Mayo Clinic Cancer Center

MC1488: A Phase 2 study of WEE1 Inhibition with AZD1775 alone or combined with Cytarabine in Patients with advanced Acute Myeloid Leukemia and Myelodysplastic Syndrome

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Drug Availability
Supplied Investigational Agents: AZD1775
Commercial Agents: Cytarabine (cytosine arabinoside or AraC)

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

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Protocol Resources

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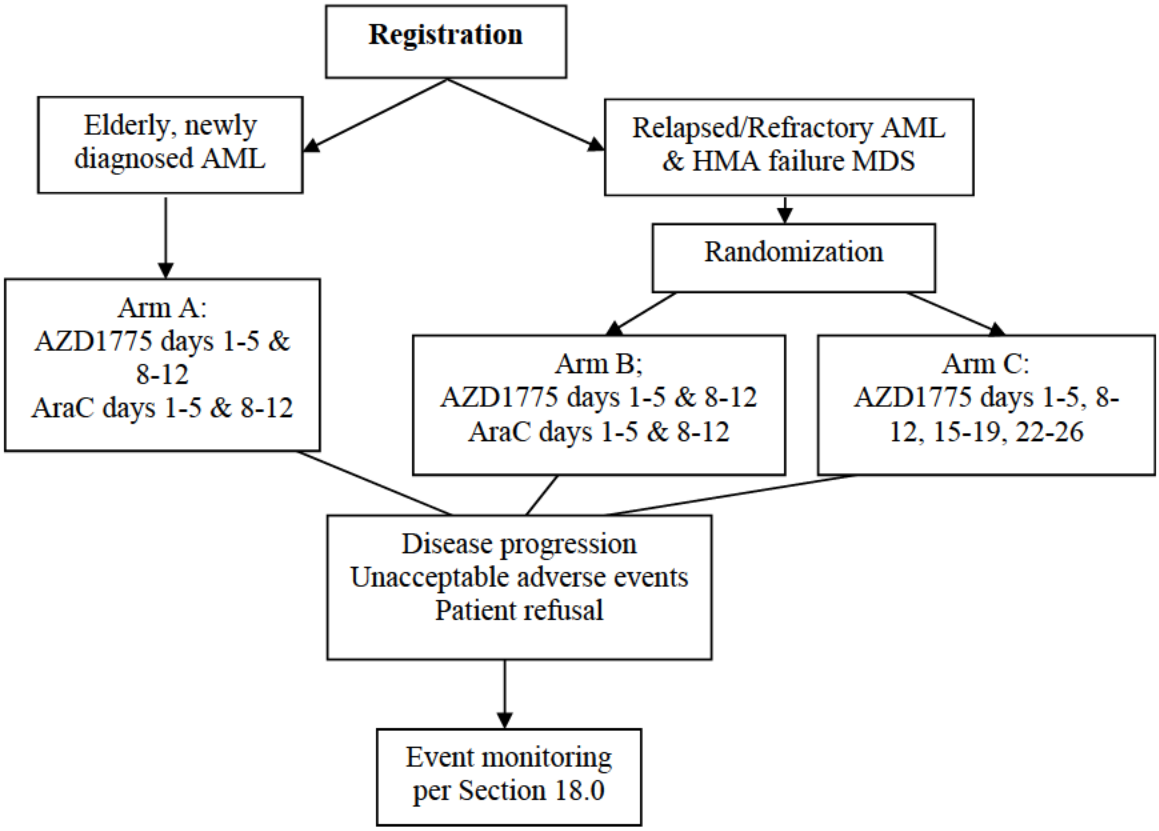
*No waivers per NCI

Index

Schema



If a patient is deemed ineligible or a cancel, please refer to Section 13.0 for follow-up information.

Cycle length=28 days
NOTE: The Safety portion of this trial will monitor six patients in each arm and observe them for a minimum of 28 days to assess toxicities.

Generic name: AZD1775 Brand name(s): n/a Mayo Abbreviation: AZD1775 Availability: Provided by AstraZeneca	Generic name: Cytosine arabinoside Brand name(s): Cytosar, ® Mayo Abbreviation: AraC (Cytarabine) Availability: Commercially available

1.0 Background

1.1 Acute Myeloid Leukemias (AML) and Myelodysplastic Syndrome (MDS)

Despite progress in the treatment of AML and MDS, most adult patients with AML and advanced/progressive MDS still do very poorly with current therapy and many die from their disease (Abe et al. 2008). Patients with primary refractory or relapsed AML have a particularly poor outcome, as well as MDS patients who are refractory, progress on or relapse after hypomethylating agents (HMA) based therapies (Duong et al. 2013, Prébet et al. 2011, Jabbour et al. 2010).

Acute myeloid leukemia patients who fail to achieve a complete remission (CR) with first induction therapy have a dismal outcome and only a small percentage of patients are alive at 1 year (Bai et al 2008). Current therapy is also inadequate for relapsed leukemias with a true long-term cure rate of less than 10% (Bai et al. 2008). Recently, comparable clinical activity and outcomes between HMAs and cytotoxic chemotherapy has been demonstrated in secondary, elderly and unfavorable cytogenetics AML in first line therapy (Quantas-Cardama et al. 2012 and Fenaux et al. 2010). Thus, many experts feel that these patients are candidates for lower intensity treatment as first line intervention.

The outcome of patients with advanced myelodysplastic syndrome (MDS) in the intermediate and IPSS intermediate-2, and especially high-risk category is almost as poor as for patients with acute leukemia. Median survival in this group is 4-5 months (Schanz et al. 2012 and Greenberg et al. 1997). The two approved HMAs Azacytidine (AZA) and Decitabine (DAC) have activity, however CR rates are less than <10-18% and overall responses (CR, PR and clinical benefit such as hematological improvements) approach at a maximum 40-50% in the first line treatment setting in MDS (Fenaux et al 2009 and Lubbert et al 2011).

Responses and survival in MDS patients having failed or progressed on or after HMA treatment is very poor with a median survival of 4.3-5.8 months and a 1-year survival of 17-29% in the largest three studies to date (Duong et al. 2013, Prébet et al. 2011, Jabbour et al. 2010). Accordingly HMA failure MDS represents one of the largest patient groups in need for novel agents and investigational therapies. In fact, apart from allogeneic transplant for which most patients do not qualify, investigational regimens were as good and better than cytotoxic chemotherapy after HMA failure (Prébet et al. 2011).

Azacitidine (AZA) and Decitabine (DAC) are often used as lower intensity, outpatient treatments in elderly AML patients not fit for induction treatment. Responses are comparable to somewhat lower than in treating MDS patients with CR/CRi rates ranging from 7-18% (Fenaux et al. 2010 and Kantarjian et al. 2012). Thus, there is likewise a great demand for improvement of alternative lower intensity, outpatient regimens and new combinations for elderly AML patients. Interestingly, in several earlier studies low-dose s.c. Cytarabine (AraC) alone or with growth factor support exhibited quite encouraging response rates of CR and PR of 18% and 8% respectively (Aul & Schneider 1989). Several studies also have been reported on the potential activity of low-dose AraC (AraC) in MDS (Visani et al. 2004). However, if the natural history of the disease is changed, and there is significant room for improvements in therapeutic options for these patients. In summary, available data demonstrates the clinical activity of HMAs and AraC specifically in elderly AML and MDS patients, providing additional clinical

justification, in addition to our pre-clinical data, to combine AZD1775 with AraC as one of the treatment arms in this protocol.

In conclusion, improvements in therapy for elderly AML patients, patients with relapsed/refractory AML as well as advanced MDS patients, particularly those who failed HMA treatments are urgently needed. Investigational therapies are one of the recommended therapeutic choices for these patients due to the lack of effective standard salvage treatments. Pre-clinically, inhibition of WEE1 kinase is one of the most potent sensitizers to AraC, identified in large-scale RNAi screens. Sensitization potential between WEE1 inhibtion and AraC was best at lower AraC concentrations as we have published (Tibes et al. 2012), and hence lower dose AraC will be used in the combination arm as proposed in this protocol. The design of this trial offers relapsed and progressive AML and MDS patients novel treatment options in the form of either single agent AZD1775 or in combination with AraC, and thereby will investigate for the first time the clinical activity of WEE1 inhibition in AML and MDS.

1.2 Overview of the WEE1 pathway and DNA damage and Cell cycle inhibition

1.21 Background on Cytarabine, DNA Damage Repair and Cell Cycle Checkpoints

Cytarabine (AraC) has been the most widely used and is still the overall most active drug in AML (Rowe 2009). It forms the backbone of most AML and MDS induction and consolidation regimens, and has activity at low doses given subcutaneously both in AML and MDS (Bolwell et al. 1987, Kantarjian et al 2012). AraC is an S-phase (cell cycle) specific agent that undergoes conversion and phosphorylation to a tri-phosphate form with subsequent incorporation into DNA of dividing cells. This usually triggers activation of the intra-S phase checkpoint a genotoxin-activated signaling pathway that stabilizes stalled replication forks and slows cell cycle progression by blocking the firing of late-acting DNA replication origins (Dimitrova & Gilbert 2000, Morita 1976, Grant 1998). AraC incorporation leads to single stranded DNA damage that later is converted to double strand DNA damage ultimately leading to cell cycle arrest and repair of damaged DNA (Do et al. 2013). Many of these processes are either directly regulated by WEE1 kinase, i.e. cell cycle arrest via CDK1/2, as well as WEE1 kinase plays a central role in the networks of DNA repair initiation, i.e. via its effect on CHEK1 or PLK1. Thus WEE1 kinase has a central role in many checkpoint and DNA damage mediated processes (Langerak & Russell 2011, Perry & Kornbluth 2007).

Interfering with cell cycle checkpoints and DNA damage repair (DDR) in combination with agents that cause DNA single or double strand breaks has been proposed as a promising concept in cancer therapy and captures a potential synthetic lethal molecular interaction. Advanced leukemias are genomically highly unstable, with cell cycle checkpoints and DDR pathways impaired causing these cells to rely on a fewer number of checkpoints, and highly reliant on the later stage in the cycle cycle at the G2/M transition (Cavelier et al. 2009, Didier et al. 2008). Thus, interfering with cell cycle checkpoints and DNA damage repair (DDR) in combination with agents that cause DNA single or double strand breaks such as AraC has been proposed as a promising concept in

cancer therapy and captures a potential synthetic lethal molecular interaction (Tibes et al. 2012).

1.22 Inhibition of WEE1 kinase by AZD1775

WEE1 kinase is an evolutionary conserved kinase regulating late G2/M cell cycle checkpoints, directing cells either towards cell cycle arrest allowing time for DNA repair or proceeding with progression into mitosis. However, emerging data indicates that WEE1 kinase activates the intra S-phase checkpoint as well and interferes with DNA damage response through various processes. WEE1 kinase acts through and is the main kinase phosphorylating and regulating Cdc2 (CDK1), which in complex with Cyclin B is a master regulator of mitotic entry at G2/M. However, WEE1 is also the main kinase phosphorylating CDK2 (assembles with cyclin A) and exerts a prominent function in G1/S phase and the intra S-phase checkpoint (Perry & Kornbluth 2007).

Based on two unbiased RNAi screens and subsequent validation studies, inhibition of WEE1 kinase was identified as one of the most potent sensitizers to AraC in AML cells (Tibes et al 2012, Porter et al. 2012). We postulate that WEE1 kinase is the essential kinase that governs the "escape pathway" in malignant myeloid cells under cellular stress with cytotoxic agents, particularly AraC. Therefore inhibition of WEE1 kinase by AZD1775 with AraC is a rational combination in aggressive myeloid malignancies and leukemias. In addition, AZD1775 had single agent activity and its anti-leukemic activity alone or in combination is independent of p53 mutation and function in AML (Van Linden et al. 2013 and Tibes, unpublished observation).

1.23 Rational of combined Cytarabine (AraC) and WEE1 inhibition

AraC is the backbone of therapy for myeloid leukemias. It is used in regimens for lymphoid leukemias as well as for patients with advanced MDS. Pre-clinically WEE1 inhibition sensitized cells to several cytotoxic agents including AraC. In Phase 1 and 2 ongoing clinical trials in solid tumor patients, the only in class Wee1 inhibitor AZD1775 is well tolerated with platinum compounds and gemcitabine providing a therapeutic window even at full standard doses of cytotoxic chemotherapies. We have generated preliminary data that shows potent sensitization with synergy combining AraC and AZD1775 in vitro and ex vivo. In addition WEE1 is highly expressed in myeloid leukemias (Tibes et al. 2012). Based on these data we hypothesize that the combination of AraC and WEE1 inhibitor AZD1775 will have strong synergistic clinical activity with manageable toxicity (given the already existing clinical data of tolerability in solid tumors) in patients with advanced myeloid malignancies. We further postulate that there will be single agent anti-leukemic activity of AZD1775 providing a rationale to compare AZD1775 single agent vs. in combination with AraC in patients with MDS and AML that would otherwise have limited therapeutic options remaining.

1.3 Overview of AZD1775

The following overview of AZD1775 (Section 1.3 and 1.4) is based on the knowledge available at the time this protocol was first finalized and is based on Edition 12 of the Investigator's Brochure, Edition 12, dated 18 February 2015, with a data cut-off date of 11 November 2014. For more detailed and up-to-date information, please consult the current Investigator's Brochure.

AZD1775 is a highly selective, adenosine-triphosphate (ATP) competitive, small-molecule inhibitor of the WEE1 kinase that sensitizes tumour cells to cytotoxic agents and is being developed for the treatment of advanced solid tumours with p53 pathway deficient malignancies. Inhibition of the DNA damage checkpoint kinase WEE1 potentiates genotoxic chemotherapies by abrogating cell-cycle arrest and eliminating the opportunity for proper DNA repair to occur. From a therapeutic standpoint, inhibition of checkpoint kinases that mediate cell-cycle arrest may force tumour cells to continue cell division before chemically induced DNA damage is repaired, eventually causing apoptosis or mitotic catastrophe (Medema and Macurek 2012).

The primary objective of the clinical development of AZD1775 is its use as a chemosensitizing drug in combination with a cytotoxic agent (or combination of agents) for treatment of advanced tumors. In vitro experiments demonstrate that AZD1775 has synergistic cytotoxic effects when administered in combination with various DNA damaging agents that have divergent mechanisms of action. In studies with matched ovarian cell lines (p53 WT and shRNA p53 knockdown), AZD1775 enhanced cell death induction by gemcitabine in p53-deficient but not in p53 positive control cells. AZD1775 also demonstrates synergistic effects on cell death induction when used in combination with cisplatin and carboplatin in a p53-dependent manner. Cervical cancer cells with HPV induced inactivation of p53 demonstrated chemosensitization to cisplatin and topotecan by AZD1775.

The ability of AZD1775 to affect tumour growth as monotherapy or to enhance the antitumor effects of gemcitabine, carboplatin, cisplatin, capecitabine, 5-fluorouracil, and gamma irradiation was evaluated in immunocompromised host animals bearing human xenograft tumors.

The anti-tumor effect of AZD1775 dosed as monotherapy was investigated in the A427 non small-cell lung cancer nude mouse xenograft model. Daily treatment with AZD1775 led to 51% tumour regression (n=10) and mean body weight loss did not exceed 5% over the course of the study. AZD1775 single agent treatment also led to tumour growth inhibition in additional xenograft models: 92% TGI (Day 28) in SKMES1 model of non-small-cell-lung cancer (NSCLC), 13% regression (day 11) in LoVo colorectal cancer model and 64% TGI(day 19) in NCI-H2122 NSCLC.

The anti-tumour effect of AZD1775 in combination with gemcitabine was investigated in the WiDr (human colorectal adenocarcinoma) nude rat xenograft model. Several schedules of gemcitabine + AZD1775 were explored. A 10 mg/kg dose of AZD1775 significantly enhanced the anti-tumour effect of gemcitabine in WiDr tumours with % treated/control (T/C) = -2%.

The anti-tumour effect of AZD1775 in combination with carboplatin was investigated in the HeLa-luc (human cervical adenocarcinoma) nude rat xenograft model. AZD1775

dose-dependently enhanced the anti-tumour effect of carboplatin tumors with %T/C = 85, 39 and 28% at doses of 10, 20 and 30 mg/kg, respectively.

The anti-tumour effect of AZD1775 in combination with carboplatin or cisplatin was also investigated in the HeLa-luc model. These experiments showed that AZD1775 dose-dependently enhanced the anti-tumour effect of cisplatin with %T/C = -5 and -16% at doses of 10 and 20 mg/kg respectively, compared to or cisplatin alone (24% T/C)

AZD1775 enhanced the anti-tumour efficacy of 5-FU and capecitabine when used in combination with these agents, as well; and experiments with nude mouse xenograft models of A549 (p53 wild type) and Calu-6 (p53 null) NSCLC cell lines showed enhanced antitumour growth effect of radiotherapy preferentially in p53 mutant xenograft tumors. Please refer to the AZD1775 Investigator Brochure (IB) for more detailed information regarding these experiments and findings.

1.31 Pharmcodynamics

Inhibition of CDC2 (Y15) phosphorylation and induction of Histone H3 (Ser10) phosphorylation were observed upon Wee1 inhibition *in vitro*. *In vivo* PD effects of AZD1775 were evaluated using the WiDr xenograft nude rat model. AZD1775 (0.5, 1.0, 3.0 and 7.0 mg/kg/hr) was intravenously infused for 8 hrs, 24 hrs after administration of gemcitabine (50 mg/kg, IV). PD marker analyses were performed on tumor tissue isolated immediately after the infusion. Continuous infusion of AZD1775 caused reduction of phospho-CDC2 (Y15) in WiDr xenograft tumor tissue in a dose-dependent manner. The EC50 value was 0.28 μM, and about 80% inhibition was achieved at 1.0 mg/kg/hr (0.45 μM at 8hr). Similar dose dependency was observed in the CEA (induction of phospho-Histone H3 [Ser10]) in tumor tissue. EC50 value was 0.21 μM, and maximal effect was observed at approximately 1.0 mg/kg/hr. These data suggest that phopsho-CDC2 (Y15) and phospho-Histone H3 (Ser10) could be useful as AZD1775 PD biomarkers in tumors.

Similar PD marker changes were observed in surrogate tissues, such as skin hair follicle and peripheral blood cells in the presence or absence of the DNA damaging agent gemcitabine. Strong pCDC2 (Y15) immunopositivity was observed in skin hair bulb in the hair follicle. In combination with gemcitabine (50 mg/kg, IV: 24 hr before AZD1775 dosing), AZD1775 (0.5, 1.0 and 3.0 mg/kg/hr x 8 hrs, IV-infusion) dose-dependently decreased these signals. An almost complete reduction of phospho-CDC2 signal was achieved at 3.0 mg/kg/hr. Phospho-CDC2 (Y15) signal in peripheral blood cells were also reduced in a dose dependent manner with significant reduction at 1.0 and 3.0 mg/kg/hr. These results suggest that hair follicles and blood cells are surrogate tissues in which phospho-CDC2 (Y15) can be used as a marker to predict PD/efficacy in tumors treated with gemcitabine.

Similar dose dependent reduction of phospho-CDC2 (Y15) by AZD1775 (0.5, 1.0 and 3.0 mg/kg/hr x 8 hrs, IV-infusion) was observed without gemcitabine pretreatment in both skin hair follicles and tumors. Thus, these PD markers may be available to predict PD/efficacy in tumors in the presence or absence of DNA damaging agents.

1.32 Nonclinical pharmacokinetics and metabolism

The pharmacokinetics of AZD1775 were evaluated in male Sprague-Dawley rats and

Beagle dogs following intravenous (IV) and oral administration. AZD1775 exhibited high plasma clearance in rats and dogs (57.3 and 50.8 mL/min/kg, respectively). The terminal half-life (T1/2) of the compound was short in both species (1.6 and 1.1 hr in rats and dogs, respectively). The oral bioavailability was 59.7% in rats and 33.6% in dogs. AZD1775 (1 μM) was moderately bound to plasma proteins from rat, dog and human, with the unbound fractions being 23.2, 40.0, and 39.5%, respectively. Metabolism was the major route of elimination of AZD1775 in rat and dog. The major metabolic pathway of AZD1775 in human liver preparations was oxidative metabolism. All metabolites observed in human liver preparations were also formed in the rat and dog. Oxidative metabolism of AZD1775 was mediated predominantly by cytochrome P450 (CYP) 3A4 and FMO3. AZD1775 was a weak reversible inhibitor of CYP2C8, CYP2C9, CYP2C19 and CYP3A4. In addition, AZD1775 was a time-dependent inhibitor of CYP3A4. Collectively, these data indicate that the pharmacokinetics of AZD1775 could be altered if AZD1775 is coadministered with CYP3A4 inhibitors and/or inducers, and depending on AZD1775 therapeutic plasma concentrations, there is also some potential for drug drug interactions when coadministered with CYP3A4 substrates.

1.33 Toxicity and safety Studies with AZD1775

In rats, the toxicologic profile of AZD1775 was evaluated using 4 separate dosing schedules; single-dose, once weekly for three weeks, and daily for 7 consecutive days or one month. A recovery (treatment-free period) was included in each study design. With each of these schedules, the toxicologic profile for physical signs, hematological changes, and gross and histomorphological findings were expected based on the cytotoxic mechanism of action of AZD1775. The major organs affected were proliferation dependent organs such as lymphoid and hematopoietic organs and gastrointestinal tract. Evidence of reversibility of all changes, based on drug-related effects observed in early death rats, was generally demonstrated by the end of the 2-week recovery period.

In the fifteen-day oral toxicity study, conducted in rats dosed once weekly for three weeks (Study Days 1, 8, and 15), no mortality or severe toxicity was observed at 300 mg/kg/day, however treatment-related body weight changes and hematological changes were observed. The magnitude of change of absolute reticulocyte counts and white blood cell parameters observed after the first dose were generally similar to those after the third dose, indicating that additive toxicity was not seen after 3 weekly intermittent doses. A trend toward recovery was observed for many of these changes following the 14 day recovery period, however recovery of hematological changes appeared to be delayed. Since the histomorphologic examination of the bone marrow revealed normal cellularity at the completion of the 14 day recovery, it is expected that hematological changes will fully recover as was seen in dogs. The magnitude of the decreased WBC and erythroid parameters were close to those observed in the previous single dose toxicity study in rats. No histological findings were noted at the completion of the 14 day recovery period in the 300 mg/kg/day once weekly group.

In a single dose oral toxicity study, severe irreversible toxicity (mortality) was seen in 1 female out of 10 female rats after a single dose at 300 mg/kg (1800 mg/m2). In a consecutive 7-day repeat dose study, expected toxicity of hematopoietic organs and the gastrointestinal tract were observed and associated with mortality at 75 mg/kg/day and 300 mg/kg/day. At 25 mg/kg/day (14.2 μM/hr), all animals survived to scheduled necropsies and all observed toxicities were demonstrated to be reversible during the 2-week recovery period. In a 1 month rat toxicity study, toxicity of hematopoietic organs

(including bone marrow, spleen, thymus, mesenteric lymph nodes, Peyer's patches) were observed at ≥10 mg/kg/day in females and at 25 mg/kg/day in males. Serum biochemical changes were observed at 25 mg/kg/day only and consisted mainly of very slight to slight decreases in total protein, albumin, and globulin and very slight increases in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in females whereas in males, the changes were limited to a slight decrease in globulin. Very slight to slight liver hepatocellular vacuolation, very slight centrilobular degeneration and slight cardiomegaly were observed at ≥10 mg/kg/day in females (except for cardiomegaly observed in females at 25 mg/kg/day only) and at 25 mg/kg/day in males; these findings in the liver and heart were considered to be correlated with anemia. At 25 mg/kg/day (25 μM/hr in females and 10 μM/hr in males), all animals survived to scheduled necropsies. In general, findings at 25 mg/kg/day were more marked in females compared to males, and all findings showed a trend towards recovery following a 4-week recovery period.

In dogs, similar dosing schedules as studied in rats were evaluated (single dose, once weekly for three weeks, and daily for 7 consecutive days or one month). Severe irreversible toxicity was not observed at the highest dose tested of 36 mg/kg (720 mg/m2) after a single dose and 3-intermittent doses (mean area under the plasma concentration time curve [AUC] from time 0 to 24 hours postdose [AUC0-24hr] for sexes combined = 17.4 μM/hr and 17.6 μM/hr, respectively). As reported in rats studies, similar hematologic changes were observed in dogs as was reported in rats; notably decreases in reticulocytes. In both the single-dose and 3 intermittent dose studies, reticulocyte counts returned to baseline following the 14-day recovery period and histomorphologic examination revealed normal cellularity in the bone marrow in these studies. In dogs receiving AZD1775 for 7 days, 15 mg/kg/day (9.5 μM/hr) was well tolerated and demonstrated expected toxicities to hematopoietic organs and the gastrointestinal tract. During a 2-week recovery period, test article-related changes recovered or showed a trend towards reversibility. In a 1-month dog repeat dose study, dose levels of 3 mg/kg/day and 10 mg/kg/day were administered daily for about 1 month. Dose dependant toxicity of hematopoietic organs (including bone marrow, thymus, lymph nodes, Peyer's patches), gastrointestinal tract, testes as well as serum biochemical changes (very slight or slight decreases in total protein, albumin, and globulin) were observed. At 10 mg/kg/day (3.60 μM/hr), all animals survived to scheduled necropsies. All findings showed a trend towards recovery following the 4-week recovery period.

AZD1775 was negative in the Microbial Mutagenesis Assay but positive in *in vitro* Chromosomal Aberrations Assays and in vivo micronucleus assay. These positive results in the chromosomal aberration assay were not unexpected based on the mechanism of action of AZD1775.

Combination treatment with AZD1775 and 5-FU was administered to tumor-bearing female nude rats for up to 5 days with several administration schedules. Test article related hematological changes (decreases in erythroid parameters, reticulocytes, leukocytes, and/or platelets) were observed with the combination treatment with AZD1775 plus 5-FU. There were no remarkable differences in the severity of these changes across the different AZD1775 administration schedules in combination with 5-FU.

These preclinical observations were expected based on the intended pharmacologic action (cytotoxicity) of the compound. The toxicity profile of AZD1775 in rats and dogs following 1 month of daily dosing was generally consistent with the toxicity profile

previously observed in studies of shorter duration. Observations in rat and dog toxicology studies are generally monitorable and with evidence of reversibility within a 4-week recovery period were demonstrated to be reversible following a 2 week recovery period. These studies support continued clinical trials in subjects with advanced stage cancer.

1.4 Clinical Experience with AZD1775

The following overview of the clinical experience with AZD1775 is based on the knowledge available at the time this protocol was written and is based on Edition 12 of the Investigator's Brochure, Edition 12, dated 18 February 2015, with a data cut-off date of 11 November 2014. For more detailed and up-to-date information, please consult the current Investigator's Brochure.
As of 11 November 2014, a total of 372 patients have been exposed to AZD1775 in AstraZeneca-sponsored or Merck-sponsored clinical studies, which include 1 completed study (Study PN001), 2 studies that were terminated early (Studies PN005 and PN008) and 3 ongoing studies (Studies PN004, D6011C00001, and D6011C00002).

- PN001: a first-time-in-patients (FTIP), Phase I, dose-escalation study evaluating AZD1775 both as monotherapy and combination therapy with gemcitabine, cisplatin, or carboplatin in adult patients with advanced solid tumours. This study has been completed except for Part 3.
- PN005: a Phase I, dose-escalation study evaluating AZD1775 as monotherapy (Part 1), combination therapy with 5-FU (Part 2), and combination therapy with 5-FU plus cisplatin (Part 3) in adult Japanese patients with advanced solid tumours. This study was terminated early due to portfolio prioritization in oncology at Merck after 3 patients had been enrolled in Part 1 and 8 patients had been enrolled in Part 2. Part 3 was not initiated.
- PN008: a Phase I/IIa, dose-escalation study evaluating AZD1775 in combination with topotecan plus cisplatin in adult patients with cervical cancer was terminated early due to portfolio prioritization in oncology at Merck after 7 patients had been enrolled in the dose-escalation part of the study. The Phase IIa part was not initiated.
- PN004: a Phase II study evaluating AZD1775 combined with carboplatin and paclitaxel in patients with platinum-sensitive p53-mutant ovarian cancer. This study is ongoing.
- D6011C00001: a lead-in Phase II multicentre, randomised, double-blind study comparing AZD1775 plus docetaxel with placebo plus docetaxel in previously treated patients with non-small-cell lung cancer. This study is ongoing.
- D6011C00002: a Phase II study of AZD1775 plus pemetrexed and carboplatin followed by a randomised comparison of pemetrexed and carboplatin with or without AZD1775 in patients with previously untreated stage IV non-squamous non-small cell lung cancer. This study is ongoing.

In addition, 93 patients have also received AZD1775 as part of externally-sponsored scientific research. These patients have received single doses per cycle as high as 1300 mg of AZD1775 as monotherapy, 325 mg of AZD1775 in a single-dose in combination

with chemotherapy, and 325 mg twice a day (BID) in a multiple-dose regimen in combination with chemotherapy.

1.41 Clinical Safety

Several clinical studies with AZD1775 alone and in combination with (mostly full dose) cytotoxic chemotherapy have been conducted.

The MTD of AZD1775 monotherapy has not been established. Single doses of AZD1775 up to 1300 mg were well tolerated.

The MTDs of AZD1775 when administered with chemotherapy are presented in the table below:

Maximum-tolerated doses of AZD1775 when administered in combination with chemotherapy

Chemotherapy	Dose Frequency	AZD1775 (mg)
Gemcitabine 1000 mg/m^2 on Day 1 weekly for 3 consecutive weeks out of every 4 weeks	Single-dose	200
Cisplatin 75 mg/m2 on Day 1 every 21 days	Single-dose	200
Carboplatin AUC 5 on Day 1 every 21 days	Single-dose	325
Gemcitabine 1000 mg/m2 on Day 1 weekly for 3 consecutive weeks out of every 4 weeks	Multi-dose	175 QD x2 days
Cisplatin 75 mg/m2 on Day 1 every 21 days	Multi-dose	200 BID x 2.5 days
Carboplatin AUC 5 on Day 1 every 21 days	Multi-dose	225 BID x 2.5 days

BID = twice daily; QD = once daily

There were no DLTs observed for patients receiving monotherapy with AZD1775. For combination treatments, DLT rates at all MTDs were less than 30% in Study PN001. Oral administration of AZD1775 both as monotherapy and in combination with gemcitabine, cisplatin, or carboplatin in patients with advanced solid tumours was tolerable.
Based on the safety data from the 3 completed clinical studies, the most frequently reported drug-related AEs were blood and lymphatic disorders (neutropenia, thrombocytopenia, anaemia), gastrointestinal disorders (diarrhoea, vomiting, nausea), and general disorders and administration site conditions (fatigue, pyrexia).

The most frequently reported SAEs occurred within the gastrointestinal, infections and infestations, and blood and lymphatic disorders system organ classes. The SAEs reported to be related to study medication in PN001 included nausea, vomiting, diarrhoea, gastric ulcer, colitis, dehydration, pyrexia, hemiparesis, renal impairment, renal failure, urinary tract infection, dyspnoea, recall phenomenon, palpitations, lung infiltration, agitation, neutropenia, febrile neutropenia, anaemia, thrombocytopenia, leukopenia, increased blood creatinine, and hyperbilirubinaemia. Events that led to death in that study included malignant neoplasm progression, renal failure, respiratory failure, pneumonia, hypoxia, and lung infiltration.
Cardiac disorders (tachycardia, palpitations, QTc prolongation) and gastrointestinal haemorrhage were not observed frequently, but are considered to be important potential risks.

The single-dose maximum tolerated dose (MTD) for both the gemcitabine and cisplatin combination therapies was 200 mg of AZD1775. Dose-limiting toxicities (DLTs) tended to be hematological in nature (i.e. leukopenia, neutropenia and thrombocytopenia) in the gemcitabine group and constitutional (i.e. diarrhea and dehydration) in the cisplatin group. The single-dose MTD for the combination with carboplatin was 325 mg of AZD1775. DLTs in this group were related to serum chemistry.

Hematologic DLTs were most commonly observed in the multiple-dose AZD1775 combination treatment groups with gemcitabine and cisplatin. An MTD of AZD1775 in combination with gemcitabine was established with a dose of 175 mg QD x 2 days during Week 1 with gemcitabine 1000 mg/m2 once weekly for 3 consecutive weeks, every 4 weeks. Two DLTs (grade 3 febrile neutropenia and grade 3 aspartate aminotransferase and alanine aminotransferase (AST/ALT) increase) were observed at 200 mg once daily for 2 days with the regimen in combination with gemcitabine. The dose was adjusted to 175 mg once daily for 2 days, and one DLT has been reported to date. The MTD for combination with cisplatin has been exceeded at the 250 mg dose level, and tolerability of the MTD at 200 mg BID has been confirmed. DLTs observed in the multiple-dose carboplatin combination have been both hematological and constitutional in nature. The multiple-dose MTD in combination with carboplatin is AZD1775 225 mg BID.

The MTD for AZD1775 in combination with 5-FU was not reached due to early study termination. DLTs of encephalopathy and hyponatraemia were observed in the AZD1775 20 mg BID in combination with 1000 mg/m2 5-FU treatment group.

Triplet-based therapy was administered in the study PN004 with AZD1775, carboplatin, and paclitaxel. The starting dose of 225 mg AZD1775 (BID for 5 total doses) in Part 1 was generally well tolerated. One patient experienced a DLT in the first six patients treated and the dose remained at 225 mg BID 2.5 days. A total of 15 patients were treated, with 3 DLTs being reported (grade 3 and 4 febrile neutropenia and grade 4 thrombocytopenia). The study expanded to Part 2 and is ongoing. Other toxicities were generally blood and lymphatic system disorders and gastrointestinal.

For the combination of AZD1775 with topotecan plus cisplatin in study PN008, toxicities were generally hematological and gastrointestinal in nature. No unexpected toxicities were observed.

In study PN009 and the premarket formulation substudy to PN001 (both combining 2.5-day BID dosing of AZD1775 with carboplatin) there were no appreciable differences in toxicity from the ones observed in the carboplatin arm of study PN001. However, increased hematological toxicity was observed, which is considered to be a function of a drug-drug interaction with aprepitant (which was administered as anti-nausea medication in these studies).

1.42 Clinical Efficacy

In Study PN001, of 176 evaluable patients who received AZD1775 (either single or multiple doses) as monotherapy or in combination with gemcitabine, cisplatin, or carboplatin, a partial response (PR) (confirmed and unconfirmed) was observed in 17 (9.7%) patients, and stable disease (SD) was observed in 94 (53.4%) patients. The table below shows the efficacy outcome in Study PN001 by treatment arm.

Study PN001 Efficacy

Treatment	Dose Frequency	Patients Treated (N)	Patients Evaluable [N (%)]	PR* (N)	SD (N)	PD (N)	NE
AZD1775 in combination with gemcitabine	Single-dose	14	12 (85.7)	1 c	8	2	1
AZD1775 in combination with cisplatin	Single-dose	13	12 (92.3)	1 c 1 u	7	3	
AZD1775 in combination with carboplatin	Single-dose	16	15 (93.8)	1 c 1 u	3	9	1
AZD1775 in combination with gemcitabine	Multi-dose	67	55 (82.1)	1 c 2 u	35	17	
AZD1775 in combination with cisplatin	Multi-dose	45	38 (84.4)	3 c 4 u	16	15	
AZD1775 in combination with carboplatin	Multi-dose	46	44 (95.6)	2 u	25	16	

* confirmed and unconfirmed
NE=not evaluable, c=confirmed, u=unconfirmed

No complete or PRs were observed in either of Studies PN005 or PN008 at the time that they were terminated.

1.43 Clinical Pharmacokinetics and pharmacodynamics

Clinical Pharmacokinetics

The PK data of AZD1775 following a single oral administration showed a moderate rate of absorption with a Tmax occurring at 3 to 4 hours. Post-peak plasma concentrations declined essentially in a mono-exponential manner with a t1/2 in the region of 10 hours. Exposure as measured by maximum plasma drug concentration observed (Cmax) and area under the curve (AUC 0-∞) increased in a dose-proportional manner over the dose range of 325 to 1300 mg. The urinary excretion of AZD1775 was investigated after single-oral-dose monotherapy at 325, 650, and 1300 mg of AZD1775. The mean percent of total AZD1775

excreted unchanged in urine over a 24-hour period was 5.15% to 11.9%, which is comparable to what was observed in preclinical species (urinary excretion as unchanged drug in rats and dogs were ~8.6% and 11.8% of the dose, respectively, over a 48-hour collection interval). The mean renal clearance of AZD1775 at 325 mg was approximately 2.30 L/hour (38.3 mL/minute), below that expected due to filtration alone (based on an AZD1775 unbound fraction of 39.5% and a typical glomerular filtration rate of 120 mL/minute) of 47.4 mL/minute, indicating that net reabsorption appeared to be taking place. Overall, the results suggest that urinary excretion is not the major route of AZD1775 elimination

Following single (100 to 325 mg) and multiple dose administrations of AZD1775 (25 to 325 mg BID and 100 to 200 mg once daily [QD]) with carboplatin, cisplatin, and gemcitabine, plasma exposure of AZD1775 was consistent with predictions based on the single-dose regimen. The median Tmax values were between 1.02 and 4.25 hours. Given a t1/2 of approximately 10 hours, steady-state was expected to be achieved after approximately 3 days of AZD1775 treatment in adult patients. Accumulation ratios for AZD1775 BID doses (geometric mean ratio = Day 3/Day 1) for the area under the plasma-concentration time curve from time 0 to 8 hours post dose ($AUC_{0\text{-}8hr}$), Cmax, and plasma drug concentration observed at 8 hours post dose (C_{8hr}) averaged 0.991 to 3.82, 0.928 to 3.32, and 1.01 to 2.98, respectively, across tested AZD1775 BID doses in combination with gemcitabine, cisplatin, and carboplatin.

Preliminary investigation of drug-drug interactions in Study PN001 suggest a 40 up to 60% increase in the exposure of AZD1775 in the presence of aprepitant (moderate CYP3A4 inhibitor), but no effect with the concomitant administration of steroids (moderate CYP3A4 inducers).

Preliminary studies also suggested that the Pre-marketed Oral Formulation (PMF) of AZD1775 was similar to that of the Fit-For-Purpose (FFP) formulation.
Preliminary PK data from a Japanese combination study with AZD1775 and 5-fluorouracil (5-FU)5FU suggests that the pharmacokinetics of AZD1775 in Japanese and Caucasian patients were similar, however, further work is ongoing.

Pharmacodynamics evaluation

PN001: Skin biopsies were obtained from patients in all 3 parts of this study in order to evaluate the pharmacodynamic effects of AZD1775 in a surrogate tissue. Formalin-fixed and paraffin- embedded skin samples were analysed by immunohistochemical staining for the presence of total and phosphorylated levels of the WEE1 kinase substrate CDC2 (CDK1). Since CDC2 is expressed and phosphorylated in a cyclical manner and only observed in proliferating cells in the epidermis, the data was reported as the percent of CDC2-positive cells that were also pCDC2 positive. Post treatment values obtained from this scoring method were divided by the same value derived from the matched pre-treatment biopsy to generate a post/pre ratio to reflect the changes in pCDC2 observed at the time of the biopsy (see IB v12 for additional details).

Additional pharmacodynamic inhibtion has been observed in a monotherpy study run by the NCI. Paired tumor biopsies were obtained at baseline and 2 to 5 hours

after the 5th dose of the first week of administration of drug. Dramatic reductions in pY15-Cdk levels (67%, 84%, and 90% compared to baseline) were found in 3 of 5 paired tumor biopsies with 2 of those patients also demonstrating concurrent evidence of DNA damage response based on increased levels of γH2AX level on post-treatment tumor biopsy (Do et al., ASCO 2014)

1.5 Study Design and Treatment

This study is a phase II study testing the clinical efficacy of combined AZD1775 with AraC or single agent activity of AZD1775 in three patient strata: Elderly newly diagnosed AML patients (Arm A) will only receive the combination; whereas relapsed/refractory AML patients and HMA failure MDS patients will be allocated to either the combination (Arm B) or single agent AZD1775 (Arm C). The study will have a run in safety cohort of six patients in each of the three arms to determine the safe use of combined AraC /AZD1775 or single agent AZD1775 in the patient populations. This will be followed by an expansion phase where elderly patients with newly diagnosed AML will receive a combination of AZD1775 and AraC (Arm A) while patients with relapsed or refractory AML or HMA failure MDS will be allocated to receive either AZD1775 with AraC (Arm B) or AZD1775 alone (Arm C). An early toxicity check will be conducted to determine safety and tolerability. If indicated, dose levels will be reduced. The study will continue to enroll the rest of the patients at the tolerated dose.

1.51 Rationale for AraC and AZD1775 Dosing Schedule, Dose Selection and Dosing Arms:

Dose and Schedule of AraC: AraC is a pyrimidine nucleoside analog that functions as an antimetabolite in a cell cycle specific manner. When given in high doses and in combination with other cytoreductive agents AraC can be too toxic in the elderly patients and those with relapsed/refractory disease. Subcutaneously administered low-dose AraC (defined as 5 to 10 mg/m^2/d or 20mg fixed dose twice daily) for 10-12 days has been employed for specific AML subpopulations since 1979, with complete remission (CR) rates generally around 10-20% at maximum (Cheson et al. 1986). It is frequently used as a comparator arm in large phase 3 trials or in combination studies with other agents (Kantarjian et al. 2012, Fenaux et al. 2009). Clinically, AraC can be given at low, intermediate and high dose concentration schedules. The strongest pre-clinical ex vivo and in vitro sensitization effects to AZD1775 occurred at low to moderate AraC concentrations of 9-120 nanoMolar (nM) (Tibes et al.2012). Whereas, antagonism was seen at very high concentrations (AraC > 1000nM, AZD1775 > 1000nM), concentrations that clinically resemble high dose AraC dosing schedules. As the mechanism is not yet fully understood, we postulate that for maximum anti-leukemic activity of combined AraC and AZD1775, there has to remain a low level proliferation with incorporation of AraC into the DNA of dividing leukemia cells upon concurrent inhibition of WEE1 kinase. This results in abrogation of the DNA repair machinery (Tibes, ASCO 2014) and cell death/apoptosis originating directly from the damaged DNA. Therefore, we propose to use AraC that can achieve concentrations of up to 50-100nM, comparable to the pre-clinical data.

Dose and Schedule of AZD1775: The optimal concentrations of AZD1775 in combination with AraC are in the range of 100-600nM based on in vitro and ex vivo data (Tibes et al, Blood 2012). Pharmacokinetic data for AZD1775 is available from the ongoing solid tumor Phase 1 trial. AZD1775 at once or twice daily dosing up to 200 to 225 mg orally (po) in combination with full doses of Gemcitabine, Cisplatin or Carboplatin was well tolerated (Schellens et al. 2011, Leijenet al. 2010, IB AZD1775, Feb. 2015). The half-life of AZD1775 is ~9 hrs and C trough for AZD1775 is up to 570nM, with an unbound concentration of 225nM. Based on preliminary population PK model, simulations suggest that once daily dosing of 200 mg AZD1775 for 7 days will result in a median plasma trough total concentration level of 172 nM (range: 8-740 nM) for AZD1775. The unbound concentration will be 67 nM (3-292 nM). This would be in the range at which pre-clinically single agent activity and sensitization has been observed (Tibes et al, Blood 2012).

AZD1775 dosing length: Currently there is a Phase 1/2 solid tumor single agent AZD1775 trial ongoing at the National Cancer Institute at 225mg orally BIDX5 for 2 weeks of a 3 week schedule. Additional studies investigating alternative more prolonged dosing schedules are underway. Information of toxicity and tolerability will be available prior to opening this protocol and inform the initial AZD1775 dosing strategies: to be given either 225mg continuously for 28 days as proposed; or alternative dosing may be explored i.e. 5 days on, 2 days off, 5 days on or only concurrently for 10 days during days of AraC administration. Either scheduling has a clinical rationale as there are 10 and 12 day dosing schedules for AraC given subcutaneously. If continuous dosing of AZD1775 is tolerated in ongoing studies, AraC will be given for 10 days. If a 5 on, 2 off, 5 on AZD1775 schedule is selected than AraC will be given for 12 days.

1.52 AZD1775 alone vs. AZD1775 plus AraC

AZD1775 ex vivo and in vitro has single agent activity against myeloid malignant cells from relapsed/refractory AML and HMA failure MDS patients. Pre-clinical data provides support for concurrent exposure to both agents at lower AraC and moderate AZD1775 concentrations and these dosing concentrations are hypothesized to have the optimal biological effect. Further, the available clinical trial data of AZD1775 alone or with full dose cytotoxic chemotherapies is showing good tolerability without excessive cytopenias [cytopenias are less concern for the patient population under study in this protocol]. Clinical trials, including single investigational agents are recommended standard interventions for the patient population in this protocol. Therefore patients with relapsed or refractory AML or HMA failure MDS will be allocated to, either AZD1775 or AZD1775 plus AraC treatment. For patients with newly diagnosed AML, AraC should in most cases be part of the upfront therapeutic regimens, therefore newly diagnosed untreated elderly AML patients will only be allocated to the combination treatment Arm of AZD1775 plus AraC. The dosing length/schedule of AZD1775 will be informed by the emerging clinical data from the ongoing continuous single agent AZD1775 trial.

2.0 Goals

2.1 Primary Goals

2.11 To estimate the clinical efficacy of AZD1775 in combination with AraC in patients with newly diagnosed AML by assessing complete response (CR plus CRi) rates

2.12 To estimate the clinical efficacy of AZD1775 alone or in combination with AraC in patients with relapsed/refractory AML and hypomethylating agent failure MDS by assessing complete response (CR plus CRi) rates

2.2 Secondary Goals

2.21 To determine the safety and tolerability of AZD1775 alone or combined with AraC in the study population.
2.22 To estimate additional measures of clinical benefit (i.e. hematological improvements, transfusion requirements).
2.23 To measure the duration of response of AZD1775 alone or combined with AraC.
2.24 To measure time to response of AZD1775 alone or combined with AraC.
2.25 To measure time to progression of AZD1775 alone or combined with AraC.
2.26 To measure overall survival of AZD1775 alone or combined with AraC.
2.27 To measure time to AML (for MDS subjects) of AZD1775 alone or combined with AraC

2.3 Correlative Research

2.31 To determine the pharmacokinetics (PK) of AZD1775 alone or combined with AraC in the study population

2.32 To conduct correlative research studies characterizing underlying molecular events and solidifying putative mechanism of action in vivo and to identify potential pharmacodynamic/biomarkers of response to AZD1775 alone or combined with AraC

2.33 To evaluate quality of life (QOL) and patient-reported symptoms in subjects treated with AZD1775 alone or combined with AraC.

3.0 Patient Eligibility

3.1 Inclusion Criteria

3.11 Age ≥18 years.

3.12 Patient population (histological or cytologically confirmed diagnosis):

- untreated elderly (>60 years) AML if in the intermediate and poor-risk cytogenetic group (please reference Appendix V) and not candidates (as judged by treating MD) for or willing to undergo standard induction therapy (i.e. elderly unfavorable cytogenetic AML) or any untreated AML age > 65 years

  Note: previous therapy with a hypomethylating agent (HMA) for a diagnosis of MDS is allowed

- relapsed or refractory AML (≥ 18 years)

- any MDS (≥ 18 years) having failed or been intolerant to prior hypomethylating agent (HMA) treatment.
    - Failure is defined as any disease progression while on HMA, relapse after HMA treatment or no response after 4 cycles of 5-Azacitidine or decitabine
    - Patients with isolated 5q-/5q- syndrome must have failed, not tolerated, or progressed on lenalidomide in addition to having failed or been intolerant to HMA treatment.

  Note: Patients with CMML and MDS/MPN overlap are allowed if meeting other study eligibility criteria.

NOTE: For all patient groups, therapy as part of a plan as a bridge to transplant is allowed.

3.13 The following laboratory values obtained ≤7 days prior to registration.
- Total bilirubin ≤ 1.5 mg/dL (except Gilbert's syndrome or known hemolysis or leukemic infiltration)
- AST (SGOT) and ALT (SGPT) ≤ 2.5 x Upper Limit normal (ULN) or < 5 x ULN if organ involvement
- Alkaline Phosphatase < 5 x ULN
- Serum creatinine ≤ 2 x ULN or 24 hour Cr clearance >30ml/min

3.14 ECOG Performance Status (PS) 0, 1 or 2 (Appendix I).

3.15 Ability to provide informed written consent and be able to adhere to the study visit schedule and other protocol requirements.

3.16 Willing to return to enrolling institution for follow-up (during the Active Monitoring Phase of the study).

3.17 Willing to provide blood and bone marrow aspirate samples for correlative research purposes

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

3.19a Men and women must be willing to use appropriate contraception throughout study and for 6 months after (please refer to Appendix IX for details).

3.19b Male patients who are sexually active with a female partner of childbearing potential must be either surgically sterilized or agree to use barrier contraception (ie, condoms) for the duration of study participation, and for 90 days after the final dose of study drug; cessation of birth control after this point should be discussed with a responsible physician.

3.19c Patients who have undergone stem cell transplantation (SCT), autologous or allogeneic, are eligible provided that they are > 60 days from stem cell infusion,

have GVHD ≤ grade 1 and are off immunosuppressive agents for > 28 days at time of registration.

3.2 Exclusion Criteria

3.21 Uncontrolled intercurrent illness including, but not limited to, active uncontrolled infection, known positive for active infectious hepatitis, type A, B or C (past infection allowed), or psychiatric illness/social situations that would limit compliance with study requirements.

Note: ongoing infection controlled on antibiotics/antifungal/antiviral medications are allowed.

3.22 Any of the following prior therapies:
- Cytotoxic Chemotherapy ≤14 days prior to registration
- Immunotherapy ≤14 days prior to registration
- Biologic therapy (i.e. antibody therapies) ≤14 days prior to registration
- Radiation therapy ≤14 days prior to registration
- Targeted therapies (i.e. kinase inhibitors, ≤7 days or 5 half-life's whichever is shorter)
- For steroids or other non-cytotoxics given for blast count control, patient must be off for > 24 hrs before starting therapy. NOTE: Hydroxyurea (HU) is allowed for blast count control throughout study
- Receiving any other investigational agent which would be considered as a treatment for the primary neoplasm ≤ 14 days prior to registration

3.23 Active uncontrolled CNS leukemia. NOTE: Positive (cyto)pathology is allowed and patient can receive intrathecal chemotherapy

3.24 Immunocompromised patients and patients known to be HIV positive and currently receiving antiretroviral therapy. NOTE: Patients known to be HIV positive, but without clinical evidence of an immunocompromised state, are eligible for this trial.

3.25 Any previous treatment with AZD1775 or allergic reactions to excipients of AZD1775.

3.26 Acute Promyelocytic Leukemia (APL, M3) unless failed regimens that included tretinoin, arsenic trioxide, anthracyclines and cytarabine and currently NOT candidates for stem cell transplantation.

3.27 Major surgery ≤ 28 days prior to registration

3.28 Clinically significant heart disease, including the following:
- Active severe angina pectoris within 3 months prior to registration
- Acute myocardial infarction within 3 months prior to registration
- New York Heart Association classification IV cardiovascular disease or symptomatic class III disease (Appendix III).

Note: patients with any of the above may be allowed after discussion amongst the investigators including the principal investigator

3.29a Any of the following because this study involves an investigational agent whose genotoxic, mutagenic and teratogenic effects on the developing fetus and newborn are unknown:

- Pregnant women
- Nursing women
- Men or women of childbearing potential who are unwilling to employ adequate contraception

3.29b Subject has had prescription or non-prescription drugs or other products known to be sensitive CYP3A4 substrates or CYP3A4 substrates with a narrow therapeutic index, or to be moderate to strong inhibitors / inducers of CYP3A4 which cannot be discontinued two weeks prior (alternatively 5 half lives if T1/2 is known) prior to Day 1 of dosing and withheld throughout the study until 2 weeks after the last dose of study drug (Appendix VI).

NOTE: Co-administration of aprepitant or fosaprepitant during this study is prohibited.

Note: Individiual drugs exerting CYP interactions as listed in tables in Appendix VI may be continued on a case by case basis if felt essential for patient managment, after discussions and discretion of the treating physician.

The preferred azole anti-fungal medication is Fluconazole (alternatively Posaconazole) which can be given during treatment with AZD1775 at the treating physician's discretion, however with dose reductions of AZD1775 by 25-75% (i.e. from AZD1775 200mg to 150 or 100mg).

3.29c Pateints may not be on an inhibitor of BCRP as outlined in Appendix VI.

NOTE: AZD1775 is an inhibitor of breast cancer resistance protein (BCRP). The use of statins including Atorvastatin which are substrates for BCRP are therefore prohibited and patients should be moved on to non-BCRP alternatives.

3.29d Not willing to avoid grapefruit, grapefruit juices, grapefruit hybrids, Seville oranges, pummelos, and exotic citrus fruits from 7 days prior to the dose of study medication and during the entire study due to potential CYP3A4 interaction with the study medication. NOTE: Orange juice is allowed.

3.29e Corrected QT interval (QTc) >470 msec (as calculated per institutional standards) at study entry or congenital long QT syndrome.

4.0 Test Schedule

Table 4.1: Test Schedule for Elderly Newly Diagnosed and Relapsed/Refractory AML patients

			Active Monitoring Phase			
Tests and procedures	≤14 days prior to registration	≤7 days prior to registration	Cycle 1 Days 1, 8, 15, & 28(+3 days)[5, 13]	Cycles 2 & 3 Days 1, 15, and 28 (+ 3 days)[5, 13]	Cycles 4+Day 1 and Beyond(+/-3 days)[5, 17]	EOS[2]
History and exam, wt, PS, vitals	X		X	X[13]	X[13]	X
Height	X					
Adverse event assessment	X		X	X	X	X
Hematology group WBC ANC Hgb PLT		X	X	X	X[9]	X
Chemistry group: sodium, potassium, calcium, creatinine, bicarbonate, glucose, chloride, albumin, BUN, alkaline phosphatase, AST, ALT, total protein, total bilirubin, LDH		X	X	X	X	X
TLS monitoring (for selected AML patients): BUN, calcium, CO2, chloride, creatinine, glucose, potassium, sodium, uric acid, phosphorus			X[14]	X[14]		

PT/PTT and D-dimer			X[19]			
Serum pregnancy test[1]		X				
Bone marrow biopsy and aspirate procedure (cytogenetics or FISH)[15]	X[11]		X[3]	X[3]	X[3]	X[10]
Bone marrow aspirate research specimens (see Section 14.0)[R]	X[8]		X[7]	X[7]	X[7]	X[6]
Blood specimens (see Section 14.0)[R]	X[4, 8]		X[4]	X[4]	X[4]	X[4]
Buccal swab (optional, see Section 14.0) [R]	X					
Patient Questionnaire Booklet[12]	X		X[16]	X[16]	X[16]	X[16]
ECG (12 lead)[18]		X				X

1. For women of childbearing potential only.
2. 30 days (+/-3days) post last dose of study drug
3. For AML patients: marrow aspirates and biopsies will be done after cycle 1 (at physician's discretion), after cycles 2 and 4, and then at physician discretion). In addition, bone marrow should be performed at time of suspected CR/CRi and/or disease progression and at physician discretion as clinically indicated. Note: Bone Marrow Aspirate and biopsy may be avoided aftercycle 4 as determined by study chair or treating physician (i.e. if determined to not yield information that would change clinical management).
4. Blood specimens for research sampling to be collected as defined in Section 14.
5. Day 1 assessments do not need to be repeated if pre-registration or Day 28 assessments completed within 48 hours of Day 1.
6. Only if an EOS marrow sample is collected as part of standard of care
7. Research bone marrow samples are to be collected at the same time when a marrow is done as part of patient's standard of care, including research marrow samples should be drawn at time of marrow for suspected CR/CRi and/or disease progression.
8. To be collected on all patients at baseline. If no baseline marrow samples obtained, the peripheral blood research sample becomes mandatory to participate in study. Bone marrow aspirate specimens must be collected and submitted per section 14.0. If bone marrow performed at end of study as part of patient's standard of care, collect sample per section 14.0.
9. After 2 cycles, CBC frequency may be reduced at treating physician's discretion but needs to be performed at least once per cycle.
10. Progression marrow can be used as EOS marrow if patient is taken off trial based on the results. Additional EOS bone marrow can be omitted if a progression marrow was done, or an EOS marrow can be done at physician discretion and is optional.
11. ≤ 28 days prior to registration; for patients with an outside marrow biopsy within 4 weeks of study start, outside results can serve as baseline after review at Mayo Clinic. In these cases, samples for correlative laboratory studies will be drawn from peripheral blood prior to study start.
12. Patient questionnaire booklet must be used; copies are not acceptable for this submission. Booklet should be completed by patient prior to review of treatment response and discussions of patient's general health since last treatment evaluation.

13. Patients with AML will be monitored per Table 4.1 during cycle 1 and 2, and thereafter at least once per cycle, i.e. at end of each/prior to a new cycle or as otherwise defined in the test schedule.
14. For patients at high risk of tumor lysis syndrome (TLS), that is for selected AML patients as determined by the treating physician, a daily basic metabolic panel, uric acid, and phosphoruswill be drawn for three to five days in Cycle 1, when maximum TLS risk is usually abated. Additional monitoring will be done on a case by case basis.
15. Either conventional cytogenetics or FISH panel (or both based on physician discretion) for AML to be performed at baseline. If diploid cytogentics at baseline, subsequent cytogenetic studies may be omitted unless disease progression is suspected. For FISH based cytogenetic assessments, an entire AML FISH panel should be performed at baseline, while on subsequent marrows only positive specific FISH abnormalities/probes need to be repeated unless disease progression is suspected, in which case a full AML FISH panel or instead conventional cytogenetics should be performed.
16. Booklets to be completed at end of all cycles.
17. Prior to the start of treatment.
18. ECG to be repeated if cardiac symptoms occur or as otherwise defined in the test schedule.
19. To be tested on C1D1 prior to treatment.

R Research funded (see Section 19.0).

Table 4.2: Test Schedule for HMA failure MDS patients

			Active Monitoring Phase			
Tests and procedures	≤14 days prior to registration	≤7 days prior to registration	Cycles 1 Days 1, 8, 15, & 28(+3 days)[12]	Cycle 2 Days 1, 15, and 28 (+ 3 days)[10, 12]	Cycles 3+ Day 1 and Beyond(+/-3 days)[10, 12]	EOS[2]
History and exam, wt, PS, vitals	X		X	X	X	X
Height	X					
Adverse event assessment	X		X	X	X	X
Hematology group WBC ANC Hgb PLT		X	X	X	X	X
Chemistry group: sodium, potassium, calcium, creatinine, bicarbonate, glucose, chloride, albumin, BUN, alkaline phosphatase, AST, ALT, total protein, total bilirubin, LDH		X	X	X	X	X
Serum Erythropoietin level	X					
Serum pregnancy test[1]		X				
Bone marrow biopsy and aspirate procedure (cytogenetics or FISH[9])	X[6]			X[3]	X[3]	X[7]
Blood specimens (see Section 14.0)[R]	X[4]		X[4]	X[4]	X[4]	X[4]
Buccal swab (see Section	X					

14.0) [R]						
Bone marrow aspirate research specimens (see Section 14.0)[R]	X[6]			X[5]	X[5]	X[5]
Patient Questionnaire Booklet[8]	X		X[8]	X[8]	X[8]	X
ECG (12 lead)[11]		X				X

1. For women of childbearing potential only
2. 30 days (+/-3days) post last dose of study drug
3. Marrow biopsies only to be done at End of cycle 2, End of cycle 4, at time of suspected CR/CRi and thereafter at physician discretion as clinically indicated (i.e. disease progression).
4. Blood specimens for research sampling to be collected as defined in Section 14.
5. Research marrow samples (aspirates) to be done at times of clinical standard of care marrow tests at End of cycle 2, End of cycle 4, at time of suspected CR/CRi or disease progression and thereafter at physician discretion as clinically indicated. A sample will be obtained at end of study (EOS) if a bone marrow is performed as part of patient's standard of care; sample will be collected per section 14.0
6. ≤ 28 days prior to registration; for patients with an outside marrow biopsy within 4 weeks of study start, outside results can serve as baseline after review at Mayo Clinic. In these cases, samples for correlative laboratory studies will be drawn from peripheral blood prior to study start.
7. Bone marrow at end of study is only to be done at physician discretion and is optional. Progression marrow can be used as EOS marrow if patient is taken off trial based on the results.
8. Patient questionnaire booklet must be used; copies are not acceptable for this submission. Booklet should be completed by patient prior to review of treatment response and discussions of patient's general health since last treatment evaluation. Booklets to be completed at end of all cycles.
9. Either conventional cytogenetics or FISH panel (or both based on physician discretion) for MDS should be performed at baseline. If diploid cytogentics at baseline subsequent cytogenetic studies may be omitted unless disease progression is suspected. For FISH based cytogenetic assessments, an entire MDS FISH panel should be performed at baseline, while on subsequent marrows only positive specific FISH abnormalities/probes need to be repeated unless disease progression is suspected.
10. Prior to the start of treatment.
11. ECG to be repeated if cardiac symptoms occur or as otherwise defined in the test schedule.
12. Day 1 assessments do not need to be repeated if pre-registration or Day 28 assessments completed within 48 hours of Day 1.

R Research funded (see Section 19.0).

5.0 Grouping Factors:

5.1 Grouping Factor

Study Stage: 1=Safety Portion vs 2=Expansion Portion

5.2 Grouping Factor

Disease type: 1=Elderly newly diagnosed AML (assigned treatment Arm A) vs 2=Relapsed/refractory AML vs 3=HMA failure MDS

NOTE: HMA failure MDS patients include MDS, CMML and MDS/MPN overlap patients.
NOTE: Disease type groups 2 and 3 are randomized to arm B or C.

6.0 Registration/Randomization Procedures

6.1 Safety Portion

Prior to discussing protocol entry with the patient, call the MCCC Registration Office [redacted] for dose level and to insure that a place on the protocol is open to the patient.

6.11 Registration Procedures

6.111 To register a patient, [redacted] a completed eligibility checklist to the Mayo Clinic Cancer Center (MCCC) Registration Office between 8 a.m. and 4:30 p.m. central time Monday through Friday.

6.2 Expansion Portion

6.12 Registration Procedures

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

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

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

6.3 Safety & Expansion Portions

6.31 Correlative Research

A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Section 3.17 and 14.0).

6.32 Prior to accepting the registration, registration/randomization application will verify the following:
- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

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

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

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

6.34 At the time of registration, the following will be recorded:
- Patient has/has not given permission to store and use his/her sample(s) for future research of Acute Myeloid Leukemia or Myelodysplastic Syndrome at Mayo.
- Patient has/has not given permission to store and use his/her sample(s) for future research to learn, prevent, or treat other health problems.

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

6.36 Treatment cannot begin prior to registration and must begin ≤ 14 days after registration.

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

6.38 All required baseline symptoms (see Section 10.3) must be documented and graded.

6.39a Study drug is available on site.

6.39b Blood draw kit is available on site.

6.39c Patient questionnaire booklet is available on site; copies are not acceptable for this submission.

6.39d Randomization Procedures

6.39d1 The factors defined in Section 5.0, together with the registering membership, will be used as stratification factors.

6.39d2 After the patient has been registered into the study, the values of the grouping factors will be recorded. An elderly newly diagnosed AML patient will be allocated to arm A. A relapsed/refractory AML or HMA failure MDS patient will be randomized to treatment group arm B or arm C.

- Elderly (age 60+) newly diagnosed AML patients assigned to Arm A: AZD1775 days 1-5, 8-12 and AraC days 1-5, 8-12
- Relapsed/Refractory AML and HMA failure MDS patients randomized to Arm B: AZD1775 days 1-5, 8-12 and AraC days 1-5, 8-12 or Arm C: AZD1775 days 1-5, 8-12, 15-19, 22-26.

All patients must be registered within 14 days before starting therapy or within 72 hours after starting treatment if over a weekend or holiday. If therapy must be started when the MCCC Registration Office is closed, a telephone call must be made when the MCCC Registration Office is open as indicated above. Under no circumstances will non-registered patients be retrospectively eligible for the study.

7.0 Protocol Treatment

7.1 Pre-Treatment Recommendation

7.11 Pretreatment medication recommendations (this is not a study requirement, but simply a recommendation)

Agent	Dose	Route	Day(s)
Ondansetron*	8mg	PO or IV	1-5, 8-12, ^

- *Or serotonin receptor antagonist of institutional standard
- ^ if study drug schedule is days 1-10, give Ondansetron days 1-10

7.2 Treatment Plan for AZD1775 combined with AraC (Arms A and B)

7.21 Safety Portion

The Safety portion of this trial will treat and monitor six patients in each arm and observe them for a minimum of 28 days to assess toxicities.

Starting Doses: Multiple Agent

Agent	Dose	Route	Day(s)	ReRx
AraC[1]	20 mg	SC twice daily [2]	1-5 & 8-12[3]	Every 28 days[4]
AZD1775	200 mg	PO daily	1-5& 8-12[3]	Every 28 days[4]

[1] Starting dose of a fixed dose of 20 mg s.c. per a single dose twice daily. In individual cases, a 10 mg/m^2 not to exceed 20 mg can be used alternatively.
[2]SC administration preferred
[3] AZD1775 should be taken either 2 hours before or 2 hours after a meal. A day 1-10 dosing schedule will be permitted as an alternative, in which case AZD1775 should be given days 1-10 as well.
[4]Cycle length can be increased to 6 and maximally 8 weeks
Note: if the starting dose is not tolerated, lower dosing cohort combination will be explored and follow the recommendation as outlined in Section 8.1 Table 8.11.

7.22 Expansion Portion

The Expansion portion of this trial will commence for Arms A and B as soon as the Safety portions for BOTH arms are completed.

Starting Doses

Agent	Dose	Route	Day(s)	ReRx
AraC[1]	20 mg	SC twice daily [2]	1-5 & 8-12[3]	Every 28 days[4]
AZD1775	MTD	PO daily	1-5& 8-12[3]	Every 28 days[4]

[1] Starting dose of a fixed dose of 20 mg s.c. per a single dose twice daily. In individual cases, a 10 mg/m^2 not to exceed 20 mg can be used.
[2]SC administration preferred

[3]AZD1775 should be taken either 2 hours before or 2 hours after a meal. A day 1-10 dosing schedule will be permitted as an alternative, in which case AZD1775 should be given days 1-10 as well.
[4]Cycle length can be increased to 6-8 weeks (maximally)

7.3 Treatment Plan for AZD1775 single agent (Arm C)

7.31 Safety Portion

The Safety portion of this trial will treat and monitor up to six patients in the arm and observe them for a minimum of 28 days to assess toxicities.

Starting Dose: Single Agent

Agent	Dose	Route	Day(s)	ReRx
AZD1775	200 mg	PO daily	1-5, 8-12, 15-19, 22-26[2]	Every 28 days[1]

[1]Cycle length can be increased to 6-8 weeks
[2]AZD1775 should be taken either 2 hours before or 2 hours after a meal.
Note: if the starting dose is not tolerated, a lower dosing cohort will be explored and follow the recommendation as outlined in Section 8.1 Table 8.12.

7.32 Expansion Portion

The Expansion portion of this trial will commence for Arm C as soon as the Safety Portion is completed.

Starting Dose

Agent	Dose	Route	Day(s)	ReRx
AZD1775	MTD	PO daily	1-5, 8-12, 15-19, 22-26[2]	Every 28 days[1]

[1]Cycle length can be increased to 6-8 weeks
[2]AZD1775 should be taken either 2 hours before or 2 hours after a meal.

7.4 Toxicity Assessment for Safety Portion (first 6 patients on each Arm)

Toxicity will be measured per NCI-CTCAE version 4. DLT is defined as an adverse event occurring during the first cycle of treatment that is not clearly related to the patient's underlying disease and that meets one of the following:

- Grade 3 AST, ALT or alkaline phosphatase will be considered dose limiting on an individual basis or if lasts more than 5 days
- Grade 3 as per NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 that does not resolve to ≤ Grade 2 within 7 days
- Grade 4 as per NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0

NOTE: Myelosuppression will not be considered in evaluating toxicity in patients with acute leukemias or similar advanced myeloid malignancies

(MDS) except where bone marrow (BM) hypoplasia occurs for >52 days with BM cellularity <5% in the absence of residual leukemia/disease.

7.7 MTD Determination

This MTD determination applies to the Safety Portion. MTD for the safety portion will be defined as the dose level below the lowest dose that induces dose-limiting toxicity in at least one-third of patients (at least 2 of a maximum of 6 new patients).

7.71 Three patients will be treated at a given dose level combination and observed for one cycle to assess toxicity (accrual will be suspended while the 3 patients are treated and observed).

7.72 If DLT is not seen in any of the 3 patients or in 1 of 3 patients, 3 new patients will be accrued. and treated at the same dose level (accrual will be suspended while the 3 patients are treated and observed). If DLT is seen in 2 or 3 of 3 patients treated at a given dose level, then the next 3 patients will be treated at the next lower dose level, if only 3 patients were enrolled and treated at this lower dose level.

7.73 After enrolling 6 patients on a specific dose level, if DLT is observed in 1 or less patients, then the combination dose will be considered safe for the expansion phase. If DLT is observed in at least 2 of 6 patients, then the MTD will have been exceeded and defined as the previous dose unless only 3 patients were treated at the lower dose level. In that case, 3 additional patients will be treated at this lower dose level.

7.75 If a patient fails to complete one cycle of treatment for reasons other than dose-limiting toxicity defined adverse events, the patient will be regarded as non-informative for estimating the MTD and an additional patient will be treated at the current dose level.

7.8 Dose escalation will not be allowed for a patient.

7.9a For this protocol, the patient must return to the consenting institution for evaluation at least once every 28 days.

7.9b Local Medical Doctor (LMD)
When it has been determined that a patient's malignant disease is stable or objective tumor regression has been observed and the patient is tolerating therapy without excessive toxicity, the drug(s) may be administered by the patient's Local Medical Doctor (LMD). The registering physician retains responsibility for the patient.

In this case, a written statement outlining drug dosage, method of administration, follow-up tests required, and telephone number to call to discuss any questions with the responsible investigator must be sent with the patient to provide necessary information to the LMD. The LMD will be required to supervise the administration of the study drugs as stipulated in the protocol and provide written documentation that the drug was administered.

8.0 Dosage Modification Based on Adverse Events - If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. Dose modifications apply to the treatment given in the preceding cycle and are based on adverse events observed since the prior dose. Dose modification recommendations listed below are general guidelines, and appropriate dose adjustments for patient safety should be done if needed per treating physician discretion.

ALERT: *ADR reporting may be required for some adverse events. See Section 10.0.*

8.1 Dose Modification

Individual drugs can be dose reduced as per the table below depending on the adverse event attribution. The dose reductions outlined in table 8.11 should first generally apply to AraC. If, however, the AE is clearly due to AZD1775, then AZD1775 can be reduced first as indicated in Table 8.11. After cycle 2, these modifications should be regarded as guidelines to produce mild-to-moderate, but not debilitating, side effects. If multiple adverse events are seen, administer dose based on greatest reduction required for any single adverse event observed. The treating MD, in discussions with the principal investigator, may reduce dose of either AraC or AZD1775 or the length of treatment at his or her discretion if it is in the best interest of the patient.

8.11 AZD1775 combined with AraC (Arms A and B)

Dose	***AZD1775*** *[reduce AZD1775 first if clearly attributed to AZD1775]*	***Cytarabine (AraC)*** *[generally reduce AraC first]*
0*	200 mg PO daily days 1-5 & 8-12	20 mg SC twice daily days 1-5 & 8-12
-1	200 mg PO daily days 1-5 & 8-12[1]	10 mg SC twice daily days 1-5 & 8-12
-2	100 mg PO daily days 1-5 & 8-12	10 mg SC twice daily days 1-5 & 8-12[2]

*Starting dose.
[1]If toxicity is clearly attributable to AZD1775, AZD1775 can be reduced to 100 mg PO daily (days 1-5, 8-12) with continued AraC 20 mg SC twice daily days 1-5, 8-12.
[2]AraC may be decreased to 10 mg or 20 mg SC once daily at physicians discretion based on myelosupression, tolerance and clinical benefit (see also dose modification section .
NOTE: intermediate dose levels of 150 mg PO daily days 1-5 & 8-12 may be explored as a dose level -1a, based on PK, PD and clinical efficacy parameters and evaluation.

8.12 AZD1775 single agent (Arm C)

Dose	**AZD1775**
0*	200 mg PO daily days 1-5, 8-12, 15-19, 22-26

-1a**	200 mg PO daily days 1-5, 8-12, 15-19 or further reduction to 200 mg PO daily days 1-5 & 8-12 as tolerated
-1b**	100 mg PO daily days 1-5, 8-12, 15-19, 22-26
-2	100 mg PO daily days 1-5 & 8-12

*Presumed starting dose of Expansion Portion.
**If at dose -1a leukemia breakthrough is observed, dose modification level -1b can be explored if continuous treatment may bring clinical benefit.
Note: Further dose modifications can be made, i.e. 150 mg PO daily days 1-5, 8-12, 15-19.
The above dose modification tables consider the fact that at present it is not clear if dose (Cmax) or continuous (length of therapy, AUC, trough) dosing of AZD1775 will achieve better disease control, hence the dose modification scheme (i.e. compared to hydroxyurea experience and titration).

8.2 A new course of treatment may begin on the scheduled Day 1 of a new cycle if:

- AZD1775 or AraC related adverse event that may have occurred has resolved to ≤ Grade 2 severity, except hematological adverse events for WBC, ANC, Platelets and Hemoglobin, or per table 8.3 guidelines.

If these conditions are not met on scheduled Day 1 of a new cycle, the subject will be evaluated as clinically indicated (i.e. weekly but at least every other week) and the new cycle of treatment will not be initiated until the adverse event has resolved as described above. **Treatment can be continued on the drug that is not associated with the adverse event at the start of the following cycle and the drug associated with the adverse event may be reintroduced when the adverse event resolves to ≤ Grade 1 if the treating physician feels that the patient will derive benefit from continued AraC or AZD1775 alone.**

NOTE: If the patient experiences a significant adverse event requiring a dose reduction at the start of the next cycle, then the dose will remain lowered for that entire subsequent cycle. If that cycle is completed with no further adverse events >Grade 2, then the dose may be increased, at the investigator's discretion, one level at a time, in the following cycles.

8.3 Dose modification table for adverse events felt to be at least possibly related to AZD1775*** or AraC

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

CTCAE System/Organ/Class (SOC)	ADVERSE EVENT	AGENT	ACTION**
BASED ON INTERVAL ADVERSE EVENT***			
Blood and Lymphatic System Disorders	Febrile neutropenia ≥ Grade 3	AZD1775	May omit dose until fever has resolved or returned to baseline. Dose to be restarted at same dose level. Treating physician may discuss possible dose reduction with one of the study chairs after the patient has completed 2 cycles. If clinical benefit is derived, AZD1775 may be continued if the infection is managed/controlled with appropriate medical intervention.
Investigations	Neutrophil & Platelet count decrease ≥ grade 4 NOTE: only applicable for patients with absolute neutrophil count (ANC) of ≥ 1000mm^3 and platelet count > 100,000/mm^3 at baseline (i.e. MDS patients)	AZD1775	Omit dose until ANC and/or platelets have recovered to ≤ grade 2. If clinical benefit is derived, AZD1775 may be continued if not recovered to ≤ grade 2 after discussion of treating physician and study chair.
Investigations	Creatinine increased Grade 2	AZD1775	Omit dose until resolved to ≤ grade 1, then: • If resolved in ≤ 7 days, then maintain dose level • If resolved in > 7 days, then decrease by 1 dose level *[If clinical benefit is derived, AZD1775 may be continued if the renal function is elevated but stable]*
		AraC	Continue treatment at current dose level
	Creatinine increased Grade 3 and 4	AZD1775	Omit dose until resolved to ≤ grade 1, then decrease dose by 1 dose level
		AraC	Omit dose until resolved to ≤ grade 1, then decrease dose by 1 dose level

→ → *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified* ← ←			
CTCAE System/Organ/Class (SOC)	**ADVERSE EVENT**	**AGENT**	**ACTION****
Investigations	Blood bilirubin increased ≥ Grade 3	AZD1775	Omit dose until resolved to ≤ grade 1, then: • If resolved in ≤ 7 days, then maintain dose level • If resolved in > 7 days, then decrease by 1 dose level
		AraC	Continue treatment at next lower dose level
	Blood bilirubin increased Grade 4	AZD1775	Omit dose until resolved to ≤ grade 1, then decrease dose by 1 dose level
		AraC	Continue treatment at Dose level -1once resolved to ≤ Grade 1
	Alanine aminotransferase (ALT) increased and/or Aspartate aminotransferase (AST) increased Grade 3	AZD1775	Omit dose until resolved to ≤ grade 1 or baseline then: • If resolved in ≤ 7 days, then maintain dose level • If resolved in > 7 days, then decrease dose by 1 dose level
		AraC	Continue treatment at next lower dose level
	Alanine aminotransferase (ALT) increased and/or Aspartate aminotransferase (AST) increased Grade 4	AZD1775	Omit dose until resolved to ≤ grade 1 or baseline, then decrease dose by 1 dose level
		AraC	Continue treatment at Dose level -1
Cardiac Disorders	Cardiac disorders – Other ≥ Grade 4	AZD1775	Omit dose and discontinue patient from study
Other Non-Hematologic events	≥ Grade 4	AZD1775	Omit drug and follow patient at least weekly or at least every other week until adverse event has resolved to grade ≤ 2, restart drug at next lower dose level.
AT TIME OF RETREATMENT			

→ → *Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0* unless otherwise specified* ← ←			
CTCAE System/Organ/Class (SOC)	**ADVERSE EVENT**	**AGENT**	**ACTION****
Non Hematologic	All Non-Hematologic Toxicities Grade ≥ 4 (With the exception of nausea, vomiting or diarrhea controlled with appropriate medications.)	AZD1775	Hold AZD1775 and AraC. Re-check patient at weekly or at least every other week. When event returns to ≤ grade 2, resume AZD1775 and AraC treatment at next lower dose level.

* Located at http:/ctep.cancer.gov/protocolDevelopment/electronic_applications.ctc.htm

** Use the following to describe actions in the Action column:

- Omit = Treatment is not given for the day that is omitted, but treatment may resume once the adverse event has resolved as mandated in Table 8.2. For example, if treatment is omitted on Day 8, but the adverse event has resolved by Day 10, treatment may resume on Day 10 to complete the cycle. Missed doses will not be made up.
- Hold/Delay = Treatment can be made up as part of this cycle
- Discontinue = Treatment is totally stopped

***AraC can be continued each cycle regardless of dose hold or adjustments for AZD1775, if patient is presumed to derive clinical benefit from the intervention.

Patients whose treatment is interrupted or permanently discontinued due to an adverse event including abnormal laboratory values must be followed by their treating physician as clinically indicated. The maximum time allowed for treatment interruption due to toxicity is 6 weeks from the intended dosing day. If interruption is > 6 weeks, continuation of treatment is allowed on a case by case basis (i.e. clinical benefit) as determined by the treating physician. However, the patient will continue to be followed for toxicity. Dose interruptions should be reported on the appropriate Dosage Administration CRF.

9.0 Ancillary Treatment/Supportive Care/Concomitant Medications

9.1 Routine use of colony-stimulating factors (G-CSF or GM-CSF) is not recommended. Prophylactic use of colony-stimulating factors should be discussed with the study chair. Therapeutic use in patients with serious neutropenic complications may be considered at physician discretion. Recombinant erythropoietin to maintain adequate hemoglobin levels is discouraged and only allowed after discussion with the study chair.

9.2 Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, anti-emetics received from the first administration of study drugs until 30 days after the final dose are to be recorded in the medical record. For more severe diarrhea (i.e. Grade 3 or 4) supportive measures such as hydration and anti-diarrheals are recommended according to instutitional practice guidelines and as deemed appropriate by the medical situation (i.e. IV rehydration as in- or outpatient, antibiotics).

9.3 Anti-emetic therapy (excluding aprepitant) may be used in accordance with standard practice and/or the discretion of the investigator.

9.4 Hydroxyurea is allowed during the trial to control blast counts/WBC if the patient is felt to derive benefit from study treatment.

9.5 Patients on metformin should be closely watched for elevated metformin levels that could put them at risk for hypoglycemia.

10.0 Adverse Event (AE) Reporting and Monitoring

10.1 Adverse Event Characteristics

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

10.11 Adverse event monitoring and reporting is a routine part of every clinical trial. First, identify and grade the severity of the event using the CTCAE version 4.0. Next, determine whether the event is expected or unexpected (see Section 10.2) and if the adverse event is related to the medical treatment or procedure (see Section 10.3). With this information, determine whether the event must be reported as an expedited report (see Section 10.4). Expedited reports are to be completed within the timeframes and via the mechanisms specified in Section 10.4. All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

10.12 Each CTCAE term in the current version is a unique representation of a specific event used for medical documentation and scientific analysis and is a single MedDRA Lowest Level Term (LLT). Grade is an essential element of the Guidelines and, in general, relates to **severity** for the purposes of regulatory reporting to NCI.

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

10.13 Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment (any procedures specified in the protocol). Adverse events occurring before starting study treatment but after signing the informed consent form are not recorded. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, and are recorded.

10.14 Any serious adverse event occurring after the patient has provided informed consent, has started taking the study medication, and until 4 weeks after the patient has stopped study participation must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication). The period after discontinuing study drug may be extended if there is a strong suspicion that the

drug has not yet been eliminated.

10.2 **Expected vs. Unexpected Events**

- The determination of whether an AE is expected is based on agent-specific information provided in Section 15.0 of the protocol and the study specific consent form.

- Unexpected AEs are those not listed in the agent-specific information provided in Section 15.0 of the protocol and the study specific consent form.

NOTE: "Unexpected adverse experiences" means any adverse experience that is neither identified in nature, severity, or frequency of risk in the information provided for IRB review nor mentioned in the consent form.

10.3 **Assessment of Attribution**

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

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

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

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

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

<u>Routine Reporting</u>

- Routine AE reporting for Phase 1 and Phase 2 clinical studies using an investigational agent /intervention in combination with a commercial agent is stated in the protocol. See Section 10.6.

- Routine AE reporting using an investigational agent/intervention and a commercial agent in combination must be reported as defined by the general guidelines provided by sponsors, Groups, Cancer Centers, or Principal Investigators. See Section 10.6.

<u>Expedited Reporting</u>

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

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

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

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

10.4 Expedited Reporting Requirements for IND/IDE Agents

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

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

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

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

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	7 Calendar Days	24-Hour 3 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in Section 10.41 of the protocol. These only apply to those events that are not attributed to AZD1775 (Unlikely or Unrelated as defined in Section 10.3).

Expedited AE reporting timelines are defined as:

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

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade ≥2 AEs resulting in hospitalization or prolongation of hospitalization

Additional Instructions:

1. Use the AdEERS Multiple Agent Template. :

   Mayo Clinic Cancer Center (MCCC) Institutions: Provide copies of the AdEERS Multiple Agent Template, along with the UPIRTSO cover sheet, by fax [redacted] to the MCCC Regulatory Affairs Unit (RAU) Risk Information Specialist who will determine and complete IRB reporting. The RAU will submit to the MCCC SAE Coordinator and the MCCC IND Coordinator to determine if FDA submission is needed.

2. **Reporting of SAEs to AstraZeneca**
   Fax or email a copy of the AdEERs report to SCRI at [redacted] or [redacted] within 24 hour of the event. The investigator must then ensure that the form and coversheet are accurately and fully completed with follow-up information and fax or email those to SRCI within 2 to 3 calendar days for deaths or life-threatening events and 5 calendar days for other serious adverse events. The original and the duplicate copies of the AdEERs report, SRCI SAE coversheet, and the fax confirmation sheet must be kept with the case report forms at the study site.

   Follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or discontinued study participation. The AdEERs report, SRCI SAE coversheet, and fax confirmation sheet must be retained. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects.

EVENT TYPE	REPORTING PROCEDURE

Other Grade 4 or 5 Events and/or Any Hospitalizations During Treatment Not Otherwise Warranting an Expedited Report	Complete a Notification Form*: Grade 4 or 5 Non-AER Reportable Events/Hospitalization Form electronically via the MCCC Remote Data Entry System or paper form within 5 working days of the date the clinical research associate (CRA) is aware of the event(s) necessitating the form. If an expedited written report has been submitted, this form does not need to be submitted.

* This form is not required for those adverse events listed in section 10.41

10.41 Special Situations for Expedited Reporting

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

An expedited report or notification form may not be required for specific Grade 1, 2 and 3 Serious Adverse Events where the AE is **EXPECTED**. Any protocol specific reporting procedures MUST BE SPECIFIED BELOW and will supercede the standard Expedited Adverse Event Reporting Requirements (Note: These adverse events must still be reported through the routine reporting mechanism [i.e. Nadir/adverse events form]; see footnote 1):

NOTE: These only apply to those events that are not attributed to AZD1775 (Unlikely or Unrelated as defined in Section 10.3).

System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be expeditedly reported[1]
General disorders and administrations site conditions	Fatigue	Grade 3
Gastrointestinal Disorders	Nausea	Grade 3
	Vomiting	
	Diarrhea	
Investigations	Neutrophil count decreased	Grade 3 and Grade 4
	White blood cell count decreased	
	Platelet count decreased	
Blood and lymphatic system disorders	Anemia	Grade 3 and Grade 4
	Febrile Neutropenia (in AML patients only)	Grade 3 and Grade 4

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

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

10.5 **Other Required Reporting**

10.51 Persistent or Significant Disabilities/Incapacities

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

10.52 Death

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

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

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) – Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.53 Secondary Malignancy

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

- All secondary malignancies that occur following treatment with an agent under an IND/IDE must be reported.

- Any malignancy possibly related to cancer treatment should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

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

10.55 Pregnancy

To ensure patient safety, each pregnancy occurring while the patient is on study treatment and for up to 20 months from the patient's last dose must be reported to the SRCI Database within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Study Pregnancy Form and reported by the investigator to SCRI Safety Database. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the AstraZeneca study drug of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

In addition, each pregnancy occurring for a female partner of a male study participant must be reported while the patient is on study treatment and for up to 6 months after the patient's last dose. Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

10.6 **Required Routine Reporting**

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

System Organ Class (SOC)	Adverse event/Symptoms	Baseline	Each evaluation
General disorders and administrations site conditions	Fatigue	X	X
	Fever (Pyrexia)	X	X
Blood and lymphatic system disorders	Anemia	X	X
Gastrointestinal Disorders	Nausea	X	X
	Vomiting	X	X
	# of stools	X	
	Diarrhea		X

Investigations	Neutrophil count decreased	X	X
	Platelet count decreased	X	X
	AST Increased	X	X
	ALT Increased	X	X

10.61 Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.611 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.612 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.613 Grade 5 AEs (Deaths)

10.6131 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6132 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

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

11.0 Treatment Evaluation Guideline

Note: Novel combinations in AML/MDS may be expected to result in different response kinetics than "conventional" cytotoxic regimens. Thus initial blast % increase may not accurately reflect later stage responses. AraC, if given SC may lead to differentiation. There will be no pre-specified number of cycles and patients can remain on study as long as they tolerate the combination and/or derive clinical benefit.

11.1 AML response criteria

11.11 Complete hematologic response (CR)
Less than 5% blasts in a non-hypocellular marrow with a granulocyte count ≥ 1.0, and a platelets count of ≥ 100 with complete resolution of extramedullary disease and absence of peripheral blood blasts.

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

11.111 Complete cytogenetic remission (CCyR)

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

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

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

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

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

11.2 MDS and CMML response criteria

Category	**Hematologic Response** **Response Criteria (responses must last at least 4 weeks)[a]**
Complete remission (CR)	Bone marrow: ≤5% myeloblasts with maturation of all cell lines. If present, persistent dysplasia will be noted (dysplastic changes should consider the normal range of dysplastic changes.) Peripheral blood: • Hemoglobin (Hgb) ≥11 g/dL (untransfused, patient not on erythropoietin) • Neutrophils ≥1.0 x 10^9/L (not on myeloid growth factor) • Platelets ≥100 x 10^9/L (not on a thrombopoietic agent) • Blasts 0%;
Partial remission (PR)	All CR criteria (if abnormal prior to treatment), except: Bone marrow blasts decreased by ≥50% compared with pretreatment but still >5%. Cellularity and morphology not relevant.
Marrow CR	Bone marrow: ≤ 5% myeloblasts and decrease by ≥50% over pretreatment if > 10% at baseline Peripheral blood: if hematologic improvement (HI) responses, they will be noted in addition to the marrow CR
Stable disease (SD)	Failure to achieve at least PR, but no evidence of progression for >8 weeks.
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to an MDS FAB subtype more advanced than pretreatment after 6 months/cycles of therapy

Relapse after CR or PR	At least one of the following: • Return to pretreatment bone marrow blast percentage • Decrement of ≥50% from maximum remission/response levels in granulocytes or platelets • Reduction in hemoglobin concentration by ≥1.5 g/L or transfusion dependence if previously had become transfusion independent for > 8 weeks[c]
Cytogenetic response	Complete: Disappearance of the chromosomal abnormality without appearance of new ones Partial: At least 50% reduction of the chromosomal abnormality
Disease progression	For patients with: • Less than 5% blasts: ≥50% increase in blasts to >5% blasts • 5%-10% blasts: ≥50% increase in blasts to >10% blasts • 10%-20% blasts: ≥50% increase in blasts to >20% blasts • 20%-30% blasts: ≥50% increase in blasts to >30% blasts Any of the following: • At least 50% decrement from maximum remission/response levels in granulocytes or platelets • Reduction in hemoglobin concentration by ≥2 g/dL • Transfusion dependence if previously had become transfusion independent for > 8 weeks[c]
a For a designated response (CR, PR), relevant response criteria must be noted on at least 2 successive determinations at least 1 week apart after an appropriate period following therapy (e.g., 1 month or longer). c In the absence of another explanation, such as acute infection, gastrointestinal bleeding, hemolysis, etc.	

Hematologic Improvement*	**Response Criteria (responses must last at least 8 weeks)[a]**
Erythroid response (pretreatment, Hgb < 11 g/dL)	Hgb increase by ≥1.5 g/dL Relevant reduction of units of red blood cell (RBC) transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for an Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation.
Platelet response (pretreatment, < 100 x 10^9/L)	Absolute increase of ≥ 30 x 10^9/L for patients starting with >20 x 10^9/L Increase from < 20 x 10^9/L to >20 x 10^9/L and by at least 100%
Neutrophil response (pretreatment, < 1.0 x 10^9/L)	At least 100% increase and an absolute increase >0.5 x 10^9/L.
Progression or relapse after HI	At least 1 of the following: • At least 50% decrement from maximum response levels in granulocytes or platelets • Reduction in Hgb by ≥1.5 g/dL • Transfusion dependence[b]

* Pretreatment count averages of at least 2 measurements (not influenced by transfusions) ≥1 week apart (modification) a For a designated response (CR, PR, HI), relevant response criteria must be noted on at least 2 successive determinations at least 1 week apart after an appropriate period following therapy (e.g., 1 month or longer). b In the absence of another explanation, such as acute infection, gastrointestinal bleeding, hemolysis, etc.

12.0 Descriptive Factors

12.1 Transfusion Dependent: Yes vs No

12.2 MDS subcategory: MDS vs MDS/MPN overlap vs CMML

12.3 Previous cytotoxic chemotherapy: yes vs no

12.4 AML subcategory: without prior HMA vs prior HMA

12.5 HMA failure: refractory vs relapsed vs progressed on HMA

12.6 AML: De novo vs secondary AML

12.7 AML: newly diagnosed vs relapsed/refractory

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Patients who have not developed PD will continue treatment per protocol.

13.2 Patients who develop PD while receiving therapy will go to the event monitoring phase.

13.3 Patients who go off protocol treatment for reasons other than PD will go to the event monitoring phase per Section 18.0.

13.4 If a patient in the initial safety portion fails to complete 28 days of treatment for reasons other than dose-limiting toxicity defined adverse events, the patient will be regarded as uninformative in regard and an additional patient will be treated; however, all toxicity information will be utilized in the analysis.

13.5 A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient will go directly to the event monitoring phase of the study (or off study, if applicable).

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

is necessary.

13.6 A patient is deemed a *major violation*, if protocol requirements regarding treatment in cycle 1 of the initial therapy are severely violated that evaluability for primary endpoint is questionable. All data up until the point of confirmation of a major violation must be submitted. The patient will go directly to the event monitoring phase of the study. Event monitoring will be required per Section 18.0 of the protocol.

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

14.0 Body Fluid Biospecimens

Body Fluid Biospecimen Submission

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

Correlative Study (Section for more information)	**Mandatory or Optional**	**Blood or Body Fluid being Collected**	**Type of Collection Tube (color of tube top)**	**Volume to collect per tube (# of tubes to be collected)**	**Baseline**	**Cycle 1 Day 2 (+1 Day)**	**Cycle 1 Day 5 (+1 Day)**	**Cycle 2 Day 5 (+1 Day) & Day 5 of Subsequent Odd Cycles**	**At time of bone marrow after cycles 1 (AML only), 2 and 4[3]**	**EOS**	**Process at site? (Yes or No)**	**Temperature Conditions for Storage /Shipping**
Biomarkers	Mandatory	Whole Blood	1 x Red top 2 x Na Heparin Green top	10 mL (3)	X	X[1]			X	X	Yes	Cold Pack
Biomarkers	Mandatory	Bone marrow aspirate	Heparinized Green top	10 mL (2)	X[4]				X	X[2]	Yes	Cold Pack
Buccal swab	Optional	Saliva	n/a	n/a	X						No	ambient
Pharmacokinetics	Mandatory	Plasma	Lavender Top	2 ml			Pre-Dose, 1, 2, 4, 6, & 8 hrs post-dose	Pre-Dose			Yes	-20°C

1. Only to be done in those patients with circulating tumor cells in their blood

2. End of study bone marrow sample is optional and to be collected only if bone marrow performed as part of patient's standard of care.
3. Bone marrow aspirates will be done at times of standard clinical marrow tests. Follow Tables 4.1. (AML) and 4.2. (MDS) for study schedule. In brief after cycle 1 (for AML only, at physician's discretion can be omitted, see Table 4), after cycles 2 and 4, and then at physician discretion.
4. This is only mandatory IF a BM biopsy has not been done within 4 weeks of study start.

NOTE: marrow bisopies will not be done soley for the purpose of drawing a research sample , but should be performed to coincide at times of standard clinical marrow biopsies/aspirates.

14.2 Collection and Processing

14.21 Biomarkers: Process on site per lab manual. Send all samples in original tubes to [redacted] lab per section 14.32.

14.22 Pharmacokinetics: Samples are collected at the time intervals presented in the protocol. Whole blood will be collected in 2 mL BD Vacutainer tubes containing K2EDTA (lavender top) as anti-coagulant for the analysis of AZD1775. Following collection, gently invert the samples 10 times and immediately place on ice. Within 30 minutes of blood collection centrifuge at 1500 xg, at 4°C for 10 minutes. From each blood sample, transfer roughly equal volumes into a total of two 1.8 mL polypropylene cryovials using a disposable polypropylene pipette. Store plasma samples at -20°C in an upright position within 30 minutes of plasma preparation and keep frozen at this temperature until shipment and during shipment.

Labels will be provided. Tubes should be labeled with the following information: Study, Subject ID, Visit, Draw Time, and Biological Matrix (e.g., blood or plasma). When applying the label, place the label in a vertical position. Do not wrap the label around the tube horizontally. Place the label as close to the cap as possible, but do not adhere the label to the cap of the tube. Do not cover any written information with the label.

14.3 Shipping and Handling

14.31 Kits will be used for this study for the pharmacokinetics.

14.32 Shipping Specimens

14.321 For Biomarkers including saliva: Verify ALL sections of the Specimen Submission Forms (i.e. blood, bone marrow, saliva – see Forms Packet) are completed and filled in correctly.

For Pharmacokinetics: Ship the aliquots frozen on dry ice. The samples must be securely packed in boxes to avoid breakage during transit, double-bagged to contain leaks, and where applicable, packed with a sufficient quantity of dry ice to ensure they remain frozen for at least 72 hours.

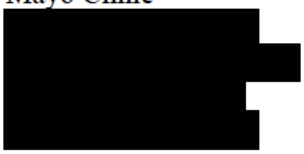
Samples should be placed in a courier box with a paper copy of the Sample Inventory. Samples should be boxed up with each subject's samples in profile order and listed in the same order on the Sample Inventory for ease of checking at the bioanalytical laboratory (BioA Lab).

Once the courier has collected the samples, the sample receiver at the BioA Lab should be notified via email of the courier name, airway bill number, expected delivery date/time and shipment contact. An electronic sample inventory (Excel format, Request file) should also be attached to this email.

14.322 Ship specimens via Priority Overnight service, **Monday – Wednesday ONLY,** to:

For Biomarkers:

Mayo Clinic



For Phamacokinetics:

Do not send samples the day before, the day of, or the observed day of a national holiday.

All specimens must be shipped Monday – Wednesday ONLY.

14.4 Suggested Correlative Studies and Experiments:

The correlative studies are designed based on the pre-clinical data in AML, MDS and other tumor types and the general mechanisms of WEE1 and AraC in pathophysiology of cancer and the diseases under study. These studies are suggestions at the time of protocol development. Specific assays may change based on the current knowledge at time of biomarkers analysis.

Patients will be asked in the informed consent to provide a research bone marrow aspirate and blood samples as outlined in the schedule of events. Research bone marrow and aspirates will be collected as outlined in Table 14.1 in conjunction with a patient's standard of care bone marrow biopsy or aspirate procedures. Peripheral blood research samples will be drawn at times of routine clinical draws, in parallel with the marrow biopsies as defined in Table 14.1.

Objectives and overview of correlative studies:
The below assays and studies to be performed are suggested at the time of protocol writing. Based on novel insights into the biology of WEE1 inihibition and AML/MDS that will arise during the protocol duration, below experiments may be adjusted to reflect the current state of the art knowledge.

1. WEE1 inhibition causes DNA damage and apoptosis. The degree of DNA damage in combination with AraC is unknown. Assessment of DNA damage induction by γH2AX and apoptosis by cleaved caspase 3 (CC3) as measured by flow cytometry on patient specimens at baseline and on follow up specimens, preferably on marrow aspirates, alternatively on peripheral blood samples will be conducted. Cell cycle distribution analysis and co-staining for total and phosphorylated CDK1/2 may be performed (using propidium iodide staining) at the same time points for samples with sufficient material.

2. Preclinical experiments (R.Tibes Lab) suggest that the damage and cell death induction is mainly/stronger in an earlier, leukemia "stem cell like" population. Therefore the "stemness" of a γH2AX/CC3 dual positive population will be assessed by co-staining for CD34+/38- cell surface markers in parallel with γH2AX/CC3 by multi-color flow cytometry. Alternatively, selection of CD34+/38- cells can be performed with subsequent staining for γH2AX/CC3.

3. In vitro we have shown that WEE1 protein expression may correlate with sensitivity to the AraC/AZD1775 combination. WEE1 protein expression levels in baseline marrow or peripheral blood may be assessed as well as WEE1 mRNA expression levels (could be deduced from RNAseq experiments below).

4. In unpublished work we have found a potential modulation of DNA damage response genes/proteins by WEE1 kinase inhibition. We will examine changes in total and phosphorylation forms of essential DNA repair proteins within the HR repair pathway, transcript genes changes under treatment with single agent AZD1775 and in the combination with AraC.

5. The role of myeloid specific mutations is unknown. Hence mutational/targeted sequencing of hematology/myeloid specific genes will be performed by targeted sequencing of gene panels or WES at baseline, at time of best response and at disease progression. A saliva sample will be used as germline control.

6. It is hypothesized that AZD1775 activity is independent of mutated/functional p53. p53 mutation status will be assessed by direct sequencing of the p53 gene (i.e. Sanger, Roche AmpliChip). Potentially, induction of p21 in fresh samples will be assessed as a transcriptional readout of functional p53 status.

7. Next Generation Sequencing - RNA sequencing: WEE1 target genes are master transcriptional regulators. Therefore we will use next generation sequencing for RNA and microRNAs to assess transcriptional changes before, during and after therapy. Expression and differential regulation for the WEE1 published signature genes [claspin, FBXO5, MCM10, CCNE 1 and 2] in patient specimens at baseline and follow up samples will be assessed, as well as p21 and HR pathway genes.

15.0 Drug Information

15.1 AZD1775

15.11 **Background**: AZD1775 is a highly selective, adenosine-triphosphate (ATP) competitive, small molecule inhibitor of Wee1 kinase, that is involved in regulation of intra-S and G2 cell cycle checkpoints through phosphorylation and inhibition of CDK2 and CDK1, respectively. AZD1775 has significant selectivity over other tested protein kinases. In vitro, AZD1775 inhibits Wee1 activity and induces DNA damage as well as G2 checkpoint escape in cell based assays. AZD1775 increases cytotoxicity when used in combination with DNA damaging agents, such as gemcitabine, cisplatin, carboplatin and topotecan, in p53-deficient cell lines. In vivo, AZD1775 was well tolerated and showed enhancement of anti-tumor efficacy by gemcitabine, carboplatin, cisplatin, 5-fluorouracil (5-FU) and capecitabine in nude rat xenograft tumor models. Similarly, in nude mouse xenograft models, AZD1775 treatment resulted in significant tumor growth inhibition at tolerated doses, and also enhanced the anti-tumor growth effect of gemcitabine, carboplatin, and radiation therapy.

15.12 **Formulation**: AZD1775 is currently available as dry filled capsules for oral administration containing 25, 100 or 200 mg of AZD1775 with the following excipients: lactose, microcrystalline cellulose, croscarmellose sodium, and magnesium stearate.

15.13 **Preparation and storage**: Capsules are packaged in high density polyethylene (HDPE) bottles, and should be stored at room temperature, no more than 30° C. For further information, refer to the investigational product label. Provided by AstraZeneca.

15.14 **Administration:** 200 mg PO (or respective dose) once daily either 2 hours before or 2 hours after a meal

15.15 **Pharmacokinetic information**: The PK data of AZD1775 following a single oral administration showed a moderate rate of absorption with a Tmax occurring at 3 to 4 hours. Post-peak plasma concentrations declined essentially in a mono-exponential manner with a t1/2 in the region of 10 hours. Exposure as measured by maximum plasma drug concentration observed (Cmax) and area under the curve (AUC)0-∞ increased in a dose-proportional manner over the dose range of 325 to 1300 mg. Following single (100 to 325 mg) and multiple dose administrations of AZD1775 (25 to 325 mg BID and 100 to 200 mg once daily [QD]) with carboplatin, cisplatin, and gemcitabine, plasma exposure of AZD1775 was consistent with predictions based on the single-dose regimen. AZD1775 was moderately bound to plasma proteins in all species tested, with the unbound fractions (at AZD1775 concentration of 1 μM) in plasma from the rat, dog and human being 23.2, 40.0, and 39.5%, respectively. Binding to plasma proteins was independent of AZD1775 concentration (0.1-10 μM) in the rat and human, but an increase in unbound fraction from 30.6% at 0.1 μM to 45.4% at 10 μM was observed in the dog. CYP3A4 is the

major CYP isoform involved in the oxidative metabolism of AZD1775, to the N-demethylated product. In addition, studies with flavin-containing monooxygenase (FMO) enzymes indicated that FMO3 and FMO5 were involved in formation of the N-oxide derivative of AZD1775.

15.16 **Potential Drug Interactions**: The following treatments and all the medications listed in Appendix VI are prohibited while in this study. Any further questions regarding concomitant treatments should be referred to the sponsor:

- No other investigational therapy should be given to patients. No anticancer agents other than the study medications should be given to patients. If such agents are required for a patient, then the patient must first be withdrawn from the study.

- No formal clinical drug interaction studies have been performed with AZD1775. An exploratory assessment of the effect of aprepitant on AZD1775 exposure in oncology patients suggests that there is a drug interaction between AZD1775 and aprepitant, as exposure to AZD1775 increased by ~60% when aprepitant was co-administered with AZD1775. The observed increase in AZD1775 exposure is likely the result of CYP3A4 inhibition by aprepitant. This increase in exposure is statistically significant. At the selected MTDs, this increase may also be of clinical importance. Therefore, concomitant treatment with aprepitant and fosaprepitant is not allowable per protocol until further evaluation.

  Potent or moderate inhibitors or inducers of CYP3A4, sensitive CYP3A4 substrates, and CYP3A4 substrates with a narrow therapeutic window should be avoided until additional data on drug-drug interaction becomes available (Appendix VI).

- *In vitro* data suggests that AZD1775 may also be a weak reversible inhibitor of CYP2C19. Caution should be exercised with concomitant administration of AZD1775 and agents that are sensitive substrates of CYP2C19, or substrates of this enzyme with narrow therapeutic range; refer to Appendix VI for a list of sensitive substrates of CYP2C19, or substrates of this enzyme with narrow therapeutic range.

- AZD1775 has been shown to be a weak inducer of CYP1A2 *in vitro* with a maximum measured response between donors of 39.9% to 93.1% (at 10 μM) and 18.6% to 32.5% (at 5 μM) of the positive control omeprazole (50 μM), respectively. Given the nature of the AZD1775 dosing schedule, however, the risk of induction in the clinic is considered low. No specific precautions are recommended at this time, except to be initially vigilant when using substrates of CYP1A2 with a narrow therapeutic range.

- *In vitro* studies have shown that AZD1775 may be a substrate and inhibitor for human P-glycoprotein (P-gp). Caution should be exercised when agents that are inhibitors or substrates of P-gp are administered concomitantly with AZD1775 (see Appendix VI).

- Recent *in vitro* transporter studies have shown AZD1775 to be an inhibitor of BCRP (IC50 5.1 μM). This finding is particularly relevant for drugs administered orally where exposure is normally limited by BCRP-mediated efflux, in particular some statins. Modelling has predicted a substantial increase in the exposure of Atorvastatin when co-administered with AZD1775 and the

use of Atorvastatin is therefore prohibited in the current study. Other drugs where the disposition is mediated via BCRP should be administered with caution, dose modification considered or substituted by an alternative drug (Appendix VI).

- AZD1775 has been shown to be an inhibitor or MATE1 and MATE2K transporters. A drug interaction with substrates of either transporter cannot be ruled out, the most important substrate known to date being **metformin**.

- Herbal preparations/medications are not allowed throughout the study. These herbal medications include, but are not limited to: St. John's wort, kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. Patients should stop using these herbal medications 14 days prior to first dose of AZD1775.

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15.17 **Known potential toxicities**: Based on the preliminary safety data available, the most frequent adverse events observed were blood and lymphatic disorders (anemia, febrile neutropenia), gastrointestinal disorders (diarrhea, vomiting, nausea, abdominal pain, constipation), general disorders and administration site conditions (fatigue, fever, chills), and investigation findings (thrombocytopenia, neutropenia, hematology and serum chemistry).

15.18 **Drug procurement:** Drug will be provided by AstraZeneca.

15.2 Cytarabine (AraC)

15.21 **Background**: Cytarabine (cytosine arabinoside) inhibits DNA synthesis. Cytosine gains entry into cells by a carrier process, and then must be converted to its active compound, aracytidine triphosphate. Cytosine is a pyrimidine analog and is incorporated into DNA; however, the primary action is inhibition of DNA polymerase resulting in decreased DNA synthesis and repair. Cytarabine is specific for the S phase of the cell cycle (blocks progression from the G1 to the S phase).

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

15.23 **Preparation, storage, and stability**: Store intact vials of powder at room temperature 15ºC to 30ºC (59ºF to 86ºF). Reconstitute with bacteriostatic water for injection. Reconstituted solutions are stable for up to 8 days at room temperature, although the manufacturer recommends use within 48 hours. Further dilution in 250-1000 mL of D5W or 0.9% NaCL is stable for 8 days at room temperature (25C). Note: Solutions containing

bacteriostatic agents should not be used for the preparation of either high doses or intrathecal doses of cytarabine.

15.24 **Administration:** Low-dose cytarabine (LD-AraC) will be administered at a starting dose of 20 mg BID subcutaneously (sc) for 10 days of a 28-day cycle., with dosing adjustments as outlined in section 8.

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

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

15.27 **Known potential adverse events:** Consult the package insert for the most current and complete information.
Warnings/Precautions: Potent Myelosuppressive agent
Common known potential toxicities, frequency not defined:
Other: Fever
Dermatologic: Rash
Gastrointestinal: Anal inflammation, anal ulceration, anorexia, diarrhea, mucositis, nausea, vomiting
Hematologic: Myelosuppression, neutropenia, anemia, thrombocytopenia, bleeding, leukopenia, megaloblastosis, reticulocytes decreased
Hepatic: Hepatic dysfunction, transaminases increased (acute)
Local: Thrombophlebitis

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

Infrequent and/or case reports:
Amylase increased, aseptic meningitis, cardiopulmonary arrest (acute), cerebral dysfunction, cytarabine syndrome (bone pain, chest pain,

conjunctivitis, fever, maculopapular rash, malaise, myalgia); exanthematous pustulosis, hyperuricemia, injection site inflammation (SubQ injection), injection site pain (SubQ injection), interstitial pneumonitis, lipase increased, paralysis, rhabdomyolysis, veno-occlusive liver disease

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

16.0 Statistical Considerations and Methodology

16.1 Overview:

This study is a phase II screening study. It is divided into two portions. The safety portion is designed to determine the dose of the treatment agents. The expansion portion is designed to continue enrollment at the acceptable dose levels to determine efficacy of treatment in elderly (age 60+) newly diagnosed AML patients (Arm A: AZD1775 days 1-5 & 8-12) and AraC days 1-5 & 8-12) and to make a selection of one of the two randomized arms for Relapsed/Refractory AML and HMA failure MDS patients (Arm B: AZD1775 days 1-5 & 8-12) and AraC days 1-5 & 8-12; Arm C: AZD1775 days 1-5, 8-12, 15-19, 22-26).

The purpose of this screening design is NOT TO ensure a high probability that the very best treatment is selected, but to ensure a low probability that a poor treatment is selected. In other words, at the conclusion of this trial, we cannot make a definitive conclusion about the superiority of one treatment compared to the other, but we can ensure that there is a small probability of bringing the inferior treatment forward.

16.2 Statistical Design and Analysis for the Primary Endpoint

16.21 Primary Endpoint

The primary endpoint of this trial is the rate of complete response (CR plus CRi). Throughout Section 16.0, complete response will be considered synonymous with "success", unless specified otherwise. All patients meeting the eligibility criteria, who have signed a consent form, and have begun treatment at the study MTD will be evaluable for complete response, with the exception of patients who are determined to be a major treatment violation.

Treatment Responses will be assessed by standard criteria for the respective disease per NCCN guidelines or according to specific criteria from expert panels (i.e. the International Working Group Criteria for the response in acute myeloid leukemias). Response will be assessed by peripheral blood criteria at the end of each cycle and every other cycle (for MDS) thereafter (Burnett et al. 2007. Gileset al. 2005, Kantarijian et al. 2012, Fenaux et al. 2009).
Bone marrow tests to assess responses will be carried as outlined in the study tables Table 4.1 and 4.2.

16.22 Arm A: Efficacy and safety of AZD1775 + AraC in elderly newly diagnosed AML

Statistical Design, Analysis and Decision Rule

Efficacy and safety of AZD1775 + AraC will be tested in elderly patients with newly diagnosed AML using a single-arm single-stage binomial design. The primary endpoint will be the complete response rate, which will be defined as an objective status of CR or CRi. Complete response rate will be evaluated over all cycles of study treatment. The proportion of CR/CRi responses will be estimated by the number of CR/CRi responses divided by the total number of evaluable patients. Two-sided 95% confidence intervals will be computed using an exact binomial confidence interval. The frequency and relative frequency of individual response categories will also be computed. For a subject to be considered evaluable for statistical analysis, the subject must be eligible, provide consent, initiate treatment and not experience a major treatment violation during the first cycle of treatment.

The largest CR/CRi response proportion where the proposed treatment regimen would be considered ineffective in this population is 15% (Burnett et al. 2007. Gileset al. 2005, Kantarijian et al. 2012, Fenaux et al. 2009) and the smallest CR/CRi response proportion that would warrant subsequent studies with the proposed regimen in this patient population is 40%.

The following one-stage binomial design uses 21 patients to test the null hypothesis that the true CR/CRi response proportion in a given patient population is at most 15%.

Decision Rule: Enter 21 patients into the study. If 5 or fewer CR/CRi responses are observed in the first 21 evaluable patients, we will consider this regimen ineffective in this patient population. Otherwise, if 6 or more CR/CRi responses are observed in the first 21 evaluable patients, we may recommend further testing of this regimen in subsequent studies in this population. We anticipate accruing an additional 2 patients to account for ineligibility, cancellation, major treatment violation, or other reasons. Thus, the total maximum accrual to this arm is 23 patients.

Assuming that the number of CR/CRi responses is binomially distributed, the significance level within this arm is </=10% and the probability of declaring that this regimen warrants further studies (i.e., statistical power) under various CR/CRi response proportions can be tabulated as a function of the true CR/CRi response rate as shown in the following table.

If the true CR/CRi rate is…	0.15	0.20	0.25	0.30	0.35	0.40
Then the probability of declaring that the regimen warrants further studies is…	0.08	0.23	0.43	0.64	0.80	0.90

16.23 Arm B and C: Efficacy and safety of AZD1775 in combination with AraC compared to AZD1775 alone in relapsed/refractory AML or HMA failure MDS patients.

Statistical Design, Analysis and Decision Rule

Efficacy and safety of AZD1775 alone or with AraC will be tested in patients with relapsed/refractory AML and patients with HMA failure MDS using a flexible randomized phase II selection design. Patients will be randomized to AZD1775 alone or with AraC in a 1:1 fashion using a dynamic allocation procedure (Pocock & Simon 1975). The primary endpoint will be the complete response rate, which will be defined as an objective status of CR or CRi. Complete response rate will be evaluated over all cycles of study treatment. The proportion of CR/CRi responses will be estimated by the number of CR/CRi responses divided by the total number of evaluable patients (by arm). Two-sided 95% confidence intervals will be computed using an exact binomial confidence interval. The frequency and relative frequency of individual response categories will also be computed. Estimates will also be computed by arm separately for each disease. For a subject to be considered evaluable for statistical analysis, the subject must be eligible, provide consent, initiate treatment and not experience a major treatment violation during the first cycle of treatment.

The selected design is a flexible randomized phase II selection design (Sargent & Goldberg 2001). This study will randomize 40 evaluable patients (20 per arm). The minimum required number of CR/CRi responses for an arm to be considered as having evidence of efficacy is 3 (out of 20 evaluable patients). This decision rule is based on a single-arm single-stage binomial design testing the null hypothesis that the true CR/CRi response proportion in a given arm is at most 5% (Tawfik et al. 2014, Giles et al. 2005, Jabour et al. 2010, Prevet et al. 2011) with the smallest CR/CRi response proportion warranting subsequent studies being 25%.

In the event that both arms meet this decision rule for efficacy, the combination arm will be considered as having additional efficacy over the single-agent arm if the CR/CRi response rate on the combination arm is at least 10% greater than the single-agent arm. If the difference is <10%, then the trial is considered statistically ambiguous and the selection between the combination and single-agent arm will be allowed to include other factors (e.g., adverse event data) in addition to the CR/CRi response rate. We anticipate accruing an additional 4 patients (2 patients per arm) to account for ineligibility, cancellation, major treatment violation, or other reasons. Thus, the total maximum accrual to Arm B is 22 patients and to Arm C is 22 patients.

Assuming that the number of CR/CRi responses is binomially distributed, the significance level for the single-arm decision rule requiring a minimum of 3 (out of 20 evaluable patients) within an arm is </=10% and the probability of declaring that a given arm's treatment regimen warrants further studies (i.e., statistical power) under various CR/CRi response proportions can be tabulated as a function of the true CR/CRi response rate as shown in the following table.

If the true CR/CRi rate is…	0.05	0.10	0.15	0.20	0.25
Then the probability of declaring that a given arm's treatment regimen warrants further studies is…	0.08	0.32	0.60	0.79	0.91

Probabilities for the combination arm being selected as the overall winner are provided in the following table.

CR/CRi rate (AZD1775 alone)	CR/CRi rate (AZD1775 + AraC)	Probability combo selected over single-agent arm
0.05	0.05	0.07
0.25	0.25	0.30
0.20	0.25	0.44
0.15	0.25	0.61
0.10	0.25	0.77
0.05	0.25	0.88

16.3 Sample Size, Accrual Rate, and Study Duration

This design is expected to accrue 18 patients to the safety portion with the possibility of enrolling up to 54. Six patients in each of the safety arms will be treated at the MTD and thus will be eligible for primary endpoint analysis. An additional 15 patients in Arm A and 14 patients in both arm B and C will be enrolled per treatment arm in the expansion portion with the addition of another 2 per arm to account for ineligibility, cancellation, major treatment violation, or other reasons. Thus the final accrual may be as large as 102 or as low as 67 (Arm A: 23; Arm B: 22; Arm C: 22).

The anticipated accrual rate is 4-6 evaluable patients per month. Therefore, the accrual period is expected to be approximately 1.5-2 years. The primary endpoint will be evaluated approximately 2.5 years after the trial opens, or after the last patient accrued has been observed for at least 4 months. The total study duration is expected to be approximately 2.5 years.

16.4 Secondary Endpoints

16.41 Safety and tolerability data will be compiled. The maximum grade for each type of adverse event will be recorded for each patient, and frequency tables will be reviewed to determine patterns (by arm, disease and overall). Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration. The rate of grade 3 or higher non-hematologic adverse events, and the rate of grade 4 or higher adverse event (hematologic and non-hematologic) will be computed each with a 95% exact binomial confidence interval.

16.42 Summary statistics for clinical benefit (mean and standard deviations for continuous variables and frequencies and percentages for discrete variables) will be compiled for hematologic improvements and transfusion requirements.

16.43 Duration of response is defined for all evaluable patients who have achieved a response as the date at which the patient's earliest best objective status is first noted to be a CR/CRi response to the earliest date progression is documented. If a patient dies subsequent to the response without a documentation of disease progression, the patient will be censored at the last date disease was assessed. In the case of a patient failing to return for evaluations before a documentation of disease progression, the patient will be censored for progression on the date of last evaluation. The distribution of duration of response will be estimated using the method of Kaplan-Meier (1958).

16.44 Time to response/progression is defined as the time from registration to the earliest date of documentation of response/disease progression. If a patient dies without a documentation of disease progression the patient will censored at the last date disease was assessed. In the case of a patient starting treatment and then never returning for any evaluations, the patient will be censored for progression 1 day post-registration. The distribution of time to progression will be estimated using the method of Kaplan-Meier (1958).

16.45 Survival time is defined as the time from registration to death due to any cause. The distribution of survival time will be estimated using the method of Kaplan-Meier (1958).

16.46 Time to AML or death is defined for all evaluable patients with MDS as the time from registration to leukemic transformation or death due to any cause. The distribution of time to AML or death will be estimated using the method of Kaplan-Meier (1958).

16.5 Exploratory Analysis

An exploratory analysis will be conducted, if there is indication, to determine any differences in study endpoints for subgroups of patients within each treatment arm as defined by the grouping factors (section 5.0), descriptive factors (section 12.0), dose level or any other patient demographics.

16.6 Correlative Research

16.61 Statistical analysis of pharmacokinetics and biomarkers will be primarily descriptive. Continuous biomarker levels will be explored in a graphical manner including mean plots and plots of change and percent change from baseline and other summary measures. Any potential relationships between the baseline level or change in the level of each biomarker and clinical outcome such as overall response, 6-month progression and survival, and adverse event incidence will be further analyzed using Wilcoxon rank sum tests or logistic regression methods, as appropriate. Association between a dichotomized biomarker and overall response will be assessed using a chi-squared test. Comparisons with 1-sided p-values ≤ 0.10 are considered significant. As this correlative component is exploratory in nature, we have not adjusted for multiple comparisons.

16.62 Patient-reported outcomes will be assessed at baseline and at the end of each cycle of treatment. MPN-SAF TSS was created to address the constellation of

symptoms. The MPN-SAF includes 1 item measuring fatigue from the previously validated Brief Fatigue Inventory (BFI), as well as linear analog scales capturing early satiety, abdominal discomfort, inactivity, concentration problems, numbness/tingling in the hands/feet, night sweats, itching, bone pain, fever, and weight loss. The MPN-SAF TSS has previously been validated for use at a single time point by co-administration with previously validated instruments.

Quality of life will be assessed prior to review of treatment response and discussions of patient's general health since last treatment evaluation. QOL will be measured using the EORTC QLQ-C30, a 30-item patient-reported questionnaire about patient ability to function, symptoms related to the cancer and its treatment, overall health and quality of life, and perceived financial impact of the cancer and its treatment. 28 of the 30 items are measured on a 1-4 scale (1=not at all; 4=very much) with the remaining two items (overall health and overall quality of life) scored on a 1-7 numeric analogue scale (1=very poor; 7=excellent). The recall period for the EORTC QLQ-C30 is one week. The EORTC QLQ-C30 is the product of more than a decade of collaborative research and to date, more than 2200 studies using the EORTC QLQ-C30 have been registered with the EORTC (Fayers et al, 2001 [EORTC Scoring Manual]). The patient booklet containing this questionnaire will be administered to all willing patients via a paper booklet in clinic at baseline and on day 1 of every cycle starting with Cycle 2, and will be scored according to the published scoring algorithms.

Scale score trajectories over time and changes from baseline over time will be examined using repeated measures or growth curve models, as appropriate, stream plots and mean plots with standard deviation error bars overall. Scores and changes at each cycle will be statistically tested using paired t-tests, and standardized response means (i.e. effect sizes) (mean of the change from baseline scores at a given cycle, divided by the standard deviation of the change scores) will be interpreted (after applying Middel's (2002) adjustment) using Cohen's (1988) cut-offs: <0.20 = trivial; 0.20-<0.50 = small; 0.50-<0.80 = moderate; and >=/0.80 = large.

16.7 Early Safety Analysis

An early safety analysis will be performed after 6 patients have been accrued to each arm of the study and observed for one cycle. Accrual will be temporarily halted while these patients are evaluated. If 2 or more of the first 6 patients experience a DLT, as defined below, then the dose level will be reduced as defined in Section 8.0 and another six patients will be treated. If < 2/6 patients experience toxicities defined as DLT, then this dose level will be defined as the operational MTD and moved into the expansion portion. Further dose de-escalation to dose level -2 will follow the same guidelines.

Should additional modification be necessary, the study will be closed and evaluated by the study team regarding continuation. Upon determination of the safe dose level, the study will re-open for accrual.

16.8 Data & Safety Monitoring:

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

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

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

- if 3 or more patients in the first 10 treated patients in each arm experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment.

- if after the first 10 patients in each arm have been treated, 30% of all patients experience a grade 4 or higher non-hematologic adverse event at least possibly related to treatment.

  NOTE: Only the arm(s) that crosses the stopping boundary rule will be closed, not then overall study accrual.

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

16.9a Results Reporting on ClinicalTrials.gov: At study activation, this study will have been registered within the "ClincialTrails.gov" website. The Primary and Secondary Endpoints along with other required information for this study will be reported on www.ClinicalTrials.gov. For purposes of timing of the Results Reporting, the initial estimated completion date for the Primary Endpoint of this study is 2.5 years after the study opens to accrual. The definition of "Primary Endpoint Completion Date" (PECD) for this study is at the time the last patient registered has been followed for at least 4 months.

16.9b Inclusion of Women and Minorities

16.9b1 This study will be available to all eligible patients, regardless of race, gender, or ethnic origin.

16.9b2 There is no information currently available regarding differential effects of this regimen in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analysis will, as always, look for differences in treatment effect based on racial and gender groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.9b3 The geographical region served by MCCC has a population which includes approximately 3% minorities. Based on prior MCCC studies involving similar disease sites, we expect about 3-5% of patients will be classified as minorities by race and about 33% of patients will be women. Expected sizes of racial by gender subsets are shown in the following table:

Accrual Estimates by Gender/Ethnicity/Race

Ethnic Category	Sex/Gender			
	Females	**Males**	**Unknown**	**Total**
Hispanic or Latino	2	3	0	5
Not Hispanic or Latino	32	65	0	97
Ethnic Category: Total of all subjects*	34	68	0	**102**
Racial Category				
American Indian or Alaskan Native	0	0	0	0
Asian	0	1	0	1
Black or African American	1	1	0	2
Native Hawaiian or other Pacific Islander	0	0	0	0
White	33	66	0	99
Racial Category: Total of all subjects*	34	68	0	**102**

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rico, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

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

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

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

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

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

17.0 Pathology Considerations: None

18.0 Records and Data Collection Procedures

18.1 Submission Timetable (need to be updated)

Initial Material(s)

Case Report Form (CRF)	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)
On-Study	≤2 weeks after registration
Baseline Adverse Event	
Measurement - Baseline	
Bone marrow biopsy report with cytogenetics and/or FISH	
Research Blood Submission (see Section 14.0)	
Research Bone Marrow Aspirate Submission (see Section 14.0)	
Research Saliva Submission -Baseline	
Patient Questionnaire Booklet	≤2 weeks after registration – Patient questionnaire booklet **must** be used; copies are not acceptable for this submission.
Patient Questionnaire Booklet Compliance	≤2 weeks after registration – This form must be completed only if the Patient Questionnaire Booklet contains absolutely NO patient provided assessment information.
End of Active Treatment/Cancel Notification	Submit ≤2 weeks after registration if withdrawal/refusal occurs prior to beginning protocol therapy

Test Schedule Material(s)

CRF	**Active-Monitoring Phase** (Compliance with Test Schedule Section 4.0)	
	At each evaluation during treatment	At end of treatment
Evaluation/Treatment	X	X
Nadir/Adverse Event	X	X
Measurement	X[3]	X[3]
Bone marrow biopsy report with cytogenetics and/or FISH	X[3]	X[3]
Patient Questionnaire Booklet	X[1]	X

CRF	Active-Monitoring Phase (Compliance with Test Schedule Section 4.0)	
	At each evaluation during treatment	At end of treatment
Patient Questionnaire Booklet Compliance	X[2]	X
Research Blood Submission	X (see Section 14.0)	X
Research Bone Marrow Aspirate Submission	X (see Section 14.0)	
PK Specimen Submission (Cycle 1)	X (see Section 14.0) (Cycle 1 only)	
PK Specimen Submission (Cycle 2+)	X (see Section 14.0) (Cycle 2+)	X
End of Active Treatment/Cancel Notification		X
Notification– Grade 4 or 5 Non-AER Reportable Events/Hospitalization	At each occurrence (see Section 10.0)	
ADR/AER	At each occurrence (see Section 10.0)	

1. Patient questionnaire booklet **must** be used; copies are not acceptable for this submission. To be completed prior to treatment every cycle.
2. This form must be completed only if the patient questionnaire booklet contains absolutely NO patient provided assessment information.
3. Submit copy of documentation of response (CR, CRi, PR, CI, MLFS, Marrow CR) or PD/Relapse/Fail/Failure, Attention [redacted].

Follow-up Material(s)

CRF	Event Monitoring Phase[1]				
	q. __3__ months until PD[2]	At PD[2]	After PD q. __6__ mos.	Death	New Primary
Event Monitoring	X	X	X	X	At each occurrence

1. If a patient is still alive 2 year after registration, no further follow-up is required.
2. Submit copy of documentation of response or progression to the MCCC Operations Office, Attention: [redacted]

19.0 Budget Considerations

19.1 Costs charged to patient: all standard of care tests and procedures such as physical exams, blood CBC and chemistries, bone marrow biopsies, AraC drug and administration costs, etc

19.2 Tests to be research funded: research blood and bone marrow collection and processing, AZD1775 drug dispensing

19.3 Other budget concerns: Drug and PK analysis will be supported by AstraZeneca.

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Appendix I

ECOG Performance Status Scale

SCORE	DESCRIPTION
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Appendix II: MEDICATION DIARY

Name _____

Mayo Clinic No_____

Study Name/Number_____

Patient Instructions

- Please indicate on the calendar below *every* day that you take your study medication by placing a check mark for each day that you take AZD1775.
- Avoid grapefruit, grapefruit juices, grapefruit hybrids, Seville oranges, pummelos, and exotic citrus fruits.
- Please take your medication either 2 hours before or 2 hours after a meal.

Start Date: _____

Medication(s)	Dose
AZD1775	MG

Study Drug	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
AZD1775							

Study Drug	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
AZD1775							

Study Drug	**Day 15**	**Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 20**	**Day 21**
AZD1775							

Study Drug	**Day 22**	**Day 23**	**Day 24**	**Day 25**	**Day 26**	**Day 27**	**Day 28**
AZD1775							

Date:_____ Participants Signature:_____

Area Below Only To Be Completed only by Coordinator

Number of pills returned_____ Study Coordinator Initials_____

Date_____ Discrepancy Yes____ No_____

Appendix III

New York Heart Association Classification of Cardiac Disease

The following table presents the NYHA classification of cardiac disease:

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Appendix IV: Revised International Prognostic Scoring System (IPSS-R) for MDS

IPSS-R Cytogenetic risk groups*,**

Cytogenetic prognostic subgroups	Cytogenetic abnormalities
Very good	-Y, del(11q)
Good	Normal, del(5q), del(12p), del(20q), double including del(5q)
Intermediate	del(7q), +8, +19, i(17q), any other single or double independent clones
Poor	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex: 3 abnormalities
Very poor	Complex: >3 abnormalities

IPSS-R Prognostic Score Values*

Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very Good		Good		Intermediate	Poor	Very Poor
BM Blast %	<=2		>2-<5%		5-10%	>10%	
Hemoglobin	=>10		8-<10	<8			
Platelets	=>100	50-<100	<50				
ANC	=>0.8	<0.8					

IPSS-R Prognostic Risk Categories/Scores*

RISK CATEGORY	RISK SCORE
Very Low	<=1.5
Low	>1.5 - 3
Intermediate	>3 - 4.5
High	>4.5 - 6
Very High	>6

*Greenberg, Tuechler, Schanz et al, Revised International Prognostic Scoring System (IPSS-R) for Myelodysplastic Syndrome, Blood 120: 2454, 2012.
**Schanz J et al, J Clin Oncology 2012; 30:820

Appendix V:

RISK STATUS BASED ON VALIDATED CYTOGENETICS AND MOLECULAR ABNORMALITIES[1]

RISK STATUS	CYTOGENETICS	MOLECULAR ABNORMALITIES
Better-risk	inv(16)[2,3] or t(16;16)[2] t(8;21)[2] t(15;17)	Normal cytogenetics: NPM1 mutation in the absence of FLT3-ITD or isolated biallelic CEBPA mutation
Intermediate-risk	Normal cytogenetics +8 alone t(9;11) Other non-defined	t(8;21), inv(16), t(16;16): with c-KIT[5] mutation
Poor-risk	Complex (≥3 clonal chromosomal abnormalities) Monosomal karyotype -5, 5q-, -7, 7q- 11q23 - non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)[4]	Normal cytogenetics: with FLT3-ITD mutation[6]

[1] The molecular abnormalities included in this table reflect those for which validated assays are available in standardized commercial laboratories. Given the rapidly evolving field, risk stratification should be modified based on continuous evaluation of research data. Other novel genetic mutations have been identified that may have prognostic significance.

[2] Other cytogenetic abnormalities in addition to these findings do not alter better risk status.

[3] Paschka P, Du J, Schlenk RF, et al. Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML study group (AMLSG). Blood 2013;121:170-177.

[4] For Philadelphia+ AML t(9;22), manage as myeloid blast crisis in CML, with addition of tyrosine kinase inhibitors.

[5] Emerging data indicate that the presence of c-KIT mutations in patients with t(8;21), and to a lesser extent inv(16), confers a higher risk of relapse. These patients should be considered for clinical trials, if available.

[6] FLT3-ITD mutations are considered to confer a significantly poorer outcome in patients with normal karyotype, and these patients should be considered for clinical trials where available. There is controversy as to whether FLT3-TKD mutations carry an equally poor prognosis.

Version 2.2014, 03/28/2014. National Comprehensive Cancer Network, Inc. 2014.

Appendix VI: List of prohibited concomitant medications and concomitant medications requiring caution

DISALLOWED MEDICATIONS AND MEDICATIONS TO BE ADMINISTERED WITH CAUTION

Formal drug-drug interaction studies have not yet been performed with AZD1775, therefore, the potential for drug-drug interaction described in this protocol are based on findings from in vitro studies and clinical experience.

In vitro data has shown that AZD1775 is metabolised predominantly by CYP3A4, with an FMO3 and/or FMO5 component. As a result, there is potential for the exposure of AZD1775 to be effected by drugs which inhibit or induce the metabolism of CYP3A4. In the clinic, coadministration of AZD1775 with the moderate CYP3A4 inhibitor, aprepaitant, resulted in a 60% increase in the plasma levels of AZD1775. Drugs known to be moderate to strong inhibitors/inducers of CYP3A4 are therefore prohibited for use in the current study, including aprepitant.

In vitro data suggests that AZD1775 may be a weak reversible inhibitor of CYP2C19 (IC_{50} 12 uM). Caution should therefore be exercised when AZD1775 is coadministered with agents that are sensitive substrates of CYP2C19, or substrates of this enzyme with a narrow therapeutic range.

Based on in vitro studies, AZD1775 has been show to be a weak reversible inhibitor (IC_{50} 14 μM) and a time-dependent inhibitor of CYP3A4 (K_{inact} 0.061/min, K_i 6.04 μM). The full impact of the time dependent inhibition is currently unknown, however, modelling data has predicted an 8-10 fold increase in the exposure of sensitive CYP3A4 substrates when administered with AZD1775 (250 mg BID for 5 doses). To date, no significant DDI effects have been reported in the clinic that may be related to the TDI finding. However, sensitive CYP3A4 substrates or substrates of CYP3A4 with a narrow therapeutic window are prohibited.

AZD1775 has been shown to be a weak inducer of CYP1A2 in vitro (39% increase in activity of positive control). Given the nature of the AZD1775 dosing schedule, however, the risk of induction in the clinic is considered low. No specific precautions are recommended at this time, except to be initially vigilant when using substrates of CYP1A2 with a narrow therapeutic range.

Transporter studies (in vitro) have shown that AZD1775 is both a substrate and inhibitor (IC_{50} 20 μM) of P-gp. Maximum impact of these finding is likely to occur for drugs administered orally at the same time as AZD1775. Caution should therefore be exercised when agents that are inhibitors or substrates of P-gp are administered concomitantly with AZD1775.

Recent invitro transporter studies have shown AZD1775 to be an inhibitor of BCRP (IC_{50} 5.1 μM). This finding is particularly relevant for drugs administered orally where exposure is normally limited by BCRP-mediated efflux, in particular some statins. Modelling has predicted a substantial increase in the exposure of Atorvastatin when coadministered with AZD1775 and the use of Atoravastatin is therefore prohibited in the current study. Other drugs where the disposition is mediated via BCRP should be administered with caution, dose modification considered or substituted by an alternative drug.

Herbal preparations/medications can be substrates, inhibitors and inducers, similar to any registered medication. Herbal preparations are therefore not allowed throughout the study.

These herbal medications include, but are not limited to: St. John's wort, kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone (DHEA), yohimbe, saw palmetto, and ginseng. In addition, any other drugs should be avoided at the Investigator's discretion if, in their opinion, the co-administration with AZD1775 may increase the risk of a clinically significant drug interaction.
A list of the main CYP3A4 substrates, inhibitors (strong and moderate) and inducers, CYP2C19 substrates, P-gp substrates and inhibitors and BCRP substrates are shown below. This is not an exhaustive list and further details can be found at Expert Opin. Drug Metab. Toxicol. (2013) 9(6):737-751.

CYP3A4 Inhibitors

Strong

Boceprevir
Clarithromycin
Cobicistat (GS-9350)
Conivaptan
Danoprevir
Elvitegravir
Fosamprenavir
Grapefruit juice
Idelalisib
Indinavir
Itraconazole
Ketoconazole
LCL161
Lopinavir
Mibefradil
Nefazodone
Nelfinavir
Posaconazole
Ritonavir
Saquinavir
Telaprevir
Telithromycin
Tipranavir
Troleandomycin
Voriconazole

Moderate

ACT-178882
Amprenavir
Aprepitant
Atazanavir
Casopitant
Ciprofloxacin
Crizotinib
Darunavir
Dronedarone
Diltiazem
Erythromycin
FK1706
Fluconazole*
Fosamprenavir
Imatinib
Ledipasvir
Lomitapide
Netupitant
Schisandra sphenanthera
Tofisopam
Verapamil

*The preferred azole anti-fungal medication is Fluconazole (alternatively Posaconazole) which can be given during treatment with AZD1775 at the treating physician's discretion, however with dose reductions of AZD1775 by 25-75% (i.e. from AZD1775 200mg to 150 or 100mg).

CYP3A4 Inducers (Strong and Moderate)

Avasimibe
Bosentan
Carbamazepine
Nafcillin
Phenobarbital
Phenytoin
Rifabutin

Efavirenz
Enzalutamide
Etravirine
Genistein
Lersivirine
Lopinavir
Mitotane
Modafinil
Rifampin
Ritonavir
Semagacestat
St John's Wort
Thioridazine
Tipranavir

CYP3A4 Sensitive Substrates or Substrates with a Narrow Therapeutic Range

ABT-384
Alfentanil
Aprepitant
Alfuzosin
Almorexant
Alpha-Dihydroergocryptine
Amiodarone
Aplaviroc
Aprepitant
Astemizole
Atazanavir
Atorvastatin
Avanafil
Bexarotine
BIRL 355
Bortezomib
Bosutinib
Brecanavir
Brotizolam
Budesonide
Buspirone
Capravirine
Carbamazepine
Casopitant
Cisapride,
Conivaptan
Cyclophosphamide
Cyclosporine
Danoprevir
Darifenacin
Darunavir
Ranolazine
Ridaforolimus
Romidepsin
Saquinavir
Sildenafil
Simeprevir
Simvastatin
Sirolimus
Tacrolimus
Temsirolimus
Terfenadine
Ticagrelor
Theoophylline
Thioridazine
Thiotepa
Tilidine
Tipranavir
Tolvaptan
Triazolam
Tretinoin
Ulipristal
Vardenafil
Vicriviroc
Voclosporin
Elvitegravir
Eplerenone
Ergotamine
Erlotinib
Etoposide
Everolimus
Felodipine
Fentanyl
Fluticasone
Gefitinib
Halofantrine
Ibrutinib
Ifosfamide
Imatinib
Indinavir
Ironotecan
Ivacaftor
Ixabepilone
L-771,688
Lapatinib
Levomethadyl (LAAm)
Lomitapide
Lopinavir
Lovastatin
Lurasidone
Maraviroc,
Midazolam
Midostaurin
Mosapride
Neratinib
Nilotinib

Dasatinib
Dihydroergotamine
Disopyramide
Dronedarone
Docetaxol
Dofetilide
Doxorubicin
Ebastine
Eletriptan
Nisoldipine
Paclitaxel
Pazopanib
Perospirone
Pimozide
Propafenone
Propofol
Quetiapine
Quinidine

CYP2C19 Sensitive Substrates or Substrates with a Narrow Therapeutic Range

Diazepam
Gliclazide
Lansoprazole
(R)-Lansoprazole
(S)-Lansoprazole
(S)-Mephenytoin
(R)-Mephobarbital
Omeprazole
(R)-Omeprazole
Pantoprazole
(+)-Pantoprazole
Rabeprazole
Tilidine

CYP1A2 Sensitive Substrates or Substrates with a Narrow Therapeutic Range

Alosetron
Caffeine
Duloxetine
Melatonin
Ramelteon
Tacrine
Theophylline
Tizanidine

P-gp Substrates

^Colchicine
*Digoxin
Fexofenadine
Indinavir
Paclitaxel
Toptecan
Vincristine

*If a patient requires initiation of digoxin during the study, or is already receiving treatment with digoxin, monitoring of digoxin levels is recommended according to local practice (as the levels of digoxin may increase). Monitoring of digoxin levels is also recommended when the patient has completed dosing with study treatment (as the levels of digoxin may then decrease).

^Colchicine may be given during study, with expected less clinical efficacy and patients should be carefully monitored clinically.

P-gp Inhibitors (Strong)

Cyclosporine
Elacridar
Erythromycin
Itraconazole
Ketocoanzole
LY335979Quinidine
Ritonavir
Valspodar
Verapamil

BCRP Substrates

Daunorubicin
Doxorubicin
Rosuvastatin
Sulfasalazine
Topotecan

Appendix VII: Patient Information Sheet

PATIENT INFORMATION SHEET
Patient Completed Quality of Life Booklet

You have been given a booklet to complete for this study. The booklet contains some questions about your 'quality of life' as a patient receiving treatment for cancer. Your answers will help us to better understand how the treatment you are receiving is affecting the way you feel.

1. This booklet contains two sets of questions:
   - EORTC QLQ-C30 (30 questions)
   - MPN-SAF TSS (11 questions)

2. Directions on how to complete each set of questions are written on the top of each set.

3. Please complete the booklet during your scheduled clinical visit and return it to your nurse, physician, or research coordinator.

Thank you for taking the time to help us.

Appendix VIII: EORTC QLQ-C30 and
Myeloproliferative Neoplasm Symptom Assessment Form
Total Symptom Score (MPN-SAF TSS)

EORTC QLQ - C30 (Version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
16. Have you been constipated?	1	2	3	4
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you.

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

Myeloproliferative Neoplasm Symptom Assessment Form
Total Symptom Score (MPN-SAF TSS)

Symptom	**1 to 10 (0 if absent) ranking** **1 is most favorable and 10 least favorable**
Please rate your fatigue (weariness, tiredness) by circling the one number that best describes your WORST level of fatigue during past 24 hours	(No Fatigue) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Circle the one number that describes, <u>during the past week</u> how much difficulty you have had with each of the following symptoms	
Filling up quickly when you eat (Early satiety)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Abdominal discomfort	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Inactivity	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Problems with concentration - Compared to prior to my MPD	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Numbness/ Tingling (in my hands and feet)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Night sweats	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Itching (pruritus)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Bone pain (diffuse not joint pain or arthritis)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)
Fever (>100 F)	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Daily)
Unintentional weight loss last 6 months	(Absent) 0 1 2 3 4 5 6 7 8 9 10 (Worst Imaginable)

Thank you for taking the time to help us.

Appendix IX: Childbearing Potential & Contraception

Female patients are considered to be of childbearing potential unless

- they are post-menopausal (defined as older than 50 years and amenorrhoeic for at least 12 months following cessation of all exogenous hormonal treatments),
- there is documentation of irreversible surgical sterilisation by hysterectomy, bilateral oophorectomy or bilateral salpingectomy (but not tubal ligation), or
- they are 50 years or younger but have been amenorrhoeic for at least 12 months following the cessation of exogenous hormonal treatments, and have serum follicle-stimulating hormone (FSH) and luteinising hormone (LH) levels in the postmenopausal range for the institution.

Female patients who are of childbearing potential must agree to use adequate contraceptive measures (as defined below) for the duration of study participation, and for 6 months after the final dose of study drug; cessation of birth control after this point should be discussed with a responsible physician. They also may not be breast feeding and must have a negative serum or urine pregnancy test within 72 hours prior to start of study treatment.

Acceptable methods of contraception include true abstinence in line with the preferred and usual lifestyle ch[redacted]mised partner, and methods listed in [redacted]
All methods of contraception (with the exception of total abstinence) should be used in combination with the use of a condom by their male sexual partner for intercourse. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control.
Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

Effective Methods of Contraception

Barrier Methods	Intrauterine Device Methods	Hormonal Methods
• Cap plus spermicide • Sponge plus spermicide • Diaphragm plus spermicide	• Copper T • Levonorgestrel-releasing intrauterine system (eg, Mirena®)[a]	Any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents) such as • Implants • Hormone shot or injection • Combined pill • Minipill • Patch

a This is also considered a hormonal method.