection before initiating treatment with nilotinib. Patients currently on nilotinib should have baseline testing for hepatitis B infection in order to identify chronic carriers of the virus. Experts in liver disease should be consulted before treatment is initiated in patients with positive hepatitis B serology (including those with active disease) and for patients who test positive for HBV infection during treatment. Carriers of HBV who require treatment with nilotinib should be closely monitored for signs and symptoms of active hepatitis B infection such as liver injury or progression of liver injury throughout therapy and for several months following termination of therapy.

6 Data management

The data collection plan for this study is to utilize REDCap to capture all protocol mandated data for all enrolled patients. Once the last patient has ceased protocol treatment and the database has been declared to be complete and accurate, the database will be locked.

6.1.1 REDCap

REDCap (Research Electronic Data Capture) is a free data management software system that is fully supported by the Weill Cornell Medical College Clinical and Translational Science Center (CTSC). It is a tool for the creation of customized, secure data management systems that include Web-based data-entry forms, reporting tools, and a full array of security features including user and group based privileges, authentication using institution LDAP system, with a full audit trail of data manipulation and export procedures. REDCap is maintained on CTSC-owned servers that are backed up nightly and support encrypted (SSL-based) connections. Nationally, the software is developed, enhanced and supported through a multi-institutional consortium led by the Vanderbilt University CTSA.

6.2 Regulatory Considerations

All protocol amendments and consent form modifications will be made by the Prinicipal Investigator. All changes will be approved by the IRB prior to institution.

Investigators must enter the information required by the protocol. Subsequently, the information entered into the database is systematically checked by Data Management staff. Obvious errors will be corrected by study personnel. Other errors or omissions will be documented and maintained with the patient files.

When the database has been declared to be complete and accurate, the database will be locked.

7 Statistical Methods

This is an open label, hypothesis-generating study. The purpose of this initial study is to describe the quantitative and qualitative changes in the leukemic stem cell population of chronic phase CML patients during therapy with nilotinib. It is anticipated that a detailed, prospective analysis of approximately 40 uniformly treated chronic phase CML patients, will allow the detection of significant variation among them, with regard to their stem cell populations. It is hoped that these qualitative and quantitative changes in stem cell populations will lead to the ability to predict the success of discontinuation of TKI therapy in CML patients, which can be tested in a subsequent study. Because this is a pilot study, no formal sample size calculation is required. However, with 40 subjects in the study, exact two-sided 95% confidence intervals for event proportions of interest can be constructed to be within +/- 15.5% of the true proportions of interest. This calculation assumes event proportions of 50% to conservatively maximize the width of the obtained confidence intervals. Event proportions for this study will constitute the proportion of subjects that demonstrate various patterns/changes in the leukemic stem cell population. Descriptive statistics will be utilized to describe the event proportions of interest (i.e., frequency, percent). Estimated differences in stem cell characteristic proportions between exploratory subgroups of interest will serve as preliminary data (i.e., hypothesis-generating) for future studies. All p-values will be two-sided with statistical significance evaluated at the 0.05 alpha level. Exact 95% confidence intervals for all proportions of interest will be calculated to assess the precision of the obtained estimates. All analyses will be performed in SAS Version 9.3 (SAS Institute Inc., Cary, NC).

Safety evaluation

The assessment of safety will be based mainly on the frequency of adverse events, particularly adverse events leading to discontinuation of treatment and on the number of significant laboratory abnormalities.

Adverse events will be summarized by presenting the number and percentage (as appropriate) of patients having any adverse event by body system, type of adverse event, and maximum severity according to the CTC grade. Those adverse events which result in death, discontinuation or are otherwise classified as dose limiting will be presented separately.

Laboratory data will be summarized using the NCI CTC version 4 criteria.

8 Administrative Procedures

8.1.1 Chanes to the Protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by Novartis and the investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study, require additional approval by the IRB/IEC/REB. A copy of the written approval of the IRB/IEC/REB, must be sent to Novartis.

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB/IEC/REB approval but the IRB/IEC/REB of each center must be kept informed of such administrative changes.

8.1.2 Discontinuation of Study

Novartis reserves the right to discontinue support for any study under the conditions specified in the clinical trials agreement.

8.1 Ethics and good clinical practice

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
3. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
4. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

8.2 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC/REB). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to Novartis before study initiation. The name and occupation of the chairman and the members of the IRB/IEC/REB must be supplied to Novartis. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

8.3 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB/IEC/REB approval.

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