Mayo Clinic Cancer Center

A Phase II Study of Combination Daunorubicin and Cytarabine (Ara-c) and Nilotinib (Tasigna) (DATA) in Patients Newly Diagnosed with Acute Myeloid Leukemia and KIT Overexpression

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Drug Availability
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√Study contributor(s) not responsible for patient care.

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Protocol Resources

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*No waivers of eligibility per NCI

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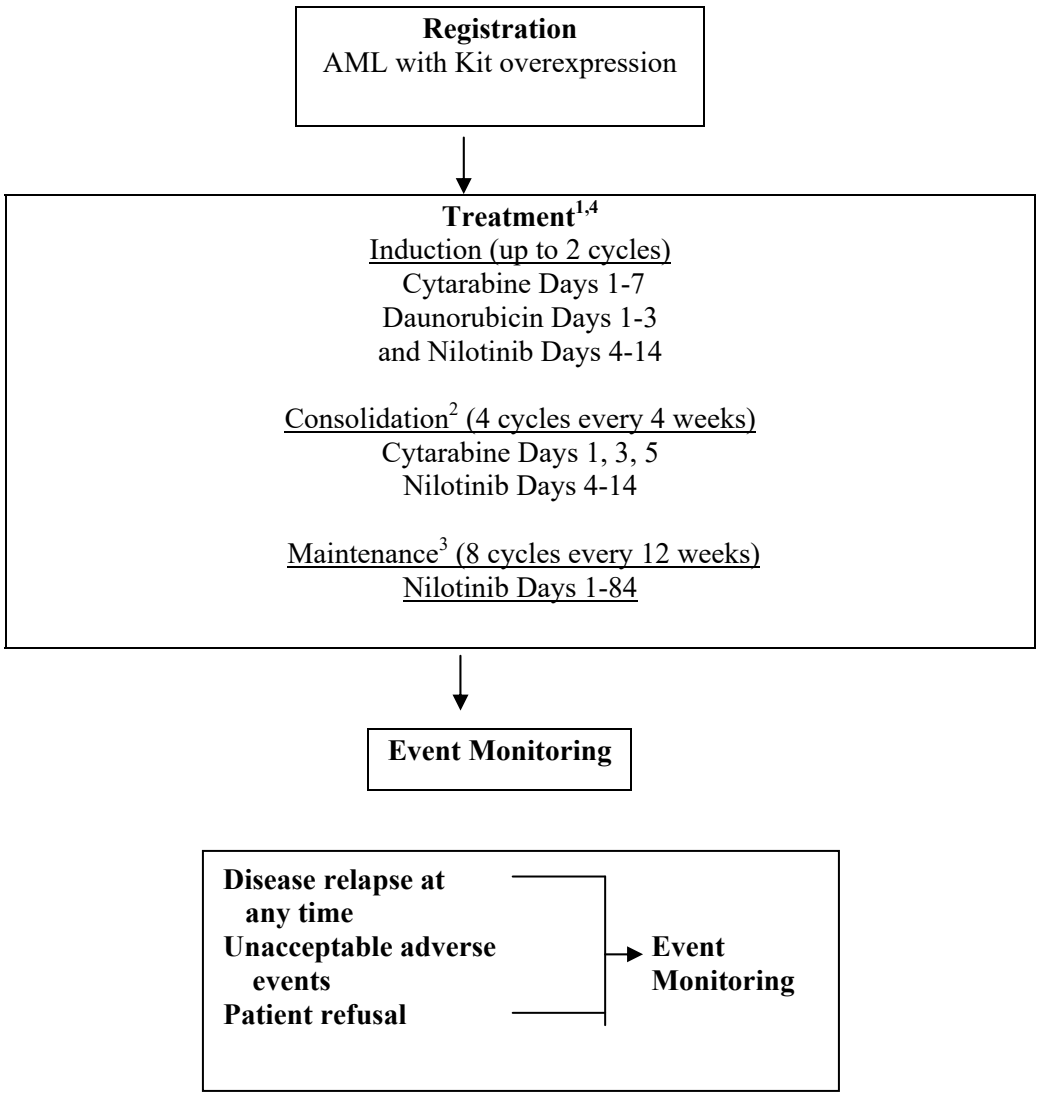
Schema



[1] Cycle length during Induction = 14-28 days; Cycle length during Consolidation = 28 days (see Section 7.12); Cycle length during Maintenance = 84 days. The next cycle of treatment may be delayed up to 56 days to achieve bone marrow recovery or meet CBC criteria for retreatment. If the next cycle is delayed beyond 56 days, the patient will go to event monitoring per Section 18.0.
[2] If the patient has not achieved a complete response (CR or CRi) after the 2nd cycle of induction, the patient should go to event monitoring.
[3] The patient must have a sustained complete response (CR or CRi) after consolidation to initiate the maintenance phase; otherwise the patient should go to event monitoring.
[4] See Section 7.14

Generic name: Nilotinib Brand name: Tasigna® Mayo abbreviation: AMN107 Availability: Novartis	Generic name: Cytarabine Brand name: Cytosar Mayo abbreviation: ARAC Availability: Commercial
Generic name: Daunorubicin Brand name: Cerubidine® Mayo abbreviation: DNM Availability: Commercial	

List of Abbreviations

AE	adverse event
ALL	Acute lymphoblastic leukemia
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

1.0 Background

Thirteen thousand new cases of acute myeloid leukemia (AML) are diagnosed every year (Last accessed May 27, 2011). Prognosis and treatment of this blood cancer remain challenging in adult patients compared to pediatric patients. Remission rates are about 65% for front-line therapy and relapse rate is about 50-60% at 4 years (Robak and Wrzesien-Kus 2002; Burnett, Wetzler et al. 2011). Both cytogenetic and molecular features predict favorable versus adverse prognosis and govern our therapy options for these patients (Byrd, Mrozek et al. 2002; Marcucci, Haferlach et al. 2011). Hematopoietic stem cell transplant is recommended for adverse prognosis group at diagnosis but is deferred to the time of relapse in patients with favorable prognosis (Dohner, Estey et al. 2010). Cytarabine and anthracycline (7+3 regimen) is the most used regimen and all other regimens have failed to show any survival advantage (Dohner, Estey et al. 2010).

Kit is a receptor tyrosine kinase and is vital in leukemic cells. It is expressed in more than 10% of the blasts in 95% of relapsed AML cases and mediates leukemic proliferation and has anti-apoptotic effects (Domen and Weissman 2000; Hans, Finn et al. 2002). AML with high c-kit expression is associated with lower complete remission (CR) rates, shorter CR duration, and shorter overall survival (OS) (Del Poeta, Venditti et al. 2003). On multivariate analysis, c-kit mean fluorescent index >20.3 correlates with a decreased progression-free survival and overall survival, independent of known prognostic factors (Advani, Rodriguez et al. 2008). Patients with core-binding factor (CBF) AML carry a superior outcome compared to other AML subgroups, but patients positive for c-kit mutation (for example D816F) carry a worse prognosis evidenced by higher relapse rate and inferior survival to wild-type kit CBF AML (Bloomfield, Lawrence et al. 1998; Paschka, Marcucci et al. 2006). Schnittger et colleagues studied 1940 AML patients for kit D816 mutations and found it positive in 33 cases (1.7%) {Schnittger,2006 #40}. Eight out of 33 patients were positive for t(8,21). Kit mutations were found to have an independent negative impact on event free and overall survival in patients with t(8,21). The D816V receptor expressed in Ba/F3 cells were fully sensitive to PKC412. On the expression level of kit, a semi-quantitative RT-PCR done on 60 acute leukemia cases correlated with complete remission rates {Han, 2006 #41}. Further research showed down-regulation of miRNA-193b in AML cells where kit was mutated or overexpressed {Gao, 2011 #43}. Restoration of MiR-1933b expression in AML cells resulted in reduced c-Kit expression and inhibited cell growth. Finally, expression of c-kit has been linked to increased P-glycoprotein, a major mediator of multi-drug resistance (MDR) in de novo AML{Sincock, 1997 #39}.Therefore, targeting kit expression seems to be a of great impact in this group of patients.

Tyrosine kinase inhibitors (TKI) (including imatinib, nilotinib, and dasatinib) are important drugs that are used for ABL inhibition in patients with chronic myeloid leukemia (CML) or Philadelphia (Ph) positive acute lymphoblastic leukemia (ALL) (Kantarjian, Shah et al. 2010; Saglio, Kim et al. 2010). They are FDA approved agents for the management of CML in all its phases (including blast phase) (Sawyers, Hochhaus et al. 2002; Kantarjian, Giles et al. 2006; Cortes, Rousselot et al. 2007). Kit is also inhibited by these TKI, more so with a second generation agents like nilotinib (Kantarjian, Giles et al. 2006). They have also been recently tried in combination with chemotherapy with increased efficacy and synergy in patients with Ph+ ALL.

Imatinib has been given to a patient with refractory AML and c-kit positive overexpression (no mutation) who achieved complete hematological remission followed by complete clearance of leukemic blasts in bone marrow lasting for more than 5 months (Kindler, Breitenbuecher et al. 2003). Imatinib has been given in combination with chemotherapy achieving very encouraging

results (Walker, Komrokji et al. 2008; Brandwein, Hedley et al. 2011). Dasatinib is now under study with combination chemotherapy in CALGB phase II study in patients with core binding factor AML (NCT01238211). Nilotinib is currently given in addition to chemotherapy (mitoxantrone, etoposide, and high dose cytarabine, NOVE-HiDAC) in the setting of relapsed and refractory AML (NCT01222143). In addition, nilotinib is given to relapsed Ph+ALL patients and is well tolerated (including combination with high dose cytarabine). All of these active trials reflect the great interest of having those effective agents tested in patients diagnosed with AML.

Chronic myelogenous leukemia (CML) is caused by the reciprocal translocation of the ABL kinase on chromosome 9 to the BCR region on chromosome 22. This results in a novel oncoprotein with constitutively activated ABL kinase activity leading to downstream pathway activation and uncontrolled myeloproliferation. The BCR-ABL fusion oncogene (the Philadelphia chromosome, Ph+) is the hallmark of Ph+ CML and is the driving characteristic molecular event in this diseases (Druker 2008) (Vardiman 2009). The other disease in which the BCR-ABL translocation plays an essential role in pathogenesis is Ph+ acute lymphoblastic leukemia (Ph+ALL), an aggressive form of blood cancers. Chemotherapy agents or single agent tyrosine kinase inhibitors (TKI) alone can induce high response rates but the response is often temporary. Therefore, combination of a targeted agent (TKI) with chemotherapy may provide a more durable response (Stock 2010).

Imatinib, an adenosine tri-phosphate (ATP)-competitive specific inhibitor of the protein tyrosine kinase activity associated with Bcr-Abl, has revolutionized treatment of CML and significantly improved the prognosis of patients since its approval in 2001. It is effective in most patients with CML with minimal toxicity, and is now frontline drug therapy for CML. However, despite the remarkable success of imatinib, a percentage of patients are intolerant to the drug and resistance can develop. Some patients do not tolerate imatinib due to the development of SAEs, including grade 3 or 4 skin rash, fluid retention, cardiopulmonary events, thrombocytopenia, liver function abnormalities, and diarrhea, which persist in spite of optimal supportive care measures; other patients do not tolerate due to prolonged AEs of lower grade which do not meet criteria of a SAE (O'Hare 2006). Imatinib resistance is primarily due to mutation of the Bcr-Abl gene, impairing the ability of imatinib to bind to the tyrosine kinase domain of Bcr-Abl. Overexpression of the Bcr-Abl protein may also cause resistance. Rates of resistance increase with each stage of progression of CML (CP < AP < BC) (Apperley 2007).

Nilotinib is a synthetic aminopyrimidine, which was developed as a second-generation inhibitor of Bcr-Abl tyrosine kinase. It is a more potent and selective inhibitor of Bcr-Abl than imatinib in vitro, and has shown efficacy in patients harboring a variety of Bcr-Abl mutations associated with imatinib resistance, except T315I. Nilotinib has an impressive anti-leukemia activity in mouse models of 190 BCR/ABL positive lymphoblastic leukemia (Kaur, Feldhahn et al. 2007). Its concentration in cell lines and primary CD34+ CML cells is not mediated by active uptake or efflux by major drug transporters (a known mechanism of resistance to imatinib (Davies, Jordanides et al. 2009). Nilotinib induces apoptosis in imatinib-resistant K562 CML cells via increase of caspase-3 enzyme and decrease in mitochondrial membrane potential (Ekiz, Can et al. 2010). Most importantly, it poses synergistic activity with chemotherapeutic drugs in imatinib-sensitive and –resistant BCR/ABL + cells (Radujkovic, Fruehauf et al. 2010). Nilotinib is approved for use in the treatment of chronic phase and accelerated phase Philadelphia chromosome positive CML in adult patients resistant to or intolerant to prior therapy including imatinib and newly diagnosed adult patients with Philadelphia chromosome positive CML.

Nilotinib also inhibits the platelet-derived growth factor receptor (PDGFR), stem cell factor (Kit), colony stimulating factor receptor (CSF-1R), discoidin domain receptor (DDR), and ephrin-A4

receptor (EphA4) kinases, and blocks the downstream cellular events mediated by these enzymes. Nilotinib was given to patients with metastatic melanoma harboring Kit gene mutation, where 22% achieved partial response and 55.6% had stable disease {Cho, 2011 #45}. Nilotinib was tested as third-line therapy in patients with gastrointestinal stromal tumor (GIST), where disease control rate at week 24 was 29% and 66% patients had stable disease >6 weeks as best response{Sawaki, 2011 #46}. In mast cells, nilotinib, as well as imatinib, was potent against mast cell with wild type c-kit and D560G c-kit mutant but no significant activity against cells expressing c-kit D816V mutant tyrosine kinase {Verstovsek, 2006 #48}. However, Von Bubnoff showed that nilotinib did suppress the growth of Ba/F3$^{\text{c-kit D814V}}$ at concentrations of 1-2 μm {von Bubnoff, 2005 #53}. Similar results were found in cells expressing the human $^{\text{c-kit D816V}}$.

1.1 Toxicology in animals

Nilotinib was not considered genotoxic, but was embryolethal and produced fetotoxicity in embryo-fetal development studies in rats and rabbits at doses that also produced maternal toxicity. There were no signs of teratogenicity. In the pre- and postnatal study, administration of nilotinib to female rats was associated with decreased pup body weight and changes in some physical development parameters at a dose that also produced maternal effects. Carcinogenicity studies are currently ongoing.

The fertility of male and female rats was not affected. Nilotinib is considered to be embryolethal, because of an increased post-implantation loss observed in both the fertility study, with treatment of both males and females, and in the embryotoxicity study with the treatment of females. It is recommended that women of childbearing potential should use effective contraceptive measures during the entire treatment period. Any women in early pregnancy when treatment starts should be informed about the potential risk to the fetus. Since nilotinib is transferred into the milk in lactating rats with a milk/plasma concentration ratio of approximately 2, women who are using the drug should not breast feed.

1.2 Pharmacokinetics and drug metabolism

The extent of nilotinib absorption following oral administration was estimated to be approximately 30%. The bioavailability of nilotinib was increased when given with a meal. Compared to the fasted state, the systemic exposure [area under the plasma drug concentration time curve (AUC)] in the fed state increased by 15% (drug administered 2 hours after a light meal), 29% (30 minutes after a light meal), or 82% (30 minutes after a high fat meal), and the maximum concentration (C_{max}) increased by 33% (2 hours after a light meal), 55% (30 minutes after a light meal), or 112% (30 minutes after a high fat meal).

With once daily (q.d.) dosing at steady-state, C_{max} and AUC increased with increasing dose from 50 mg to 400 mg in a generally dose-proportional manner, but appeared to plateau at dose levels starting at 400 mg, remaining relatively constant over the dose range from 400 mg to 1200 mg. Dividing the daily dose in a twice daily schedule overcame the dose-limiting exposure to some extent with daily steady-state serum exposure to nilotinib with 400 mg twice daily dose being approximately 35% greater than with 800 mg q.d. dose. However, there was no further relevant increase in exposure to nilotinib when given 600 mg dose with the twice daily schedule (1200 mg/day). With multiple oral doses of nilotinib, steady-state conditions were achieved by day 8 after initiating nilotinib treatment. There was a 2-fold or 3.8-fold accumulation with q.d.

dosing or twice daily dosing, respectively, in serum concentrations between the first-dose and steady-state. The median time to reach C_{max} of nilotinib (t_{max}) was 3 hours. Drug elimination half-lives calculated during the dose interval averaged 17 hours for q.d. dosing, consistent with the observed accumulation in serum concentrations.

Exposure to nilotinib in female patients was approximately 20% greater than in male patients. There was no difference in nilotinib apparent clearance between Caucasians and non-Caucasians. The population pharmacokinetic (PK) analysis showed that nilotinib PK is not affected by age. Hepatic impairment has a modest effect on nilotinib pharmacokinetics; however, impaired renal function is not expected to influence nilotinib PK.

Results of a Phase I, open-label, multicenter, dose-escalation study in patients with GISTs who progressed on previous imatinib therapy suggest that nilotinib might have inhibitory effects on the metabolism of imatinib. The increases in serum concentration levels of imatinib on Day 8 were, in general, within the variability of fluctuation of serum imatinib concentrations in a dosing interval, suggesting that the inhibitory effects of nilotinib on the metabolism of imatinib might not be clinically significant.

Coadministration of ketoconazole with nilotinib increased nilotinib C_{max} by 84% and AUC by 3-fold on average. Single-dose nilotinib did not appear to affect ketoconazole concentrations. Single-dose administration of nilotinib (600 mg) with a single dose of midazolam resulted in mean increase in midazolam C_{max} and AUC of 20% and 31%, respectively.

Rifampin 600 mg, administered as a pretreatment for 8 days, had a pronounced impact on a single 400 mg dose of nilotinib. Apparent clearance of nilotinib was increased by 4.8-fold on average. Induction with rifampin significantly reduced Cmax and AUC of nilotinib by 64% and 80%, respectively.

Concurrent intake of grapefruit juice increased the nilotinib C_{max} by 60% and $AUC_{0-\infty}$ by 29%, but the median time to reach peak concentration (t_{max}) and the mean elimination half-life ($t_{1/2}$) were not altered.

Concurrent administration of nilotinib with a single oral dose of 25 mg warfarin in healthy subjects showed a lack of interaction between two drugs. Pharmacokinetic parameters of S- and R-warfarin as well as pharmacodynamic parameters of warfarin were all found to be similar between two treatments (warfarin+nilotinib versus warfarin alone), with the geometric mean ratios and 90% CIs all being within 80-125% range.

Co administration of esomeprazole with nilotinib caused a modest reduction in the rate and extent of nilotinib absorption. The mean C_{max}, $AUC_{0-tlast}$ and $AUC_{0-\infty}$ of nilotinib were decreased by about 27%, 34% and 33%. The mean $T_{1/2}$ of nilotinib, however, was found to be similar between two treatments.

1.3 Efficacy

1.31 Efficacy in CML

1.311 Imatinib resistant and imatinib intolerant CML-CP and CML-AP

A Phase II study [CAMN107A2101], was conducted to evaluate the efficacy and safety of nilotinib in CML-CP and CML-AP patients with imatinib-intolerant or imatinib-resistant (due to either a primary resistance to imatinib or due to acquired resistance primarily due to known Bcr-Abl mutations).

A total of 321 CML-CP patients in the Phase II study were evaluated for efficacy. Of these, 71.4% were imatinib-resistant and 29.6% were imatinib-intolerant. Previous therapies, in addition to imatinib, which 72.2% of patients had received at doses ≥ 600 mg, included prior interferon (64.4% of patients). Patients were treated with nilotinib 400 mg b.i.d. for 6 months, and could be dose-escalated to 600 mg b.i.d. for lack of efficacy. Fifty-one of 321 patients (15.9%) were dose-escalated due to lack of efficacy. Dose reduction from either 400 or 600 mg b.i.d. occurred in 34.1% of all patients treated, primarily due to AEs.

The median duration of exposure in CML-CP patients was 341 days (exposure range of 1 - 624 days). The median average daily dose of nilotinib for CML-CP patients was 792 mg which is indicative of relatively infrequent dose interruptions and reductions. At the time of data cutoff, 188 (58.8%) patients were ongoing and 132 (41.3%) had discontinued treatment. The reasons for discontinuation were mainly due to AEs (51 patients, 15.9%) and disease progression (51 patients, 15.9%).

The achievement of major cytogenetic response (MCyR) is associated with a survival benefit in CML patients treated with imatinib. For nilotinib-treated CML-CP patients, the percentage of patients who achieved MCyR by intent-to-treat (ITT) analysis was 56.3% (median exposure of 341 days; range 1 to 624 days). This is indicative of the durability of the response in CML-CP. A complete hematological response (CHR) was achieved in 76.2% of CML-CP patients.

Table 1-1 Response rates in CML-CP patients

Best Response		ITT population N=320
Major Cytogenetic Response (complete + partial)		
	n (%)	180 (56.3)
	95% CI	50.6 - 61.8
Complete Cytogenetic Response		
	n (%)	128 (40.0)
	95% CI	34.6 - 45.6
Partial Cytogenetic Response		
	n (%)	52 (16.3)
	95% CI	12.4 - 20.8
Complete Hematologic Response (patients not in CHR at baseline)		**N=206**
	n (%)	157 (76.2)
	95% CI	69.8 - 81.9

CHR = complete hematologic response, CI = confidence interval, ITT = intent-to-treat.
* Group A

The patients evaluated for efficacy consisted of 119 out of 127 enrolled CML-AP Phase II patients of which 80.7% were imatinib-resistant and 19.3% were imatinib-intolerant. Previous therapies included interferon (58.0% of patients) and imatinib (82.9% of patients received doses ≥ 600 mg). Patients were treated with nilotinib 400 mg b.i.d. for 4 months and could be dose-escalated to 600 mg b.i.d. for lack of efficacy.

The median duration of exposure was 202 days for the overall enrollment population (N = 119; exposure range of 2 - 611 days). Notably, 67 (56%) and 29 (24%) CML-AP patients have been exposed for longer than 6 and 12 months, respectively. Twenty-nine of 119 patients (24.4%) were dose-escalated due to lack of efficacy. Dose reduction from either 400 or 600 mg b.i.d. occurred in 42.0% of all patients treated, primarily due to AEs. Sixty-seven (56.3%) of CML-AP patients enrolled have received nilotinib for 6 months or more. The median average daily dose of nilotinib for CML-AP patients was 790.0 mg, and is indicative of relatively infrequent dose interruptions and reductions throughout the treatment period. At the time of data cutoff, 48 (40.3%) patients were still on treatment and 71 (59.7%) had discontinued treatment. The reasons for discontinuation included disease progression (35 patients, 29.4%) and AEs (15 patients, 12.6%).

The anticipated prognosis for imatinib-resistant or -intolerant CML-AP patients is poor since all will eventually progress to CML-BC, typically within 6 month's time. Despite this, nilotinib-treated CML-AP patients achieved high rates of hematological response (HR) for the ITT population. The percentage of patients who achieved confirmed HR by ITT analysis was 47.1% (median exposure of 202 days; range 2 to 611 days). The current estimate for median time to progression (TTP) has been reached at 16.4 months in CML-AP patients.

Table 1-2 Response rates in CML-AP patients

Best Response	ITT population N=119
Confirmed Hematologic Response	
n (%)	56 (47.1)
95% CI	37.8 - 56.4
Complete Hematologic Response	31 (26.1)
Marrow response/No evidence of leukemia (NEL)	11 (9.2)
Return to chronic phase	14 (11.8)
Major Cytogenetic Response (complete + partial)	
n (%)	35 (29.4)
95% CI	21.4-38.5

CI = confidence interval, ITT = intent-to-treat, NEL = no evidence of leukemia.
* Group A

1.312 **Newly diagnosed CML-CP**

Study [CAMN107A2303] is a Phase III, randomized, open-label, multicenter study comparing the efficacy of nilotinib 300 or 400 mg b.i.d. to imatinib 400 mg qd in adult patients with newly diagnosed Ph+ CML in CP. A total of 846 patients with newly diagnosed Ph+ CML-CP, diagnosed within 6 months, and stratified by Sokal risk score, were randomized 1:1:1 to nilotinib 300 mg bid (n=282), nilotinib 400 mg bid (n=281), and imatinib 400 mg qd (n=283) arms.

The primary endpoint was rate of major molecular response (MMR) at 12 months (mos). All patients had a minimum of 12 months of treatment or discontinued early; median follow-up was 14 mos. MMR was defined as a value of ≤ 0.1% of BCR-ABL/ABL ratio on the International Scale. Molecular response was assessed by RQ-PCR at baseline, monthly for 3 months and every 3 months thereafter. Samples were analyzed at a central PCR laboratory. The major secondary endpoint was rate of complete cytogenetic response (CCyR) by 12 mos based on bone marrow cytogenetics.

Baseline demographics, disease characteristics, and Sokal scores were well balanced among the 3 arms; patients with high-risk Sokal scores were 28% in all arms. Median dose intensities of nilotinib delivered were 592 mg/day for 300 mg bid and 779 mg/day for 400 mg bid; imatinib dose intensity was 400 mg/day. Overall, 84%, 82%, and 79% of patients remained on the study for 300 mg bid nilotinib, 400 mg bid nilotinib, and 400 mg qd imatinib, respectively. Rates of MMR at 12 mos were superior for nilotinib 300 mg bid compared with imatinib 400 mg qd (44% vs. 22%, $P < .0001$) and also for nilotinib 400 mg bid compared with imatinib 400 mg qd (43% vs. 22%, $P < .0001$). Median time to MMR among patients who achieved MMR was faster for nilotinib 300 mg bid (5.7 mos) and nilotinib 400 mg bid (5.8 mos) compared with imatinib 400 mg qd (8.3 mos). Rates of CCyR by 12 months were

significantly higher for both nilotinib at either 300 mg bid compared with imatinib 400 mg qd (80% vs. 65%, $P < .0001$) and for nilotinib 400 mg bid compared with imatinib 400 mg qd (78% vs. 65%, $P = .0005$). Overall, progression to advanced disease was lower for nilotinib 300 mg bid (2 pts) and nilotinib 400 mg bid (1 pt) compared with imatinib 400 mg qd (11 pts). Overall, both drugs were well-tolerated. Rates of discontinuation due to adverse events or laboratory abnormalities were 7% for nilotinib 300 mg bid, 11% for nilotinib 400 mg bid, and 9% for imatinib 400 mg qd. Patients were monitored for QT prolongation and LVEF. No patients in any treatment arm showed a QTcF interval > 500 msec. There was no decrease from baseline in mean LVEF anytime during treatment in any arm. The study is ongoing.

Nilotinib at both 300 mg bid and 400 mg bid induced significantly higher and faster rates of MMR and CCyR compared with imatinib 400 mg qd, the current standard of care in patients with newly diagnosed CML. Nilotinib was effective across all Sokal scores. After only one year of treatment, both nilotinib arms resulted in a meaningful clinical benefit compared to imatinib, with reduction of transformation to AP/BC. Nilotinib exhibited a favorable safety and tolerability profile. The superior efficacy and favorable tolerability profile of nilotinib compared with imatinib suggests that nilotinib may become the standard of care in newly diagnosed CML (Saglio et al, 2009). It was approved in 2010 for the management of newly diagnosed CML CP patients.

1.313 **CML-BC and Ph+ ALL**

In a landmark study (Kantarjian, Giles et al. 2006), 119 patients with imatinib-resistant CML or Ph+ ALL were enrolled in a phase I dose-escalation study at doses of 50 mg, 100 mg, 200 mg, 400 mg, 600 mg, 800 mg, and 1200 mg once daily and at 400 mg and 600 mg twice daily. Of the 33 patients with blast phase, 13 had a hematological response and 9 had a cytogenetic response. Of the 46 patients with accelerated phase, 33 had a hematologic response and 22 had a cytogenetic response. A total of 51 ABL mutations were observed in 37 of 91 patients who had a baseline assessment for mutational status. Nilotinib was effective in both groups (regardless of mutation analysis) with no significant differences in response rates. T315I mutation was found in 2 patients and did not respond to nilotinib. Frequently noted side effects were mild-moderate rashes; transient, clinically insignificant elevations of indirect bilirubin levels and myelosuppression. The maximum tolerated dose was selected as 600 mg twice daily, though 400 mg twice daily showed similar response rates.

A similar efficacy was reported in a small phase I/II of nilotinib in Japanese patients with imatinib-resistant or –intolerant Ph+ CML or relapsed/refractory Ph+ALL (Tojo, Usuki et al. 2009Tojo, Usuki et al. 2009). Three out of seven patients with Ph+ALL had a complete hematologic response and 2 out of 4 patients with CML-BC had hematologic response (1 did achieve complete hematologic response).

1.314 **AML**

Three current studies have been activated to study the clinical effect of adding nilotinib in patients with acute myeloid leukemia.

Brandwein and colleagues are checking the safety and efficacy of nilotinib combined with mitoxantrone, etoposide, and high-dose cytarabine (NOVE-HiDAC) induction chemotherapy for high risk AML patients (NCT01222143); which include relapsed and refractory AML in addition to secondary AML from myeloproliferative neoplasm or chronic myelomonocytic leukemia. Patients need to be positive for c-kit (CD117) in at least 30% of the blasts measured by flow cytometry. This is a phase I/II study where nilotinib dose is escalated in the first phase to get the maximum tolerated dose, and then proceed with phase II to assess the clinical efficacy. No results have been reported yet.

Duyster and colleagues are studying the combination of nilotinib and RAD001 in patients diagnosed with AML. Patients could be newly diagnosed but unfit for chemotherapy or have a relapsed/ refractory AML diagnosis. No results have been reported yet on this group of patients.

Kim et al will be studying the combination of cytarabine and daunorubicin (7+3) in combination with nilotinib at a higher dose (400mg orally bid) in patients diagnosed with previously untreated CML myeloid BP or Ph+AML (NCT01690065). This is a phase II study using a higher dose of nilotinib than our proposed dose (300mg bid). The primary goal is to increase complete remission rates with secondary goals of safety and survival.

In the clinical practice, we have given nilotinib in combination with chemotherapy (7+3) for newly diagnosed or relapsed refractory Ph+ AML with no excessive toxicity (personal experience). Due to rarity of Ph+AML cases, no protocol have yet published for these cases, therefore, we have been treating them as AML cases with adding tyrosine kinase inhibitor.

1.4 **Nilotinib safety**

In Phase II study [CAMN107A2101], nilotinib given at the recommended dose of 400 mg b.i.d. was well tolerated in imatinib-resistant and -intolerant CML-CP (study [CAMN107A2101E2], Group A) and CML-AP (study [CAMN107A2101E1], Group A) patients as evidenced by the high actual median dose intensities of 797 mg/day and 780 mg/day for both populations. In addition, the median cumulative duration of dose interruption was low relative to the median exposures in both CML-CP and CML-AP populations. Adverse events (AEs) were generally managed either with dose reduction, dose interruption, or supportive care. Discontinuation for drug related adverse reactions was observed in 16% of CML-CP and 10% of CML-AP patients.

1.41 **Frequent adverse events**

Table 1-3 lists the adverse events for the Phase II of study [CAMN107A2101]. The most frequently reported AEs overall included thrombocytopenia, rash, headache, nausea, pruritus, fatigue, anemia, diarrhea, constipation, neutropenia, vomiting and arthralgia. These AEs were generally mild to moderate (CTC grade 1 or 2), were manageable with symptomatic treatment, and were reversible.

Table 1-3 Frequent adverse events (>5%) in CML patients

	2101E1[1]	2101E2[1]
Preferred Term	**All grades CML-AP N=137 n (%)**	**All grades CML-CP N=321 n (%)**
Any event	120 (87.6)	304 (94.7)
Thrombocytopenia	52 (38.0)	90 (28.0)
Neutropenia	31 (22.6)	48 (15.0)
Rash	29 (21.2)	99 (30.8)
Anemia	24 (17.5)	42 (13.1)
Pruritus	24 (17.5)	84 (26.2)
Lipase increased	18 (13.1)	41 (12.8)
Fatigue	14 (10.2)	65 (20.2)
Constipation	13 (9.5)	43 (13.4)
Diarrhea	13 (9.5)	39 (12.1)
Leukopenia	13 (9.5)	13 (4.0)
Muscle spasms	13 (9.5)	24 (7.5)
Nausea	13 (9.5)	79 (24.6)
Alopecia	12 (8.8)	27 (8.4)
Myalgia	12 (8.8)	33 (10.3)
Blood bilirubin increased	11 (8.0)	22 (6.9)
Headache	11 (8.0)	57 (17.8)
Hyperbilirubinemia	11 (8.0)	23 (7.2)
Abdominal pain	10 (7.3)	17 (5.3)
Pyrexia	9 (6.6)	13 (4.0)
Anorexia	8 (5.8)	23 (7.2)
Pain in extremity	8 (5.8)	17 (5.3)
Arthralgia	7 (5.1)	24 (7.5)
Hypophosphataemia	7 (5.1)	8 (2.5)
Peripheral edema	7 (5.1)	20 (6.2)
Vomiting	5 (3.6)	41 (12.8)
Alanine aminotransferase increase	5 (3.6)	34 (10.6)
Bone pain	5 (3.6)	24 (7.5)
Erythema	2 (1.5)	23 (7.2)
Asthenia	5 (3.6)	21 (6.5)
Aspartate aminotransferase increase	4 (2.9)	20 (6.2)
Dry skin	5 (3.6)	20 (6.2)
Dyspnea	0 (0.0)	17 (5.3)
Weight decreased	3 (2.2)	17 (5.3)

AE = adverse event, AP = accelerated phase, CML = chronic myeloid leukemia, CP = chronic phase, na = not applicable
[1]The source used for 24-month 2101E1 data (cut-off 29-Aug-2008) and 24-month 2101E2 data (cut-off 20-Apr-2008
Source: CAMN107A2101E1 and CAMN107A2101E2 CSR

1.42 **Myelosuppression**

Myelosuppression, as expected for this class of drug in a CML population, was seen with nilotinib, with CTC grade 3/4 thrombocytopenia, neutropenia, and anemia reported in 29.38%, 31%, and 10.7% in CML-CP and 41.9%, 42.1%, and 27.4% in CML-AP patients (Table 1-4). Treatment emergent grade 3/4 cytopenias were reported in higher proportion of CML-AP patients than CML-CP. The majority of first episodes of grade 3 and 4 neutropenia and thrombocytopenia occurred within the first 2 months of therapy, and were generally manageable with dose adjustment or study drug interruption, only rarely requiring treatment discontinuation. Severe clinical consequences such as febrile neutropenia, sepsis, pneumonia and bleeding associated with thrombocytopenia, occurred infrequently in patients in both disease categories.

Table 1-4 Myelosuppression and abnormal blood chemistry

Hematological abnormality	CML-CP Total (N=321) n/N* (%)	CML-AP Total (N=137) n/N* (%)
Absolute Lymphocytes	85/316 (26.9)	51/132 (38.6)
Absolute Neutrophils (Seg. + Bands)	98/316 (31.0)	56/133 (42.1)
Fibrinogen	8/187 (4.3)	2/ 77 (2.6)
Haemoglobin	34/318 (10.7)	37/135 (27.4)
Partial thromboplastin time	1/199 (0.5)	1/ 91 (1.1)
Platelet count (direct)	95/319 (29.8)	52/124 (41.9)
Prothrombin time (INR)	11/198 (5.6)	5/ 86 (5.8)
WBC (total)	61/318 (19.2)	46/135 (34.1)

AP = Accelerated Phase, BID = Twice daily, CP = Chronic Phase, CTC = Common Toxicity Criteria, QD = Once daily.
n=number of subjects who had less than grade 3 at baseline, and worsened to grade 3 post-baseline, or who had less than grade 4 at baseline, and worsened to grade 4 post-baseline.
N*=total number of subjects evaluable post-baseline, who had less than grade 4 at baseline.
Source: [CAMN107A2101 E2 CSR] and [CAMN107A2101 E1 CSR]

1.43 Blood chemistry

Serum lipase and amylase
Lipase and amylase were observed in clinical trials but not in preclinical toxicology studies. Increased lipase was reported in 13.1% and 12.8% of CML–CP and CML-AP patients, respectively. Lipase (11.3%) is one of the most frequently reported grade 3 or 4 biochemical laboratory abnormalities. As for amylase it was reported in 2.6% and 2.3% of CML-CP and CML-AP patients, respectively.

Hepatotoxicity
Low grade increases in bilirubin and hepatic transaminases were frequently observed in CML-CP and CML-AP patients. However, grade 3/4 events occurred in 7.3% and 9% for bilirubin, 2.5% and 2.3% for AST, and 4.1% and 3.7% for ALT in CML-CP and CML-AP patients, respectively. Elevations of bilirubin occurred early, were transient, and the more severe cases were managed with brief treatment interruptions or dose reductions, rarely requiring treatment

discontinuation. Patients with normal LFTs at baseline infrequently went on to develop grade 3 or 4 bilirubin or hepatic transaminase values on treatment. Of interest, a significant increase in relative risk of hyperbilirubinemia was seen in patients with the (TA) 7(TA) 7 genotype at the (A (TA) nTAA) element of the UGT1A1, suggesting that genetic susceptibility may contribute to the development of hyperbilirubinemia in some patients (Kantarjian 2006). Elevations of bilirubin and transaminases were observed in animal models, with histologic lesions in monkey showing bile duct hyperplasia, sinusoidal cell hyperplasia, and periportal fibrosis, partially reversible at 4 weeks.

The incidence of grade 3 or 4 hyperglycemia was 12.2% and 6.1% in CML-CP and CML-AP patients. No CML-CP or CML-AP patients required dose adjustment and/or study drug interruption due to hyperglycemia. No CML-CP or CML-AP patients discontinued nilotinib due to hyperglycemia. Furthermore there was no evidence that insulin requirements increased in insulin-dependent diabetics receiving nilotinib.
The incidence of Grade 3 or 4 hypophosphatemia was 15.1% in CML-CP and CML-AP patients each. There were no reports of clinical symptoms related to hypophosphatemia.

Table 1-5 Abnormal blood chemistry

Biochemistry abnormality	CML-CP (E2) Total (N=321) n/N* (%)	CML-AP (E1) Total (N=137) n/N* (%)
Albumin	11/301 (3.7)	4/131 (3.1)
Alkaline phosphatase, serum	2/313 (0.6)	2/135 (1.5)
Amylase	8/302 (2.6)	3/131 (2.3)
Bilirubin (total)	23/316 (7.3)	12/133 (9.0)
Calcium (hyper)	10/315 (3.2)	1/134 (0.7)
Calcium (hypo)	5/315 (1.6)	7/134 (5.2)
Creatinine	4/317 (1.3)	1/135 (0.7)
Glucose (hyper)	38/311 (12.2)	8/131 (6.1)
Glucose (hypo)	5/311 (1.6)	0/131 (0.0)
Lipase (Blood)	54/301 (17.9)	23/130 (17.7)
Magnesium (hyper)	13/289 (4.5)	6/126 (4.8)
Magnesium (hypo)	1/289 (0.3)	0/126 (0.0)
Phosphate (Inorganic Phosphorus)	51/301 (16.9)	20/130 (15.4)
Potassium (hyper)	19/315 (6.0)	6/135 (4.4)
Potassium (hypo)	7/315 (2.2)	12/135 (8.9)
SGOT (AST)	8/316 (2.5)	3/133 (2.3)
SGPT (ALT)	13/318 (4.1)	5/135 (3.7)
Sodium (hyper)	3/317 (0.9)	0/135 (0.0)
Sodium (hypo)	21/317 (6.6)	9/130 (6.9)

AP = Accelerated Phase, CP = Chronic Phase, CTC = Common Toxicity Criteria,
n=number of subjects who had less than grade 3 at baseline, and worsened to grade 3 post-baseline, or who had less than grade 4 at baseline, and worsened to grade 4 post-baseline.
N*=total number of subjects evaluable post-baseline, who had less than grade 4 at baseline.
Source: [CAMN107A2101 E2 CSR] and [CAMN107A2101 E1 CSR]

1.44 Clinically significant adverse events

Rash and pruritus

Rash and pruritus were frequently reported by patients in the pivotal phase II study (2101): rash (CML-CP: 30.8%, CML-AP: 21.2%) and pruritus (CML-CP: 26.2%, CML-AP: 17.5%). Four CML-CP patients (1.3%) discontinued due to skin toxicity: two cases of rash or rash generalized, one case of dermatitis allergic, one case of pruritus, all suspected of study drug relationship. Two CML-AP patients (1.7%) discontinued due to 4 AEs related to skin toxicity: 2 cases of rash, one case of urticaria and one case of skin burning sensation, all but the latter suspected of study drug relationship.

Fluid retention

The rates for all CTC grades of effusions (pleural, pericardial) and edema were low. Peripheral edema was seen in 5.1% and 6.2% for CML-AP and CML-CP, respectively. AE related to pleural effusion was 2.7% and pericardial effusion <1%. The incidence of fluid retention appeared to be slightly higher in elderly (>65 years old).

Bleeding-related events

Bleeding-related events are not uncommon in CML patients with compromised marrow function at baseline. GI and CNS hemorrhages were included as the indicators for significant bleeding. GI hemorrhage was common in both newly diagnosed CML-CP and imatinib-resistant/intolerant CML-AP and CML-CP patients treated with nilotinib. However, the incidence of SAEs indicative of GI hemorrhage was 1% in each of these CML patients' populations. CNS hemorrhage was infrequent but medically significant. All reports of CNS hemorrhage were SAEs.

QT prolongation

In the phase III study in newly diagnosed Ph+ CML-CP patients the change from baseline in mean time-averaged QTcF interval at steady-state observed in the nilotinib 300 mg twice daily group was 6 msec. In the 300 mg twice daily treatment group, no patient had an absolute QTcF of >480 msec. No events of Torsade de Pointes were observed in any of the treatment groups.

In the phase II study in imatinib-resistant and intolerant CML patients in chronic and accelerated phase, treated with nilotinib 400 mg twice daily, the change from baseline in mean time-averaged QTcF interval at steady state was 5 and 8 msec, respectively. QTcF of >500 msec was observed in 4 patients (<1% of these patients).

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, CHF, unstable angina or clinically significant bradycardia

Left ventricular function

AEs related to cardiac failure or dysfunction occurred in 3 CML patients (1.0%) overall in Phase II of study 2101. Patients who presented with cardiac events within 12 months prior to nilotinib administration, such as myocardial infarction,

unstable angina, clinically significant atrial and ventricular arrhythmias and congestive heart failure (CHF) were excluded from nilotinib trials. It is therefore unknown whether patients with these concomitant conditions may be safely treated with nilotinib.

1.45 **Serious adverse events**

In the CML-CP study (2101E2) 22.6% of patients experienced an SAE. The most frequent SAEs were thrombocytopenia (3.4%), neutropenia (2.2%), angina pectoris (2.8%), pyrexia (2.5%), and myocardial infarction (1.3%). In the CML-AP study (2101E1) 30.8% of patients experienced an SAE. The most frequent SAEs were thrombocytopenia (8.0%), neutropenia (6.7%), pneumonia (5.8%), and pyrexia (4.4%). Other SAEs occurred in less than 1% of patients and are listed in detail in the Investigator's Brochure.

Deaths and Sudden Cardiac Deaths (SCD)
Two deaths in CML-CP patients and 2 deaths in CML-AP have a cause of death suspected of study drug relationship by the investigator (multi-organ failure, sudden death, CNS hemorrhage and pneumonia.) These four cases are briefly described in the Investigator's Brochure.
10 deaths in CML-CP and CML-AP were related to progressive disease or underlying medical condition rather than the study drug (sepsis, multi-organ failure, cardiac disorders, CNS hemorrhage) and are briefly summarized described in the Investigator's Brochure.

A review of all cases received to date from all sources has identified a total of 12 cases that meet the conservatively modified definition of sudden cardiac death (SCD). These 12 cases have all been reported in the context of adverse event reporting for clinical trials including the Compassionate Use Program. No reports of sudden death have been received from post marketing sources. A total of 7170 patients have received nilotinib in clinical trials through 31-Jan-2010 for an incidence of sudden death of 0.21%. 15 of these 16 cases have been reported as sudden death from a cardiac or presumed cardiac cause while 1 were reported as sudden death from unknown cause. The sudden deaths are summarized in the Investigator's Brochure. So far data indicated, the relative early occurrence of some of these deaths relative to the initiation of Nilotinib suggests the possibility that ventricular repolarization abnormalities may have contributed to their occurrence.

Safety in Phase I Study
In the Phase I CAMN107A2103 study [overall safety population (N=53)], AEs were reported in 100% of patients in each of the 5 dosing groups. Rash was the most common AE overall as well as the most common drug-related AE in the safety population. There were two deaths during the study (one in the nilotinib monotherapy group and one in the selected phase II dose group nilotinib 400 mg bid + imatinib 400 mg qd), both of which were assessed by the investigator to be due to disease progression and were not suspected to be related to study drug. SAEs occurred in 32.1% of patients; none of these were suspected by the investigator to be study drug related.

Overall, six patients (11.3%) experienced AEs associated with study treatment discontinuation. In addition to the two patients discontinuing due to death, two patients discontinued due to SAEs (abdominal pain and alteration of general status due to progressive disease) and two patients due to study drug-related AEs (hyperbilirubinemia and rash). The event of hyperbilirubinemia in the nilotinib monotherapy patient improved after discontinuation of study drug, but was reported as ongoing at the time of discontinuation from the study. The rash in the nilotinib 400 mg bid + imatinib 400 mg bid patient resolved after 34 days. Both events were considered as DLTs.

Seventeen patients (32.1%) experienced a total of 52 SAEs, with gastrointestinal disorders the most frequently affected system organ class. Of these patients, two died. No SAEs were suspected by the investigator to be study drug related.

Table 1.6 Frequent adverse events occurring in at least 5% of GIST patients by preferred term (Study 2103 safety population)

	AMN 400 mg BID n=18 n (%)	AMN 200 mg QD / IM 400 mg BID n=7 n (%)	AMN 400 mg QD / IM 400 mg BID n=7 n (%)	AMN 400 mg BID / IM 400 mg BID n=5 n (%)	AMN 400 mg BID / IM 400 mg QD n=16 n (%)	Total N=53 n (%)
Any drug-related event	16 (88.9)	7 (100)	6 (85.7)	5 (100)	15 (93.8)	49 (92.5)
Rash	3 (16.7)	2 (28.6)	3 (42.9)	3 (60.0)	10 (62.5)	21 (39.6)
Pruritus	3 (16.7)	1 (14.3)	2 (28.6)	0	9 (56.3)	15 (28.3)
Fatigue	9 (50.0)	1 (14.3)	2 (28.6)	1 (20.0)	0	13 (24.5)
Nausea	4 (22.2)	1 (14.3)	3 (42.9)	1 (20.0)	4 (25.0)	13 (24.5)
Diarrhea	5 (27.8)	1 (14.3)	2 (28.6)	1 (20.0)	2 (12.5)	11 (20.8)
Abdominal pain	6 (33.3)	2 (28.6)	1 (14.3)	0	1 (6.3)	10 (18.9)
Anorexia	5 (27.8)	1 (14.3)	2 (28.6)	1 (20.0)	0	9 (17.0)
Edema peripheral	0	3 (42.9)	3 (42.9)	0	3 (18.8)	9 (17.0)
Eczema	3 (16.7)	1 (14.3)	3 (42.9)	1 (20.0)	0	8 (15.1)
Anemia	1 (5.6)	2 (28.6)	1 (14.3)	1 (20.0)	2 (12.5)	7 (13.2)
Asthenia	1 (5.6)	1 (14.3)	2 (28.6)	0	3 (18.8)	7 (13.2)
Flatulence	4 (22.2)	2 (28.6)	1 (14.3)	0	0	7 (13.2)
Myalgia	5 (27.8)	0	0	1 (20.0)	1 (6.3)	7 (13.2)
Vomiting	3 (16.7)	1 (14.3)	1 (14.3)	1 (20.0)	1 (6.3)	7 (13.2)
Alopecia	2 (11.1)	1 (14.3)	0	0	3 (18.8)	6 (11.3)
Headache	2 (11.1)	2 (28.6)	0	0	2 (12.5)	6 (11.3)
Sleep disorder	6 (33.3)	0	0	0	0	6 (11.3)
Abdominal pain upper	3 (16.7)	1 (14.3)	0	0	1 (6.3)	5 (9.4)
Constipation	3 (16.7)	0	1 (14.3)	0	1 (6.3)	5 (9.4)
Dyspepsia	3 (16.7)	1 (14.3)	1 (14.3)	0	0	5 (9.4)
Erythema	0	0	3 (42.9)	0	2 (12.5)	5 (9.4)
Pain in extremity	4 (22.2)	1 (14.3)	0	0	0	5 (9.4)
Dry skin	3 (16.7)	1 (14.3)	0	0	0	4 (7.5)
Face edema	0	0	0	1 (20.0)	3 (18.8)	4 (7.5)
Hyperbilirubinemia	3 (16.7)	0	1 (14.3)	0	0	4 (7.5)
Muscle spasms	1 (5.6)	1 (14.3)	1 (14.3)	0	1 (6.3)	4 (7.5)
Night sweats	3 (16.7)	1 (14.3)	0	0	0	4 (7.5)
Restlessness	3 (16.7)	0	1 (14.3)	0	0	4 (7.5)
Weight decreased	2 (11.1)	1 (14.3)	1 (14.3)	0	0	4 (7.5)
Abdominal discomfort	3 (16.7)	0	0	0	0	3 (5.7)
Arthralgia	2 (11.1)	0	1 (14.3)	0	0	3 (5.7)
Corrected QT interval prolonged	0	0	0	0	3 (18.8)	3 (5.7)
Dysgeusia	1 (5.6)	0	1 (14.3)	1 (20.0)	0	3 (5.7)
Dysphonia	3 (16.7)	0	0	0	0	3 (5.7)
Rash pruritic	2 (11.1)	0	0	1 (20.0)	0	3 (5.7)

1.5 **Study rationale/ purpose**

AML has been a challenging cancer for hematologists and adult patients. Combination chemotherapy using cytarabine and anthracycline (known as 7+3) induction success and relapse rates have been challenged by many concepts but yet remains the standard therapy. These strategies included using higher doses of anthracycline or cytarabine, addition of novel agents (like proteosome inhibitors or monoclonal antibodies (like gemtuzumab ozogamicin), or even using agents directed against drug-resistance targets. Fernandez H et colleagues randomized 657 patients diagnosed with AML between the ages of 17-60 to receive 7+3 but at different doses for daunorubicin (90 mg/m2 vs 45 mg/m2). Patients who received high dose daunorubicin had higher complete remission rates (70% vs 57%, p<0.001) and better median overall survival (23.7 months vs 15.7 months, p=0.003){Fernandez, 2009 #54}. In a similar study reported by Lowenberg et al, 813 patients were randomized to the same treatment but in patients equal or older than 60 years. The complete remission rate favored the higher dose daunorubicin (64% vs 54%, p=0.002), however the overall survival did not differ significantly {Lowenberg, 2009 #55}. Dose intensification of cytarabine was studied compared to standard dose (2gm/m2 IV q12 hours x 12 doses vs. 200 mg/m2/day continuos IV daily x 7 days) in addition to daunorubicin 45 mg/m2 daily x 3 days in AML patients less than 65 years old. A total of 665 patients were treated where CR rates 55% vs 58% (high vs standard dose cytarabine) for patients aged less than 50 and there was no significant difference in 4-year overall survival (32% vs 22%, p=0.41){Weick, 1996 #56}. The British hematologists tested the role of adding gemtuzumab ozogamicin 3mg/m2 on day 1 of induction and course 3 of consolidation) to induction chemotherapy in their MRC AML 15 trial in 1113 patients diagnosed with AML and age less than 60{Burnett, 2011 #58}. Unfortunately, no overall survival benefit was found to adding this treatment in neither induction nor consolidation. On subsequent analysis, patients with favorable cytogenetics had a favorable effect from adding gemtuzuamb (p=0.001). Finally, in randomized study done by ECOG (3999), the addition of zosuquidar (a modulator of P-glycoprotein), did not improve the outcome of older patients newly diagnosed with AML (499 patients){Cripe, 2010 #59}. Remission rate was 52% vs 49% (p0.158) on placebo, while median overall survival was 7.2 vs 9.4 months on placebo compared to patients who received zosuquidar.

Over the last decade, molecular breakthroughs in understanding the genes role and pathways governing the cell cycles, proliferation, apoptosis and transcription have revolutionized our understanding and treatment of myeloid disease, especially chronic myeloid leukemia, myeloproliferative neoplasms and acute myeloid leukemias. Based on this molecular profiling we are able to identify high risk AML cases for low chance of remission and high risk of relapse. Consequently this affected our treatment recommendation, especially with regards to whether to proceed with allogeneic transplantation or not. For example patients harboring CEBPA mutation (single vs double mutations) had a favorable outcome compared with wild type AML cases {Taskesen, 2011 #62}. In fact double mutation CEBPA was the only prognostic factor for overall survival on multivariate analysis (hazard ratio 0.36, p<0.0001). On the other hand, high EVI1 expression in young patients diagnosed with AML predicts worse outcome by having low CR rates (odd ratio 0.54, p=0.002) and adverse event-free survival (hazard ratio 1.46, p<0.001){Groschel, 2010 #63}. Patients who received allogeneic stem cell transplantation in CR1 had a better 5-year relapse free survival (33% vs 0%) compared to without transplantation. FLT3 is another potential gene, where mutations (internal tandem duplicate or tyrosine kinase domain mutation) predicts worse clinical outcome (especially in patients with normal cytogenetics){Kottaridis, 2001 #64}{Whitman, 2008 #65}{Stirewalt, 2006 #67}. This became of utmost significance since many FLT3 inhibitors have been tested in trials with encouraging results. This include both as a single

agent and in combination with chemotherapy {Fischer, 2010 #68}{Levis, 2011 #69}{Borthakur, 2011 #71}{Al-Kali, 2011 #70}{Zarrinkar, 2009 #72}.

Kit encodes for a transmembrane protein, which has tyrosine kinase activity. It is also thought to be a proto-oncogene with important role in hematopoiesis. Kit is activated by SCF (stem Cell factor) and results in proliferation of blast cells in many cases at diagnosis and at relapse. There has been a strong correlation between levels of Kit phosphorylation and AML cell lines proliferation {Kanakura, 1993 #76}. Many mutations in c-Kit are seen in core binding factor AML cases and predict worse clinical outcome. Paschka P et al assessed Kit mutations in exon 8 and 17 in 61 cases with AML harboring inv(16) and 49 AML cases with t(8;21) treated with standard chemotherapy and high dose cytarabine consolidation{Paschka, 2006 #78}. Kit mutations were detected in 30% of AML inv(16) and 22% with AML t(8;21). In inv(16), cumulative incidence of relapse for both Kit mutations compared to Kit wild-type (56% vs 29%, p=0.05); mutated Kit also predicted worse overall survival (after adjusting to sex). Additionally, Kit intensity (<20.3) assessed by fluorescence was found to be correlated with decreased progression-free and overall survival on multivariate analysis (independent of age, white blood count, and cytogenetics){Advani, 2008 #79}. Finally, a variant of t(8;21) called AML1-ETO9a was found to be correlated with c-kit overexpression/mutations and predicts poor prognosis{Jiao, 2009 #80}.

Tyrosine kinase inhibitors (TKI) (including imatinib, nilotinib, and dasatinib) have changed the prognosis and therapy paradigms of patients diagnosed with CML, as well as Ph-positive acute lymphoblastic leukemia (ALL). Imatinib was first approved in patients diagnosed with chronic phase CML based on the landmark study IRIS, while both second generation TKIs (nilotinib and dasatinib) were approved for second line therapy in patients with imatinib-resistant or intolerant CML-CP (O'Brien, Guilhot et al. 2003; Hochhaus, Kantarjian et al. 2007; Kantarjian, Giles et al. 2007). However, recently and based on randomized phase III studies, both nilotinib and dasatinib have been approved for frontline therapy of patients diagnosed with chronic phase CML (Kantarjian, Shah et al. 2010; Saglio, Kim et al. 2010). In more advanced stages of CML (accelerated and blast phases), second generation TKIs have been more effective in achieving hematological and cytogenetic remissions due to their improved potency (Cortes, Rousselot et al. 2007le Coutre, Ottmann et al. 2008).

Nilotinib (Kantarjian, Giles et al. 2006), a second generation TKI, has been tested as single agent in imatinib-failure patients diagnosed with CML-BC with encouraging responses. Nilotinib has been approved for the treatment of CML as first line therapy and after imatinib failure as second line therapy in CML-CP. Therefore, a combination of chemotherapy regimen with nilotinib should be an effective strategy, better than chemotherapy alone, and may be superior to other combination (like imatinib and chemotherapy). Nilotinib is 20-50-fold more potent than imatinib in its ABL inhibition. It also is more effective than imatinib especially in the presence of tyrosine kinase mutations (except for T315I), frequently seen in Ph+ALL. In addition, nilotinib concentration in cell lines and primary CD34+ CML cells is not mediated by active uptake or efflux by major drug transporters (a known mechanism for imatinib resistance). Most importantly, it has a synergistic activity with established chemotherapeutic drugs in BCR-ABL positive cells. Additionally, nilotinib does not increase stem cell transplantation toxicity in patients with Ph+ acute lymphoblastic leukemia (Shimoni, Leiba et al. 2009). In a case report, nilotinib restored long-term full-donor chimerism in Ph+ ALL relapsed after allogeneic transplantation (Merante, Colombo et al. 2009).

We plan to study the clinical efficacy of nilotinib in combination with standard chemotherapy (7+3) in patients with Kit+AML as a frontline therapy. We will assess their CR rates, survival outcome (DFS and OS) in addition to predictive factors for superior outcome. Cytarabine and daunorubicin regimen (7+3) is one of the well-established and widely used chemotherapy regimens for the treatment of adult AML. In addition, it has been successfully combined with other TKIs (imatinib, dasatinib) in the management of both newly diagnosed and relapsed AML. Cancer and Leukemia Group B (CALGB) is currently studying combination dasatinib and 7+3 in a phase II study (CALGB 110801) for core binding factor positive AML patients. Finally imatinib has been combined with mitoxantrone, etoposide and cytarabine (MEC) in patients with relapsed/refractory c-Kit+ AML.

2.0 Goals

2.1 Primary

2.11 To determine the complete response rates of combination Nilotinib, cytarabine, and daunorubicin in patients newly diagnosed with AML and Kit overexpression

2.2 Secondary

2.21 Determine the 2-year overall survival (OS) and disease-free survival (DFS) rates.

2.22 Determine the complete response duration in patients treated with this regimen.

2.23 Assess the safety and toxicity of this regimen based on NCI CTCAE version 4.0.

2.3 Correlative Research

2.31 Assess the prognostic and predictive factors (Kit mutation/expression level, Flt3 mutation) for patients treated with this regimen.

2.32 Assess the patterns of molecular response and relapse for Kit

2.33 Assess the effect on minimal residual disease (MRD) by PCR or flow cytometry

3.0 Patient Eligibility

3.1 Inclusion Criteria

3.11 Age ≥ 18 years and < 70 years.

3.12 Untreated, histological confirmed acute myeloid leukemia (AML) based on WHO 2008 criteria (See Appendix V) with Kit expression (CD117) of myeloblasts ≥ 20% by flow cytometry from bone marrow aspirate at diagnosis.

3.13 ECOG Performance Status (PS) 0, 1, or 2 (Appendix3).

3.14 The following laboratory values obtained ≤ 7 days prior to registration.
- Magnesium/potassium/phosphorus WNL
- Serum amylase and lipase ≤ 1.5 x ULN
- Total Bilirubin ≤1.5 x ULN [Does not apply to patients with isolated hyperbilirubinemia (e.g., Gilbert's disease), in that case direct bilirubin should be ≤ 2 x ULN]
- Alkaline phosphatase ≤3 x ULN
- SGOT (AST) ≤3 x ULN
- Creatinine ≤1.5 x ULN

3.15 Negative pregnancy test done ≤7 days prior to registration, for women of childbearing potential only.

3.16 Provide informed written consent.

3.17 Willing to return to consenting Mayo Clinic (Mayo Clinic's campus in Rochester, Mayo Clinic's campus in Arizona, or Mayo Clinic's campus in Florida) institution for follow-up during the Active Monitoring Phase of the study.

3.18 Willing to provide bone marrow aspirate and blood samples for correlative research purposes (see Sections 6.*2 and 14.1).*

3.2 Exclusion Criteria

3.21 Any of the following because this study involves investigational agent(s) whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown
- Pregnant women
- Nursing women
- Men or women of childbearing potential who are unwilling to employ adequate contraception throughout the study and for 3 months after completion of study treatment

3.22 Co-morbid systemic illnesses or other severe concurrent disease which, in the judgment of the investigator, would make the patient inappropriate for entry into this study or interfere significantly with the proper assessment of safety and toxicity of the prescribed regimens.

3.23 Immunocompromised patients (other than that related to the use of corticosteroids) including patients known to be HIV positive.

3.24 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, or psychiatric illness/social situations that would limit compliance with study requirements.

3.25 Receiving any other investigational agent which would be considered as a treatment for the primary neoplasm.

3.26 Other active malignancy ≤3 years prior to registration. EXCEPTIONS: Non-melanotic skin cancer or carcinoma-in-situ of the cervix.

3.27 Previous treatment with chemotherapy (cytarabine, idarubicin, daunorubicin) for a hematological disorder. Exceptions: Patients with prior diagnosis of MDS and/or treatment with hypomethylating agent (azacytidine or decitabine or lenalidomide) are not excluded, prior hydroxyuria allowed.

3.28 Impaired cardiac function including any one of the following:

- Inability to monitor the QT interval on ECG
- Congenital long QT syndrome or a known family history of long QT syndrome.
- Clinically significant resting brachycardia (<50 beats per minute)
- QTc > 450 msec on baseline ECG. If QTc >450 msec and electrolytes are not within normal ranges, electrolytes should be corrected and then the patient re-screened for QTc
- Myocardial infarction ≤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,atrial tachyarrhythmias or ejection fraction cutoff
- Left ventricle ejection fraction <45%
- History of, Congestive heart failure requiring use of ongoing maintenance therapy for life-threatening ventricular arrhythmias.

3.29a Patients currently receiving treatment with strong CYP3A4 inhibitors and treatment that cannot be either discontinued or switched to a different medication prior to starting study drug. Patients receiving any medications or substances that are strong or moderate inhibitors of CYP3A4.

Use of the following strong or moderate inhibitors is prohibited ≤ 7 days prior to registration. Concurrent use is not allowed simultaneously with nilotinib during the study.

Strong Inhibitors of CYP3A4/5 > 5-fold increase in the plasma AUC values or more than 80% decrease in clearance
Boceprevir (Victrelis®)
Clarithromycin (Biaxin®, Biaxin XL®)
Conivaptan (Vaprisol®)
Grapefruit juice
Indinavir (Crixivan®)
Itraconazole (Sporanox®)
Ketoconazole (Nizoral®)
Lopinavir/Ritonavir (Kaletra®)
Mibefradil
Nefazodone (Serzone®)
Nelfinavir (Viracept®)
Posaconazole (Noxafil®)
Ritonavir (Novir®, Kaletra®)
Saquinivir (Fortovase®, Invirase®)

Telaprevir (Incivek®)
Telithromycin (Ketek®)
Voriconazole (Vfend®)
Moderate Inhibitors of CYP3A4/5 > 2-fold increase in the plasma AUC values or 50-80% decrease in clearance
Amprenavir (Agenerase®)
Aprepitant (Emend®)
Atazanavir (Reyataz®)
Ciprofloxacin (Cipro®)
Darunavir (Prezista®)
Diltiazem (Cardizem®, Cardizem CD®, Cardizem LA®, Cardizem SR®, Cartia XT™, Dilacor XR®, Diltia XT®, Taztia XT™, Tiazac®)
Erythromycin (Erythrocin®, E.E.S. ®, Ery-Tab®, Eryc®, EryPed®, PCE®
Fluconazole (Diflucan®)
Fosamprevnavir (Lexiva®)
Imatinib (Gleevec®)
Verapamil (Calan®, Calan SR®, Covera-HS®, Isoptin SR®, Verelan®, Verelan PM®)

3.29b Receiving any medications or substances that are **inducers** of CYP3A4. Use of the following inducers is prohibited ≤ 7 days prior to registration. Concurrent use is not allowed simultaneously with nilotinib during the study.

Strong Inducers of CYP3A4/5 > 80% decrease in AUC
Avasimibe
Carbamazepine (Carbatrol®, Epitol®, Equetro™, Tegretol®, Tegretol-XR®)
Phenytoin (Dilantin®, Phenytek®)
Rifampin (Rifadin®)
St. John's wort
Moderate Inducers of CYP3A4/5 50-80% decrease in AUC
Bosentan (Tracleer®)
Efavirenz (Sustiva®)
Etravirine (Intelence®)
Modafinil (Provigil®)
Nafcillin
Nevirapine (Viramune®)
Phenobarbital (Luminal®)
Rifabutin (Mycobutin®)
Troglitazone

3.29c 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 (See Appendix1). Prohibited medications are listed in Appendix Ia "Drugs With Risk of Torsades de Pointes." Appendix Ib contains drugs that should be used with caution due to possible or conditional risk of Torsades de Pointes.

3.29d 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).

3.29e Acute or chronic pancreatic disease.

3.29f Known cytopathologically confirmed CNS infiltration.

3.29g Acute or chronic liver disease or severe renal disease considered unrelated to the cancer.

3.29h History of significant congenital or acquired bleeding disorder unrelated to cancer.

3.29i Major surgery ≤4 weeks prior to registration of the study or who have not recovered from prior surgery.

3.29j Treatment with other investigational agents ≤14 days of registration.

3.29k Diagnosis of AML-M3 (or acute promyelocytic leukemia).

4.0 Test Schedule

All routine assessments must be performed within ± 3 days of the day indicated on the Visit Schedule.

	Active Monitoring Phase								
Tests and procedures	≤7 days prior to registration*	Day 1 of induction/ re-induction or SCT	Cycle 1, Day 4	Cycle 1, days 8,14, and upon bone marrow recovery	Cycle 2 if Re-induction: day 14 and upon bone marrow recovery	Day 1 of each consolidation cycle	Every 3 months during maintenance	For patients who go to SCT: End of stem cell transplant (prior to start of maintenance after SCT)	Early Discontinuation or End of Study
History and exam, weight, PS	X	X				X	X	X	X
Height	X								
Adverse event assessment	X	X[11]				X	X	X	X
BM testing[2, 10]	X			X[9]	X[9]		X[2]		X
Biomarkers CD117/ Flow cytometry[R]	X[2]			X[2]	X[2]		X[2]		X[2]
Biomarkers Kit Mutation by PCR [„R]	X						X[2,7]		X
Hematology group HgB, WBC, PLT, WBC with diff.	X	X				X	X	X	X
Serum Chemistry (magnesium [R], potassium, phosphorus, total bilirubin, alkaline phosphatase, AST, ALT, creatinine)	X	X				X	X	X	X
Serum Chemistry [R] (Serum Amylase & Lipase)	X	X				X	X	X	X
MUGA/Echo	X*								
Direct bilirubin	X[6]								
LDH	X								
Hepatitis B surface Antigen, Anti-HBc	X								
Serum pregnancy test[1]	X								
Urine analysis [R] (proteinuria, hematuria)	X								
ECG (QTcF results preferred) [R ,3]	X	X		X[3]	X[3]	X	X	X	X
Patient Medication Diary (Appendix IV)[4]		X				X	X	X	X
Research Correlatives [,8,R]	X		X[5]	X[5]					

1. For women of childbearing potential only. Must be done ≤7 days prior to registration.
2. Bone marrow (BM) sampling will be done upon screening, bone marrow recovery of Cycle 1 (Standard of Care), upon bone marrow recovery of Cycle 2 if patient receives re-induction (Standard of Care), Day 1 Cycle 1 of maintenance (Standard of Care), and end of maintenance (Research Funded) and upon relapse (Standard of Care). Bone marrow MRD assessment for CD117 (Kit) by flow cytometry will be done at screening (Standard of care), upon recovery of Cycle 1 (**Research funded**), upon bone marrow recovery of Cycle 2 if patient receives re-induction (**Research funded**). Bone marrow MRD assessment for CD117/Kit by flow cytometry will be done at day 1 Cycle 1 of maintenance (Standard of Care), end of maintenance (Standard of Care) and upon relapse (Standard of care) if deemed needed by the pathologist. Bone marrow MRD assessment for Kit mutation by PCR (if positive at diagnosis) will be done at screening (Research funded), Day 1 of cycle 1 of maintenance (if positive at screening) (Research funded), and upon relapse (if positive at screening) (Research funded). Bone marrow aspirate/biopsy done for cycle 1 day 1 of maintenance could be done up to 3 weeks prior.
3. ECGs will be performed locally at screening, Day 1 (prior to dosing) of each cycle, Day 8 of induction/re-induction, and when there's any dose change or re-initiation after a dose interruption. When a dose change occurs or upon re-starting nilotinib, an ECG must be obtained at 7 days afterwards. QTcF results are preferred, however all reporting methods will be accepted.
4. The diary must begin the day the patient starts taking nilotinib and must be completed per protocol and returned to the treating institution OR compliance must be documented in the medical record by any member of the care team. Patients will be asked to return all unused medication at each visit.
5. Serum nilotinib levels (research) will be checked only on cycle 1 days 4, 8, and 14 (30 minutes pre-dosing.) See section 14.0
6. Only if total bilirubin > upper normal limit.
7. Only if positive at diagnosis.
8. See section 14.0
9. Bone marrow aspirate and biopsy will be done on Day 14 of Cycle 1 Induction and Day 14 of Cycle 2 if patient receives Re-Induction to assess for cellularity and blasts assessment (Standard of Care).
10. Additional bone marrow testing may be done as clinically indicated
11. If the patient goes to stem cell transplant, the cycle length of the cycle proceeding transplant will be extended until the SCT (pre-transplant conditioning chemotherapy plus stem cell infusion).

R Research funded (see Section 19.0). This will be charged to study and not to patient's account.

* Labs done same day of consenting (prior to consenting) do not need to be repeated. ≤14 days prior to registration for bone marrow biopsy (regardless of consent date). MUGA/Echocardiogram done ≤ 28 days prior to screening is allowed. Correlative studies at screening could be done via a peripheral blood sample to avoid bone marrow resampling.

5.0 Grouping Factors: None.

6.0 Registration Procedures

6.1 To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration/randomization application. The registration/randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the Web site. If unable to access the Web site, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the registration/randomization application are available on the MCCC web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and a MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office [redacted]. If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to "Instructions for Remote Registration" in section "Finding/Displaying Information about A Registered Subject."

6.2 Correlative Research
A mandatory correlative research component is part of this study. The patient will be automatically registered onto this component (See Sections 3.18 and 14.0).

6.3 Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office [redacted] If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.4 Prior to accepting the registration, registration application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.5 At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use his/her sample(s) for future

research of AML at Mayo.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.
- Patient has/has not given permission for MCCC to give his/her sample(s) to researchers at other institutions.

6.6 Treatment cannot begin prior to registration and must begin ≤ 7 days after registration.

6.7 Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.8 All required baseline symptoms (see Section 10.5) must be documented and graded.

6.9a Treatment on this protocol must commence at Mayo Clinic Rochester, Mayo Clinic Arizona, or Mayo Clinic Florida institution under the supervision of a hematologist.

6.9b Study drug is available on site.

7.0 Protocol Treatment

7.1 Treatment Schedule

7.11 **Induction (Cycle 1; repeat for Cycle 2 for patients who require re-induction):** Patients eligible for the study based on screening tests will be enrolled on the study. During Cycle 1, patients will be assessed for hypocellularity on Day 14. If the patient has a decrease in bone marrow blasts, they will be assessed for response upon marrow recovery (End of Cycle 1). Patients who achieve a CR/CRi will proceed to consolidation. Patients who fail to achieve a significant decrease in bone marrow blasts on Day 14 of Cycle 1 or fail to achieve a CR/CRi upon bone marrow recovery at the end of Cycle 1will proceed to re-induction Cycle 2 (follow same treatment schedule as Cycle 1). Treating physician could repeat other bone marrow(s) on day 21 (and later) if felt best for patient interest and if felt that blast reduction might still happen then. These patients will get repeat bone marrow evaluation for hypocellularity on Day 14 of Cycle 2 and will be assessed for response upon marrow recovery (End of Cycle 2). Patients who fail to achieve a decrease in bone marrow blasts on Day 14 of Cycle 2 or fail to achieve a CR/CRi upon bone marrow recovery at the end of Cycle 2 will go to event monitoring. Idarubicin (12mg/m2) instead of daunorubicin is allowed a substitute in cases of daunorubicin national shortages (Not a preferred first line anthracycline).

Agent	Dose	Route	Day	Comments
Daunorubicin	60 mg/m^2	IV	1-3	IV Push through the Y-site of a running IV over 10 minutes
Cytarabine	25 mg/m^2/day	IV	1	IV Push through the Y-injection site of a running IV prior to continuous infusion

Cytarabine	100 mg/m^2/ day	IV	1-7	Continuous infusion
Nilotinib	300 mg twice/day**	Oral	4-14	Take on an empty stomach 1 hour before or 2 hours after food.

** See Section 7.15

7.12 **Consolidation (Cycles 2-5 or Cycles 3-6 for patients who received re-induction):** Patients who lose sustained CR/CRi will discontinue treatment and go to event monitoring. Cycles will be repeated every 28 days for a total of 4 cycles. .

CBC criteria to receive subsequent cycles of consolidation:

- ANC $\geq$ 1.0 x10^9/L
- Platelets $\geq$80 x10^9/L

NOTE: If CBC criteria is not met, the next cycle of treatment may be delayed up to 56 days. If CBC criteria is still not met, the patient will go to event monitoring.

NOTE: Patients > 60 years or serum creatinine (Scr) > 2 mg/dL should get 50% dose reduction of their cytarabine, for a total of 2-4 cycles, based on treating physician judgment

Agent	Dose	Route	Day	Comments
Cytarabine	3000 mg/m^2 q 12 hours	IV	Day 1, 3, 5	
Nilotinib	300 mg twice/day**	Oral	Day 4-14	Take on an empty stomach 1 hour before or 2 hours after food.

** See Section 7.15

7.13 **Maintenance (Cycles 6-13 or Cycles 7-14 for patients who received re-induction):** Prior to commencement of this phase, patients will get a repeat bone marrow evaluation. Patients who lose their sustained CR/CRi will discontinue treatment and go to event monitoring. Cycles will be repeated every 84 days for a total of 8 cycles.

NOTE: If toxicity occurs, the next cycle of treatment may be delayed up to 56 days. If toxicity is not resolved, the patient will go to event monitoring.

Agent	Dose	Route	Day	Comments
Nilotinib	300 mg twice/day**	Oral	1-84	Take on an empty stomach 1 hour before or 2 hours after food.

** See Section 7.15

7.14 **Stem Cell Transplantation (SCT):** Allogeneic stem cell transplantation for intermediate and high risk patients (based on treating MD assessment) will be allowed at any time if a suitable donor is found and no chemotherapy will be given. In this case, the cycle length of the cycle proceeding transplant will be extended until the SCT (pre-transplant conditioning chemotherapy plus stem cell infusion). Patients who undergo SCT will receive maintenance with nilotinib per Section 7.13 for 8 cycles starting day +100 (after achieving hematological recovery as defined by CR, or after day +100 as deemed appropriate per treating MD). The SCT until the start of maintenance will be defined as a separate cycle, where the stem cell transplant form will be used instead of the evaluation/treatment form for that cycle. Post-transplant maintenance will be allowed between day100-200.

7.15 Nilotinib dose will be increased to 400 orally twice daily in the following cases:

1. Conversion of negative RT-PCR (CMR) for Kit mutation to positive on 2 readings (if mutation was present at diagnosis).
2. Failure to achieve negative RT-PCR (CMR) for Kit at the end of consolidation therapy.
3. Failure to achieve negative flow cytometry for persistent AML (MRD) at the end of consolidation therapy.
4. Failure to achieve CR at the end of cycle 1 of induction.

NOTE: If nilotinib is dose escalated (e.g. from 300 mg b.i.d daily to 400 mg b.i.d.), an ECG should be repeated approximately 7 days after the dose adjustment.

8.0 Dosage Modification Based on Adverse Events

Dose reduction for nilotinib is required in cases of grade 3 or 4 hematologic adverse events (AE) concerning white blood cells (WBC) and platelets (PLT) (not concerning hemoglobin level) during maintenance therapy, and in cases of grade 2, 3 or 4 non-hematologic AEs. The dose reduction schedule used in this study differs from the label recommendations, but has been successfully pioneered by the GIMEMA study group in a recently reported nilotinib trial (Rosti 2008), which included similar patients treated with nilotinib for CML-CP.

ALERT: *ADR reporting may be required for some adverse events (See Section 10)*

- Omit = The current dose(s) for the specified drug(s) during a cycle is skipped. The patient does not make up the omitted dose(s) at a later time
- Hold/Delay = The current dose(s) of all drugs during a cycle is delayed. The patient does make up the delayed dose(s) when the patient meets the protocol criteria to restart drugs.
- Discontinue = The specified drug(s) are totally stopped.

8.1 Dose Levels (Based on Adverse Events in Tables below)

Dose Level	Dose	Drug Name
+1**	400 mg orally bid	Nilotinib
0*	300 mg orally bid (also dose for maintenance)	Nilotinib
-1	200 mg orally bid	Nilotinib
-2	150 mg orally bid	Nilotinib

*Dose level 0 refers to the starting dose.
** If nilotinib is dose escalated (e.g. from 300 mg b.i.d daily to 400 mg b.i.d.), an ECG should be repeated approximately 7 days after the dose adjustment.

8.2 Nilotinib dose adjustments for AE at least possibly related to treatment (WBC or PLT) during **Maintenance Therapy.**

→ → ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 unless otherwise specified*** ← ←

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
AT TIME OF NEXT DOSE (All treatment days of each cycle)			
Investigations	Grade 3-4 White blood cell decreased (persisting >7days with optimal supportive care) Grade 3-4 Platelet count decreased (persisting >7days with optimal supportive care)	Nilotinib	**1st and 2nd time:** Hold nilotinib Check labs weekly until ≤ Grade 2. Resume nilotinib at 600 mg a day (300 mg b.i.d.) if recovery to ≤ Grade 2 occurs within 14 days If toxicity persists for 15-28 days, hold therapy and resume nilotinib at 400 mg a day (200 mg b.i.d.) after recovery to ≤ Grade 2 Dose re-escalation to 600 mg a day (300 mg b.i.d.) is permitted if tolerated and no recurrent Grade 3 or 4 hematological toxicities occur while on the reduced dose. **3rd and 4th time:** Hold nilotinib Check labs weekly until ≤ Grade 2. Resume nilotinib at 400 mg (200mg bid) a day. Dose re-escalation to 600 mg a day (300 mg b.i.d.) is permitted if tolerated and no recurrent Grade 3 or 4 hematological toxicities occur while on the reduced dose. **5th time:** Stop nilotinib and proceed to event monitoring.

8.3 Nilotinib dose adjustments for AE at least possibly related to treatment (Other Non-hematological) for **All Cycles.**

→ → ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 unless otherwise specified*** ← ←

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
AT TIME OF NEXT DOSE (All treatment days of each cycle)			
Other Non-hematological	Grade 2-3 Non-Hematologic (persisting >7days with optimal supportive care)	Nilotinib	**1st and 2nd time:** Hold nilotinib Check labs weekly (if applicable)until ≤ Grade 1 Resume nilotinib at 600 mg (300 mg b.i.d.) if recovered to ≤Grade 1 **3rd time:** Hold nilotinib Check labs weekly (if applicable)until ≤ Grade 1 Resume nilotinib at 400 mg (200 mg b.i.d) a day if recovered to ≤Grade 1 Resume nilotinib at 600 mg a day (300 mg b.i.d.) if patient was able to tolerate 2 weeks on 400 mg a day without the same Grade 2/3 non-hematologic AE recurring. **4th time:** Hold nilotinib Check labs weekly (if applicable) until ≤ Grade 1 Resume nilotinib at 300 mg (150mg b.i.d) a day if recovered to less than or equal to grade 1. Resume nilotinib at 400mg a day(200 b.i.d.) if patient was able to tolerate 4 weeks on 300 mg a day without the same Grade 2/3 non-hematologic AE recurring, re-escalation to 300 mg b.i.d. may be considered.
Other Non-hematological	Grade 4 Non-Hematologic	Nilotinib	Discontinue nilotinib and proceed to event monitoring.

If nilotinib is held and re-initiated, an ECG should be repeated prior to re-starting nilotinib and approximately 7 days after.

8.4 Nilotinib dose reduction for cardiac AE
If QTc > 480 msec
1. Stop nilotinib
2. Perform an analysis of serum electrolytes (including potassium and magnesium) and correct with supplements to WNL if required.
3. Review concomitant medications for drug interactions.

4. Resume nilotinib within 2 weeks at prior dose if QTc returns to < 450 msec and to within 20msec of baseline.
5. If QTc is between 450msec and 480msec after 2 weeks reduce the dose to 400mg daily
6. If following a dose-reduction to 300mg daily, the QTc returns to >480msec, nilotinib should be discontinued.
7. An ECG should be repeated 7 days after any dose adjustment.

8.5 Dose modification on chemotherapeutic agents

Chemotherapeutic agents will be given for Cycle 1 of induction (and also Cycle 2 if no CR is achieved after Cycle 1) regardless of the hematological panel results (i.e. CBC results) because it is related to the underlying disease (acute leukemia). If patient does not achieve CR by the end of Cycle 2 re-induction, the patient will discontinue treatment and go to event monitoring. Subsequent cycles of consolidation are to be given after peripheral blood recovery. Peripheral blood recovery is defined as ANC $\geq 1.0 \times 10^9$/L, platelets $\geq 80 \times 10^9$/L.

Dose modification for chemotherapeutic agents will be mainly based on non-hematological AEs as described below. Dose adjustments will be re-evaluated at the beginning of each cycle, For example, if a dose reduction was applied because of abnormal lab results on previous cycle and the lab results normalizes, then full dose will be given. Creatinine clearance will be calculated using Cockcroft formula with total body weight and race adjustment.

→ → Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 unless otherwise specified ← ←

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION
AT TIME OF NEXT DOSE (All treatment days of each cycle)			
Investigations	Grade 2 Blood Bilirubin Increased with total bilirubin >2 mg/dL	Daunorubicin	Decrease by 50%
	Grade 3 Blood Bilirubin Increased with total bilirubin 3-5 mg/dL		Decrease by 75%
	Grade 3 or 4 Blood Bilirubin Increased with total bilirubin>5 mg/dL		Omit for remainder of the cycle
Investigations	Creatinine > 2 mg/dL	Cytarabine 1.5 to 3 grams/m^2/dose	Decrease by 50%
	Grade 3 or 4 increased creatinine > 4 mg/dL		Discontinue treatment and proceed to event monitoring
	Total bilirubin > 2 mg/dL		Decrease by 50%
	AST/ALT > 2 X ULN		Decrease by 50%
	Grade 3 or 4 bilirubin >5 mg/dL, increased AST, ALT		Discontinue treatment and proceed to event monitoring

9.0 Ancillary Treatment/Supportive Care

9.1 Antiemetics may be used at the discretion of the attending physician.

9.2 Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations.

9.3 Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.4 Diarrhea: This could be managed conservatively with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2-4 hours until diarrhea free (maximum 16 mg/day).

In the event of grade 3 or 4 diarrhea, the following supportive measures are allowed:

hydration, octreotide, and antidiarrheals.
If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (grade 3 or 4), broad-spectrum antibiotics must be prescribed.
Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting **should be hospitalized** for intravenous hydration and correction of electrolyte imbalances.

10.0 Adverse Event (AE) Reporting and Monitoring

10.1 Adverse Event Characteristics

Adverse events for this study will be collected while the patient is receiving protocol chemotherapy. If the patient becomes eligible for Stem Cell Transplant, neither the hospitalization nor prolonged hospitalization for transplant will be reported as an adverse event. Adverse events related to the Stem Cell Transplant will not be collected. At the time the patient returns to treatment with nilotinib, recording of adverse events will resume.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site:
(http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

10.11 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE version 4.0. Next, determine whether the event is expected or unexpected (see Section 10.2) and if the adverse event is related to the medical treatment or procedure (see Section 10.3). With this information, determine whether the event must be reported as an expedited report (see Section 10.4). Expedited reports are to be completed within the timeframes and via the mechanisms specified in Sections 10.4. All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.52 and 18.0).

10.12 Each CTCAE term in the current version is a unique representation of a specific event used for medical documentation and scientific analysis and is a single MedDRA Lowest Level Term (LLT).

- **NOTE:** A severe AE, as defined by the above grading scale, is **NOT** the same as serious AE which is defined in the table in Section 10.4.

10.2 Expected vs. Unexpected Events

- The determination of whether an AE is expected is based on agent-specific information provided in Section 15.0 of the protocol.
- Unexpected AEs are those not listed in the agent-specific information provided in Section 15.0 of the protocol.

NOTE: "Unexpected adverse experiences" means any adverse experience that is neither

identified in nature, severity, or frequency of risk in the information provided for IRB review nor mentioned in the consent form.

10.3 Assessment of Attribution

When assigning attribution category, the attribution refers to the relationship to Cytarabine, Daunorubicin or Nilotinib (not Stem Cell Transplant). Adverse events are not collected during Stem Cell Transplant.
When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The adverse event *is clearly related* to the agent(s).
Probable - The adverse event *is likely related* to the agent(s).
Possible - The adverse event *may be related* to the agent(s).
Unlikely - The adverse event *is doubtfully related* to the agent(s).
Unrelated - The adverse event *is clearly NOT related* to the agent(s).

Events determined to be possibly, probably or definitely attributed to a medical treatment suggest there is evidence to indicate a causal relationship between the drug and the adverse event.

10.31 **AEs Experienced Utilizing Investigational Agents and Commercial Agent(s) on the SAME Arm**

NOTE: The combination of an investigational agent with a commercial agent is considered investigational.

Routine Reporting

- Routine AE reporting for Phase 1 and Phase 2 clinical studies using an investigational agent /intervention in combination with a commercial agent is stated in the protocol. See Section 10.52.

  NOTE: When a commercial agent(s) is (are) used on the same treatment arm as the investigational agent/intervention (also, investigational drug, biologic, cellular product, or other investigational therapy under an IND), the entire combination (arm) is then considered an investigational intervention for reporting.

Expedited Reporting

- An AE that occurs on a combination study must be assessed in accordance with the guidelines for CTEP investigational agents/interventions in Section 10.4, and where indicated, an expedited report must be submitted.

- An AE that occurs prior to administration of the investigational agent/intervention must be assessed as specified in the protocol. In general, only Grade 4 and 5 AEs that are unexpected with at least possible attribution to the commercial agent require an expedited report. Refer to Section 10.4 for specific AE reporting requirements or exceptions.

- Commercial agent expedited reports must be submitted by the Cooperative Group to the FDA via MedWatch.

- An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity, expedited reporting is required. The clinical investigator must determine severity.

10.32 Special Situations for Expedited Reporting and submission of Notification Forms

Exceptions to Expedited Reporting and Submission of Notification Forms: EXPECTED Serious Adverse Events [1]

An expedited report or notification form may not be required for specific Serious Adverse Events where the AE is listed in Section 15.0 of the protocol as **EXPECTED**. Any protocol specific reporting procedures MUST BE SPECIFIED BELOW and will supercede the standard Expedited Adverse Event Reporting and Notification Form Requirements (see Footnote 1):

CTCAE System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be expeditedly reported
Blood and lymphatic system disorders	Anemia	≤Grade 4
	Febrile neutropenia	≤Grade 3
Investigations	Lymphocyte count decreased	≤Grade 4
	Neutrophil count decreased	≤Grade 4
	Platelet count decreased	≤Grade 4
	White blood cell decreased	≤Grade 4
Metabolism and nutrition disorders	Hyperglycemia	≤Grade 3
	Hyperkalemia	≤Grade 3
	Hyperuricemia	≤Grade 3
	Hypocalcemia	≤Grade 3
	Hypokalemia	≤Grade 3
	Hypophosphatamia	≤Grade 3
Vascular disorders	Hypertension	≤Grade 3

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.

Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

10.321 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital abnormities or birth defects, must be reported immediately if they occur at any time following treatment with an agent under an IND/IDE since they are considered to be a serious AE and must be reported to the sponsor as specified in 21 CFR 312.64(b).

10.322 **Death**

- Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

- Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND/IDE requires expedited reporting within 24-hours.

- **Reportable categories of Death**

  - Death attributable to a CTCAE term.

  - Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.

  - Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.

  - Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.

  - Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.323 **Secondary Malignancy**

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

- All secondary malignancies that occur following treatment with an agent under an IND/IDE be reported. Three options are available to describe the event:

  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])

- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.324 **Second Malignancy**

- A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting.

10.4 Expedited Reporting Requirements for IND/IDE Agents

10.41 Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the sponsor within the timeframes detailed in the table below.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour 3 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>7 Calendar Days</td></tr>
</table>

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in section 10.32 of the protocol.

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

Additional instructions:

1. Contact Novartis within 24 hours via email using the applicable SAE Cover Sheet (see Forms Packet)

2. Use Mayo Clinic Cancer Center SAE Reporting Form: http://livecycle2.mayo.edu/workspace/?startEndpoint=MC4158-56/Processes/MC4158-56-Process.MC4158-56.

   Provide documentation to the MCCC Regulatory Affairs Unit (RAU) Risk Information Specialist who will determine and complete IRB reporting. The RAU will submit to the MCCC SAE Coordinator and the MCCC IND Coordinator to determine if FDA submission is needed.

10.5 Other Required Reporting

10.51 Adverse events to be graded at each evaluation and pretreatment symptoms/conditions to be evaluated at baseline per the CTCAE v4.0 grading unless otherwise stated in the table below:

System Organ Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
Investigations	Electrocardiogram QT corrected interval prolonged	X	X
	Blood bilirubin increased	X	X
	Alanine aminotransferase increased	X	X
	Aspartate aminotransferase increased	X	X
	Lipase increased	X	X
Skin and subcutaneous tissue disorders	Rash maculo-papular	X	X

10.52 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.5. When assigning attribution category, the attribution refers to the relationship to Cytarabine, Daunorubicin or Nilotinib (not Stem Cell Transplant).

10.521 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure (not Stem Cell Transplant).

10.522 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure (not Stem Cell Transplant).

10.523 Grade 5 AEs (Deaths)

10.5231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.5232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.53 Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 Treatment Evaluation

11.1 Complete hematologic response (CR)
Less than 5% blasts in a non-hypocellular marrow with a granulocyte count ≥ 1.0, and a platelets count ≥ 100 of with complete resolution of any extramedullary disease and absence of peripheral blood blasts. The patient is in sustained CR if they have previously achieved a CR and continue to meet the CR criteria (at least 28 days).

CR incomplete (CRi) is called if patient meets all CR criteria except for residual neutropenia (ANC<1 $x10^9$/L) or thrombocytopenia (platelets<100 $x10^9$/L)

11.11 Complete cytogenetic remission (CCyR)
The absence of chromosome abnormalities (if present at diagnosis) on conventional cytogenetic study using G-banding (at least 10 metaphases present).

11.12 Complete molecular remission (CMR)
The achievement of negative RT-PCR for Kit mutation (if present at diagnosis).

11.13 MRD: Minimal Residual Disease

Positive PCR for Kit mutation or flow cytometry for Kit overexpression from peripheral blood or bone marrow.

11.2 Morphologic leukemia-free state (MLFS):
If bone marrow blasts <5%, absence of Auer rods blasts, absence of extramedullary disease without hematological recovery.

11.3 Partial remission (PR)
The presence of trilineage hematopoiesis in the bone marrow with recovery of ANC and platelet count to above levels, but with 5-25% bone marrow blasts and ≥50% decrease in bone marrow blast percentage from baseline.

11.4 No response (NR)
Failure to achieve a PR, MLFS, CRi, or CR.

11.5 Relapse:
Disease recurrence after achieving CR. Disease recurrence is defined by blast ≥5% in the bone marrow, or recurrence of peripheral blood blasts or extramedullary involvement.

11.6 Treatment failure:

Resistant disease: Failure to achieve CR or CRi by the end of 2 cycles of induction .

Note: These patients will be recorded as an objective status of MLFS, PR, or NR to document depth of response but will be considered treatment failures at the time of analysis.

Death in aplasia: Death occurring ≥ 7 days following completion of treatment with aplastic/hypoplastic bone marrow (obtained within 7 days of death) with no evidence of persistent leukemia

Death from indeterminate cause: Death occurring during therapy or < 7 days following treatment completion, or ≥ 7 days following completion of treatment with no blast in the blood but no bone marrow examination available

12.0 Descriptive Factors

12.1 Bone marrow Kit mutation by PCR at baseline: positive vs. negative

12.2 Bone marrow transplant candidate: Yes vs. No.

12.3 Bone marrow FLT3 mutation: positive vs. negative vs. not done

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Criteria for Patient Initiation of Event Monitoring:

- Delay > 56 days for the next cycle
- Disease relapse
- Failure to achieve CR or CRi during induction/reinduction
- Patient withdraws consent to continue in the trial
- Patient develops an intercurrent illness that precludes further participation
- The Investigator withdraws the patient in the patient's best interests
- Administrative reasons (e.g., the patient is transferred to hospice care)
- An adverse event, which in the opinion of the Investigator, precludes further trial participation

All attempts should be made to complete the End of Study procedures if a patient withdraws from the trial early. The patient will go to the event-monitoring phase per Section 18.0.

13.2 Patients who achieve and sustain a CR or CRi will receive a maximum total of 13 cycles or 14 cycles for patients who received re-induction:

- If the patient has not achieved a complete response (CR or CRi) after 2 cycles of induction treatment, the patient should go to event monitoring
- The patient must have a sustained complete response (CR or CRi) after consolidation to initiate the maintenance phase; otherwise the patient should go to event monitoring
- Patients who complete all cycles of treatment will go to event monitoring

13.3 Event monitoring: Patients who are alive and recurrence-free will be followed in event monitoring every 6 months until disease recurrence. After disease recurrence, the patient will be followed in event monitoring every 6 months until death or up to 3 years from registration.

13.4 A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. The patient will go directly to the event-monitoring phase of the study (or off study, if applicable).

- If the patient received treatment, all data up until the point of confirmation of ineligibility must be submitted. Event monitoring will be required per Section 18.0 of the protocol.
- If the patient never received treatment, on-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

13.5 A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary end point is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go directly to the event-monitoring phase of the study. The patient may continue treatment off-protocol at the discretion of the physician as long as there are no safety concerns, and the patient was properly registered. Event monitoring will be required per Section 18.0 of the protocol.

13.6 A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood and Body Fluid Specimens to be collected for this Protocol

	Mandatory (M) or Optional (O)	**Blood or Body Fluid being Collected**	**Type of Collection Tube (color of tube top)**	**Volume to collect per tube (# of tubes to be collected)**	**Screening**	**Cycle 1 Day 4 (pre-nilotinib treatment, 30 mins pre-dosing)**	**Cycle 1 Day, 8, 14 and upon bone marrow recovery(30 mins pre-dosing)**	**Process at site? (Yes or No)**	**Temperature Conditions for Storage /Shipping**
Serum nilotinib level- See sect. 14.31	M	Blood	Serum Separator Vacutainer™ tube (SST)	3 ml		yes	yes[1]	yes	Stored frozen at or below -20 C until shipped.
Correlative studies - See sect. 14.32	M	Blood	BD Vacutainer EDTA 10ml 366643	30-40 ml in total	yes	yes	yes	no	Ship right away away (ice bags +4C) or pick up, room temperature
Correlative studies - See sect. 14.32	M	Bone marrow aspirate	BD Vacutainer EDTA 10ml 366643	20-30 ml in total	yes		yes[2]	no	Ship right away away (ice bags +4C) or pick up, room temperature
Biomarker-Kit mutation-- See sect. 14.33	M	Bone marrow aspirate	BD Vacutainer EDTA	3 ml minimum	yes	no	no	no	Pick up, room temperature

1. Days 4, 8 and 14 only, pre-nilotinib treatment. (30 mins pre-dosing)
2. Day 14 and upon bone marrow recovery only

When possible, samples should be obtained prior to the nilotinib dose. The exact time since the last dose should be documented. For the correlative studies, blood samples should be collected at screening, pre-treatment (day 4), day 8 day 14) and upon marrow recovery. For the correlative studies, bone marrow aspirate samples should be collected at screening, day 14, and day upon marrow recovery.

14.2 Collection and Processing

14.21 Serum Nilotinib level (PK study)

For the determination of nilotinib concentrations, 3 mL of whole blood will be drawn at each time point using a Serum Separator (SST) VacutainerTM tube. The tube will be placed vertically (avoiding prolonged contact with the rubber stopper) for about 30 minutes at room temperature and will then be centrifuged at about 5 ºC for 10 minutes at approximately 1100 x g. This should be done within 60 minutes of drawing the sample. Immediately after centrifugation, the upper serum sample (about 1.5 mL) will be transferred into a 2-mL, tapered, polypropylene screw-cap tube and stored frozen at ≤ -20 ºC until shipped (on dry-ice) to the analytical site for sample analyses.
Document the time since last nilotinib dose on the sample tube.

14.22 Correlative studies: Compare the pre-treatment with post-treatment samples for the alteration of nilotinib's direct and potential-indirect targets:

1) Manley et al (2010) demonstrated that nilotinib has inhibitory effect on c-Kit, the direct measurement of c-Kit kinase activities by Western blot, will be performed, including c-Kit phosphorylation, and its downstream effectors (e.g. AKT, ERK, STAT5);

2) Because the target patients are c-Kit overexpression that critically drives leukemogenesis (Liu et al, 2010), the expression of c-Kit mRNA and total protein will be examined by PCR and Western blot;

3) We demonstrated that c-Kit gene is regulated by Sp1 (Liu et al, 2010), therefore, Sp1 expression will be measured by PCR and Western blot;

4) Since Sp1 is also a positive regulator of DNA methyltransferases (DNMT1, DNMT3a and DNMT3b), the expression of DNMTs will be investigated;

5) If the results from "4)" are positive, the global DNA methylation will be studied, since DNA methylation is under control of DNMTs;

6) Recent reports showed that Flt3 gene regulation is controlled by Sp1/NFkB complex (Blum et al, 2012), therefore, Flt3 expression and activities will be examined if the above "Sp1" studies are positive.

14.3 Shipping and Handling

14.31 **Kits will not be used for this study.**

All serum nilotinib specimens must be shipped Monday – Wednesday ONLY.

Please batch samples every six months and ship on dry ice in cardboard boxes with a sample list. Label the individual samples with the study number MC1284 and the unique study identification number for the patient

Pharmacokinetics will be performed at the Clinical Pharmacology Analytical Facility at the University of Pittsburgh Cancer Institute by [redacted]. Please ship to the address below.

[redacted]

Contact information is listed below.

[redacted]

14.32 Correlative studies will be performed at Hormel Institute, University of Minnesota, by [redacted]. Samples can be shipped Monday-Thursday. Samples that come in on Friday will be refrigerated and shipped on the following Monday. Label the individual samples with the study number MC1284 and the unique study identification number for the patient.

Please ship to the address below.

[redacted]

Contact information is below.

[redacted]

14.33 Kit analysis gene sequencing will be performed at the Mayo Clinic in Rochester, MN by [redacted] lab contact information is below.

[redacted]

14.34 Handling Specimens

After receipt by the analytical laboratory, samples will be stored at -20 °C or less until analysis.

14.4 Background and Methodology

14.41 Nilotinib concentrations will be quantitated with a validated LC-MS/MS assay as published (J Chrom B 2009; 877(20-21): 1894-900), or a modification thereof, if required.

14.42 Both KIT and FLT3 kinase activities will be assessed by PCR and Western blot. The targets include the autophosphorylation and protein expression of FLT3 and KIT, the downstream effectors, like AKT, ERK, STAT5, STAT3, both phosphorylation and gene expression.

14.43 Cytokine profiling in serum by human cytokine array kit. This study is based on the recent report showing that cytokine abnormalities contribute to the nilotinib-dependent resistance in vitro (Klag T et al, 2012). We can make a good use of the collected serum.

14.44 Characterization of Sp1/microRNA (miR) 29b network by PCR or western blot, because our previous report showed that Sp1/miR29b network regulates both DNMT and KIT (Liu et al, Blood, 2008; Garzon and Liu et al, Blood, 2009; Liu et al, Cancer Cell, 2010).

15.0 Drug Information

15.1 Nilotinib (Tasigna®, AMN107)

Note: As per Nilotinib IB Edition 13

15.11 **Background**: Nilotinib is a protein tyrosine kinase (TK) inhibitor, which selectively inhibits the kinase activity of ABL and BCR-ABL, the sterile alpha motif and leucine zipper containing kinase (ZAK), the platelet-derived growth factor receptor (PDGFR), the stem cell factor receptor (KIT), the colony stimulating factor receptor (CSF-1R), the discoidin domain receptors (DDR) and of several ephrin receptors (e.g. EPHB4), and blocks the downstream cellular events mediated by these enzymes.

Nilotinib is currently approved for the treatment of adult patients with Philadelphia chromosome positive (Ph+) chronic myeloid leukemia (CML) in chronic phase (CP) and accelerated phase (AP) resistant to or intolerant to at least one prior therapy including imatinib and for the treatment of adult patients with newly diagnosed Ph+ CML in CP.

Age is not expected to have an effect on the PK in the age range of 15-85 years. In the pediatric population (age 1 to < 18 years), PK exposure in pediatric patients dosed at 230 mg/m^2 twice daily was comparable to the reference data for nilotinib 400 mg BID in adult Ph+ CML patients. No significant effect of ethnicity and gender on dose-adjusted exposure of nilotinib was observed. Following multiple oral doses of nilotinib, steady state concentrations were achieved as early as Cycle 1 Day 8 across the disease cohorts and age groups (2 to 12 years, 12 to <18 years). The exposure of nilotinib after single dose/multiple doses was comparable across the disease cohorts, within the respective age groups. In addition, a flat exposure-response relationship was observed for efficacy (BCRABL/ABL% ratio and MMR) and incidence of adverse events (adverse events of special interest and/or hepatic abnormalities). This would suggest that the PK fluctuations at the dose of 230 mg/m^2 bid in the age range of 1-18 years would be too small to affect response and, therefore, that dose and dosage regimen is appropriate. With regards to QT prolongation, QTcF and QTcB prolongation potential was observed with increasing exposure levels and at the median observed exposure for 230 mg/m^2 bid. No significant exposure-related changes were observed in the ECG parameters PR, QRS, and heart rate. This is consistent with the already well known safety profile of adult patients treated with nilotinib.

15.12 **Formulation**: The drug product is formulated as an immediate release (IR) solid oral dosage form (hard capsule). The HGCs contain the active drug substance nilotinib as the hydrochloride monohydrate salt at dosage strengths of 50 mg, 150 mg and 200 mg. The 150 mg and 200 mg capsules strengths are currently

approved and marketed. Dosage strengths correspond to the free base. The inactive ingredients are colloidal silicon dioxide (silica, colloidal anhydrous), crospovidone, lactose monohydrate, magnesium stearate, and poloxamer.

15.13 **Preparation and storage**: Do not store nilotinib capsules above 30°C (86°F).

15.14 **Administration:** Nilotinib should be taken twice daily approximately 12 hours apart and must not be taken with food. The capsules should be swallowed whole with water. For patients who are unable to swallow capsules, the content of each capsule may be dispersed in one teaspoon of applesauce (pureed apple) and should be taken immediately. Not more than one teaspoon of applesauce and no food other than applesauce must be used. Food increases blood levels of nilotinib. No food should be consumed for 2 hours before the dose is taken and no food should be consumed for at least one hour after the dose is taken. Due to possible occurrence of tumor lysis syndrome (TLS), correction of clinically significant dehydration and treatment of high uric acid levels are recommended prior to initiating therapy with nilotinib.

15.15 **Pharmacokinetic information**:
a) **Absorption** – The extent of nilotinib absorption following oral administration of drug was estimated to be approximately 30%. The bioavailability of nilotinib was increased when given with a meal. Compared to the fasted state, the systemic exposure (AUC) increased by 82% when the dose was given 30 minutes after a high fat meal.
b) **Distribution:** The extent of nilotinib binding to human plasma was high (98% on average), and independent of concentration tested.
c) **Metabolism**: Nilotinib is metabolized in the liver via oxidation and hydroxylation pathways, mediated primarily by CYP3A4. Nilotinib was identified as the main circulating component in the serum, while none of the metabolites was found to contribute significantly to the pharmacological activity of nilotinib.
Hepatic impairment has a modest effect on the pharmacokinetics of nilotinib. A lower starting dose is recommended for patients with mild to severe hepatic impairment (at baseline) and QT interval should be monitored closely.
d) **Excretion** – Primarily as metabolites: Feces (93%; 69% as unchanged drug). The average elimination half-life of nilotinib is 17 hours. Steady state conditions were achieved by Day 8.

15.16 **Potential Drug Interactions**:
Nilotinib is a competitive inhibitor of CYP3A4, CYP2C8, CYP2C9, CYP2D6 and UGT1A1 in vitro, potentially increasing the concentrations of drugs eliminated by these enzymes. In vitro studies also suggest that nilotinib may induce CYP2B6, CYP2C8 and CYP2C9, and decrease the concentrations of drugs which are eliminated by these enzymes.

Single-dose administration of nilotinib with midazolam (a CYP3A4 substrate) to healthy subjects increased midazolam exposure by 30%. Single-dose administration of nilotinib to healthy subjects did not change the pharmacokinetics and pharmacodynamics of warfarin (a CYP2C9 substrate). The ability of nilotinib to induce metabolism has not been determined in vivo. Exercise caution when co-administering nilotinib with substrates for these

enzymes that have a narrow therapeutic index.

Nilotinib undergoes metabolism by CYP3A4, and concomitant administration of strong inhibitors or inducers of CYP3A4 can increase or decrease nilotinib concentrations significantly. The administration of nilotinib with agents that are strong CYP3A4 inhibitors should be avoided. Concomitant use of nilotinib with medicinal products and herbal preparations 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 has pH-dependent solubility, with decreased solubility at higher pH. Drugs such as proton pump inhibitors that inhibit gastric acid secretion to elevate the gastric pH may decrease the solubility of nilotinib and reduce its bioavailability. Increasing the dose of nilotinib when co-administered with such agents is not likely to compensate for the loss of exposure. Since proton pump inhibitors affect pH of the upper GI tract for an extended period, separation of doses may not eliminate the interaction. The concomitant use of proton pump inhibitors with nilotinib is not recommended. When the concurrent use of a H2 blocker is necessary, it may be administered approximately ten hours before and approximately two hours after the dose of nilotinib. If necessary, an antacid (aluminum hydroxide/magnesium hydroxide/simethicone) may be administered approximately two hours before or approximately two hours after the dose of nilotinib.

Nilotinib inhibits human P-glycoprotein (P-gp). If nilotinib is administered with drugs that inhibit P-gp, increased concentrations of nilotinib are likely, and caution should be exercised.

The administration of nilotinib with agents that may prolong the QT interval, such as anti-arrhythmic medicines or serotonin receptor antagonists, should be avoided. Data suggests nilotinib has the potential to prolong cardiac ventricular repolarization (QT interval). This clinically meaningful prolongation of the QT interval may also occur when nilotinib is inappropriately taken with food and/or strong CYP3A4 inhibitors. The presence of hypokalemia and hypomagnesemia may place patients at risk of developing QT prolongation.

The effects of nilotinib on the pharmacokinetics of valsartan were assessed in a healthy volunteer study [CAMN107A2132]. The results showed limited effects of nilotinib on valsartan PK which indicates that nilotinib does not inhibit OATP1B1 (organic anion transporting polypeptide 1B1) uptake transporter in a competitive and time-dependent manner.

There is an ongoing study to evaluate the effects of nilotinib on the pharmacokinetics of valsartan in healthy volunteers [CAMN107A2132]. This study is intended to provide further clarification about possible nilotinib-mediated OATP1B1 (organic anion transporting polypeptide 1B1) inhibition.

Food Effect: The bioavailability of nilotinib is increased with food. Nilotinib must not be taken with food. No food should be taken at least 2 hours before and at least one hour after the dose is taken. Grapefruit products and other foods that

are known to inhibit CYP3A4 should be avoided.

15.17 **Known potential toxicities**: Consult the Investigator's Brochure for the most current and complete information.

Important Safety Information including Boxed WARNING: QT PROLONGATION AND SUDDEN DEATHS. Nilotinib prolongs the QT interval. Prior to nilotinib administration and periodically, monitor for hypokalemia or hypomagnesemia and correct deficiencies. Obtain ECGs to monitor the QTc at baseline, seven days after initiation, and periodically thereafter, and follow any dose adjustments. Sudden deaths have been reported in patients receiving nilotinib. Do not administer to patients with hypokalemia, hypomagnesemia, or long QT syndrome. Avoid use of concomitant drugs known to prolong the QT interval and strong CYP3A4 inhibitors. Patients should avoid food 2 hours before and 1 hour after taking dose.

Patients should be tested for hepatitis B infection before initiating treatment with nilotinib, as 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). Experts in liver 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 hepatitis B infection during treatment. Carriers of hepatitis B virus 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.

Very Common known potential toxicities, > 10%:
Central nervous system: Headache
Dermatologic: Rash (Includes the following PTs: Rash pustular, Genital rash, Miliaria, Rash, Rash erythematous, Rash follicular, Rash generalized, Rash macular, Rash maculo-papular, Rash papular and Rash pruritic), pruritus, alopecia
Gastrointestinal: Nausea, constipation, diarrhea, vomiting, upper abdominal pain
General disorders: Fatigue
Hematologic: Neutropenia, thrombocytopenia, anemia, myelosuppression
Hepatobiliary: hyperbilirubinemia
Investigations: ALT increased, AST increased, lipoprotein cholesterol increased, total cholesterol increased, lipase increased, blood triglycerides increased
Musculoskeletal and connective tissue disorders: Musculoskeletal pain upon discontinuation (Including myalgia, pain in extremity, arthralgia, bone pain, spinal pain) , myalgia
Metabolism and nutrition disorders: hypophosphatemia
Psychiatric disorders: Insomnia
Respiratory: Cough, oropharyngeal pain, dyspnea
Vascular disorders: Hypertension

Common known potential toxicities, 1% - 10%:
Cardiovascular: Angina pectoris, arrhythmia (including atrioventricular block, cardiac flutter, extrasystoles, atrial fibrillation, tachycardia, bradycardia),

palpitations, electrocardiogram QT prolonged, cardiac murmur
Dermatologic: Hyperhidrosis, eczema, urticaria, erythema, dermatitis, contusion, acne
Ear and Labyrinth: Vertigo
Eye disorders: eye hemorrhage, vision impairment, periorbital edema, eye pruritus, conjunctivitis, dry eye (including xerophthalmia)
Gastrointestinal: Abdominal discomfort, abdominal pain, flatulence, abdominal distension, pancreatitis, dysgeusia, dyspepsia, gastroesophageal reflux, stomatitis
General disorders and administrative site conditions: pyrexia, asthenia, peripheral edema, facial edema, chest pain or discomfort, pain, malaise, feeling body temperature changes (hot or cold)
Hematologic: Febrile neutropenia, pancytopenia, lymphopenia, eosinophilia, leukopenia
Hepatobiliary: hepatic function abnormal
Infections and infestations: Folliculitis, upper respiratory infection, nasopharyngitis, pharyngitis, rhinitis, pneumonia
Investigations: Hemoglobin decreased, blood amylase increased, gamma-glutamyltransferase increased, weight decreased, weight increased, blood alkaline phosphatase increased, blood creatine increased, , globulins decreased
Metabolism and Nutrition: Decreased appetite, electrolyte imbalance (including hyperkalemia, hypokalemia, hyponatremia, hypocalcemia, hypercalcemia, hyperphosphatemia, hypomagnesemia), hyperglycemia, diabetes mellitus, hyperlipidemia, hypercholesterolemia
Musculoskeletal: Musculoskeletal chest pain, musculoskeletal pain, neck pain, back pain, joint swelling, bone pain, pain in extremities, muscular weakness, arthralgia, muscle spasms
Neoplasms: Skin papilloma
Nervous system disorders: dizziness, peripheral neuropathy, hypoesthenia, hyperesthesia, paraesthenia, tremor, intracranial hemorrhage
Psychiatric disorders: depression, insomnia, anxiety
Renal: Pollakuria, nocturia
Reproductive system and breast disorders: Gynecomastia
Respiratory: Epistaxis, oropharyngeal pain, pleural effusion dysphonia, dyspnea (and exertional), cough
Skin and Subcutaneous Tissue Disorders: Dry skin, night sweats, eczema, urticaria, erythema, hyperhidrosis, confusion, acne, dermatitis (including allergic, exfoliative, and acneiform), skin pain
Vascular: Flushing, hypertension, peripheral arterial occlusive disease, peripheral artery stenosis

Uncommon, Rare, and Very Rare, less than 1% (limited to important or life-threatening):
Cardiovascular: Myocardial infarction, pericarditis, ventricular dysfunction, ejection fraction decrease, cardiac failure, pericardial effusion, coronary artery disease, cyanosis,
Central nervous system: Confusional state, disorientation, amnesia, dysphoria, ischemic stroke, transient ischemic attack, cerebral infarction, cerebrovascular accident, migraine, loss of consciousness (including syncope), disturbance in attention, hyperesthesia, brain edema, optic neuritis, lethargy, dysesthesia, restless legs syndrome
Dermatologic: Exfoliative rash, drug eruption, swelling face, ecchymosis,

psoriasis, erythema multiforme, erythema nodosum, skin ulcer, petechiae, photosensitivity, blister, dermal cyst, sebaceous hyperplasia, skin atrophy, skin discoloration, skin exfoliation, skin hyperpigmentation, skin hypertrophy, hyperkeratosis, palmar-plantar erythrodysesthesia syndrome
Endocrine: Hyperthyroidism, hypothyroidism, hyperparathyroidism secondary, thyroiditis
Eye Disorders: vision blurred, visual acuity reduced, eyelid edema, photopsia, hyperemia, eye irritation, conjunctival hemorrhage, papilloedema, diplopia, photophobia, eye swelling, blepharitis, eye pain, chorioretinopathy, conjunctivitis allergic, ocular surface disease
Gastrointestinal: Gastrointestinal hemorrhage, melena, mouth ulceration, esophageal pain, dry mouth, gastric ulcer, gastrointestinal ulcer perforation, retroperitoneal hemorrhage, hematemesis, esophagitis ulcerative, gastritis, subileus, enterocolitis, hemorrhoids, hiatus hernia, rectal hemorrhage, sensitivity of teeth, non-infective gingivitis
General: Gravitational edema, influenza-like illness, chills, localized edema, face edema, fluid retention
Hematologic: Thrombocythemia, leukocytosis
Hepatobiliary: Toxic hepatitis, jaundice, cholestasis, hepatotoxicity, hepatomegaly
Immune System: Hypersensitivity
Infections and infestations: Urinary tract infection, gastroenteritis, sepsis, bronchitis, candidiasis, subcutaneous abscess, anal abscess, furuncle, tinea pedis, herpes virus infection, hepatitis B reactivation
Investigations: Blood lactate dehydrogenase increased, blood bilirubin unconjugated increased, blood urea increased, troponin increased, blood insulin decreased, blood insulin increased, insulin C-peptide decreased, blood parathyroid increased
Metabolism and Nutrition: Increased appetite, dehydration, hyperuricemia, gout, hypoglycemia, dyslipidemia, hypertriglyceridemia
Musculoskeletal: Musculoskeletal stiffness, arthritis, flank pain
Neoplasms: Oral papilloma, paraproteinemia
Otic: Hearing impaired, ear pain, tinnitus
Renal: Dysuria, micturition urgency, renal failure, hematuria, urinary incontinence, chromaturia
Reproductive System and Breast: Breast pain, erectile dysfunction, breast induration, menorrhagia, nipple swelling
Respiratory: Pharyngolaryngeal pain, interstitial lung disease, pleuritic pain, pleurisy, pulmonary edema, throat irritation, pulmonary hypertension, wheezing
Vascular: Hypertensive crisis, hematoma, shock hemorrhagic, hypotension, thrombosis, arteriosclerosis, intermittent claudication, arterial stenosis limb, arterial occlusive disease, peripheral ischemia

Other Potential Toxicities:

Pregnancy: There were reports of congenital anomalies in babies, reports of premature babies, and reports of neonatal deaths. Nilotinib should not be used during pregnancy. Women of childbearing potential should be informed of the potential risk to the fetus and must be advised to use highly effective contraception during treatment with nilotinib for up to 2 weeks after ending treatment. Patients using an oral hormonal contraception method should

complete their monthly treatment course.Lactation: Excretion of nilotinib in breast milk has not been studied in humans, but is excreted in animal breast milk. Women should not breast-feed while taking nilotinib, as a risk to the infant cannot be excluded.

Ischemic vascular or ischemic cardiovascular events: Newly diagnosed or worsened Ischemic Vascular and Ischemic Cardiovascular Events such as Ischemic Heart Disease (IHD), Ischemic Cerebrovascular Event (ICVE) or Peripheral Artery Occlusive Disease (PAOD) have occurred in a relatively small number of CML chronic phase patients treated with nilotinib. The majority of reported events were in patients with associated risks.

15.18 **Drug procurement**
Drug will be provided free of charge to study participants by Novartis Pharmaceuticals.

15.19 Nursing Guidelines

15.191 Patients should take nilotinib twice daily approximately 12 hours apart on an empty stomach (no food 2 hours prior or 1 hour after a dose) with water. Capsules may be dispersed in one teaspoon of applesauce (pureed apple) and should be taken immediately. Do not use more than one teaspoon of applesauce and no food other than applesauce must be used.

15.192 Nilotinib is known to prolong the QT interval. Patients with a history of long QT syndrome should not receive nilotinib. Sudden death has been reported in patients receiving nilotinib. Assess patient's concomitant medications, for other medications that may prolong the QT interval. These medications should be avoided. Patients should undergo a baseline and 7 days post initiation ECG to assess the QTc and periodically thereafter.

15.193 Nilotinib is known to have significant drug-drug interactions with strong CYP3A4 inducers and should be avoided. Additionally, caution should be used when administered with drugs that inhibit P-glycoprotien as this can increase the levels of nilotinib. Assess patients concomitant medications, over the counter, and herbal supplements, avoid those that may interact with nilotinib.

15.194 Patients should avoid the use of protein pump inhibitors, if absolutely necessary, use with caution. If necessary to administer with an H2 blocker or antacid, doses should be separated from the nilotinib by at least 2 hours.

15.195 May cause headache. Treat symptomatically and monitor for effectiveness.

15.196 Rash and itching may occur. Instruct patients to report rash to study team. Treat symptomatically and assess for effectiveness.

15.197 GI disturbances are common (nausea, constipation, diarrhea, vomiting). Assess patients for need of premedication prior to nilotinib and/or treat symptomatically and monitor for effectiveness.

15.198 Monitor electrolytes (especially potassium and magnesium). Do not administer nilotinib to patients with hypokalemia and/or hypomagnesemia. These electrolyte disturbances should be corrected prior to initiating therapy and monitored periodically.

15.199a Monitor LFT's. Patients with elevated LFT's may need dose reductions.

15.199b Monitor CBC. Cytopenias are common. Instruct patient to report any signs or symptoms of infection, unusual bruising or bleeding to the study team.

15.199c Instruct patient to report any rash to the study team.

15.199d Headache is commonly seen. Treat patient symptomatically and monitor for effectiveness.

15.199e Monitor for eye disorders (hemorrhage, periorbital edema, itching, conjunctivitis and dryness). Instruct patients to report these symptoms to study team. Refer to Ophthalmology as necessary.

15.199f Hypertension can be seen.

15.199g Patients should undergo testing for Hepatitis B, as a reactivation of Hepatitis B can occur.

15.999h Tumor lysis syndrome can occur, correction of clinically significant dehydration and treatment of high uric acid levels should occur prior to initiation of therapy.

15.2 **Daunorubicin**

15.21 **Background**: Daunorubicin inhibits DNA and RNA synthesis by intercalation between DNA base pairs and by steric obstruction. Daunorubicin intercalates at points of local uncoiling of the double helix. Although the exact mechanism is unclear, it appears that direct binding to DNA (intercalation) and inhibition of DNA repair (topoisomerase II inhibition) result in blockade of DNA and RNA synthesis and fragmentation of DNA.

15.22 **Formulation**: Commercially available for injection as:
Injection, powder for reconstitution: 20 mg
Injection, solution: 5 mg/mL (4 mL, 10 mL)

15.23 **Preparation, storage, and stability**: Refer to package insert for complete preparation and dispensing instructions. Store intact vials of powder for injection at room temperature of 15°C to 30°C (59°F to 86°F); intact vials of solution for injection should be refrigerated at 2°C to 8°C (36°F to 46°F). Protect from light. Dilute vials of powder for injection with 4 mL SWFI for a final concentration of 5 mg/mL. May further dilute in 100 mL D5W or NS. Reconstituted solution is

stable for 4 days at 15°C to 25°C. Further dilution in D5W, LR, or NS is stable at room temperature (25°C) for up to 4 weeks protected from light.

15.24 **Administration:** Refer to the treatment section for specific administration instructions. Daunorubicin is not for I.M. or SubQ administration. Administer as a slow I.V. push over 1-5 minutes into the tubing of a rapidly infusion I.V. solution of D5W or NS or dilute in 100 mL of D5W or NS and infuse over 15-30 minutes.

15.25 **Pharmacokinetic information**:
Distribution: V_d: 40 L/kg; to many body tissues, particularly liver, spleen, kidney, lung, heart; does not distribute into the CNS; crosses placenta
Protein binding, plasma: 70% to 76%
Metabolism: Primarily hepatic to Daunorubicinol (active), then to inactive aglycones, conjugated sulfates, and glucuronides
Half-life elimination:
Distribution: 2 minutes
Elimination: 14-20 hours
Terminal: 18.5 hours
Daunorubicinol plasma half-life: 24-48 hours
Excretion: Feces (~40%); urine (~25% as unchanged drug and metabolites)

15.26 **Potential Drug Interactions**:
Avoid Concomitant Use of Daunorubicin with any of the following: BCG; Natalizumab; Pimecrolimus; Tacrolimus (Topical); Vaccines (Live).
Increased Effect: Daunorubicin may increase the levels/effects of: Leflunomide; Natalizumab; Vaccines (Live).

The levels/effects of Daunorubicin may be increased by: Bevacizumab; Denosumab; P-Glycoprotein inhibitors; Pimecrolimus; Tacrolimus (Topical); Taxane Derivatives; Trastuzumab

Decreased Effect: Daunorubicin may decrease the levels/effects of: BCG; Cardiac Glycosides; Sipuleucel-T; Vaccines (Inactivated); Vaccines (Live)

The levels/effects of Daunorubicin may be decreased by: Cardiac Glycosides; Echinacea; P-Glycoprotein Inducers

Ethanol/Herb/Nutraceutical Interactions: Avoid ethanol (Due to GI irritation).

15.27 **Known potential adverse events:** Consult the package insert for the most current and complete information.

Common known potential toxicities, > 10%:
Cardiovascular: Transient ECG abnormalities (supraventricular tachycardia, S-T wave changes, atrial or ventricular extrasystoles); generally asymptomatic and self-limiting. CHF, dose related, may be delayed for 7-8 years after treatment.
Dermatologic: Alopecia (reversible), radiation recall
Gastrointestinal: Nausea, vomiting, stomatitis
Genitourinary: Discoloration of urine (red)
Hematologic: Leukopenia, thrombocytopenia, anemia

Less common known potential toxicities, 1% - 10%:
Dermatologic: Skin "flare" at injection site; discoloration of saliva, sweat, or tears
Endocrine & metabolic: Hyperuricemia
Gastrointestinal: Abdominal pain, GI ulceration, diarrhea

Rare known potential toxicities, <1% (Limited to important or life-threatening):
Anaphylactoid reaction, bilirubin increased, hepatitis, infertility, cellulitis, pain at injection site, thrombophlebitis at injection site, MI, myocarditis, nail banding, onycholysis, pericarditis, pigmentation of nailbeds, secondary leukemia, skin rash, sterility, systemic hypersensitivity (including urticaria, pruritus, angioedema, dysphagia, dyspnea); transaminases increased.

15.28 **Drug procurement:** Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.29 **Nursing Guidelines:**

15.291 Drug is a potent vesicant. Establish patency of IV before and frequently during administration as to avoid extravasation. If drug is being administered IV push administer through side port of rapidly running IV solution. If suitable vein cannot be used, central venous access may become necessary. Discuss with treating MD. If extravasation occurs treat per your institution's policy.

15.292 Drug is not compatible with heparin. Be sure to flush heparin locked IV's or central lines before administering agent.

15.293 Moderate to severe nausea occurs in up to 50% of patient within first 24 hours. Administer antiemetics as ordered and assess for their effectiveness.

15.294 Inform patient about alopecia

15.295 Potent myelosuppressive agent. Monitor CBC closely. Inform patient to report any signs or symptoms of infection, or unusual bruising or bleeding.

15.296 Drug is cardiotoxic. Dose limit at 550mg/m2. Monitor for signs of acute cardiac toxicity, which is possible within hours after administration. This is unrelated to cumulative dose and may manifest symptoms of pump or conduction dysfunction. Watch for signs or symptoms of CHF, pericardial effusion, and transient ECG abnormalities.

15.297 Monitor liver function tests. Dose reduction is necessary in patients with impaired liver function.

15.298 Radiation recall is a possibility with this drug. Monitor patient's skin at

site of previous irradiation for damage.

15.299 Inform patient that urine may be pink or red for up to 48 hours after administration.

15.3 Cytarabine (Cytosar, ARAC)

15.31 **Background**: Cytarabine (cytosine arabinoside) inhibits DNA synthesis. Cytosine gains entry into cells by a carrier process, and then must be converted to its active compound, aracytidine triphosphate. Cytosine is a pyrimidine analog and is incorporated into DNA; however, the primary action is inhibition of DNA polymerase resulting in decreased DNA synthesis and repair. Cytarabine is specific for the S phase of the cell cycle (blocks progression from the G_1 to the S phase).

15.32 **Formulation**: Commercially available for injection, powder for reconstitution: 100 mg, 500 mg, 1 gram, 2 gram

15.33 **Preparation, storage, and stability**: Store intact vials of powder at room temperature 15ºC to 30ºC (59ºF to 86ºF). Reconstitute with bacteriostatic water for injection. Reconstituted solutions are stable for up to 8 days at room temperature, although the manufacturer recommends use within 48 hours. Further dilution in 250-1000 mL of D5W or 0.9% NaCL is stable for 8 days at room temperature (25C). Note: Solutions containing bacteriostatic agents should not be used for the preparation of either high doses or intrathecal doses of cytarabine.

15.34 **Administration:** Refer to the drug treatment section of the protocol for specific administration directions and infusion rates.

15.35 **Pharmacokinetic information**:
Distribution: V_d: Total body water; widely and rapidly since it enters the cells readily; crosses blood-brain barrier with CSF levels of 40% to 50% of plasma level
Metabolism: Primarily hepatic; metabolized by deoxycytidine kinase and other nucleotide kinases to aracytidine triphosphate (active); about 86% to 96% of dose is metabolized to inactive uracil arabinoside.
Half-life elimination: I.V.: Initial: 7-20 minutes; Terminal: 1-3 hours.
Excretion: Urine (~80%) within 24 hours

15.36 **Potential Drug Interactions**:
Decreased Effect: Cytarabine may decrease the effect of Flucytosine; cytarabine may decrease digoxin absorption.

15.37 **Known potential adverse events:** Consult the package insert for the most current and complete information.
Warnings/Precautions: Potent Myelosuppressive agent
Common known potential toxicities, frequency not defined:
Central nervous system: Fever
Dermatologic: Rash
Gastrointestinal: Anal inflammation, anal ulceration, anorexia, diarrhea, mucositis, nausea, vomiting

Hematologic: Myelosuppression, neutropenia, anemia, thrombocytopenia, bleeding, leukopenia, megaloblastosis, reticulocytes decreased
Hepatic: Hepatic dysfunction, transaminases increased (acute)
Local: Thrombophlebitis

Less common known potential toxicities:
Cardiovascular: Chest pain, pericarditis
Central nervous system: Dizziness, headache, neural toxicity, neuritis
Dermatologic: Alopecia, pruritus, skin freckling, skin ulceration, urticaria
Gastrointestinal: Abdominal pain, bowel necrosis, esophageal ulceration, esophagitis, pancreatitis, sore throat
Genitourinary: Urinary retention
Hepatic: Jaundice
Local: Injection site cellulitis
Ocular: Conjunctivitis
Renal: Renal dysfunction
Respiratory: Dyspnea
Miscellaneous: Allergic edema, anaphylaxis, sepsis

Infrequent and/or case reports:
Amylase increased, aseptic meningitis, cardiopulmonary arrest (acute), cerebral dysfunction, cytarabine syndrome (bone pain, chest pain, conjunctivitis, fever, maculopapular rash, malaise, myalgia); exanthematous pustulosis, hyperuricemia, injection site inflammation (SubQ injection), injection site pain (SubQ injection), interstitial pneumonitis, lipase increased, paralysis, rhabdomyolysis, veno-occlusive liver disease

15.38 **Drug procurement:** Commercial supplies. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler.

15.39 **Nursing Guidelines:**

15.391 Can be a potent myelosuppressive agent. Monitor CBC closely. Anemia, leukopenia, and thrombocytopenia are expected. Nadir within 5-7 days with recovery expected in 2-3 weeks. Hematological toxicity is more intense if ARA-C is given as a continuous IV infusion versus an IV bolus.

15.392 Nausea and vomiting is dose-related, common, and often preventable with antiemetic drugs. Administer as ordered and assess for their effectiveness.

15.393 Instruct patient of possibility of metallic taste. The use of sugarless hard candies may lessen this effect.

15.394 Stomatitis is possible. Instruct patient not to swallow.

15.395 May cause pancreatitis or peritonitis. Instruct patient to report any severe or worsening abdominal pain immediately.

15.396 Monitor LFT's-elevations of LFT's may occur.

15.397 Cerebellar toxicity can occur in 16-40% of patients with more severe symptoms at higher doses. Monitor for these toxicities with each dose of ARA-C. Signs and symptoms of cerebellar toxicity can include: lethargy with progressive confusion, ataxia, nystagmus, slurred speech. Report any of these or other neurological changes to the MD immediately.

15.398 Instruct patient to report any shortness of breath or difficulty breathing as this may be a sign of a rare but life threatening pulmonary complication.

15.399a Monitor for signs of conjunctivitis and/or keratitis. This is usually prevented with prophylactic glucocorticoid eye drops.

15.399b Assess for skin rash. This may present itself as a erythema without exfoliation, or a generalized rash. Report to MD.

15.399c Monitor for "ARA-C" syndrome. This is characterized by bone and muscle pain, chest pain, fever, general weakness, reddened eyes, and skin rash. Report these to the MD, as patient may need to be treated with corticosteroids.

16.0 Statistical Considerations and Methodology

16.1 Overview

This is a phase II study of a combination of Nilotinib, cytarabine, and daunorubicin in patients with newly diagnosed AML with Kit overexpression. This study will use a one-stage design with an interim analysis to assess the efficacy of this combination.

16.11 Endpoint: The primary endpoint in this trial is the proportion of complete responses during induction therapy. A success is defined as a CR or CRi as the objective status during induction therapy. Throughout Section 16.0, complete response will be considered synonymous with "success", unless specified otherwise. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response.

16.2 Statistical Design:

16.21 Decision Rule:

In a previous phase 3 randomized study including 582 patients with previously untreated AML who were treated with induction therapy of daunorubin combined with cytarabine, 64% of patients received a complete response (Fernandez et al, 2009). More specifically, the complete response rate was 57% in 293 patients treated at the standard dose (45 mg/m2) of daunorubicin and 71% in 289 patients treated at a higher dose (90 mg/m2) of daunorubicin. This study will include patients with previously untreated AML who will be treated with induction therapy of 60 mg/m2 daunorubicin combined with cytarabine and the addition of nilotinib. It is expected that a dose of 60 mg/m2 daunorubicin combined with cytarabine will have a complete response rate similar to the higher dose group of the previous phase 3 study. However, that study included only patients between 17 and 60 years of age, while the median age at diagnosis of AML is approximately 57 years. This study will include patients of all ages and thus a

lower rate of complete response is expected. Therefore, a complete response rate of 60% is a reasonable estimate for 60 mg/m2 daunorubicin combined with cytarabine in the population that will be accrued to this study. The addition of nilotinib to this combination is anticipated to increase the complete response rate.

This study will include only patients with Kit overexpression. Studies looking at the effect of Kit overexpression on complete response rate have not yet been conducted. If there is any new evidence showing an effect of Kit overexpression on complete response rate for previously untreated AML patients treated with daunorubicin plus cytarabine, it will be taken into consideration when evaluating the efficacy of this regimen.

The largest success proportion where the proposed treatment regimen would be considered ineffective in this population is 60%, and the smallest success proportion that would warrant subsequent studies with the proposed regimen in this patient population is 80%. A one-stage design with an interim analysis based on a Simon optimum design uses 39 evaluable patients to test the null hypothesis that the true complete response rate is at most 60%.

16.211 Interim Analysis: Enter 18 evaluable patients into the study. If 11 or fewer successes are observed in the first 18 evaluable patients, we will consider this regimen ineffective in this patient population and terminate this study. Otherwise, if the number of successes is at least 12, we will continue accrual.

16.212 Final Decision Rule: If 27 or fewer successes are observed in the first 39 evaluable patients, we will consider this regimen ineffective in this patient population. If 28 or more successes are observed in the first 39 evaluable patients, we may recommend further testing of this regimen in subsequent studies in this population.

16.213 Over Accrual: If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any decision making process. Analyses involving over accrued patients are discussed in Section 16.34.

16.214 NOTE: We will not suspend accrual at the interim analysis to allow the first 18 patients to become evaluable, unless undue toxicity is observed. Given the limited overall sample size and the inclusion of an adverse events stopping rule, we feel it is ethical to not halt accrual for the interim analysis. However, if accrual is extremely rapid, we may temporarily suspend accrual in order to obtain safety data on these patients before re-opening accrual to further patients.

16.22 Sample Size: This study is expected to require a minimum of 18 and a maximum of 39 evaluable patients. We anticipate accruing 4 additional patients to account for ineligibility, cancellation, major treatment violation, or other reasons. Therefore, the study is expected to accrue a maximum of 43 patients overall.

16.23 Accrual Rate and Study Duration: The anticipated accrual rate is 1-2 evaluable patients per month. Therefore, the accrual period is expected to be 2 years. The

primary endpoint will be evaluated approximately 2.5 years after the trial opens, or after the last patient accrued has been observed for at least 2 months and data collection for the induction phase is complete. The total study duration is expected to be approximately 4.5 years, or until all patients have completed all cycles of treatment.

16.24 Power and Significance Level: Assuming that the number of successes is binomially distributed, the significance level is .08, i.e. there is an 8% chance of finding the drug to be effective when it truly is not. The probability of declaring that this regimen warrants further study (i.e. statistical power) under various success proportions and the probability of stopping accrual after the interim analysis can be tabulated as a function of the true success proportion as shown in the following table.

If the true success proportion is...	0.60	0.65	0.70	0.75	0.80
Then the probability of declaring that the regimen warrants further study is…	0.08	0.22	0.45	0.71	0.90
and the probability of stopping after the interim analysis is …	0.63	0.45	0.28	0.14	0.05

16.25 Other considerations: Adverse events, quality/duration of response, and patterns of treatment failure observed in this study, as well as scientific discoveries or changes in standard care will be taken into account in any decision to terminate the study

16.3 Analysis Plan

16.31 Primary Outcome Analyses:

16.311 Definition: The primary endpoint of this trial is the proportion of complete responses to induction therapy. A success is defined as a CR or CRi as the objective status during induction therapy. All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for response.

16.312 Estimation: The proportion of successes will be estimated by the number of successes divided by the total number of evaluable patients. Confidence intervals for the true success proportion will be calculated according to the approach of Duffy and Santner (Duffy D 1987).

16.32 Secondary Outcome Analyses: These analyses will include all patients meeting the eligibility criteria who have signed a consent form and have begun treatment, including patients who fail to achieve a complete response after 2 cycles of treatment (unless noted otherwise).

16.321 Overall survival time is defined as the time from registration to death due to any cause. The distribution of survival time will be estimated using the method of Kaplan-Meier(Kaplan E 1958). In addition, the overall survival rate at 2 years after registration will be reported.

16.322 Disease free survival time is defined for all evaluable patients who have achieved a CR or CRi as the time from registration to relapse or death due to any cause. The distribution of disease-free survival will be estimated using the method of Kaplan-Meier. In addition, the disease-free survival rate at 2 years after registration will be reported.

16.323 Duration of complete response is defined for all evaluable patients who have achieved a CR or CRi as the date at which the patient's objective status is first noted to be a CR or CRi to the earliest date relapse is documented. The distribution of duration of complete response will be estimated using the method of Kaplan-Meier

16.324 Adverse Events: All eligible patients that have initiated treatment will be considered evaluable for assessing adverse event rate(s). The maximum grade for each type of adverse event will be recorded for each patient, and frequency tables will be reviewed to determine patterns. Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration.

16.33 Correlative Analyses

16.331 Prognostic and predictive factors including age, Kit mutation/expression, Flt3 mutation and whether the patient received a transplant will be assessed. These factors will be summarized and used to help characterize the types of patients accrued to this trial. In addition, we will explore differences in the distributions of these risk factors by clinical outcome (disease-free survival status at 2 years and whether the patient remains in sustained CR). Nonparametric quantitative comparisons by group will be made as appropriate (Fisher's exact or Wilcoxon rank sum). Kaplan-Meier methods and log-rank statistics will be used to compare between groups for time-to-event measures. Given the limited number of patients, the difference in risk factor distribution by outcome will be largely exploratory.

16.332 In patients who are Kit mutation positive at diagnosis and achieve a complete molecular remission (i.e. negative RT-PCR for Kit mutation) to treatment, the Kit mutation status (positive vs. negative) will be reassessed at time of relapse. Due to the small number of patients that are expected to be in this subgroup, this analysis will be primarily descriptive in nature.

16.333 MRD will be assessed by PCR or flow cytometry at the end of each phase of treatment (induction, consolidation, and maintenance) and at time of relapse. MRD status will be correlated with response using Fisher's exact test. In addition, the relationship between MRD status (positive vs. negative) and disease-free survival will be evaluated using landmark analyses.

16.34 Over Accrual: If more than the target number of patients are accrued, the additional patients will not be used to evaluate the stopping rule or used in any

decision making processes; however, they will be included in final endpoint estimates and confidence intervals.

16.4 Data & Safety Monitoring:

16.41 The principle investigator(s) and the study statistician will review the study at least twice a year to identify accrual, adverse event, and any endpoint problems that might be developing. The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least twice a year, based on reports provided by the MCCC Statistical Office.

16.42 Adverse Event Stopping Rules: The stopping rules specified below are based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e. an adverse event with attribute specified as "possible," "probable," or "definite") that satisfy one of the following:

- if 4 or more patients in the first 12 treated patients experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment excluding infections and hypophosphatamia, hypocalcemia, hypo- or hyperkalemia, hyperuricemia as these abnormal lab results tend to resolve quickly (≤7 days) without affecting or jeopardizing patient safety.

- if after the first 12 patients have been treated, 30% of all patients experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment excluding infections and hypophosphatamia, hypocalcemia, hypo- or hyperkalemia, hyperuricemia as these abnormal lab results tend to resolve quickly (≤7 days) without affecting or jeopardizing patient safety.

Grade 4 electrolyte abnormalities will be excluded only if the study PI has determined that they are not associated with any clinically significant events.

Note: Stem cell transplant will not be considered part of protocol treatment and attribution refers to the relationship to Cytarabine, Daunorubicin or Nilotinib (not Stem Cell Transplant). Adverse events are not recorded during Stem Cell Transplant (see section 10.0).

We note that we will review grade 4 and 5 adverse events deemed "unrelated" or "unlikely to be related", to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse event.

16.5 Results Reporting on ClinicalTrials.gov: At study activation, this study will have been registered within the "ClincialTrails.gov" website. The Primary and Secondary Endpoints along with other required information for this study will be reported on ClinicalTrials.gov. For purposes of timing of the Results Reporting, the initial estimated completion date for the Primary Endpoint of this study is 2.5 years after the study opens to accrual. The definition of "Primary Endpoint Completion Date" (PECD) for this study is at the time the last patient registered has been followed for at least 2 months.

16.6 Inclusion of Women and Minorities

16.61 This study will be available to all eligible patients, regardless of race, gender, or ethnic origin.

16.62 There is no information currently available regarding differential effects of this regimen in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analysis will, as always, look for differences in treatment effect based on racial and gender groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.63 The geographical region served by MCCC has a population which includes approximately 3% minorities. Based on prior MCCC studies involving similar disease sites, we expect about 3-5% of patients will be classified as minorities by race and about 30% of patients will be women. Expected sizes of racial by gender subsets are shown in the following table:

Accrual Estimates by Gender/Ethnicity/Race

Ethnic Category	Sex/Gender			
	Females	Males	Unknown	Total
Hispanic or Latino	0	1	0	1
Not Hispanic or Latino	13	29	0	42
Ethnic Category: Total of all subjects*	13	30	0	43
Racial Category				
American Indian or Alaskan Native	0	0	0	0
Asian	0	0	0	0
Black or African American	1	1	0	2
Native Hawaiian or other Pacific Islander	0	0	0	0
White	12	29	0	41
Racial Category: Total of all subjects*	13	30	0	43

Ethnic Categories: Hispanic or Latino – a person of Cuban, Mexican, Puerto Rico, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."
Not Hispanic or Latino

Racial Categories: American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens: None.

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Initial Material(s)

Case Report Form (CRF)	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)
On-Study Form	≤2 weeks after registration
Baseline Adverse Event Form	
Baseline Research Blood Submission Form	
Baseline Research Bone Marrow Submission Form	
Pretreatment Measurement and Bone Marrow Biopsy Form	
Bone Marrow Biopsy and Aspirate Report including the Kit expression by flow cytometry, Kit mutation by PCR, and cytogenetics	
End of Active Treatment/Cancel Notification Form	Submit ≤2 weeks after registration if withdrawal/refusal occurs prior to beginning protocol therapy

Test Schedule Material(s)

CRF	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)	
	At each evaluation during treatment	At end of treatment
Evaluation/Treatment Form	X[3]	X[3]
Nadir/Adverse Event Form	X[4]	X
Active Monitoring Measurement and Disease Response Form	X	X
Bone Marrow Biopsy and Aspirate Report including the Kit expression by flow cytometry, Kit mutation by PCR, and cytogenetics	X	X
Research Blood Submission Form	X[1]	
Research Bone Marrow Submission Form	X[1]	
Stem Cell Transplant Form	X[2]	
End of Active Treatment/Cancel Notification Form		X
ADR/AER	At each occurrence (see Section 10.0)	

1. Only when required by the Test Schedule (see Section 4.0 and 14.0).

2. Submit only once if a patient receives a transplant. The SCT (pre-transplant conditioning chemotherapy plus stem cell infusion) until the start of maintenance will be defined as a separate cycle, where the stem cell transplant form will be used instead of the evaluation/treatment form for that cycle.
3. Only for cycles where the patient is receiving Nilotinib (NOT a Stem Cell Transplant).
4. Not collected during Stem Cell Transplant

Follow-up Material(s)

CRF	Event Monitoring Phase[1]				
	q. 6 months until PD[2]	At PD[2]	After PD q. 6 mos.	Death	New Primary
Event Monitoring Form	X	X	X	X	At each occurrence

1. If a patient is still alive 3 years after registration, no further follow-up is required.
2. Submit copy of documentation of response or progression to the MCCC Operations Office, Attention: QAS for MC1284.

19.0 Budget

19.1 Costs charged to patient: routine clinical care

19.2 Tests to be research funded: Study drug, Magnesium, Amylase, and Lipase test, Urinalysis, ECGs Bone marrow (BM) sampling at the end of maintenance . Bone marrow MRD assessment for CD117/Kit by flow cytometry at end of maintenance. Bone marrow MRD assessment for Kit mutation by PCR (if positive at diagnosis) at screening , upon recovery of Cycle 1, upon bone marrow recovery of Cycle 2 if patient receives re-induction, Day 1 of cycle 1 of maintenance , end of maintenance, and upon relapse.

19.3 Other budget concerns: Protocol administration, data management and statistical analysis efforts will be by funded by Novartis.

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Appendix Ia: Drugs with Risk of Torsades de Pointes

This list contains medications that are generally accepted (and documented in published data) as having an increased risk of QT prolongation and/or torsades de pointes. Concomitant administration of nilotinib and a medication on this list is **prohibited**.

Generic Name	Brand Name	Comments
Amiodarone	Cordarone®, Pacerone®	Low risk torsades de pointes
Arsenic trioxide	Trisenox®	Torsades de pointes
Bepridil	Vasocor®	
Chloroquine	Aralen®	
Chlorpromazine	Thorazine®	
Cisapride	Propulsid®	Restricted availability in U.S.
Clarithromycin	Biaxin®	
Disopyramide	Norpace®	Torsades de pointes
Dofetilide	Tikosyn®	Torsades de pointes
Dolasetron	Anzemet®	
Droperidol	Inapsine®	Torsades de pointes
Erythromycin	Erythrocin®, E.E.S. ®	IV > PO
Halofantrine	Halfan®	
Haloperidol	Haldol®	IV > PO, high doses increase QT prolongation and torsades de pointes
Ibutilide	Corvert®	Torsades de pointes, female > male, non-Caucasian > Caucasian
Levomethadyl	Orlaam®	
Mesoridazine	Serentil®	
Methadone	Dolophine®, Methadose®	
Pentamidine	Pentam®, Nebupent®	
Pimozide	Orap®	
Procainamide	Pronestyl®, Procan®, Procanbid®	N-acetylprocainamide causes torsade de pointes, not parent compound
Quinidine	Cardioquin®, Quinaglute®	Torsades de pointes
Sotalol	Betapace®	Torsade de pointes female > male
Sparfloxacin	Zagam®	
Thioridazine	Mellaril®	

Note: the above list is not all-inclusive.

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http://www.azcert.org/medical-pros/druginteractions.cfm Accessed 5/13/11

Appendix Ib: Drugs with Possible or Conditional Risk of Torsades de Pointes

This list contains medications that, in some reports, have been associated or weakly associated with causing torsades de pointes and/or QT prolongation. There is insufficient data that these medications alone may cause torsades de pointes and/or QT prolongation, however when nilotinib is given concomitantly with a medication on this list (or other risk factors are present such as bradycardia, electrolyte disturbances, congenital long QT syndrome, or concomitant drugs that inhibit metabolism), there may be possible or conditional risk of torsades de pointes and/or QT prolongation. Extreme caution and careful monitoring should be instituted with concomitant administration of nilotinib and a medication on this list.

Generic Name	Brand Name	Comments
Alfuzosin	Uroxatral®	
Amantadine	Symmetrel®	Low
Amitriptyline	Elavil®	Nonspecific ECG changes reported.
Atazanavir	Reyataz®	
Azithromycin	Zithromax®	
Chloral hydrate	Noctec®	
Ciprofloxacin	Cipro®	
Citalopram	Celexa®	
Clomipramine	Anafranil®	
Desipramine	Pertofrane®	QT prolongation, VF/sudden death reported
Diphenhydramine	Benadryl®, Nytol®	
Dolasetron	Anzemet®	Granisetron < Ondansetron < Dolasetron
Doxepin	Sinequan®	
Dronedarone	Multaq®	
Escitalopram	Lexapro®, Cipralex®	
Felbamate	Felbatrol®	
Flecainide	Tambocor®	
Foscarnet	Foscavir®	
Fosphenytoin	Cerebyx®	
Fluconazole	Diflucan®	IV > PO
Fluoxetine	Prozac®, Sarafem®	1 in 10,000 ventricular arrhythmias reported
Galantamine	Reminyl®	
Gatifloxacin	Tequin®	
Gemifloxacin	Factive®	
Granisetron	Kytril®	Granisetron < Ondansetron < Dolasetron
Imipramine	Norfranil®	Nonspecific arrhythmias reported
Indapamide	Lozol®	
Isradipine	Dynacirc®	
Itraconazole	Sporanox®	
Ketoconazole	Nizoral®	
Lapatinib	Tykerb®	
Levofloxacin	Levaquin®	Lower risk than that of similar agents
Lithium	Lithobid®, Eskalith®	
Moexipril/HCTZ	Uniretic®	
Moxifloxacin	Avelox®	
Nicardipine	Cardene®	

Nilotinib	Tasigna®	
Nortriptyline	Pamelor®	Nonspecific arrhythmias reported
Octreotide	Sandostatin®	
Ofloxacin	Floxin®	
Ondansetron	Zofran®	Granisetron < Ondansetron < Dolasetron
Oxytocin	Pitocin®	
Paliperidone	Invega®	
Paroxetine	Paxel®	Lower risk than TCA's
Perflutren lipid microspheres	Definity®	
Protriptyline	Vivactil®	
Quetiapine	Seroquel®	QT prolongation
Ranolazine	Ranexa®	
Risperidone	Risperdal®	QT prolongation, sudden death reported
Ritonavir	Norvir®	
Sertraline	Zoloft®	Lower risk than TCA's
Solifenacin	VESIcare®	
Sunitinib	Sutent®	
Tacrolimus	Prograf®	
Tamoxifen	Nolvadex®	
Telithromycin	Ketek®	
Tizanidine	Zanaflex®	
Trazodone	Desyrel®	
Trimethoprim-Sulfa	Sulfa®, Bactrim®, Bactrim DS®	Low
Trimipramine	Surmontil®	
Vardenafil	Levitra®	
Venlafaxine	Effexor®	1 :1000 risk of arrhythmia reported
Voriconazole	VFend®	
Ziprasidone	Geodon®	QT prolongation, 1:1000 risk of arrhythmia

Note: the above list is not all-inclusive.

References:

Al-Khatib SM, Allen LaPointe NM, Kramer JM, Califf RM. What Clinicians Should Know About the QT Interval. *Journal of the American Medical Association* 2003;289:2120-2127.

Crouch MA, Limon L, Cassano AT. Clinical Relevance and Management of Drug-Related QT Interval Prolongation. *Pharmacotherapy* 2003;23:887-908.

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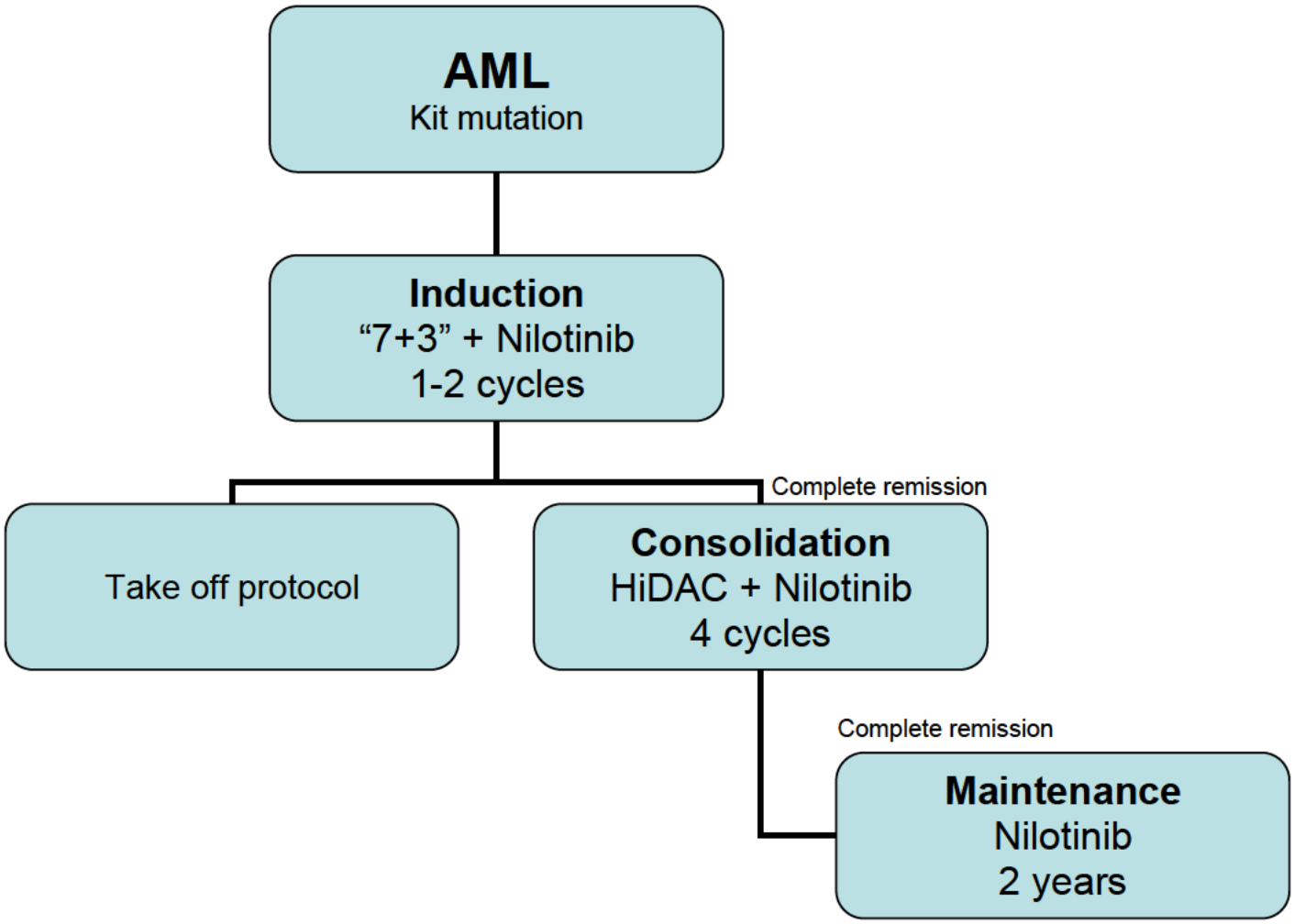
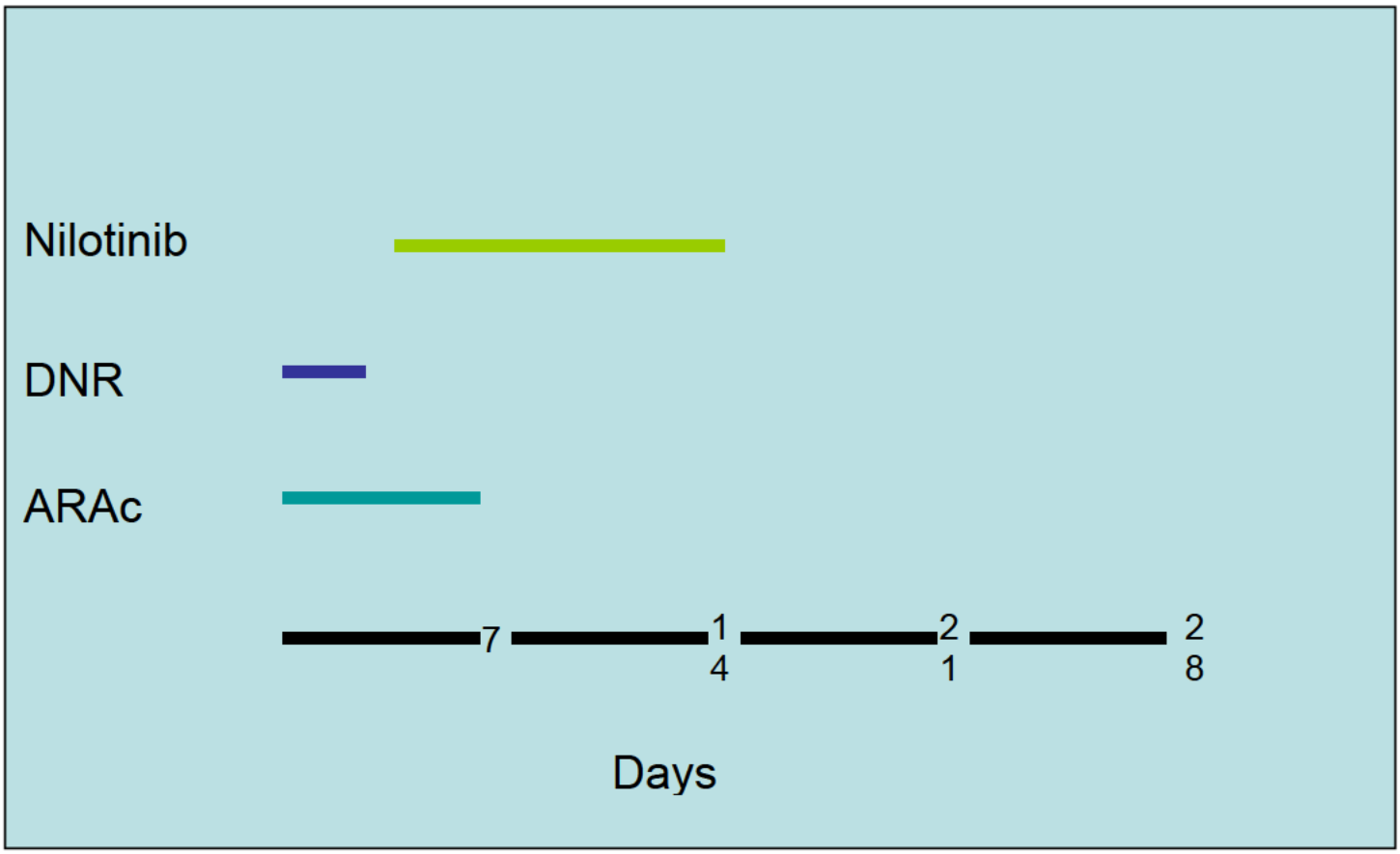
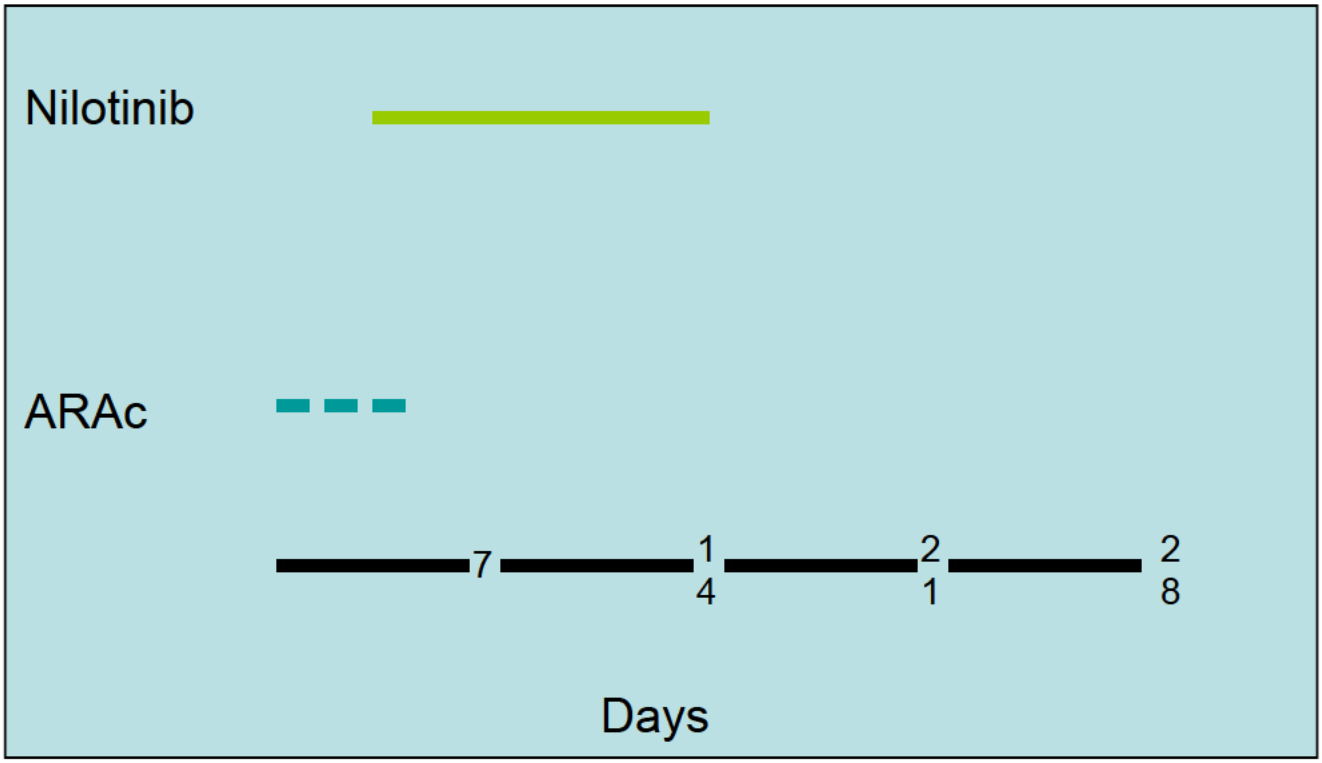
Appendix II: Protocol schema DATA





DNR: daunorubicin, ARA-c: cytarabine

Consolidation



ARA-c: cytarabine

Appendix III: ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From http://www.ecog.org/general/perf_stat.html

Appendix IV: Patient Medication Diary

Name _____ Study No. MC1284
Patient No. _____
Please complete this diary on a daily basis. You will take the study medication (nilotinib) twice a day and you should take the medicine at about the same time in the morning and in the evening each day. Do not miss any capsules. Nilotinib should be taken on an empty stomach. Whenever you take the study medication, you must not eat anything for 2 hours before you take the medicine, and for 1 hour after you have taken the medicine. Water is allowed while you are fasting; however do not eat grapefruit, star fruit, or Seville oranges at any time while taking nilotinib. If the pills are thrown up, this should be noted on your diary but you should not take another pill until your next scheduled dose.INDUCTION

Day	Date (mm/dd/yy)	Nilotinib		
		Time taken	Dose in mg	Number of tablets
1		a.m.		
		p.m.		
2		a.m.		
		p.m.		
3		a.m.		
		p.m.		
4		a.m.		
		p.m.		
5		a.m.		
		p.m.		
6		a.m.		
		p.m.		
7		a.m.		
		p.m.		
8		a.m.		
		p.m.		
9		a.m.		
		p.m.		
10		a.m.		
		p.m.		
11		a.m.		
		p.m.		
12		a.m.		
		p.m.		
13		a.m.		
		p.m.		
14		a.m.		
		p.m.		

Day	Date (mm/dd/yy)	Nilotinib		
		Time taken	Dose in mg	Number of tablets
15		a.m.		
		p.m.		
16		a.m.		
		p.m.		
17		a.m.		
		p.m.		
18		a.m.		
		p.m.		
19		a.m.		
		p.m.		
20		a.m.		
		p.m.		
21		a.m.		
		p.m.		
22		a.m.		
		p.m.		
23		a.m.		
		p.m.		
24		a.m.		
		p.m.		
25		a.m.		
		p.m.		
26		a.m.		
		p.m.		
27		a.m.		
		p.m.		
28		a.m.		
		p.m.		

Participant's Signature: _____ Date: _____

CONSOLIDATION

Day	Date (mm/dd/yy)	Nilotinib		
		Time taken	Dose in mg	Number of tablets
1		a.m.		
		p.m.		
2		a.m.		
		p.m.		
3		a.m.		
		p.m.		
4		a.m.		
		p.m.		
5		a.m.		
		p.m.		
6		a.m.		
		p.m.		
7		a.m.		
		p.m.		
8		a.m.		
		p.m.		
9		a.m.		
		p.m.		
10		a.m.		
		p.m.		
11		a.m.		
		p.m.		
12		a.m.		
		p.m.		
13		a.m.		
		p.m.		
14		a.m.		
		p.m.		

Day	Date (mm/dd/yy)	Nilotinib		
		Time taken	Dose in mg	Number of tablets
15		a.m.		
		p.m.		
16		a.m.		
		p.m.		
17		a.m.		
		p.m.		
18		a.m.		
		p.m.		
19		a.m.		
		p.m.		
20		a.m.		
		p.m.		
21		a.m.		
		p.m.		
22		a.m.		
		p.m.		
23		a.m.		
		p.m.		
24		a.m.		
		p.m.		
25		a.m.		
		p.m.		
26		a.m.		
		p.m.		
27		a.m.		
		p.m.		
28		a.m.		
		p.m.		

Participant's Signature: _____ Date: _____

MAINTENANCE

Day	Date (mm/dd/yy)	Nilotinib		
		Time taken	Dose in mg	Number of tablets
1		a.m.		
		p.m.		
2		a.m.		
		p.m.		
3		a.m.		
		p.m.		
4		a.m.		
		p.m.		
5		a.m.		
		p.m.		
6		a.m.		
		p.m.		
7		a.m.		
		p.m.		
8		a.m.		
		p.m.		
9		a.m.		
		p.m.		
10		a.m.		
		p.m.		
11		a.m.		
		p.m.		
12		a.m.		
		p.m.		
13		a.m.		
		p.m.		
14		a.m.		
		p.m.		

Day	Date (mm/dd/yy)	Nilotinib		
		Time taken	Dose in mg	Number of tablets
15		a.m.		
		p.m.		
16		a.m.		
		p.m.		
17		a.m.		
		p.m.		
18		a.m.		
		p.m.		
19		a.m.		
		p.m.		
20		a.m.		
		p.m.		
21		a.m.		
		p.m.		
22		a.m.		
		p.m.		
23		a.m.		
		p.m.		
24		a.m.		
		p.m.		
25		a.m.		
		p.m.		
26		a.m.		
		p.m.		
27		a.m.		
		p.m.		
28		a.m.		
		p.m.		
29		a.m.		
		p.m.		
30		a.m.		
		p.m.		
31		a.m.		
		p.m.		
32		a.m.		
		p.m.		
33		a.m.		
		p.m.		
Day	Date (mm/dd/yy)	Nilotinib		

		Time taken	Dose in mg	Number of tablets
34		a.m.		
		p.m.		
35		a.m.		
		p.m.		
36		a.m.		
		p.m.		
37		a.m.		
		p.m.		
38		a.m.		
		p.m.		
39		a.m.		
		p.m.		
40		a.m.		
		p.m.		
41		a.m.		
		p.m.		
42		a.m.		
		p.m.		
43		a.m.		
		p.m.		
44		a.m.		
		p.m.		
45		a.m.		
		p.m.		
46		a.m.		
		p.m.		
47		a.m.		
		p.m.		
48		a.m.		
		p.m.		
49		a.m.		
		p.m.		
50		a.m.		
		p.m.		
51		a.m.		
		p.m.		
52		a.m.		
		p.m.		

53		a.m.		
		p.m.		
54		a.m.		
		p.m.		
55		a.m.		
		p.m.		
56		a.m.		
		p.m.		
57		a.m.		
		p.m.		
58		a.m.		
		p.m.		
59		a.m.		
		p.m.		
60		a.m.		
		p.m.		
61		a.m.		
		p.m.		
62		a.m.		
		p.m.		
63		a.m.		
		p.m.		
64		a.m.		
		p.m.		
65		a.m.		
		p.m.		
66		a.m.		
		p.m.		
67		a.m.		
		p.m.		
68		a.m.		
		p.m.		
69		a.m.		
		p.m.		
70		a.m.		
		p.m.		
71		a.m.		
		p.m.		

Date (mm/dd/yy)		Nilotinib		
		Time taken	Dose in MG	Number of tablets
72		a.m.		
		p.m.		
73		a.m.		
		p.m.		
74		a.m.		
		p.m.		
75		a.m.		
		p.m.		
76		a.m.		
		p.m.		
77		a.m.		
		p.m.		
78		a.m.		
		p.m.		
79		a.m.		
		p.m.		
80		a.m.		
		p.m.		
81		a.m.		
		p.m.		
82		a.m.		
		p.m.		
83		a.m.		
		p.m.		
84		a.m.		
		p.m.		

Participant's Signature: _____ Date: _____

Appendix V Acute Myeloid Leukemia WHO 2008 Criteria

Acute myeloid leukemia and related precursor neoplasms, and acute leukemias of ambiguous lineage (WHO 2008)

Categories

Acute myeloid leukemia with recurrent genetic abnormalities

- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- APL with t(15;17)(q22;q12); *PML-RARA**
- AML with t(9;11)(p22;q23); *MLLT3-MLL*†
- AML with t(6;9)(p23;q34); *DEK-NUP214*
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
- AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
- *Provisional entity: AML with mutated NPM1*
- *Provisional entity: AML with mutated CEBPA*

Acute myeloid leukemia with myelodysplasia-related changes‡

Therapy-related myeloid neoplasms§

Acute myeloid leukemia, not otherwise specified (NOS)

- Acute myeloid leukemia with minimal differentiation
- Acute myeloid leukemia without maturation
- Acute myeloid leukemia with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Acute erythroid leukemia
  - Pure erythroid leukemia
  - Erythroleukemia, erythroid/myeloid

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute