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Title: **Phase II trial of dasatinib in patients with *isocitrate dehydrogenase (IDH)*-mutant advanced intrahepatic cholangiocarcinoma**

Study Sponsor: Massachusetts General Hospital Cancer Center

Drug Supplier: Bristol-Myers Squibb

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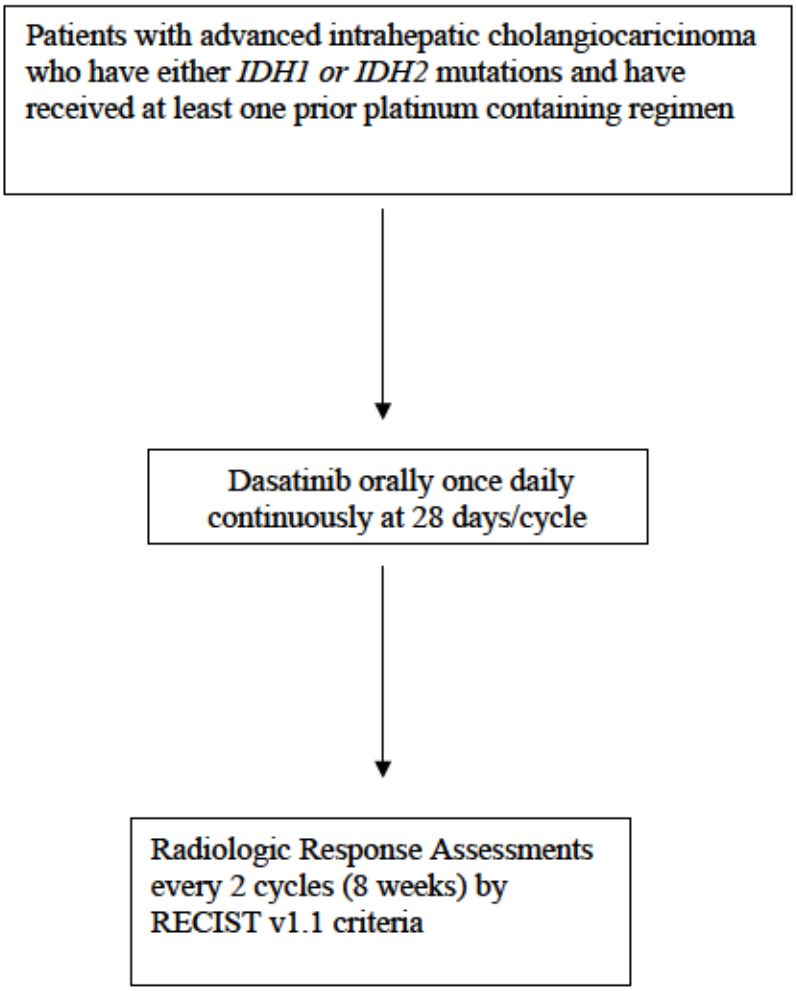
Agent(s): Dasatinib, (NSC# _____; IND # _____) – BMS

SCHEMA



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1. OBJECTIVES

1.1 Study Design

This is an open-label, single-arm Phase II study of dasatinib in patients who have progressed on first line systemic therapy for advanced intrahepatic cholangiocarcinoma (ICC) with either *IDH1 or IDH2* mutations. The phase II study would have a two-stage design, with a target accrual of 8 evaluable patients for the first stage and a total of 19 patients for the whole study. If no responses seen in the first 8 patients, the trial will be terminated. If at least 1 patient in the first stage has response, the trial would proceed to the second stage. Patients will be treated with dasatinib at 100 mg daily administered orally continuously for 28-day cycles. Tumor assessments will be performed every 8 weeks until documented disease progression by RECIST criteria or drug intolerance. The estimated time for acc rual of the patients is 12-24 months.

1.2 Primary Objectives

The primary objective of the study is to assess the objective response rate (ORR) per RECIST v1.1 criteria.

1.3 Secondary Objectives

1.3.1 Evaluate the safety and tolerability of dasatinib in patients with *IDH*-mutant advanced ICC

1.3.2 Evaluate the progression free survival (PFS) and median overall survival (OS) for patients with *IDH*-mutant advanced ICC

1.3.3 Explore molecular correlates of response and resistance including circulating biomarkers and tumor tissue biomarkers

2. BACKGROUND

2.1 Study Agent

A summary of the properties of dasatinib and the clinical and nonclinical experience with the drug are contained in the Investigator's Brochure (IB) supplied by BMS. The IB should be reviewed in conjunction with this study protocol.

2.1.1 Spectrum of Dasatinib Activity

Dasatinib potently inhibits: SRC kinases, BCR-ABL, c-KIT, PDGFRβ and EPHA and was less potent against 16 other unrelated protein tyrosine kinases (PTKs) and serine/threonine kinases. *In vitro*, dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCR-

ABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression. Dasatinib inhibits the BCR-ABL kinase with an *in vitro* IC50 of 3 nM, a potency 260-fold greater than that of imatinib mesylate (IC50 = 790 nM). In cellular assays, dasatinib killed or inhibited the proliferation of all BCR-ABL dependent leukemic cell lines tested to date. Dasatinib also demonstrated undiminished anti-tumor activity against several preclinically- and clinically-derived models of imatinib mesylate resistance. Evidence that SRC family kinase overexpression may play a role in clinical resistance to imatinib mesylate was demonstrated in three CML cell lines established from patients who failed imatinib mesylate therapy. These cells remained highly sensitive to the cell-killing effects of dasatinib. These results demonstrate that dasatinib is effective in reducing the proliferation or survival of both imatinib mesylate-sensitive and resistant cells, and its inhibitory activity is not solely dependent on BCR-ABL.

The activity of dasatinib against CML cells *in vitro* was reproduced *in vivo* against several human CML xenograft models grown subcutaneously in SCID mice. Against the K562/imatinib mesylate/R CML model, dasatinib was curative in 100% of the treated animals. In contrast, at its optimal dose and schedule, imatinib mesylate was inactive.

2.1.2 Dasatinib Preclinical Toxicology

Single or repeated oral administration of dasatinib principally affected the gastro-intestinal (GI) tract, including the liver, the hematopoietic and lymphoid systems in rats and monkeys. Other prominent effects after single oral administration of dasatinib included renal and cardiac toxicity in rats at lethal doses, and cutaneous hemorrhage in monkeys. Dasatinib can also affect the immune system and bone turnover.

Dasatinib *in vitro* activity in the HERG/IKr and Purkinje-fiber assays indicated a moderate liability for prolongation of cardiac ventricular repolarization (QT interval) in the clinic. However, there were no dasatinib -related changes observed in electrocardiograms, nervous system function, respirations and heart rate, blood pressure, or arterial oxygen saturation in single-dose, 10-day, or 1-month oral toxicity studies in monkeys.

Dasatinib was found to exhibit a profile of broad-spectrum platelet inhibition best typified by anti-platelet agents such as the GPIIb/IIIa antagonists, integrelin and abciximab. Finally, modulation of SRC kinase activity could also affect osteoclast morphology and function and bone remodeling. This effect could potentially result in an increase in bone mineral density and a phenotype analogous to osteopetrosis.

Additional toxicology information may be found in the Investigator's Brochure.

2.1.3 Clinical Pharmacokinetics

The pharmacokinetics of dasatinib have been evaluated in 229 healthy subjects and in 137 patients with leukemia (CML or Ph+ALL) from a Phase I clinical study (CA180002).

Absorption
Maximum plasma concentrations (Cmax) of dasatinib are observed between 0.5 and 6 hours

(Tmax) following oral administration. Dasatinib exhibits dose proportional increases in AUC and linear elimination characteristics over the dose range of 15 mg to 240 mg/day. The overall mean terminal half-life of dasatinib is 3–5 hours. Data from a study of 54 healthy subjects dministered a single, 100-mg dose of dasatinib 30 minutes following consumption of a high-fat meal resulted in a 14% increase in the mean AUC of dasatinib. The observed food effects were not clinically relevant.

Distribution
In patients, dasatinib has an apparent volume of distribution of 2505 L, suggesting that the drug is extensively distributed in the extravascular space. Binding of dasatinib and its active metabolite to human plasma proteins *in vitro* was approximately 96% and 93%, respectively, with no concentration dependence over the range of 100–500 ng/mL.

Metabolism
Dasatinib is extensively metabolized in humans, primarily by the cytochrome P450 enzyme 3A4. CYP3A4 was the primary enzyme responsible for the formation of the active metabolite. Flavin-containing monooxygenase 3 (FMO-3) and uridine diphosphateglucuronosyltransferase (UGT) enzymes are also involved in the formation of dasatinib metabolites. In human liver microsomes, dasatinib was a weak time-dependent inhibitor of CYP3A4. The exposure of the active metabolite, which is equipotent to dasatinib, represents approximately 5% of the dasatinib AUC. This indicates that the active metabolite of dasatinib is unlikely to play a major role in the observed pharmacology of the drug. Dasatinib also had several other inactive oxidative metabolites. Dasatinib is a time-dependent inhibitor of CYP3A3. At clinically relevant concentrations, dasatinib does not inhibit CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, or 2E1. Dasatinib is not an inducer of human CYP enzymes.1

Elimination
Elimination is primarily via the feces. Following a single oral dose of [14C]-labeled dasatinib, approximately 4% and 85% of the administered radioactivity was recovered in the urine and feces, respectively, within 10 days. Unchanged dasatinib accounted for 0.1% and 19% of the administered dose in urine and feces, respectively, with the remainder of the dose being metabolites.

2.1.4 Clinical Efficacy in CML

A Phase 2, randomized, open-label study was conducted in patients with chronic phase CML whose disease was resistant to prior imatinib therapy at doses of 400 or 600 mg. The primary endpoint was MCyR at 12 weeks. One hundred fifty patients were randomized in a 2:1 ratio to either dasatinib 70 mg twice daily or imatinib 800 mg daily (400 mg twice daily). Crossover to the alternate therapy was permitted in the event of disease progression or intolerable toxicity. Median follow-up was 15 months. Median duration of treatment prior to crossover was 14 months for dasatinib and 3 months for imatinib. Prior to crossover, 93% of the dasatinib-treated patients and 82% of the imatinib-treated patients achieved a CHR. At 12 weeks, MCyR was achieved in 36% of the dasatinib-treated patients (CCyR in 22%) and 29% of the imatinib-treated patients (CCyR in 8%). With longer treatment and follow-up, MCyR was achieved in 52% of the dasatinib-treated patients (CCyR in 40%) and 33% of the imatinib-treated patients (CCyR in

16%) prior to crossover. Since the median follow-up was 15 months, there were too few progressions to reliably estimate the duration of MCyR.

In the phase 3 DASISION trial, patients with newly diagnosed chronic-phase (CP) CML were randomized to receive dasatinib 100 mg (n = 259) or imatinib 400 mg (n = 260) once daily. Primary data showed superior efficacy for dasatinib compared with imatinib after 12 months, including significantly higher rates of complete cytogenetic response (CCyR), confirmed CCyR (primary end point), and major molecular response (MMR). The 24-month follow up showed similar results (Kantarijian et al, Blood, 2012).

A Phase 3, randomized, open-label, dose-optimization study was conducted in patients with chronic phase CML, whose disease was resistant to or who were intolerant to imatinib, to evaluate the efficacy of dasatinib administered once daily compared with dasatinib administered twice daily. Patients with significant cardiac diseases including myocardial infarction within 6 months, congestive heart failure within 3 months, significant arrhythmias, or QTc prolongation were excluded from the study. The primary endpoint was MCyR in patients with imatinib-resistant chronic phase CML. The main secondary endpoint was MCyR by total daily dose level in the same population. A total of 670 patients, of whom 498 had imatinib resistant disease, were randomized to the dasatinib 100 mg once daily, 140 mg once daily, 50 mg twice daily, or 70 mg twice daily group. Minimum follow-up was 6 months and median duration of treatment was approximately 8 months. Response rates are presented in Table 2. Efficacy was achieved across all dasatinib treatment groups with the once daily schedule demonstrating comparable efficacy (noninferiority) to the twice daily schedule on the primary efficacy endpoint (difference in MCyR 2.8%; 95% confidence interval [-6.0%–11.6%]). The main secondary endpoint of the study also showed comparable efficacy (non-inferiority) between the 100 mg total daily dose and the 140 mg total daily dose (difference in MCyR -0.8%; 95% confidence interval [-9.6%–8.0%]). Since the minimum follow-up was only 6 months, there were too few progressions to estimate the duration of MCyR. Long-term follow up at 6 years confirmed similar results (Shah et al, Blood, 2014).

2.1.5 Safety of Dasatinib in Clinical Studies in CML and Ph+ ALL

The data presented in Table 3 reflect exposure to dasatinib in 2182 patients with leukemia in clinical studies (starting dosage 100 mg once daily, 140 mg once daily, 50 mg twice daily, or 70 mg twice daily). The median duration of therapy was 11 months (range 0.03–26 months). The majority of dasatinib-treated patients experienced adverse reactions at some time. Drug was discontinued for adverse reactions in 9% of patients in chronic phase CML, 10% in accelerated phase CML, 15% in myeloid blast phase CML, and 8% in lymphoid blast phase CML or Ph+ ALL. In a Phase 3 dose-optimization study in patients with chronic phase CML, the rate of discontinuation for adverse reaction was lower in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily (4% and 12%, respectively). The most frequently reported adverse reactions (reported in ≥20% of patients) included fluid retention events, diarrhea, headache, skin rash, nausea, hemorrhage, fatigue, and dyspnea. The most frequently reported serious adverse reactions included pleural effusion (9%), pyrexia (3%), pneumonia (3%), infection (2%), febrile neutropenia (4%), gastrointestinal bleeding (4%), dyspnea (3%), sepsis (1%), diarrhea (2%), congestive heart failure (2%), and pericardial effusion (1%).

Anticipated Adverse Events

Myelosuppression

Treatment with dasatinib is associated with severe (NCI CTC Grade 3 or 4) thrombocytopenia, neutropenia, and anemia. Their occurrence is more frequent in patients with advanced CML or Ph+ ALL than in chronic phase CML. Complete blood counts should be performed weekly for the first 2 months and then monthly thereafter, or as clinically indicated. Myelosuppression was generally reversible and usually managed by withholding dasatinib temporarily or dose reduction. In a Phase 3 dose-optimization study in patients with chronic phase CML, Grade 3 or 4 myelosuppression was reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily.

Bleeding Related Events

In addition to causing thrombocytopenia in human subjects, dasatinib caused platelet dysfunction *in vitro*. In all clinical studies, severe central nervous system (CNS) hemorrhages, including fatalities, occurred in <1% of patients receiving dasatinib. Severe gastrointestinal hemorrhage occurred in 4% of patients and generally required treatment interruptions and transfusions. Other cases of severe hemorrhage occurred in 2% of patients. Most bleeding events were associated with severe thrombocytopenia.

Patients were excluded from participation in dasatinib clinical studies if they took medications that inhibit platelet function or anticoagulants. In some trials, the use of anticoagulants, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) was allowed concurrently with dasatinib if the platelet count was >50,000. Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants.

Fluid Retention

Dasatinib is associated with fluid retention. In all clinical studies, severe fluid retention was reported in 8% of patients, including pleural and pericardial effusion reported in 5% and 1% of patients, respectively. Severe ascites and generalized edema were each reported in <1% of patients. Severe pulmonary edema was reported in 1% of patients. Patients who develop symptoms suggestive of pleural effusion such as dyspnea or dry cough should be evaluated by chest X-ray. Severe pleural effusion may require thoracentesis and oxygen therapy. Fluid retention events were typically managed by supportive care measures that include diuretics or short courses of steroids.

In the Phase 3 dose-optimization study in patients with chronic phase CML, fluid retention events were reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily.1

QT Prolongation

In vitro data suggest that dasatinib has the potential to prolong cardiac ventricular repolarization (QT interval). In single-arm clinical studies in patients with leukemia treated with dasatinib, the mean QTc interval changes from baseline using Fridericia's method (QTcF) were 3–6 msec; the upper 95% confidence intervals for all mean changes from baseline were <8 msec. Nine patients

had QTc prolongation reported as an adverse event. Three patients (<1%) experienced a QTcF >500 msec.

Dasatinib should be administered with caution to patients who have or may develop prolongation of QTc. These include patients with hypokalemia or hypomagnesemia, patients with congenital long QT syndrome, patients taking anti-arrhythmic medicines or other medicinal products that lead to QT prolongation, and cumulative high-dose anthracycline therapy. Hypokalemia or hypomagnesemia should be corrected prior to dasatinib administration.

2.2 Study Disease

Biliary tract cancers (BTCs) include a spectrum of invasive adenocarcinomas encompassing both cholangiocarcinoma arising in the intrahepatic, perihilar, or distal biliary tree, as well as carcinoma arising from the gallbladder. As a subset of BTCs, intrahepatic cholangiocinoma (ICC) is the second most common type of primary liver tumor and among the most lethal of all human malignancies. It carries a median survival of <1 year[1], and has been rising in incidence worldwide for the past 3 decades[2]. An estimated 80,000 die per year die from diagnosed ICC annually, although the actual rate is likely several times higher disease, as recent molecular studies demonstrate that liver "cancers-of-unknown primary" are most commonly biliary in origin[3]. In most cases, ICC presents at advanced stage disease with a poor prognosis. Despite the current standard chemotherapy with gemcitabine/cisplatin combination for patients with unresectable or metastatic BTCs, the median survival time remains less than one year[1]. There are no standard treatments for patients who failed gemcitabine/platinum-based chemotherapy. Recently, there have been renewed interests in developing molecularly targeted therapies in BTCs[4], however, there are no studies targeting a particular molecular signature for ICC. Recent phase II studies evaluating targeted agents that inhibit EGFR or mitogen-activated protein kinase/extracellular signal–related kinase have demonstrated evidence of limited antitumor activity in unselected biliary tract carcinoma patients[5]. Several targeted agents including lapatinib and sorafenib, failed to demonstrate objective responses in advanced BTCs[6,7]. Thus, there is an immediate need to understand ICC biology and to develop improved therapeutics.

2.3 Study Rationale

Important advances have come from recent genome-wide and candidate-based sequencing studies. Work by our group and others have provided a detailed view of the genetics of ICC, and have immediate clinical implications for targeted therapeutic approaches in defined patient subsets. Based on our data and subsequent studies by other groups, specific "hot-spot" mutations in *IDH1* and *IDH2* (at IDH1R132 and IDH2R172) are now established as the most common known genetic lesions in ICC (20-40% of cases)[8-11].

While prior reports have varied in their conclusions about the potential prognostic significance of *IDH* mutation in ICC, our own larger scale study of 200 patients indicated that the IDH mutant subset of ICC have a comparable aggressive course to the IDH wild type tumors[12].

Whereas the precise mechanisms by which IDH 1 and 2 mutations promote tumorigenesis have not been elucidated, biochemical studies suggest that they act through an entirely novel cellular transformation paradigm[13]. Cancer-associated IDH mutations cause a single amino acid change at a conserved arginine residue within the isocitrate binding site of *IDH1* (R132) or *IDH2* (R172, R140), resulting in a novel enzymatic function: while the wild type enzyme catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (αKG) mutant IDH leads to the NADPH-dependent reduction of (αKG) to *R*(–)-2-hydroxyglutarate (2-HG), a proposed 'oncometabolite'. 2-HG inhibits the function of the family of αKG-dependent dioxygenase enzymes that utilize αKG as a cofactor, many of which function as epigenetic modifiers, including the Tet family hydroxylases, which convert 5-methyl-cytosine to hydroxymethyl-cytosine as a step in DNA demethylation, and members of the Jumomji family of histone demethylases. Correspondingly, IDH mutant tumors are characterized by high levels of DNA methylation and histone lysine methylation. There are a total of ~70 αKG-dependent dioxygenases that are potential 2-HG targets, and additional family members have diverse functions in cell physiology. Notably, our group has shown that circulating 2-HG is a surrogate biomarker of *IDH1* or *IDH2* mutation status in ICC and that circulating 2-HG levels may correlate directly with tumor burden[14].

Our recent studies have defined mechanisms for IDH-driven ICC progression and established novel genetically engineered mouse models (GEMMs) of ICC[15]. We found that mutant IDH impairs adult liver progenitor cells from undergoing hepatocyte differentiation via suppression of HNF4α transcription factor, a master regulator of hepatocyte identity and quiescence. In vivo, the IDH mutant liver shows aberrant response to injury, characterized by HNF4α silencing, rampant cell proliferation, and expansion of undifferentiated progenitors. We have also established a biobank of patient-derived xenograft (PDX) and early passage cell line models.

In order to develop novel strategies against ICC, we have performed screens of our new collection of ICC cell lines to 205 clinically-relevant targeted and chemotherapeutic agents, consisting of FDA approved drugs as well as innovative tool compounds. Importantly, the screening of ~1000 additional cell lines from other cancers in parallel enables us to identify compounds or classes of compounds to which ICC's in general show sensitivity relative to other tumor derived cell lines and compounds specific to defined mutational subsets. Most strikingly, these screens have identified one class of inhibitor to which IDH mutant ICCs exhibited extreme sensitivity -- SRC family kinase (SFK) inhibitors, Dasatinib (BMS-354825) and Saracatinib (AZD0530). Importantly, these two molecules are not structurally related which is consistent with on-target specificity. Of all 205 compounds tested, dasatinib demonstrated the greatest differential sensitivity between our IDH mutant ICC lines and all other ~1000 cell lines. These compelling data suggest novel synthetic lethal interactions with the IDH mutant genotype.

We have defined the IC50 of dasatinib and found the range of sensitivity was 1-10 nM in human and murine IDH mutant ICC lines, associated with a high degree of cell death. By contrast, all ICC cell lines with WT IDH were > 10 times less sensitive to the drug, with IC50's of greater 150 nM. The sensitivity was comparable to that of CML cell lines tested, and corresponds with a readily achievable plasma concentration of the drug in humans (plasma concentration with existing regimens is 143 nM). We subsequently tested the response of an IDH mutant xenograft model of ICC to dasatinib and observed a dramatic anti-tumor effect, with dasatinib treatment

leading to rapid and sustained tumor regression associated with massive tumor cell death. Further mechanistic studies revealed a potent and rapid decrease in levels of activated beta-catenin and the phospho-paxillin (a direct target of Src) within 30 min of treatment with dasatinib.

We propose to assess the initial safety and efficacy of dasatinib in a phase II study in patients with *IDH* mutant unresectable or metastatic ICC based on the following rationale: 1) our initial drug screen has identified *IDH* mutant ICC cell lines exceedingly sensitive to dasatinib (IC50 comparable to CML); 2) our xenograft experiments have confirmed this finding in vivo; and 3) *IDH* mutation is the most common gene mutation in ICC (20-40% of cases) and is routinely tested using our SNaPshot platform. Demonstrating the clinical efficacy with novel agents in this subgroup and identifying the potential predictors for response and resistance are essential steps to developing effective treatments for molecularly selected patients with advanced ICC.

2.4 Correlative Studies Background

Based on our prior and ongoing findings, we will propose the following correlative studies to assess the relevant pharmacodynamic endpoints, explore the potential mechanisms of action of dasatinib in ICC, and identify potential mechanism of resistance:
1) blood samples will be obtained at baseline, 2 weeks after treatment, and at the beginning of each cycle, and tested for serum 2-HG and CA19-9 levels; These changes will be correlated with tumor response by imaging studies;
2) patients will have mandatory core tumor biopsies at baseline, 8 weeks following dasatinib treatment and at the time of progression for those who had either SD or responses for more than 4 months. Tumor biopsies will be tested for changes in phospho-paxillin (Src kinase target), activated beta-catenin, Ki-67 (proliferation) and cleaved-caspase 3 (cell death).

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

- Participants must have unresectable or metastatic histologically confirmed intrahepatic cholangiocarcinoma
- Patients must have either *IDH1 or IDH2* mutations (any known mutations) based on the SNaPshot platform or other molecular testing platform from either archived tissue or fresh biopsy (tested in CLIA-certified lab)
- Patients with other biliary tract cancers (extrahepatic or gallbladder cancers) with IDH1 or IDH2 mutations are allowed
- Participants must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded) as $\geq$ 20 mm

with conventional techniques or as ≥ 10 mm with spiral CT scan. See section 10 for the evaluation of measureable disease.

- Participants must have received at least one prior platinum-based regimen for advanced cholangiocarcinoma and had progressive disease or become intolerable to the regimen
- Age ≥ 18 years.
- Life expectancy of ≥ 3 months.
- ECOG performance status 0 or 1 (see Appendix A).
- Participants must have adequate organ and marrow function as defined below:
    - Absolute neutrophil count ≥ 1,200/mcL
    - Platelets ≥75,000/mcL
    - Hemoglobin ≥ 9 g/dL
    - Total bilirubin ≤ 2.5 x the upper limit of normal
    - AST (SGOT)/ALT (SGPT) ≤ 5 X institutional upper limit of normal
    - PT/PTT ≤ 1.5 x ULN
    - Creatinine ≤ 1.5 or GFR ≥ 60 mL/min/1.73m^2
    - Serum Albumin ≥ 2.8 g/dl
- Prior chemoembolization, radiofrequency ablation, or radiation to the liver is allowed as long as the patient has measurable disease outside of the treated area or measurable progression at the site of the treated area
- Ability to understand and the willingness to sign a written informed consent document.
- Sexually active subjects (men and women) must agree to use medically accepted barrier methods of contraception (eg, male or female condom) during the course of the study and for 4 months after the last dose of study drug(s), even if oral contraceptives are also used. All subjects of reproductive potential must agree to use both a barrier method and a second method of birth control during the course of the study and for 4 months after the last dose of study drug(s).
- Women of childbearing potential must have a negative pregnancy test at screening. Women of childbearing potential include women who have experienced menarche and who have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or are not postmenopausal. Postmenopause is defined as amenorrhea ≥ 12 consecutive months. Note: women who have been amenorrheic for 12 or more months are still considered to be of childbearing potential if the amenorrhea is possibly due to prior chemotherapy, antiestrogens, ovarian suppression or any other reversible reason.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

- Prior treatment with dasatinib
- periampullary tumors
- Chemotherapy, within 4 weeks prior to entering the study (6 weeks for nitrosoureas or mitomycin) or those who have not recovered to less than or equal to grade 1 from adverse events due to agents administered more than 4 weeks earlier.
- The subject has received radiation therapy:

  - to bone or brain metastasis within 14 days of the first dose of study treatment
  - to any other site(s) within 28 days of the first dose of study treatment

- The subject has active brain metastases or epidural disease (Note: Subjects with brain metastases previously treated with whole brain radiation or radiosurgery or subjects with epidural disease previously treated with radiation or surgery who are asymptomatic and do not require steroid treatment for at least 2 weeks before starting study treatment are eligible. Neurosurgical resection of brain metastases or brain biopsy is permitted if completed at least 3 months before starting study treatment. (Baseline brain imaging with contrast-enhanced CT or MRI scans for subjects with known brain metastases is required to confirm eligibility.)
- The subject has uncontrolled, significant intercurrent or recent illness including, but not limited to, the following conditions:
  a. Cardiovascular disorders including
     i. Congestive heart failure (CHF): New York Heart Association (NYHA) Class III (moderate) or Class IV (severe) at the time of screening
     ii. Concurrent uncontrolled hypertension defined as sustained BP > 140 mm Hg systolic, or > 90 mm Hg diastolic despite optimal antihypertensive treatment within 7 days of the first dose of study treatment
     iii. Diagnosed or suspected congenital long QT syndrome
     iv. Any of the following within 6 months before the first dose of study treatment:
        - unstable angina pectoris
        - clinically-significant cardiac arrhythmias
        - stroke (including TIA, or other ischemic event)
        - myocardial infarction
        - thromboembolic event requiring therapeutic anticoagulation (Note: subjects with a venous filter (e.g. vena cava filter) are not eligible for this study)
        - Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes)
        - Prolonged QTc interval on pre-entry electrocardiogram (> 450 msec), may use either the Fridericia and Bazett's correction
        - Hypokalemia or hypomagnesemia that is not corrected prior to dasatinib administration
        - Patients should not be taking drugs that are generally accepted to have a risk of causing Torsades de Pointes. The following must be discontinued at least 7 days prior to starting dasatinib to be eligible:quinidine, procainamide, disopyramide, amiodarone, sotalol, ibutilide,dofetilide, erythromycins, clarithromycin, chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide, cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine,levomethadyl, pentamidine, sparfloxacin, lidoflazine

b. Other clinically significant disorders such as:

i. active infection requiring systemic treatment within 28 days before the first dose of study treatment

serious non-healing wound/ulcer/bone fracture within 28 days before the first dose of study treatment

c. Patients with known moderate/severe pleural effusions that are unrelated to malignancy or established diagnosis of pulmonary arterial hypertension

- Concurrent malignancy (other than adequately treated non-melanoma skin cancer, superficial transitional cell carcinoma of the bladder, and cervical carcinoma in situ) diagnosed within the past 3 years or any currently active malignancy
- Psychiatric illness/social situations that would limit compliance with study requirements.
- The subject has received any other type of investigational agent within 28 days before the first dose of study treatment.
- The subject is unable to swallow tablets
- Individuals who are known to be HIV-positive are excluded from this study.
- Pregnant women are excluded from this study due to the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk of adverse events in nursing infants secondary to treatment of the mother with dasatinib, breastfeeding should be discontinued if the mother is treated with dasatinib. These potential risks may also apply to other agents used in this study.
- Subjects may not be receiving any other study agents concurrently while on this study

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

There are no specific provisions for this. Both men and women and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's

protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at [redacted] and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at [redacted].

   Exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

5. TREATMENT PLAN

Treatment will be administered on an outpatient basis. Expected toxicities and potential risks as well as dose modifications for dasatinib are described in Section 6 of this protocol. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Treatment Description					
Agent	Pre-medications; Precautions	Dose	Route	Schedule	Cycle Length
dasatinib	None	100 mg	Oral	Days 1-28 continuously	28 days

5.1 Pre-treatment Criteria

Subjects must meet eligibility criteria on the first day of treatment.

5.2 Agent Administration

Currently dasatinib is available on study from Bristol-Myers Squibb as pills of 20 mg or 50 mg. The starting dose of dasatinib will be 100 mg by mouth daily generally for each 28-day cycle. The dasatinib should be taken at approximately the same time in the morning each day, but fasting before and after dosing is not considered necessary at this time. Subjects should try to avoid proton pump inhibitors and H2 antagonists. Short-acting antacid agents may be taken. If unacceptable toxicity as defined below is encountered, the dose will be reduced to 70 mg by mouth daily. If this lower dose still results in unacceptable toxicity, a second dose reduction will be instituted at 40 mg by mouth daily. If this dose results in unacceptable toxicity, the subject will be removed from the study. Scheduled doses that are missed for more than 6 hours should be omitted without an attempt to "make it up later." A vomited dose should not be made up under any circumstances. These missed and vomited doses must be recorded, however, in the patient's file.

Subjects will be monitored continuously for adverse events through 30 days (+7 days) after the date of the decision to discontinue study treatment. Subjects will be instructed to notify their physician immediately for any and all toxicities. Subjects experiencing one or more AEs due to the study treatment may require a dosing delay, or reduction(s), in their dose in order to continue with study treatment. Assessment of causality (chronology, confounding factors such as disease, concomitant medications, diagnostic tests, and previous experience with the study treatment) should be conducted by the PI when possible, before a decision is made to modify the dose or to hold dosing temporarily.

5.3 General Concomitant Medication and Supportive Care Guidelines

5.3.1 Drug-drug interactions

Because there is a potential for interaction of Dasatinib with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator (or Protocol Chair) should be alerted if the participant is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

5.3.2 Supportive medications

5.3.2.1 Antiemetics

Antiemetics should be prescribed by the treating physician as clinically indicated if a patient develops nausea and/or vomiting. Agents classified as having a high therapeutic index (such as 5-HT3 receptor antagonists, or NK-1 receptor antagonists) per ASCO or MASCC/ESMO guidelines for anti-emetics in oncology or dexamethasone are recommended (Hesketh et al. 2008, ASCO 2006; Roila et al, Annals of Oncology, 2010). Caution is recommended with the use of aprepitant, fosaprepitant and nabilone because aprepitant and fosaprepitant are both inhibitors and inducers of CYP3A4, and nabilone is a weak inhibitor of CYP3A4; thus concomitant administration of any of these medications with dasatinib can affect dasatinib exposure.

5.3.2.2 Anticoagulants and Antiplatelet therapy

See exclusion criteria section.

5.3.2.3 Growth factors

Prophylactic use of a colony-stimulating factor (G-CSF or GM-CSF) is not permitted on this trial. Nonetheless, use of a colony-stimulating factor for the treatment of febrile neutropenia as per American Society of Clinical Oncology guidelines is permissible (Bennett et al. J. Clin Oncol 1996 Sep; 14(9):2511-2520). Use of erythropoietin (including Procrit and Aranesp) for treatment of disease or treatment-related anemia is permitted.

5.4 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements,
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.

5.5 Duration of Follow Up

Participant survival information will be collected, preferably via office visit or telephone contact, every 12 weeks (±1 week) from the date of last dose of study drug until 1) the participant's death, 2) the participant is lost to follow-up, or 3) study closure (approximately 6 months after the last participant terminates treatment). Participants removed from study for unacceptable

adverse events will be followed until resolution or stabilization of the adverse event, and they will be followed up for survival as above.

5.6 Criteria for Removal from Study

Participants will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator (or Protocol Chair), Dr. Bruce Giantonio at pager 15050

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) which is available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Subjects will receive dasatinib orally at a (starting) dose of 100 mg once daily. Dose reductions and delays to manage toxicity are allowed under the guidelines below.

All adverse events (AEs) experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

Participants must be instructed to notify their physician immediately for any and all toxicities. The general adverse event profile of dasatinib includes pleural effusions, pericardial effusions, peripheral edema, and severe myelosuppression. Other toxicities likely to be encountered due to dasatinib are nausea, heartburn, diarrhea, rash, liver toxicity, and fatigue.

6.2 Dose Reductions, Interruptions, Re-Escalations, and Delays

6.2.1 Dose Reductions

Table 6-1 Dose Reductions

Dose Level	Dasatinib Dose
Starting Dose	100 mg daily
-1	70 mg daily
-2	40 mg daily

Guidelines for the management of AEs (ie, dose interruptions and dose reductions) are presented in the next sections. Each dose reduction of dasatinib should be to one dose level lower that the current dose. Dose reductions of more than one dose level are acceptable if agreed to by the Investigator. If study treatment of dasatinib is restarted after being withheld or interrupted, the subject should be instructed not to make up the missed doses of dasatinib. The reason for treatment delay and reduced dose must be recorded on the CRF.

6.2.2 Dose Interruptions

Dosing may need to be interrupted for AEs considered not related to dasatinib if this is clinically indicated or if causality is initially uncertain. Study treatment may be resumed at the same dose (or a lower dose per investigator judgment) if the AE is determined not to be related to dasatinib once the investigator determines that retreatment is clinically appropriate and the participant meets the protocol re-treatment criteria.

If study treatment is interrupted, the participant should be instructed not to make up the withheld doses, and the planned safety and tumor assessment schedule are to be maintained.

6.2.3 Dose Re-Escalations

Dose re-escalation is not allowed for dose reduction triggered treatment related toxicities.

6.2.4 Discontinuation of Study Drug

Reasons/events triggering permanent discontinuation of dasatinib:

- If the subject does not recover from his or her toxicities to tolerable Grade ≤ 2 or baseline within 6 weeks
- Subjects who cannot tolerate 40 mg daily
- An acute myocardial infarction or any other clinically significant arterial thromboembolic complication
- Patients with confirmed pulmonary artery hypertension
- Any treatment-related Grade 4 non-hematologic toxicity. However, if the patient is deriving clinical benefit from dasatinib, the toxicity must resolve to Grade ≤ 1 prior to restarting, and after approval by the principal investigator, treatment at a reduced dose can be considered.
- Treatment-related Grade 4 febrile neutropenia. However, if the patient is deriving clinical benefit from dasatinib, the toxicity must resolve to Grade ≤ 1 prior to restarting, and after approval by the principal investigator, treatment at a reduced dose can be considered.
- Necessity for treatment with other anticancer treatment prohibited by protocol
- Sexually active subjects who refuse to use medically accepted barrier methods of contraception (eg, male condom, female condom) during the course of the study and for 4 months following discontinuation of study treatment
- Women who become pregnant or are breast feeding

The reason for study treatment discontinuation will be documented. For subjects who discontinue or are withdrawn from study treatment, every effort must be made to undertake

protocol-specified follow-up procedures and end-of-treatment assessments, if possible, unless consent to participate in the study is also withdrawn.

6.3 Toxicity Management

General Guidelines for Non-Hematologic and Hematologic Adverse Events (AEs)

General guidelines for the management of non-hematologic and hematologic toxicities are provided in Table 6-2 and Table 6-3, respectively. As a general approach, it is suggested that all AEs be managed with supportive care when possible at the earliest signs of toxicity.

Table 6-2 Management of Study Treatment-Related Non-Hematologic Toxicities

CTCAE Version 4 Grade	Guidelines/Intervention
Grade 1:	Add supportive care as indicated. Continue dasatinib at the current dose level.
Grade 2:	
Grade 2 AEs considered related to dasatinib that are subjectively tolerable or easily managed	Add supportive care as indicated. Continue dasatinib at the current dose level.
Grade 2 AEs considered related to dasatinib that are intolerable to the subject or deemed unacceptable in the investigator's judgment; or are not easily managed or corrected	Dose reduce • If the AE dose not resolve to Grade ≤ 1 or baseline in 7 to 10 days or worsens at any time, dasatinib dosing should then be interrupted. Then upon resolution to baseline or Grade ≤ 1, the reduced dose should be restarted. • If the AE does resolves to resolves to Grade ≤ 1 or baseline without a dose interruption, continue the reduced dose.
Grade 3:	
Grade 3 AEs considered related to dasatinib which occurred without optimal prophylaxis or which is easily managed by medical intervention or resolved quickly	• Interrupt dasatinib and add supportive care as indicated • For AEs that are easily managed (e.g., correction of electrolytes) with resolution to baseline or Grade ≤ 1 within 24 hours, dasatinib may be resumed at either the same dose or with a dose reduction at the discretion of the investigator unless this is a recurring event at which time the dose should be reduced • For AEs that require supportive care, the dose should be held while supportive care is initiated and optimized. Then upon resolution of the AE to baseline or Grade ≤ 1, dasatinib may be resumed at either the same dose or with a dose reduction at the discretion of the investigator unless this is a recurring event at which time the dose should be reduced
Grade 3 AEs considered related to study treatment that occurred despite optimal prophylaxis or is not easily managed by medical intervention	Interrupt study treatment until recovery to ≤ Grade 1 or baseline, and resume treatment with a dose reduction
Grade 4:	
Grade 4 AEs considered related to study treatment	Permanently discontinue study treatment unless determined that the subject is unequivocally deriving clinical benefit. In this case, upon recovery to Grade ≤ 1 or baseline, the subject may be re-treated at a reduced dose that is to be determined by the investigator

Dose reductions or delays may occur in the setting of lower grade toxicity than defined above if the investigator believes that it is in the interest of the subject's safety.

Table 6-3:Management of Hematologic Toxicities

CTCAE Version 4 Grade	Intervention
Neutropenia	
Grade 3 neutropenia with documented infection Grade 3 neutropenia ≥ 5 days Grade 4 neutropenia	Interrupt dasatinib treatment until resolution to Grade ≤ 1, and resume dasatinib treatment at a reduced dose.
Thrombocytopenia	
Grade 3 thrombocytopenia with clinically significant bleeding or Grade 4 thrombocytopenia	Interrupt dasatinib treatment until platelet count is ≥ 75,000/mm^3, and resume dasatinib treatment at a reduced dose
Febrile Neutropenia	
Grade 3 febrile neutropenia	Interrupt dasatinib treatment until recovery of ANC to Grade ≤ 1 and temperature to ≤ 38.0°C and resume dasatinib treatment at a reduced dose.
Grade 4 febrile neutropenia	Permanently discontinue study treatment unless determined that the subject is unequivocally deriving clinical benefit. In this case, upon recovery to Grade ≤ 1 or baseline, the subject may be re-treated at a reduced dose that is to be determined by the investigator
Other Grade 4 Hematologic Toxicities	
Grade 4 hematologic toxicities other than anemia	Permanently discontinue study treatment unless determined that the subject is clearly deriving clinical benefit. In this case, upon recovery to Grade ≤ 1 or baseline, the subject may be re-treated at a reduced dose that is to be determined by the investigator and sponsor and only with approval by the sponsor.
Grade 4 anemia	Permanent discontinuation for Grade 4 anemia is not mandated. Dose reductions or dose delays for anemia should be applied as clinically indicated. Supportive care such as red blood cell transfusions should be managed according to institutional guidelines.

ANC, absolute neutrophil count.

Neutropenia: Grade 1 (LLN ≤ ANC < 1.5 × 10^9/L; Grade 2 (1 × 10^9/L ≤ ANC <1.5 x 10^9/L), Grade 3 (0.5 × 10^9/L ≤ ANC < 1 × 10^9/L), Grade 4 (ANC < 0.5 × 10^9/L).

Febrile Neutorpenia: Grade 3 (present); Grade 4 (Life-threatening consequences; urgent intervention indicated).

Thrombocytopenia: Grade 1 (<LLN – 75 x 10^9/L); Grade 2 (<75.0 – 50.0 x 10^9/L); Grade 3 (Platelet count ≤ 50 - 25 × 10^9/L); Grade 4 (Platelet count < 25 × 10^9/L).

Dose reductions or delays may occur in the setting of lower grade toxicity than defined above if the investigator believes that it is in the interest of the subject's safety.

Treatment-Emergent Edema

If treatment-emergent edema becomes symptomatic, dasatinib dosing will be stopped and no restarted until toxicity is reduced to a grade 1 or less.

Treatment-Emergent Pleural Effusion

If treatment-emergent pleural effusion becomes symptomatic, the patient will undergo thoracentesis as needed for symptomatic relief and be treated with prednisone 40 mg daily (or equivalent) for 2 days, followed by a taper over 7 days.

Treatment-Emergent Corrected QT (QTc) Prolongation

Treatment with dasatinib has been associated with a mild prolongation of the QTc interval. Other factors which may contribute to QTc prolongation include

- Treatment with other drugs associated with QTc prolongation (see http://www.qtdrugs.org)
- Treatment with CyP 3A4 inhibitors (which may increase dasatinib drug levels)
- Electrolyte changes (hypokalemia, hypocalcemia, hypomagnesemia)
- Medical conditions which can alter electrolyte status e.g., severe or prolonged diarrhea

Subjects having any of these additional risk factors while on dasatinib must have ECGs performed approximately one week after the onset of these factors.

If at any time on study there is an increase in QTc interval to an absolute value > 500 msec, two additional ECGs should be performed within 30 minutes after the initial ECG with intervals not less than 3 minutes apart. If the average QTcF from the three ECGs is > 500 msec, study treatment must be withheld and the following actions should be taken:

- Check electrolytes, especially potassium, magnesium and calcium. Correct abnormalities as clinically indicated
- If possible, discontinue any QTc-prolonging concomitant medications
- Repeat ECG triplets hourly until the average QTcF is ≤ 500 msec or otherwise determined by consultation with a cardiologist

The Sponsor should be notified immediately of any QTc prolongation event.

Subjects with QTc prolongation and symptoms must be monitored closely until the QTc elevation has resolved. Cardiology consultation is recommended for evaluation and subject management. Symptomatic subjects must be treated according to standard clinical practice. No

additional study treatment is to be given to the subject until after the event has resolved, the subject has been thoroughly evaluated, and further treatment has been agreed to by the Sponsor. If any additional study treatment is given (eg, after correction of electrolyte abnormalities and normalization of QTcF), it will be at a reduced dose as agreed to by the investigator and the Sponsor.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Dasatinib

Details can be found in the Investigator Brochure.

7.1.1 Description

Dasatinib tablets are white to off-white, biconvex, film-coated tablets containing dasatinib, with the following inactive ingredients: lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose, and magnesium stearate. The tablet coating consists of hypromellose, titanium dioxide, and polyethylene glycol.

7.1.2 Formulation

Dasatinib is provided in two different strengths:
20 mg film-coated tablets, biconvex, round, white to off-white in appearance with“BMS” debossed on one side and "527" on the other side
50 mg film-coated tablets, biconvex, oval, white to off-white in appearance with“BMS” debossed on one side and "528" on the other side
Dasatinib is packaged in bottles as follows:
dasatinib 20 mg film-coated tablets, 30 tabs/bottle
dasatinib 50 mg film-coated tablets, 30 tabs/bottle
Each bottle is labeled in an open label manner. Labels contain, at a minimum, the following information: product name, tablet strength, batch number, directions for use, storage conditions, and appropriate caution statements.

7.1.3 Storage and Stability

Dasatinib tablets should be stored in a secure area at 25°C (77°F); between 15°–30C°.

7.1.4 Handling

Procedures for proper handling and disposal of anticancer drugs should be considered.

Dasatinib tablets consist of a core tablet (containing the active drug substance), surrounded by a film coating to prevent exposure of pharmacy and clinical personnel to the active drug substance. If tablets are crushed or broken, pharmacy and clinical personnel should wear disposable chemotherapy gloves. Personnel who are pregnant should avoid exposure to crushed and/or broken tablets.

7.1.5 Availability

Dasatinib (Sprycel®) will be supplied by Bristol-Myers Squibb free-of-charge.

7.1.6 Administration

The dasatinib should be taken by mouth with a glass of water at approximately the same time in the morning each day, but fasting before and after dosing is not considered necessary at this time.
Subjects should try to avoid proton pump inhibitors and H2 antagonists.

7.1.7 Accountability

It is the responsibility of the Investigator to ensure that a current record of dasatinib disposition is maintained at each study site where dasatinib is inventoried and disposed.
Records or logs must comply with applicable regulations and guidelines, and should include:
Amount received and placed in storage area.
Amount currently in storage area.
Label ID number or batch number and use date or expiry date.
Dates and initials of person responsible for each dasatinib inventory entry/movement.
Amount dispensed to and returned by each subject, including unique subject identifiers.
Amount transferred to another area/site for dispensing or storage.
Non-study disposition (e.g., lost, wasted, broken).
Amount returned to BMS, if applicable.
Amount destroyed at study site, if applicable.
Retain samples sent to third party for bioavailability/bioequivalence, if applicable.
Dasatinib dispensing record/inventory logs and copies of signed packing lists must be maintained at the investigational site. Batch numbers for dasatinib must be recorded in the drug accountability records.

7.1.8 Destruction and Return

At the end of the study, unused supplies of dasatinib should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8. CORRELATIVE/SPECIAL STUDIES

8.1 Pharmacodynamic Studies

8.1.1 Laboratory Correlative Studies

8.1.1.1 Serum biomarkers for dasatinib

Our group has shown that circulating 2-HG is a surrogate biomarker of IDH1 or IDH2 mutation status in ICC and that circulating 2-HG levels may correlate directly with tumor burden (Borger 2014).

Blood samples will be obtained at baseline, 2 weeks after treatment, and at the beginning of each cycle, at time of disease progression, and tested for serum 2-HG and CA19-9 levels; These changes will be correlated with tumor response by imaging studies;

8.1.2 Tissue Biomarkers for dasatinib

8.1.2.1 Tissue biomarkers for dasatinib

Patients will have mandatory tumor core biopsies at baseline. Post-treatment biopsies at 8 weeks following dasatinib treatment and at the time of progression for those who had either SD or responses for more than 4 months are strongly encouraged but optional Tumor biopsies will be tested for changes in phospho-paxillin (Src kinase target), activated beta-catenin, Ki-67 (proliferation) and cleaved-caspase 3 (cell death).

9. CLINICAL AND LABORATORY EVALUATIONS AND STUDY CALENDAR

Baseline evaluations are to be conducted within 2-weeks prior to start of protocol therapy. Scans must be done $\leq$4 weeks prior to the start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

All assessments must be performed prior to administration of any study medication. All study assessments should be done within $\pm$ 3 days of the protocol-specified date, unless otherwise noted.

9.1 Evaluations

9.1.1 Pretreatment evaluations

Evaluations that must be performed within 1 week prior to the first dose of dasatinib:

- Pregnancy test (serum or urine) for women of childbearing potential within 7 days prior to initiation of therapy.

Evaluations that must be performed within 2 weeks prior to the first dose of dasatinib:

- Medical history
- Physical examination, including vital signs, height, weight, performance status.
- Hematology: complete blood count (CBC) with differential.
- Serum Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, SGOT(AST), SGPT (ALT), calcium, phosphorus and TSH.
- CA19-9
- PT/INR
- Urinalysis: Lab urinalysis will be obtained. Patients with a positive urinalysis for urine protein (reading of 2+ or greater) will then undergo a 24 hour urine collection for protein.
- Electrocardiogram
- Hepatitis B surface antigen, core antibody, and surface antibody, and Hepatitis C antibody must be obtained within 120 days of study enrollment

*If the screening labs have been completed within 7 days of Cycle 1 Day 1, these labs do not need to be repeated. However, baseline correlative studies need to be drawn on Cycle 1 Day 1 prior to administration of dasatinib.

Evaluations that must be performed within 28 days prior to the first dose of dasatinib:

- Imaging: Baseline radiologic evaluation of tumor burden by chest/abdomen/pelvis CT scan. MRI of the liver can be used if patients have an IV contrast allergy or the CT scan is suboptimal based on the physician's judgment.

9.1.2 On-Treatment Evaluations

- Subjects are defined as enrolled upon receipt of the first dose of study treatment.

- Each cycle is defined as 28 days.

- During cycle 1, assessments will be made on day 1, 8 and 15.
- During cycles 2-3, assessments will be made on day 1, and 15.
- In subsequent cycles, assessments will be made on day 1.

- All non-radiological assessments have a +/- 3 day window.
- All radiological assessments have a +/- 7 day window.

If the participant is unable to have a study assessment taken within the defined time window due to an event outside of his or her control (e.g., clinic closure, personal emergency, inclement weather, vacation), the assessment should be performed as close as possible to the required schedule.

See Table 9-1 for details on the timing of assessments for on-treatment evaluations.

- Physical examination, including vital signs, height, weight, performance status.
- Toxicity assessment
- Hematology: complete blood count (CBC) with differential.
- Serum Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, SGOT(AST), SGPT (ALT), calcium, phosphorus and TSH.
- CA19-9
- Urinalysis: Lab urinalysis will be obtained. Patients with a positive urinalysis for urine protein (reading of 2+ or greater) will then undergo a 24 hour urine collection for protein.

9.1.3 Post-Treatment Evaluations

Subjects will return to the study site between 30 – 37 days after the last dose of dasatinib for an end-of-treatment assessment. Laboratory and physical examinations will be performed. Remaining study treatment will be returned by the participant, and treatment compliance will be documented. Additional follow-up will occur for participants with AEs related to study treatment that are ongoing at the time of this visit, and for participants with SAEs related to study treatment that occur after the time of this visit.

All Grade 3 or 4 SAEs that are ongoing 30 days after the last dose of study treatment, and AEs assessed that led to study treatment discontinuation that are ongoing 30 days after the last dose of study treatment, are to be followed until:

- the AE has resolved, or;
- the AE has improved to Grade 2 or lower, or;
- the investigator determines that the event has become stable or irreversible.

This requirement also applies to related SAEs that occur > 30 days after last dose of study treatment. The status of all other AEs that are ongoing 30 days after the last dose of study treatment will be documented as of the Post-Treatment Follow-Up Visit.

After the end-of-treatment assessment, limited participant survival information (medical history, ECOG performance status, toxicity assessment) will be collected, preferably via office visit or telephone contact, every 12 weeks (±1 week) from the date of last dose of study drug until 1) the participant's death, 2) the participant is lost to follow-up, or 3) study closure (approximately 6 months after the last participant terminates treatment).

9.2 Study Calendar

Table 9-1: STUDY CALENDAR

	Screening (weeks)			Cycle 1			Cycle 2-3		Cycle 4 and beyond	Every 2 cycles (+/- 7 days)	Post-Treatment [9]
	-4	-2	-1	Day 1[5]	Day 8[5]	Day 15[5]	Day 1 of each cycle[5]	Day 15 of each cycle[5]	Day 1 of each cycle[5]		
Informed consent		X									
Medical History		X		X	X	X	X	X	X		X
Concurrent meds		X		X	X	X	X	X	X		X
Physical exam (including height, weight, pulse, blood pressure, O_2 saturation)		X		X	X	X	X	X	X		X
ECOG Performance Status		X		X	X	X	X	X	X		X
Toxicity Assessment					X	X	X	X	X		X
Archived Tumor Tissue		X									
EKG		X					X		X		
CBC w/differential		X		X[6]	X	X	X	X	X		X
Bilirubin, ALT, AST, Albumin, Alkaline Phosphatase		X		X[6]	X	X	X	X	X		X
Na, K, Cl, HCO3, glucose, BUN, Creatinine, Mg, PO4, Ca		X		X[6]	X	X	X	X	X		
LDH		X		X[6]			X		X		
PT/PTT/INR		X		X[6]							
TSH with reflex T4		X		X[6]			X		X		X
Urinalysis[1]		X		X[6]			X		X		
CA19-9		X		X[6]			X		X	X	X
HBsAg, HBsAb, HBcAB, HCV Ab[2]	X										
β-HCG[3]			X				X		X		
Tumor measurements	X									X	
Radiologic evaluation[4]	X									X	
Correlative Serum Studies				X[7]		X[7]	X[7]		X[7]		
Correlative Tissue Studies[8]	X										

1. Will have 24-hour urine protein collection for ≥ 2+ protein on urinalysis.
2. Hepatitis B and C serology as listed in the study calendar must be obtained within 120 days of

study enrollment.
3. Serum or urine β-HCG should be performed on women of child bearing potential.
4. Baseline measurements of tumor by radiographic means should be completed within 28 days prior to treatment and should include full evaluation of the extent of metastatic disease. Assessments are repeated every 2 cycles (8 weeks). Responses must be confirmed after 4-8 weeks with repeat radiological scans. If abdominal/pelvic CT scan or abdominal MRI is chosen as the radiographic means of tumor measurement, then the same modality should be used consistently for all future assessments.
5. Required non-radiological assessments should be performed within +/- 3 days of the scheduled day.
6. Screen assessments need not be repeated for cycle 1 day 1.
7. Correlative serum studies will be performed at baseline, 2 weeks after treatment, and at the beginning of each cycle, and tested for serum 2-HG and CA19-9 levels.
8. Correlative tissue studies will be performed on the biopsy tissues obtained at baseline, 8 weeks after dasatinib treatment and at the time of disease progression. See Appendix C for details.
9. Subjects will return to the study site between 30 – 37 days after the last dose of dasatinib for an end-of-treatment visit and undergo the assessments in this column. After that, limited participant survival information (medical history, ECOG performance status, toxicity assessment) will be collected, preferably via office visit or telephone contact, every 12 weeks (±1 week) from the date of last dose of study drug until 1) the participant's death, 2) the participant is lost to follow-up, or 3) study closure (approximately 6 months after the last participant terminates treatment).

10. MEASUREMENT OF EFFECT

10.1 Antitumor Effect– Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks following initial documentation of objective response. Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee (Therasse et al, 2000). RECIST version 1.1 will be used. Changes in the diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

10.1.1 Definitions

Evaluable for toxicity. All participants who receive at least one dose of study treatment will be evaluable for toxicity from the time of their first treatment.

Evaluable for objective response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective

disease progression or die prior to the end of cycle 1 will also be considered evaluable.}

10.1.2 Disease Parameters

Measurable disease. Measurable disease is the presence of at least one (1) lesion that can be accurately measured in at least one dimension with longest diameter ≥20 millimeters (mm) using conventional techniques (CT, MRI, x-ray) or ≥10 mm with spiral CT scan. Measurable lesions must be at least 2 times the slice thickness in mm. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Tumor lesions that are situated in a previously irradiated or embolized areas can be considered measurable if there is objective evidence of progression of the lesion prior to study enrollment.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm) or pathological lymph nodes with ≥ 10 to < 15mm short axis, are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques, and cystic lesions are all considered non-measurable.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Lesions must be accurately measured in 1 dimension with a minimum size of 10 mm by CT or MRI (slice thickness no greater than 5 mm), 20 mm by *chest* x-ray. Nodes must have a short axis ≥ 15 mm. The short axis should be included in the sum of the lesions in the calculation of response. Nodes that shrink to < 10 mm are considered normal. Target lesions should be selected on the basis of their size, be representative of all the involved organs, and should be lesions that can be followed with reproducible repeated measurements.

Lytic bone lesions or mixed lytic-blastic lesions, *with identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered target lesions if the *soft tissue component* meets the definition of measurability as defined above. Cystic lesions thought to represent cystic metastases can be considered as target lesions. However, if non-cystic lesions are present, these are preferred for selection as target lesions.

Lesions in previously irradiated areas or areas subject to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression of that lesion.

Non-target lesions. All other lesions, including small lesions < 10 mm or pathological lymph nodes measuring ≥ 10 mm to < 15 mm in short axis, as well as truly non-measurable lesions, which include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses identified by physical exam that are not measurable by reproducible imaging techniques.

10.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation, using a ruler, calipers, or digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US). US is not an acceptable method of disease assessment.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

10.1.4 Response Criteria

10.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph node must have reduction in short axis to < 10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study with at least a 5 mm absolute increase in the sum of all lesions. The appearance of one or more new lesions* denotes disease progression.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Unknown (UN): Assessment of target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

Note: If tumor response data is missing for target lesions, the overall assessment must be UN unless there is new disease that would result in an overall assessment of PD. However, if there is missing or unevaluable data for non-target lesions, but data is available for all target lesions, the overall response for that time point will be assigned based on the sum LD of all target lesions. Additionally, the assessment of CR cannot be made if there is missing or unevaluable data for non-target lesions. In this case, the overall assessment would be PR.

*Definition of New Lesion: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesions and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions* (new lesions must be > slice thickness) and/or unequivocal progression of existing non-target lesions.

Note: Increased uptake on bone scan alone is insufficient evidence of progression; additional evidence of progressive disease would be present to declare unequivocal progression.

Unknown (UN): Assessment of non-target lesions cannot be made due to insufficient or unevaluable data. In this case, a concise explanation must be given.

*Definition of New Lesion: The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (ex: new bone lesions may be healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size, etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

10.1.4.3 Evaluation of new lesions

No: There are no new lesions.

Yes: New lesions are present. Note: If new lesions are present, the patient is considered to have progressive disease overall.

10.1.4.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best response assignment will depend on the achievement of both measurement and confirmation criteria. Overall response will be determined as tabulated below, based on the evaluation of target, non-target, and new lesions:

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response for when Confirmation is Required:
CR	CR	No	CR	≥4 wks confirmation
CR	Non-CR/Non-PD	No	PR	≥4 wks confirmation
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/Not evaluated	No	PR	
SD	Non-CR/Non-PD/Not evaluated	No	SD	Documented at least once ≥4 wks from baseline
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD*	Yes or No	PD	
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as *"symptomatic deterioration"*. Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	NonCR/non-PD
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Non-CR/non-PD is preferred over stable disease for non-target disease since SD is increasingly used an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

In the case of stable disease (SD), follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 16 weeks.

Every effort should be made to document tumor measurements and extent of disease, even after discontinuation of therapy, in order to classify patients for overall response as described above. Patients who do not have tumor response assessment due to rapid progression or toxicity will be considered as non-responders, will be included in the denominator for the response rate, and will be classified into one of the following categories:

- death attributed to disease progression
- deterioration attributed to disease progression
- death attributed to drug toxicity
- early discontinuation attributed to drug toxicity

10.1.5 Definitions related to response and progression:

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started. Patients who do not relapse will be censored at the day of their last tumor assessment.

Time to response: Time from date of initial treatment until first objective documentation of response.
Time to tumor progression: Time from date of initial treatment to first objective documentation of progressive disease or death. Patients who die without a reported prior progression will be considered to have progressed on the day of their death.
Time to treatment failure: Time from date of initial treatment to first objective documentation of progressive disease, or off study date, whichever occurs first.
Time to death: Time from date of initial treatment to date of death

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the duration of time from start of treatment to time of objective disease progression or death from any cause without evidence of disease progression, whichever comes first.

Overall Survival: Overall Survival (OS) is defined as the duration of time from start of treatment to time of death.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

All laboratorydata required by this protocol and any other clinical investigations should be reviewed. Any abnormal value that leads to a change in subject management (eg, dose reduction or delay or requirement for additional medication or monitoring) or that is considered to be of clinical significance by the investigator should be reported as an AE as appropriate.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events not considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen

- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Other Safety Considerations

Pregnancy – If a subject becomes pregnant during the study, she will be taken off study treatment and will be followed through the end of her pregnancy. Forms for reporting pregnancies will be provided to the study sites upon request. The outcome of a pregnancy (for a subject or for the partner of a subject) and the medical condition of any resultant offspring must be reported to BMS or designee. Any birth defect or congenital anomaly must be reported as an SAE, and any other untoward events occurring during the pregnancy must be reported as AEs or SAEs, as appropriate.

Medication Errors/ Overdose – Any study drug administration error or overdose that results in an AE, even if it does not meet the definition of serious, requires reporting within 24 hours to BMS or designee.

11.1.4 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.4.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered <u>expected</u> when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

11.1.4.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered <u>unexpected</u> when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.5 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Follow-Up of Adverse Events

Any related SAEs or any AEs assessed as related that led to treatment discontinuation, including clinically significant abnormal laboratory values that meet these criteria, ongoing 30 days after the last dose of study treatment must be followed until either resolution of the event or determination by the investigator that the event has become stable or irreversible. This follow-up guidance also applies to related SAEs that occur > ***30 days after the last dose*** of study treatment. The status of all other continuing AEs will be documented as of 30 days after the last dose of study treatment.

11.3 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

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 website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.4 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.5 Reporting to the Study Sponsor

11.5.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention.
- All Grade 4 (life-threatening or disabling) Events – Unless expected AND specifically listed in the protocol as not requiring reporting.
- All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Principal Investigator: Bruce Giantonio, MD
Institution: Massachusetts General Hospital Cancer Center
[redacted]

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

Serious Adverse Event Reporting to BMS

All SAEs, whether considered related or unrelated to dasatinib, must be reported to BMS (by the investigator or designee) within 24 hours of study personnel becoming aware of the event.
All SEAs should be faxed or emailed to BMS at:

Global Parmacovigilance & Epidemiology
Bristol-Myers Squibb Company
[redacted]

As soon as an investigator becomes aware of an AE that meets the definition of 'serious,' this should be documented to the extent that information is available.

- This report must be submitted by the study site to BMS or designee within 24 hours, even if it is not felt to be drug related
- The investigator agrees to provide supplementary information requested by the BMS Drug Safety personnel or designee.
- Pregnancy, although not itself an SAE, should also be reported on an SAE form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

Regulatory Reporting to the FDA and BMS
All serious unexpected adverse drug reactions (unexpected related SAEs) must be reported to the Food and Drug Administration (FDA) by the investigator as required by 21 CFR 312.32.

- These reports are to be filed utilizing the Form FDA 3500A (MedWatch Form)

The final MedWatch Form must be submitted by the study site to BMS at the time of submission to the FDA to allow BMS time to cross-report to BMS IND.

11.5.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.6 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

Other investigative sites should report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional SAE form should be forwarded to:

Principal Investigator: Bruce Giantonio
Institution: Massachusetts General Hospital Cancer Center
[redacted]

The DF/HCC Principal Investigator will submit SAE reports from outside institutions to the DFCI Office for Human Research Studies (OHRS) according to DFCI IRB policies and procedures in reporting adverse events.

11.7 Reporting to the Food and Drug Administration (FDA)

(Use this section for investigator-held IND studies only, including gene transfer)
The DF/HCC Overall Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. The DF/HCC Overall Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by telephone (1-800-FDA-1088) or by fax (1-800-FDA-0178) using Form FDA 3500A (Mandatory Reporting Form for investigational agents) or FDA Form 3500 (Voluntary Reporting Form for commercial agents). Forms are available at http://www.fda.gov/medwatch/getforms.htm.

11.8 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.9 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case

report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related

activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
  www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
  - Title 21 Part 11 – Electronic Records; Electronic Signatures
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html
  - Title 21 Part 50 – Protection of Human Subjects
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
  - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
  - Title 21 Part 56 – Institutional Review Boards
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
  - Title 21 Part 312 – Investigational New Drug Application
    www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html
- State laws
- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14. STATISTICAL CONSIDERATIONS

14.1 Study Design/Endpoints

14.1.1 Study Design

This is an open-label, single-arm, two stage, Phase II study of dasatinib in patients who have progressed on first line systemic therapy for advanced intrahepatic cholangiocarcinoma (ICC) with either *IDH1 or IDH2* mutations.

This phase II study would have a two-stage design, with a target accrual of 8 evaluable patients for the first stage and a total of 19 patients for the whole study. If no responses seen in the first 8 patients, the trial will be terminated. If at least 1 patient in the first stage has response, the trial would proceed to the second stage. We hypothesized that dasatinib would improve the response rate from 2 % to 20%. As designed, the overall study with 19 evaluable patients would have an 80% power at a 0.04 level of significance to detect a 18% difference of response rate from 2% to 20%. At least 2 responses must be observed among the total study population of 19 patients at the completion of second stage in order to reject the null hypothesis (ORR $\leq$ 2%) in favor of the alternative (ORR $\geq$ 20%). Distributions of overall survival and PFS will be calculated using the Kaplan-Meier method, and confidence limits for survival estimates will be calculated using the Greenwood formula.

Based on the prevalence of ~20% of *IDH1/2* mutations, approximately 40-50 or 90-100 ICC patients will be screened depending on whether the study will be terminated after the first stage with 8 patients enrolled or completed with the targeted accrual of 19 patients.

14.1.1.2 Analysis Population

The analysis population will consist of all participants who enroll in the study.

14.1.1.3 Safety Population

The safety population will consist of all participants who receive any amount of study treatment.

14.1.1.4 Safety Analysis

Safety will be assessed by evaluation of AEs. All safety analyses will be performed using the safety population.

14.1.2 Primary Endpoint

The primary endpoint of the study is objective response rate (ORR) per RECIST v1.1 criteria. The objective response rate (ORR) is defined using the RECIST criteria as the proportion of the population with a confirmed CR or confirmed PR. The point estimate of the ORR with a 90% confidence interval based on the exact binomial distribution will be presented.

14.2 Sample Size, Accrual Rate, and Early Stopping Rule

14.2.1 Sample Size

The planned sample size for Stage I is 8 and if the study proceeds to Stage II, a total of 19 patients will be enrolled.

14.2.2 Accrual Rate and Follow-up after Termination of Accrual

An accrual rate of 1-2 patients/month is expected. Patients will be followed, as specified in Section 9, until death or until 6 months after the last enrolled patient terminates treatment, whichever comes first.

14.2.3 Rationale for Sample Size

We hypothesized that dasatinib would improve the response rate from 2 % to 20%. As designed, the overall study with 19 evaluable patients would have an 80% power at a 0.04 level of significance to detect a 18% difference of response rate from 2% to 20%. Prior studies with lapatinib and sorafenib have produced no responses in this population.

14.2.4 Early Stopping Rule

Of the 8 patients enrolled in Stage I, at least 1 patient needs to have response before the second stage can proceed.

14.2.5 Outcome Indicating Efficacy of the Drug

The overall study with 19 evaluable patients would have an 80% power at a 0.04 level of significance to detect a 18% difference of response rate from 2% to 20%. If this primary endpoint is met, the drug warrants further investigation in this population.

14.3 Analysis of Secondary Endpoints

14.3.1 Progression Free Survial (PFS)

Progression Free Survial (PFS) will be defined as the time from the start of treatment until progression or death from any cause. Kaplan-Meier estimates of PFS will be calculated along with their corresponding 95% confidence intervals.

14.3.2 Overall Survival

Overall survival (OS) will be defined as the time from the start of treatment until death from any cause. Kaplan-Meier estimates of overall survival rates will be calculated along with their corresponding 95% confidence intervals. Cox proportional hazards regression modeling of OS will be used to assess the effect of dasatinib on OS while controlling for other confounders.

14.3.3 Tolerability and safety analysis

For the safety analysis, all enrolled patients who receive at least one dose of dasatinib will be included for analysis. Adverse event terms recorded on the CRFs will be mapped to preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA). Seriousness, severity/grade, and relationship to study

treatment will be assessed by the investigator. Severity/ grade will be defined by the National Cancer Institute (NCI) CTCAE v4.0.

14.3.4 Biologic correlative studies of target inhibition (see above)
Kaplan-Meier survival curves and Cox Proportional Hazard models will be used to explore the relationship between the biomarker categories and the overall response rate as well as progression-free survival.

14.4 Reporting and Exclusions

14.4.1 Evaluation of toxicity. All participants will be evaluable for toxicity from the time of their first treatment.

14.4.2 Evaluation of response. All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.

15. PUBLICATION PLAN

The Principal Investigator (Protocol Chair) holds the primary responsibility for publication of the study results; provided that the PI will provide any such publication to BMS, Inc. for review at least sixty (60) days prior to submission and also comply with any provisions regarding publication as are agreed to between the PI's institution (eg, Massachusetts General Hospital) and BMS in the Clinical Trial Agreement related to this study. The results will be made public within 24 months of the end of data collection. However, if a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. In any event, a full report of the outcomes should be made public no later than three (3) years after the end of data collection. Authorship for abstracts and manuscripts resulting from this study will be determined according to guidelines established by the International Committee of Medical Journal Editors.

16. REFERENCES

1 Valle, J. *et al.* Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* **362**, 1273-1281, doi:10.1056/NEJMoa0908721 (2010).

2 Shaib, Y. & El-Serag, H. B. The epidemiology of cholangiocarcinoma. *Seminars in liver disease* **24**, 115-125, doi:10.1055/s-2004-828889 (2004).

3 Hainsworth, J. D. *et al.* Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon research institute. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **31**, 217-223, doi:10.1200/JCO.2012.43.3755 (2013).

4 Zhu, A. X. & Hezel, A. F. Development of molecularly targeted therapies in biliary tract cancers: reassessing the challenges and opportunities. *Hepatology* **53**, 695-704, doi:10.1002/hep.24145 (2011).

5 Bekaii-Saab, T. *et al.* Multi-institutional phase II study of selumetinib in patients with metastatic biliary cancers. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **29**, 2357-2363, doi:10.1200/JCO.2010.33.9473 (2011).

6 El-Khoueiry, A. B. *et al.* SWOG 0514: a phase II study of sorafenib in patients with unresectable or metastatic gallbladder carcinoma and cholangiocarcinoma. *Investigational new drugs* **30**, 1646-1651, doi:10.1007/s10637-011-9719-0 (2012).

7 Ramanathan, R. K. *et al.* A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. *Cancer chemotherapy and pharmacology* **64**, 777-783, doi:10.1007/s00280-009-0927-7 (2009).

8 Borger, D. R. *et al.* Frequent mutation of isocitrate dehydrogenase (IDH)1 and IDH2 in cholangiocarcinoma identified through broad-based tumor genotyping. *Oncologist* **17**, 72-79, doi:10.1634/theoncologist.2011-0386 (2012).

9 Jiao, Y. *et al.* Exome sequencing identifies frequent inactivating mutations in BAP1, ARID1A and PBRM1 in intrahepatic cholangiocarcinomas. *Nat Genet* **45**, 1470-1473, doi:10.1038/ng.2813 (2013).

10 Kipp, B. R. *et al.* Isocitrate dehydrogenase 1 and 2 mutations in cholangiocarcinoma. *Hum Pathol* **43**, 1552-1558, doi:10.1016/j.humpath.2011.12.007 (2012).

11 Wang, P. *et al.* Mutations in isocitrate dehydrogenase 1 and 2 occur frequently in intrahepatic cholangiocarcinomas and share hypermethylation targets with glioblastomas. *Oncogene*, doi:10.1038/onc.2012.315 (2012).

12 Zhu, A. X. *et al.* Genomic Profiling of Intrahepatic Cholangiocarcinoma: Refining Prognosis and Identifying Therapeutic Targets. *Annals of surgical oncology*, doi:10.1245/s10434-014-3828-x (2014).

13 Losman, J. A. *et al.* (R)-2-hydroxyglutarate is sufficient to promote leukemogenesis and its effects are reversible. *Science* **339**, 1621-1625, doi:10.1126/science.1231677 (2013).

14 Borger, D. R. *et al.* Circulating oncometabolite 2-hydroxyglutarate is a potential surrogate biomarker in patients with isocitrate dehydrogenase-mutant intrahepatic cholangiocarcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* **20**, 1884-1890, doi:10.1158/1078-0432.CCR-13-2649 (2014).

15 Saha, S. K. *et al.* Mutant IDH inhibits HNF-4alpha to block hepatocyte differentiation and promote biliary cancer. *Nature*, doi:10.1038/nature13441 (2014).

APPENDICES

Appendix A - Performance Status Criteria

ECOG Performance Status Scale	
Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Appendix B: Correlative Studies

Tissue Biomarkers for dasatinib

Exploratory analysis of potential tissue biomarkers of dasatinib will be performed in the MGH in the Bardeesy Laboratory (overseen by Dr. S. Saha and Dr. N. Bardeesy) on the formalin-fixed, paraffin-embedded diagnostic tumor tissue from baseline, 8 weeks after dasatinib treatment and at progression. Sections of all tumor samples submitted for analysis will be counterstained with hematoxylin and eosin. These slides will be reviewed by a pathologist to confirm diagnosis and to identify the region of the tissue samples that contains tumor for molecular and immunohistochemical analyses.

Shipment of Specimens to MGH:
Formalin-fixed, paraffin-embedded tumor tissue recuts should be submitted to the MGH Bardeesy Laboratory as detailed below:

a. Diagnostic cholangiocarcinoma specimens and post-treatment biopsies should be submitted as 4 micron-thick sections mounted onto microscope slides.
   - Sequential tissue sections should be cut and mounted as a single section on a positively-charged microscope slide.
   - Sections should be placed on the lower central third of the slide.
   - All sections from a given sample should be mounted in the same orientation on the microscope slide.
   - Surgical number and block should be printed or written legibly in pencil on each individual slide. Inclusion of the protocol number is highly recommended.
   - Slides should be air dried and not baked.
   - For shipment, slides should be secured in a crush-proof slide mailer.
   - To maintain the highest integrity for some labile targets of interest, it is important that the samples are shipped immediately after cutting by overnight courier. Shipments at room temperature are adequate.

b. For all samples shipped to the MGH:
   - A trial sample data sheet must be fully completed for each sample and must accompany the shipment.
   - A confirmation email must be sent to [redacted] indicating the courier, courier tracking number, clinical trial number, sample names and quantity of samples being sent. A reply to this email will indicate either receipt of all expected specimens in good condition or will list any problems/discrepancies.
   - Please ship by overnight courier to the following address. Overnight shipment should not fall on a Saturday, Sunday or holiday.

[redacted]

[redacted]

Blood Samples Shipment to MGH

The following supplies are needed for each blood sample drawn:

- EDTA blood collection tubes (Sarstedt, #03.1068)

Sample Labels

Prior to use, EDTA tubes should be labeled with the following information:

- Patient Initials
- Patient ID number
- Protocol number
- Collection date
- Institution

Collection and handling (Please see attached Biomarker Manual for full details)
Approximately 8 cc of venous blood will be collected in each of 2 EDTA blood collection tubes (Sarstedt, #03.1068). The tubes should be gently inverted several times to ensure mixing with the anticoagulant and kept on wet ice at all times. Within two hours the tubes should be sent to:

Steele Laboratory

[redacted]