17 October 2019
EMA/CHMP/602286/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Vanflyta

International non-proprietary name: quizartinib

Procedure No. EMEA/H/C/004468/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Administrative information

Name of the medicinal product:	Vanflyta
Applicant:	Daiichi Sankyo Europe GmbH Zielstattstrasse 48 81379 Munich GERMANY
Active substance:	quizartinib dihydrochloride
International Non-proprietary Name/Common Name:	quizartinib
Pharmaco-therapeutic group (ATC Code):	other antineoplastic agents, protein kinase inhibitors (L01XE)
Therapeutic indication:	Vanflyta is indicated for the treatment of adults with relapsed or refractory acute myeloid leukaemia (AML) which is FLT3-ITD positive, and for continuation/maintenance of treatment post-transplant (see section 4.2).
Pharmaceutical form:	Film-coated tablet
Strengths:	17.7 mg and 26.5 mg
Route of administration:	Oral use
Packaging:	(PCTFE/PVC/Alu)
Package sizes:	14 x 1 tablets (unit dose) and 28 x 1 tablets (unit dose)

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List of abbreviations

AC220 = quizartinib
ADME = absorption, distribution, metabolism, excretion
AE = adverse event
AESI = Adverse Events of Special Interest
AhR = aryl hydrocarbon receptor
AFND = acute febrile neutrophilic dermatosis
ALT = alanine transaminase
AML = acute myeloid leukaemia
ANC = absolute neutrophil count
AST = aspartate transaminase
ASXL1 = additional sex combs like-1
ATP = adenosine triphosphate
AUC= area under the curve
AUC0-24 = area under the plasma concentration-time curve from time zero to 24 hours
AUCinf = area under the plasma concentration-time curve from time zero to infinity
AUClast = Area under the plasma concentration-time curve fromtime zero to time of last measurable concentration
AUCtau = Area under the plasma concentration-time curve during a dosage interval (tau)
B/R = benefit-risk
BCRP = Breast Cancer Resistance Protein (ABCG2)
BCS = Biopharmaceutics Classification System
BID = Bis-in-diem; twice daily
BMI = Body mass index
B/P = blood to plasma
BSA = Body surface area
Caco2 = human colonic adenocarcinoma cells
CAR = constitutive androstane receptor
CEBPA = CCAAT/enhancer-binding protein alpha
Cmax = Maximum plasma concentration after a single dose
CFU = Colony Forming Units
CHMP = Committee for Medicinal Products for Human Use
CI = confidence interval
CL/F = Apparent systemic clearance
CPP = Critical process parameter
CQA = Critical Quality Attribute
CR = complete remission
CRc = CR+ complete remission with incomplete platelet recovery (CRp) + complete remission with incomplete hematologic recovery (Cri)
CRi = complete remission with incomplete hematologic recovery
CRp = complete remission with incomplete platelet recovery
CSF = cerebrospinal fluid
CSF1R = colony stimulating factor 1 receptor
Ctrough = trough concentration
CYP = cytochrome P450
DDR = discoidin domain receptor
DMC = data monitoring committee
DMSO = dimethyl sulfoxide
DoE = Design of experiments
DPBS = Dulbecco’s phosphate buffered saline supplemented with Ca2+ and Mg2+
DS = differentiation syndrome
DSp = Design Space
D180 JAR = Day 180 joint assessment report
ECG = Electrocardiogram
ECOG = Eastern Cooperative Oncology Group
EFS = event-free survival
eGFR = epidermal growth factor
ELN = European LeukemiaNet
EMA = European Medicines Agency
ERA = environmental risk assessment
FAB = French–American–British

FDA = Food and Drug Administration
FGFR = fibroblast growth factor receptor
FLAG-IDA = fludarabine, cytarabine, idarubicin
FLT3 = Fms-like tyrosine kinase 3
FMEA = Failure mode effects analysis
GC = Gas Chromatography
GCP = good clinical practice
GI = gastro-intestinal
GLP = Good Laboratory Practice
GMP = good manufacturing practice
GVHD = graft versus host disease
HEK = human embryonic kidney
hERG = human ether-a-go-go-related gene
hFL = human FLT3 ligand
HIPAA = health insurance portability and accountability act
HPLC = High performance liquid chromatography
HR = hazard ratio
IC50 = half maximal inhibitory concentration
IC90 = concentration inhibiting 90% of kinase activity
ICH = International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC = independent ethics committee
ILS = increased life span
IR = Infrared
IRB = institutional review board
IRT = interactive response technology
ITD = internal tandem duplication
ITT = intention to treat
IT50 = plasma titer that results in 50% inhibition of kinase activity
IV = intravenous
IWG = international working group
Kd = dissociation constant
KF = Karl Fischer titration
KIT = receptor tyrosine kinase c-KIT
LC-MS/MS= liquid chromatography coupled with tandem mass spectrometry
LDPE = Low Density Polyethylene
LFS = leukemia-free survival
LLOQ = lower limit of quantitation
LoDAC = low Dose Cytarabine
LoOI = list of outstanding issues
LQTS = long QT syndrome or LQTS
MATE = multi-anion and toxin extrusion protein
MDD = maximum daily dose
MDS = myelodysplastic syndrome
MEC = mitoxantrone, etoposide and cytarabine
MO = major objection
MSD = meso scale discovery
MTD = maximum tolerated dose
MTV = median tumor volume
NMR = Nuclear Magnetic Resonance
NOAEL = no observed adverse effect
NOR = Normal Operating Range
NPM1 = nucleophosmin-1
NR = no response
NZW = new Zealand white
OATP = organic anion transporting polypeptide
OCT = organic cation transporter
OECD = organisation for economic co-operation and development
OS = overall survival
PAR = Proven Acceptable Range
PBT = persistent, bioaccumulative and toxic
PBPK = physiologically based pharmacokinetic
PCTFE = Polychlorotrifluoroethylene
PD = pharmacodynamics

PD = progressive disease
PDGFR = platelet-derived growth factor receptor
PEC = predicted environmental concentration
P-gp = p-glycoprotein
Ph. Eur. = European Pharmacopoeia
PIF = photo irritation factor
PIP = paediatric investigational plan
PK = pharmacokinetics
Papp = apparent permeability coefficient
pFLT3 = phospho-FLT3
PIA = plasma inhibitory assay
PO = per os, oral
PopPK = population pharmacokinetics
PPS = per-protocol analysis set
PR = partial response
PRAC = pharmacovigilance risk assessment committee
PS = performance status
PT = preferred term
PVC = polyvinyl chloride
PXR = pregnane X receptor
QbD = Quality by design
QD = quaque die; once daily
QTc interval = interval between the start of the Q wave and the end of the T wave (QT)
QTcF = QT interval corrected using Fridericia's formula
RBC = red blood cell
RET = rearranged during transfection
RLU = relative light unit
R-N-T = randomized-not-treated
RR or R/R = relapsed/refractory
RTK = receptor tyrosine kinase
SAE = serious adverse event
SD = stable disease
SDD = Spray Dried Dispersion
SmPC = summary of product characteristics
SOC = system organ class
STAT5 = signal transducer and activator of transcription 5
T1/2 = apparent terminal elimination half-life
TGR = toxicological transgenic rodent
TAMC = Total Aerobic Microbial Count
TEAE = treatment-emergent adverse event
TKD = tyrosine kinase domain
Tmax = time to the maximum observed serum concentration
TYMC =Total Combined Yeasts/Moulds Count
ULN = upper limit of normal
UV = ultraviolet
UVA = ultraviolet A
UVA = ultraviolet B
Vd/F = apparent volume of distribution
WBC = white blood cell
WHO = World Health Organisation
WT = wild type
XRPD = X-Ray Powder Diffraction
%CV = coefficient of variation

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Daiichi Sankyo Europe GmbH submitted on 11 October 2018 an application for marketing authorisation to the European Medicines Agency (EMA) for Vanflyta, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 May 2016.

Vanflyta was designated as an orphan medicinal product EU/3/09/622 on 23 March 2009 in the following condition: treatment of acute myeloid leukaemia.

The applicant applied for the following indication:

Vanflyta is indicated for the treatment of adults with relapsed or refractory acute myeloid leukaemia (AML) which is FLT3-ITD positive, and for continuation/maintenance of treatment post-transplant.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicant's own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0268/2018 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

New active Substance status

The applicant requested the active substance quizartinib contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

Protocol assistance

The applicant received Protocol assistance for the development programme supporting the indication "treatment of adults with relapsed or refractory acute myeloid leukaemia (AML) which is FLT3-ITD positive, and for continuation/maintenance of treatment post-transplant" as granted by the CHMP on 23 June 2011 (EMEA/H/SA/2143/1/2011/PA/II), 20 February 2014 (EMEA/H/SA/2143/1/FU/1/2013/PA/II), 28 January 2016 (EMEA/H/SA/2143/3/2015/PA/I) and 21 July 2016

(EMEA/H/SA/2143/3/FU/1/2016/PA/I). The Scientific advice pertained to the following quality and clinical aspects:

Quality:

- The Applicant sought agreement from CHMP on a number of aspects relating to the drug substance, drug product intermediate and drug product for quizartinib tablets. The questions covered genotoxic impurity control strategy, GMP starting materials, control of metal impurities in drug substance, drug substance specification, drug product intermediate specifications, drug product intermediate method of manufacture, drug product specifications, dissolution analytical procedure, drug product and drug product intermediate shelf-life, GMP starting materials and GTI control strategy.

Clinical:

- Phase 1 relative bioavailability study in healthy volunteers (Study AC220-014) comparing quizartinib oral solution and a quizartinib tablet formulation to support the use of the tablet formulation.
- Consideration was also sought if the Phase 2 Study AC220-002 uncontrolled efficacy and safety data in comparison to a historical control group might be supportive for a conditional marketing authorisation.
- The Applicant initially requested protocol assistance in relation to the proposed Phase 3 confirmatory study, Study AC220-007, regarding aspects of the protocol design, study population, endpoints, comparators, dose and statistical analysis.
- A question was also asked regarding the development of a companion diagnostic test for FLT3-ITD status in parallel with the clinical development of Quizartinib.
- Further questions (after the completion of the AC220-002 Phase 2 Study and an additional Phase 2b (2689-CL-2004) Study) covered the potential for the Phase 2 dataset to support conditional approval and the design of the Phase 3 (AC220-007) Study. Questions included inclusion/exclusion criteria, a new proposal for a starting dose, proposed dose-adjustments for drug-drug interactions, confirmation of OS as the primary endpoint, physician's best choice (one of 3 salvage regimens MEC, FLAG-IDA or low-dose cytarabine) as the comparator and statistical analysis plan. Also, the historical data for different degrees of responses was discussed.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Alexandre Moreau Co-Rapporteur: Paula van Hennik

The application was received by the EMA on	11 October 2018
Accelerated Assessment procedure was agreed-upon by CHMP on	20 September 2018
The procedure started on	1 November 2018
The Rapporteur's first Assessment Report was circulated to all CHMP members on	3 January 2019
The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on	7 January 2019

The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	8 January 2019
In accordance with Article 6(3) of Regulation (EC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	N/A
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	31 January 2019
The applicant submitted the responses to the CHMP consolidated List of Questions on	26 April 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CHMP members on	7 June 2019
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	13 June 2019
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	27 June 2019
The applicant submitted the responses to the CHMP List of Outstanding Issues on	20 August 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	4 September 2019
The Rapporteurs circulated updated Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP members on	16 September 2019
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	17 September 2019
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Vanflyta on	17 October 2019

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Vanflyta (quizartinib) is proposed for the treatment of adults with relapsed or refractory acute myeloid leukaemia (AML) which is FLT3-ITD positive, and for continuation/maintenance of treatment post-transplant.

2.1.2. Epidemiology and risk factors, screening tools/prevention

In Europe the incidence of AML is 5 to 8 cases per 100,000 adults per year, with approximately 4 to 6 deaths per 100,000 per year. Estimated 5-year survival rate is 26%. AML is predominantly a disease in older adults with an incidence of 15-25 per 100,000 newly diagnosed adults of 70 years and older per year and is more common in men than in women. Yearly 4-6 adults per 100,000 die due to AML (1, 2). The mortality rate increases dramatically with higher age, 5-year survival rates of 3% to 8% are reported in patients ≥60 years compared with rates of up to 50% for younger patients.

The incidence of AML is rising, which can be explained by the increased life expectancy in general and the increase in therapy-related AML caused by the use of cytotoxic chemotherapy in patients cured of their primary malignancy. Genetic and environmental risk factors, such as cigarette smoking and pesticides, have been identified to predispose to AML development. Also, previous haematological diseases, including myelodysplastic syndromes (MDS) or myeloproliferative neoplasms, are associated with increased AML risk. In most patients a clear predisposing factor for AML can however not be identified. In 30% of patients with newly diagnosed AML a mutation in the FLT3 gene can be found in the malignant cells, with ±25% having a FLT3-ITD and ±5% having a mutation in the TKD (2).

2.1.3. Biologic features-Aetiology and pathogenesis

AML is a heterogeneous hematologic malignancy that is characterized by clonal expansion of myeloid blasts in the bone marrow and frequently also in the peripheral blood and/or other tissues. It is characterized by clonal heterogeneity at the time of diagnosis, with the presence of both a founding clone and at least 1 subclone. The clonal heterogeneity has a different pattern at diagnosis compared to relapse. AML relapse is associated with the addition of new mutations and clonal evolution, which is shaped in part by the chemotherapy that the patients receive to establish and maintain remissions (3).

Among molecular abnormalities, FLT3 mutations are the most common. The FLT3 gene encodes a receptor tyrosine kinase involved in haematopoiesis. Two major classes of activating FLT3 mutations have been identified in patients with AML, which include the internal tandem duplications (ITD) and tyrosine kinase domain (TKD) point mutations. The mechanism of activation for these mutations is different, which is thought to account for the biological differences observed between them. FLT3-ITD mutations occur in approximately 30% of cases and are more common than FLT3-TKD mutations which occur in approximately 10% of patients.

The incidence of FLT3-ITD mutations decreases with age, with an incidence of up to 35% patients between 20 and 59 years of age compared with 16% to 20% in patients older than 60 years. The FLT3-ITD allelic ratio reflects the clonal burden of the leukemia cell population. In most cases, the FLT3-ITD-containing clone or clones that emerge at relapse appear to be more "FLT3addicted" with a higher mutant allelic burden. In fact, FLT3-ITD AML evolves from diagnosis to relapse, with the allelic ratio most often increasing upon relapse. Occasionally, the mutation is lost at relapse, or is present at too low a level for conventional assays to detect.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

AML (including the FLT3-ITD positive AML) is characterised by rapid, uncontrolled proliferation of malignant clonal hematopoietic stem cells that accumulate as immature, undifferentiated cells (blasts) and lead to impaired production of normal hematopoietic elements which in turn leads to anaemia, neutropenia, and thrombocytopenia. This is associated with symptoms of fatigue, shortness of breath, disturbed wound healing, infections and bleedings (2).

Diagnosis is based on the examination of peripheral blood and bone marrow including morphology, cytochemistry, immunophenotyping, cytogenetics and molecular genetics (nucleophosmin-1 (NPM1), CCAAT/enhancer binding protein alfa (CEBPA), runt-related transcription factor 1 (RUNX1), FLTS, tumor

protein 53 (TP53), additional sex combs like-1(ASXL1)) (1). The diagnosis is based on the identification of 20% or more myeloid blasts in the peripheral blood or bone marrow.

Among disease specific risk factors, the presence of FLT3-ITD has been proposed as one of the most important prognostic factors in AML. Numerous studies have shown the negative prognostic influence of FLT3-ITD in patients with AML, resulting in shorter remission durations and poorer survival outcomes compared with patients who have wild-type FLT3 (Median OS from the time of diagnosis ranged from 6 to 12 months). FLT3-ITD AML is associated with a shorter duration of response, a greater cumulative incidence of relapse, and shorter survival after relapse. The median time to relapse is 6 to 7 months in patients with FLT3-ITD AML compared with 9 to 11 months for patients with other AML subtypes, and the response rate to salvage therapy for FLT3- ITD AML in first relapse is lower than that in non-mutated cases (22% versus 64%).

Interestingly, a study in patients with NK-AML showed that prognosis was worse among patients with FLT3-ITD without wild-type FLT3, compared with those with FLT3-ITD with wild type FLT3 in the second allele. The median OS was 7 months in the absence of a wild-type FLT3 compared with 46 months among wild-type FLT3 patients with or without FLT3-ITD.

FLT3-ITD AML carries an even worse prognosis in patients who have experienced resistance or relapse after prior chemotherapy. Patients with AML who are refractory to initial induction therapy as well as those who relapse within 6 months after initial response or after allogeneic transplantation have a median survival of <6 months. Presence of the FLT3-ITD mutation is associated with decreased overall survival (OS) in relapsed or refractory AML.

2.1.5. Management

There is no standard treatment in relapsed or refractory AML for salvage therapy and if possible, patients should be enrolled in clinical trials. If first remission lasted more than 1 year, retreatment with intermediate- of high-dose cytarabine-based therapy can be used. In patients with shorter remission duration or when the primary induction failed, there is no consensus on the next-line treatment. In general, an objective response after salvage chemotherapy is observed in only 20-25% and median survival is 3-4 months. In older patients (>70 years) or unfit patients, treatment is mainly palliative (2). Poor outcome is associated with a shorter duration of remission, increasing age, non-favourable karyotype at initial diagnosis, and previous allogeneic HSCT (4).

Median survival in relapsed AML is about 6 months with 10% of patients achieving long-term survival high rate of relapse and short relapse-free and overall survival after chemotherapy and after transplantation (5, 6).

In view of the inherent poor prognosis of RR FLT3-mutation positive AML and as no therapies are approved in the EU, there is an unmet medical need in FLT3-ITD RR AML.

About the product

Quizartinib is an inhibitor of the receptor tyrosine kinase FLT3. Quizartinib and its major metabolite AC886 competitively bind to the adenosine triphosphate (ATP) binding pocket of FLT3 with high affinity (Kd=1.3 nM and 0.54 nM, respectively). Quizartinib and AC886 inhibit FLT3 kinase activity, preventing autophosphorylation of the receptor, thereby inhibiting further downstream FLT3 receptor signalling and blocking FLT3-ITD-dependent cell proliferation.

The sponsor applied for the following indication: Vanflyta is indicated for the treatment of adults with relapsed or refractory acute myeloid leukaemia (AML) which is FLT3-ITD positive, and for continuation/maintenance of treatment post-transplant.

During the evaluation the applicant revised the proposed indication as follows: Vanflyta is indicated as monotherapy for the treatment of adult patients with early relapsed or refractory FLT3 ITD positive acute myeloid leukaemia (AML) suitable for intensive first line treatment, and for continuation of treatment post-transplant. See section 2.5.3 for relevant CHMP discussion.

Type of Application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on data showing that patients with FLT3-ITD positive R/R acute myeloid leukemia (AML) have a very poor diagnosis with no approved targeted therapies and that quizartinib showed relevant preliminary efficacy data in these patients with prolonged OS (HR=0.76, 1-sided p-value from stratified log-rank test = 0.0177) compared to salvage standard chemotherapy.

However, during assessment the CHMP concluded that it was no longer appropriate to pursue accelerated assessment, since major objections were raised.

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as film-coated tablets containing 17.7 mg or 26.5 mg of quizartinib, which is present as quizartinib dihydrochloride.

Other ingredients are: (tablet core): hydroxypropylbetadex, microcrystalline cellulose, magnesium stearate, (film-coating): hypromellose, talc, triacetin, titanium dioxide, yellow iron oxide (26.5 mg tablet only). The product is available in aluminium/aluminium perforated unit dose blisters or PCTFE/PVC//aluminium perforated unit dose blisters.

2.2.2. Active Substance

General information

The chemical name of quizartinib dihydrochloride is 1-(5-tert-butyl-1,2-oxazol-3-yl)-3-(4-{7-[2-(morpholin-4-yl)ethoxy]imidazo[2,1-b][1,3]benzothiazol-2-yl}phenyl)urea corresponding to the molecular formula $C_{29}H_{32}N_6O_4S*2HCl$. It has a relative molecular mass of 633.59 g/mol and the following structure:

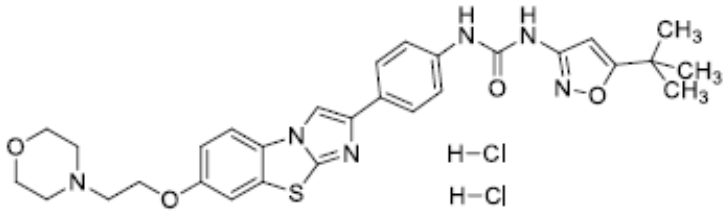


Figure 1: Quizartinib dihydrochloride active substance structure

The chemical structure of quizartinib was elucidated by a combination of halide titration, ultraviolet (UV) spectrum, infrared (IR) spectroscopy, ^{1}H-NMR spectroscopy, ^{13}C NMR spectroscopy, proton-proton correlation spectroscopy (COSY), heteronuclear multiple-bond correlation spectroscopy (HMBC), heteronuclear single-quantum correlation spectroscopy (HSQC), mass spectrometry and single crystal X-Ray structure determination.

The active substance is a white to off-white solid, slightly hygroscopic, very slightly soluble in acidic aqueous medium and its solubility decreases with pH. Quizartinib has a non-chiral molecular structure.

Polymorphism has been observed for quizartinib dihydrochloride. The active substance is manufactured as a consistent polymorphic form.

Manufacture, characterisation and process controls

The commercial active substance manufacturing process consists of three chemical transformations (with isolation of intermediates) followed by a final salt formation step. It is a convergent synthesis with both arms of equal length (two steps).

The process uses well defined starting materials with acceptable specifications. The proposed starting materials were the subject of pre-submission Scientific Advice from EMA and were re-defined as recommended. However, during the procedure a major objection was raised on the choice of one starting material as the information provided on potential impurities, including fate and purge of potential region-isomers and their control, was not sufficient and was not fully in line with the Scientific Advice. Following the provision of additional data and supportive information, the justification of the choice of starting material was considered acceptable and it was concluded that the proposed starting materials ensure sufficient portion of the process is performed under GMP.

The manufacturing process is sufficiently well described and the overall control strategy was considered adequate to control the process leading to an active substance of intended and consistent quality. Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The manufacturing process has been developed using a combination of conventional univariate studies and elements of Quality by Design (QbD). The critical process parameters were identified by a combination of prior knowledge and multivariate Design of Experiments (DoEs). Based on these studies, proven acceptable ranges (PARs) were proposed for a number of unit operations of the manufacturing process of the active substance. In response to questions raised during the procedure, and in line with EMA Q&A on "Improving the understanding of NORs, PARs, DSp and normal variability of process parameters" (EMA/CHMP/CVMP/QWP/354895/2017), the applicant revised the proposed PARs so that the flexibility in the commercial manufacturing process of the active substance is limited to a single PAR for each unit operation (i.e. a Design Space is not claimed). A traditional approach is maintained in the control strategy (with in-process controls for reaction monitoring and intermediates' specifications). The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the finally approved PARs.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development program. The proposed commercial version of the manufacturing process (B3) was developed at the proposed manufacturing site and it has undergone only minor adjustments from previous process version which produced stability and toxicology batches. Late stage clinical batches were obtained from these processes at the proposed commercial scale. The quality of the active substance used in the various phases of the development is considered to be comparable with that produced by the proposed commercial process.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

During the procedure a major objection was raised regarding the presence of potential mutagenic impurities. Considering that the proposed indication for advanced cancer also included 'continuation/ maintenance of treatment post-transplant', impurities with known mutagenicity (class 1) and impurities

with structural alerts different from the active substance (class 3) should be evaluated as described in ICH M7, unless justified. Following assessment of the applicant's' responses it was agreed that ICH M7 is not applicable in this case. Furthermore, it was accepted that the definition of advanced cancer per the ICH S9 guideline, was applicable. The major objection was considered to be resolved. The active substance is packaged in is a low density polyethylene (LDPE) sleeve packaged inside a LDPE bag, which comply with Ph. Eur. chapter 3.1.3 and Regulation EC 10/2011 as amended.

Specification

The active substance specification includes tests for: appearance, identity (IR, HPLC), assay (HPLC), impurities (HPLC), residual solvents (GC), water content (KF), counter-ion assay (Ph. Eur.), and residue on ignition (Ph. Eur.).

The tests included in the specifications are in line with the requirements of ICH Q6A. Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. The results from forced degradation, confirming the stability-indicating power of the HPLC impurity test procedure, were provided. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data (including commercial scale batches) of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from three commercial scale batches of active substance from the proposed manufacturer stored in the intended commercial package for up to 48 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The primary batches are manufactured according to process B2 while the proposed commercial process is the more recent process B3 however, considering the minor differences between the two versions of the process this was not considered to have a significant effect on the stability profile of the active substance.

The following parameters were tested: appearance, organic impurities assay, moisture (every time point), microbial limits and X-ray powder diffraction (annually). The analytical methods used were the same as for release and were stability indicating.

All tested parameters were within the specifications. Despite a somewhat variable impurity profile at time zero, the study results show that the active substance quizartinib dihydrochloride is highly stable and no clear trends are observed, notably towards degradation.

Photostability testing following the ICH guideline Q1B was performed on one batch. Results on stress conditions (Acidic, Basic, Oxidative, Thermal) were also provided on one batch. The results show no significant changes after storage. The physical and chemical properties, appearance, IR, XRPD pattern, assay, related substance and water remain the same compared to unstressed control.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 48 months with no special storage conditions in the proposed container.

2.2.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The finished product is presented as immediate release film-coated tablets containing 17.7 mg or 26.5 mg of quizartinib, which is present as quizartinib dihydrochloride. The 17.7 mg tablets are white, round shaped film-coated tablets, 8.9 mm in diameter and debossed with "DSC 511" on one side and the 26.5 mg tablets are yellow, round shaped film-coated tablets, 10.2 mm in diameter and debossed with "DSC 512" on one side.

The product is packaged in aluminium/aluminium perforated unit dose blisters or PCTFE/PVC//aluminium perforated unit dose blisters.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in paragraph 2.2.1 of this report

The formulation development was supported by clinical development. At the highest dosage strength of 26.5 mg the active substance is not completely soluble in 250 mL of aqueous media over the range from pH 2.2 to 8.0. Quizartinib demonstrates low permeability and has thus been classified as a BCS class 4 compound (low solubility/low permeability).

The major excipient is the hydroxypropyl-β-cyclodextrin (HPβCD) which increases the quizartinib solubility and prevents gel formation. The other inactive ingredients are microcrystalline cellulose, magnesium stearate and colour coating mixture. It is considered that there is no significant difference between commercial formulation and clinical trial formulations.

Vanflyta 17.7 mg and 26.5 mg tablets are manufactured from the same blend by adjusting the compression weight. The manufacturing process is considered as a non-standard process. The tablet formulation is based on an amorphous solid dispersion of quizartinib dihydrochloride and hydroxypropyl-β-cyclodextrin (HPβCD), mixed with microcrystalline cellulose and magnesium stearate to form the common final blend. The final blend is compressed to manufacture the core tablets which are then coated.

The active substance in crystalline form is dissolved with HPβCD in water), and subsequently spray dried to form a spray dried dispersion (SDD). With regards to the active substance polymorphic form in the SDD and tablets, verification of the crystallinity of quizartinib in the SDD has been monitored as part of release and stability testing. The variation in the particle size of the active substance is not a critical material attribute for the final dosage form. Pharmaceutical development of the finished product contains QbD elements. The starting point for the process development work was based on an initial risk assessment identifying the potential interactions between unit operations and critical quality attributes (CQAs). It was estimated what factors could be judged less important and what factors required further studies. All parameters relevant to QTPP were identified from an Ishikawa diagram, and analysed using a detailed risk assessment failure mode and effects analysis (FMEA) approach to establish those process parameters (CPPs) associated with critical material attributes (CMAs) affecting the critical quality attributes (CQAs).

The acceptance criteria for the critical material attributes, were determined by examination of the manufacturing capability of the Phase 3 clinical and registration stability batches and performance of structured experimental studies. The process understanding have been generated through prior knowledge and use of multivariate experimental plans (DoEs). To establish critical process parameters (CPPs), many potential–CPPs have been selected and studied with well described design of experiments (DoEs) (full factorial designs at two levels with center points) and with well-defined ranges.

The development of the dissolution method was conducted in two steps, the first step was the selection of the testing conditions and the second step was the evaluation of the discriminating power. To evaluate the discriminating power of the proposed dissolution method, studies assessing purposeful changes in the ratio of active substance to HPβCD, crystallinity (amorphous or crystalline) in the finished product and differences in tablet hardness and film coat level were conducted using quizartinib tablets 26.5 mg. The results demonstrated the discriminating power of the method.

During the procedure a major objection was raised with regards to the originally proposed dissolution release limit , its discrepancy with the shelf-life limit at and need to address the observed decrease in dissolution rate upon storage in particular how it is ensured / demonstrated that quizartinib tablet batches upon storage are bioequivalent with the batches used in clinical trials. The applicant confirmed that the dissolution specification was based on the data of release testing of the clinical batches and the registration batches. Based on these data and in line with EMA Reflection Paper, the dissolution specification was tightened to reflect the shelf-life limit for both release and shelf-life. All stability results met this specification. With regards to the differences observed on storage, the applicant used *in-silico* simulation studies (based on GastroPlus software) in order to make assumable that the decrease dissolution results for both strength tablets on storage are still results that can be considered as bio-relevant. Humidity transmission through the PCTFE/PVC//Al blisters was identified as a potential cause for the slowing down of dissolution. This slowing down of dissolution was not seen with Al/Al blisters. Since the Al/Al is a more protective blister, the applicant's proposal that the 36 months shelf life assigned to the PCTFE/PVC//Al blister is also applicable to the Al/Al blisters was accepted. It was recommended to finally replace the PCTFE/PVC//Al blister by the Al/Al blister taking into account the differences in dissolution profiles resulting from the two packaging types.

The choice of materials for the container and closure for spray dried dispersion intermediate, bulk tablets and finished product have been adequately justified with stability study. The primary packaging of the finished product is aluminium/aluminium perforated unit dose blisters or PCTFE/PVC//aluminium perforated unit dose blisters. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Manufacture of the product and process controls

The manufacturing process consists of four main steps: manufacture of the amorphous solid dispersion intermediate (SDD), blending, tabletting and film-coating. The solid dispersion intermediate is prepared from quizartinib dihydrochloride and hydroxypropylbtadex, and further blended with excipients and compressed into tablets that are finally film-coated.

A design space of the CPPs in the Spray Drying Process was proposed and considered to be acceptable. The design space is the mathematically determined range within which the water content of SDD satisfies the acceptance criterion. The in-process controls and specification of the quizartinib solid dispersion intermediate are adequately described.

A design space of the CPPs in the tabletting process was proposed and considered to be acceptable. Proven acceptable ranges have been defined for the CPPs in the film coating process.

The available development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed PARs and Design Spaces. A process validation protocol summarizing the full studies intended to be conducted at commercial scale, has been provided in section 3.2.R.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form; appearance, identification (HPLC, UV), uniformity of dosage units (Ph. Eur.), assay (HPLC), related substances (HPLC), dissolution (Ph. Eur.), water content (KF) and microbiological quality (Ph. Eur.).

The finished product is released based on the release specifications, through traditional final product release testing. The specifications are in line with ICH Q6A and Ph. Eur. general monograph for film-coated tablets. The limits proposed for the specified impurity in the finished product at release and end of shelf-life are considered justified based on the increase of said impurity upon storage. Furthermore, the impurity is considered qualified through toxicology studies and hence there is no safety concern. The limit for unspecified impurities is in line with ICH Q3B identification threshold (MDD 60 mg) and therefore acceptable. The proposed limits for total impurities in the tablets at release correspond to the maximum allowed content in spray-dried dispersion intermediate after two years of storage.

A risk assessment for potential elemental impurities in quizartinib 2HCl tablets has been performed according to ICH Q3D.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis results are provided for multiple batches of both 17.7 mg tablets and26.5 mg tablets batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data from three batches of finished product stored for up to 36 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The batches of finished product are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, dissolution, assay, impurities and water content. The analytical procedures used are stability indicating. The stability results show relatively little degradation and only one impurity increases over time; this increase is more pronounced under accelerated conditions but its content remains within the specification limit. A similar trend is noticed for content in total impurities. As discussed above, a downward trend in dissolution results was observed on storage for product packaged in PCTFE/PVC//Al blisters. Nevertheless the product remained within specification at long term conditions. This slowing down of dissolution was not seen in product packed in Al/Al blisters.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products, and one batch was subjected to thermal cycling (freeze and thaw) study.

The applicant proposed to calculate the shelf-life starting with compression of SDD with the other excipients. To support this proposal, the applicant has put on stability one batch of each strength of tablets manufactured from "aged" SDD. The data provided show a stability profile similar to the other stability batches. Based on this extended stability data, this approach to calculating the start of shelf-life can be accepted. The Applicant further committed to continue for at least 3 years the stability program on the tablets made with aged SDD.

Based on available stability data, the proposed finished product shelf-life of 36 months with no special storage condition are acceptable.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The applicant has applied QbD principles in the development of the active substance and finished product and their manufacturing process. Design spaces have been proposed for two steps in the manufacture of the finished product. The design spaces have been adequately verified.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used as proposed. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.2.6. Recommendation(s) for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

The nonclinical safety profile of quizartinib has been characterized in *in vitro* and *in vivo* pharmacological, pharmacokinetic and toxicological studies in mice, rats, rabbits, guinea pigs, dogs, and monkeys. Safety pharmacology and pivotal toxicology studies were conducted in compliance with Good Laboratory Practice (GLP) and the test facilities are globally conform to GLP requirements.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro pharmacology of quizartinib

Biochemical potency and selectivity of quizartinib (Study NR0119)

Quizartinib binds with the highest affinity to FLT3 (Kd = 1.3 nM) and with less affinity to receptor tyrosine kinase c-KIT (KIT) (Kd = 4.9 nM). It also binds to a few other class III RTKs including CSF1R/FMS (Kd = 9.6 nM), PDGFRα (Kd = 14 nM), and PDGFRβ (Kd = 8.4 nM), and non-class III RTK, RET (Kd = 7.1 nM). Of the 441 kinases tested, FLT1 (Kd = 44 nM), FLT4 (Kd = 49 nM), and DDR1 (Kd = 81 nM) were also found to bind quizartinib, and 10 non-class III RTKs bound quizartinib with moderate affinity (Kd between 100 and 1000 nM).

Biochemical potency and selectivity of metabolite AC886 (Study NR0118)

AC886 binds with the highest affinity to FLT3 (Kd = 0.54 nM) and with less affinity to KIT (Kd = 0.97 nM). It also binds to a few other class III RTKs including CSF1R/FMS (Kd = 8.6 nM), PDGFRα (Kd = 3.6 nM), and PDGFRβ (Kd = 1.8 nM), and non-class III RTK, RET (Kd = 14 nM). Of the 391 kinases tested, FLT1 (Kd

= 94 nM) and FLT4 (Kd = 69 nM) were also found to bind AC886, and 5 non-class III RTKs bound AC886 with moderate affinity (Kd values between 100 and 1000 nM).

Intracellular inhibition of flt3 activity by quizartinib (Study NR0006)

The data below shows an inhibition of FLT3 phosphorylation at concentrations of quizartinib from 0.8 to 20 nM in the MV4-11 cells (Figure 2).

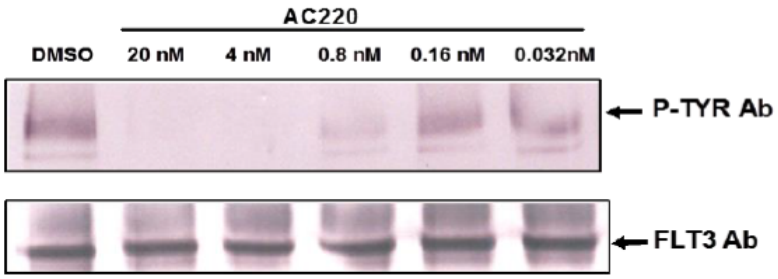


Figure 2: Inhibition of FLT3-ITD Autophosphorylation in MV4-11 Cells

Ab: Antibody; AC220: Quizartinib; DMSO: Dimethyl sulfoxide; ITD: Internal tandem duplication; P-TYR Ab: Phospho-tyrosine specific antibody. The upper panel shows phospho-FLT3, determined with a phospho-tyrosine specific antibody. The lower panel shows total FLT3, determined with a FLT3 specific antibody.

Inhibition of leukemia cell proliferation by quizartinib (Study NR0007)

The ability of quizartinib to inhibit the proliferation of the MV4-11 leukemia cell line was determined. Cell proliferation was measured using a standard, nonradioactive, MTS tetrazolium compound assay. Quizartinib was found to be a highly potent inhibitor of FLT3-dependent cell proliferation in the MV4-11 cell line (half maximal inhibitory concentration (IC50) = 0.3 nM) and to have more than 1000-fold weaker activity against the FLT3 independent cell proliferation of the RS4;11 control cell line (IC50 = 990 nM).

In Vivo Pharmacology of quizartinib

Pharmacodynamic analysis of quizartinib (Study NR0112)

The temporal effect of quizartinib treatment on the expression of p-FLT3 and total FLT3 protein was examined in MV4-11 tumors in mice. The % p-FLT3 for vehicle-treated animals remained stable over time and ranged from 70% to 126%. A time-dependent reduction of p-FLT3 was observed following a single dose of 10 mg/kg quizartinib dihydrochloride (Figure 3). The maximal effect was observed at 1, 2, and 6 h post-dose with % p-FLT3 value of 6%, 4%, and 7%, respectively. Levels of % p-FLT3 rebounded at 48 and 96 h post-dose but leveled off at approximately 60%.

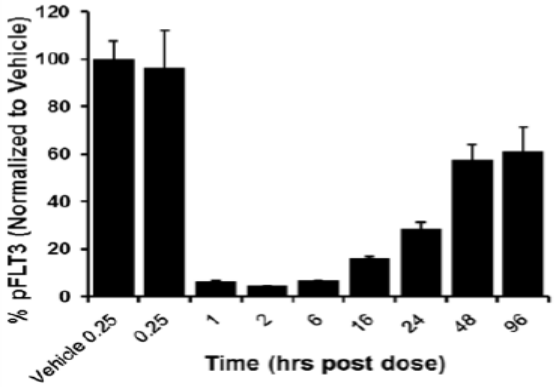


Figure 3 Effect of 10 mg/kg Quizartinib Dihydrochloride on % p-FLT3 Levels in MV4-11 Tumors

p-FLT3: Phosphorylated FLT3; p/tFLT3 ratio: Ratio of p-FLT3 to total FLT3. % p-FLT3 (Normalized to Vehicle) = (p/tFLT3 ratio) / (p/tFLT3 ratio of Vehicle0.25 h) × 100. N = 4 animals per group. Data are reported as mean and standard error.

Efficacy of quizartinib in tumour xenograft model (Study NR0009)

The effect of quizartinib on tumour growth was determined in a mouse tumor xenograft model using MV4-11 cells, which were grown as subcutaneous solid tumors in female athymic nude mice. Quizartinib showed strong dose-dependent antitumor activity in this model (Figure 4). Significant activity was observed at 1 mg/kg QD, with a median tumor volume (MTV) of 411 mm^3 (based on 4 surviving mice) and one partial regression (PR) of the tumor on Day 60. The activity was nearly maximal at 3 mg/kg QD, with an MTV of 63 mm^3 (based on 10 mice) and 7 PRs. Maximal activity was produced at 10 mg/kg QD (MTV of 52 mm^3 [based on 10 mice] and 9 PRs). Quizartinib dihydrochloride dosed at 10 mg/kg QD resulted in no tumor regrowth after dosing discontinuation and during the entire 32-day follow-up observation period. In addition, no group mean body-weight loss or other toxicity was observed for any of the treatment groups.

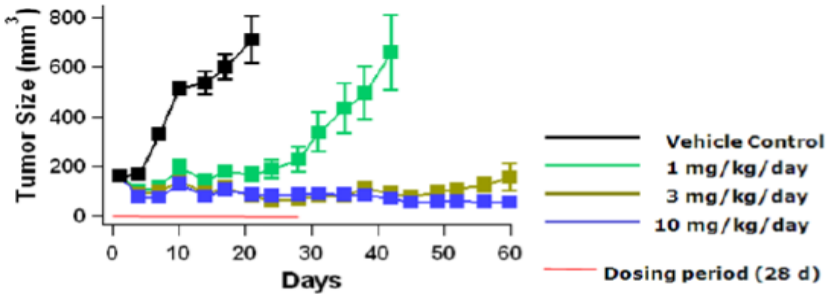


Figure 4: Efficacy of quizartinib dihydrochloride in the MV4-11 tumor xenograft model

d: days. N = 10 animals per group. Data are reported as mean and standard error. 22% HPβCD was used as a vehicle control.

Efficacy of quizartinib in disseminated engraftment model (Study NR0116)

The activity of quizartinib was determined using the non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice in which MV4-11 cells were intravenously inoculated and disseminated to bone marrow. Single-cycle 5-azacytidine (0.125, 0.25, or 0.375 mg/kg BID) showed no survival benefit. Single-cycle cladribine at doses of 10 and 20 mg/kg BID provided a marginal improvement in median survival time. Quizartinib treatment provided a dose-dependent survival advantage in the mice (Figure 5). Quizartinib dihydrochloride dosed for 31 days at 0.1, 1, and 10 mg/kg QD provided an increased life span (ILS) of 12%, 55%, and >250%, respectively, compared to vehicle. At 1 mg/kg QD, quizartinib dihydrochloride dosed continuously for 150 days gave an ILS of 153% and provided a markedly higher survival advantage compared to 31-day dosing. The study was terminated prior to reaching 50% mortality in the quizartinib dihydrochloride 10 mg/kg QD continuous dosing group, yielding an ILS of >250% for this dosing regimen.

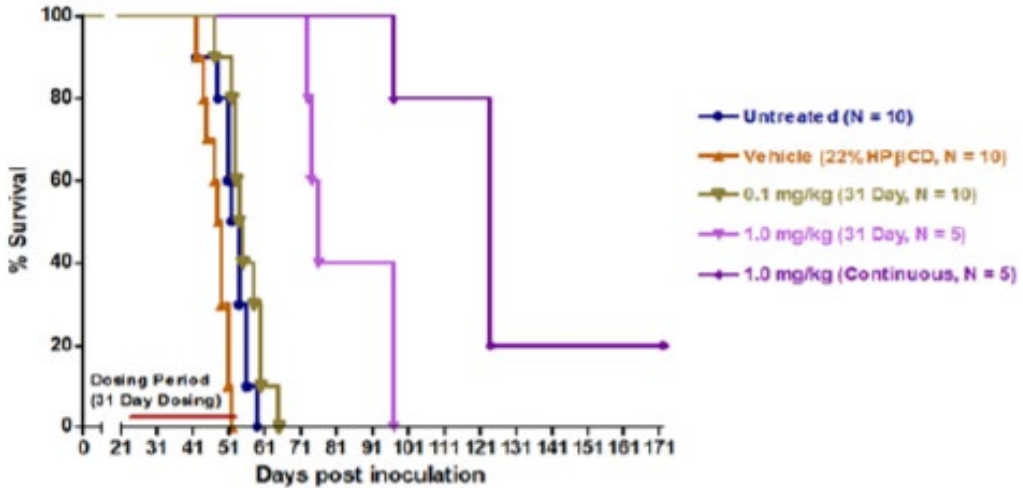


Figure 5 Effect of 31-Day or Continuous Dosing With Quizartinib Dihydrochloride on Survival in a Disseminated MV4-11 Leukemia Model. Continuous: Treatment groups dosed for 150 days.

Secondary pharmacodynamic studies

The potential off-target activity of quizartinib against "non-kinases" was determined in biochemical assays (Study NR0011). Quizartinib at 10 µM was incubated with a diverse panel of 118 non-kinase enzymes, receptors, channels, and transporters in a primary screen. Six assays showed >50% inhibition in the primary screen, and the IC50 for quizartinib was subsequently determined for those six primary screen hits. Table 6 shows the percent inhibition observed in the primary screen and the IC50 determined from five concentrations (1 to 100 µM) of quizartinib in the follow-up assay.

Table 1 Quizartinib inhibitory activity in non-kinase assays

Assay	% Inhibition at 10 µM	IC_{50} (µM)
Lipoxygenase 15-LO	68	5.12
Peptidase, Renin	67	10.3
Prostanoid EP_4	70	5.55
Sigma σ_1	57	7.16
Sigma σ_2	62	5.04
Sodium Channel, Site 2	81	2.71

IC_{50}: 50% inhibitory concentration.

Safety pharmacology programme

In vivo and in vitro safety pharmacology study with quizartinib are presented in Table 7 and Table 8.

Table 2 *In vitro* safety pharmacology studies with quizartinib

Type of Channel Model	Test items concentration	Findings AC220	Findings AC886
human ether-a-go-go-related gene (hERG) Potassium	Quizartinib and AC886: 0.1, 0.3, 1, and 3 µM	Inhibition 1.8 ± 1.6% at 0.1 µM 3.8 ± 0.5% at 0.3 µM 4.3 ± 0.9% at 1.0 µM 16.4 ± 2.1% at 3.0 µM	Inhibition -0.4 ± 0.8% at 0.1 µM , 3.3 ± 0.1% at 0.3 µM 3.9 ± 1.6% at 1.0 µM 12.0 ± 2.0% at 3.0 µM

	Quizartinib and AC886: 1, 3, 10, and 30 µM	no statistically significant inhibition	plateau max inhibition of 36.5%
Sodium (INa), potassium (IKs), and calcium (ICa-L) currents dispersed canine cardiomyocytes and IKs–expressing CHO cells ref 20100519-4, GLP	Quizartinib and AC886: 1, 3, 10, and 30 µM	ICa-L and INa current density: no statistically significant inhibition IKs current plateau inhibition of 37% (1 µM)	 ICa-L 23.6 and 17.3% at 10 and 30 µM IKs plateau inhibition of 29% (1-30µM) INa:inhibition dose dependent (IC_{50}: 24.4µM)
Potassium (IKs) Currents Cloned hKvLQT1/minK Potassium Channels Expressed in Human Embryonic Kidney Cells ref 170506.EBH, GLP	Quizartinib and AC886: 0.1, 0.3, 1, and 3 µM	Inhibition IKs by: 28.9 ± 7.5% at 0.1 µM 55.2 ± 4.2% at 0.3 µM 56.5 ± 2.5% at 1.0 µM 67.5 ± 3.3% at 2.9 µM	Inhibition IKs by: 11.0 ± 3.3% at 0.1 µM 20.6 ± 4.0% at 0.3 µM 21.2 ± 2.7% at 1.0 µM 26.9 ± 2.7% at 2.9 µM
Sodium (INa) currents Cloned Sodium Channels Expressed in Human Embryonic Kidney Cells ref 170507-EBH, GLP	Quizartinib and AC886: 0.1, 0.3, 1, and 3 µM	Inhibition INa by: -3.1 ± 1.2% at 0.1 µM, 0.8 ± 0.2% at 0.3 µM, 1.0 ± 1.0% at 1 µM 0.9 ± 0.4% at 3 µM	Inhibition INa by: 1.2 ± 0.7% at 0.1 µM, 2.6 ± 0.3% at 0.3 µM, -0.2 ± 1.1% at 1 µM 2.8 ± 1.5% at 3 µM
Sodium-late (INa,L) currents Cloned Channels Expressed in Human Embryonic Kidney Cells ref 170508-EBH, GLP	Quizartinib and AC886: 0.1,0.3, 1, and 3 µM	statistically significant inhibition at 3 µM	no effect
Calcium (ICa-L) currents Cloned L-type Calcium Channels Expressed in Chinese Hamster Ovary Cells ref 170505-EBH, GLP	Quizartinib and AC886: 0.1, 0.3,1 and 3 µM	no effect	no effect
Cardiac action potential rabbit ventricular tissues. ref 20100519-3, GLP	Quizartinib and AC886: 1, 3, 10, and 30 µM	no significant effect	significant prolongation of the APD60 and 90
Cardiac electrophysiological and mechanical parameters of isolated rabbit hearts. ref 20100519-1, GLP	Quizartinib and AC886: 1, 3, 10, and 30 µM	prolongation of the RR, PR and QT intervals, MAP ↓ contraction rate of the heart QT and MAP prolongations stopped not reversed after wash out RR and PR intervals still increased after washout period.	prolongation of the RR, PR and QT intervals, MAP ↓ contraction rate of the heart QT and MAP prolongations stopped not reversed after wash out RR and PR intervals still increased after washout period.

Table 3 *In vivo* safety pharmacology study with quizartinib

Type of Study Study reference	Purpose	Test system	Main findings
Cardiovascular system			

A Cardiovascular Telemetry Study in the Unrestrained Conscious Non-Naïve Cynomolgus Monkey ref 692985, GLP	to evaluate the potential acute pharmacological effects of AC220 on the hemodynamic and electrocardiographic parameters	Cynomolgus Monkey telemetered Oral gavage, single dose Phase 1: 0(vehicle), 30, 100, 200 mg/kg Phase 2 ‡: 0(vehicle), 30, 10, 3 mg/kg	Quizartinib: marked, dose related increase in QTc interval over a 24-h monitoring period at ≥10 mg/kg. Quizartinib at 100 or 200 mg/kg: significant dose-related effects on systemic blood pressure characterized by transient elevations in blood pressure over a 24-h monitoring period. Quizartinib at 30 mg/kg produced significant dose-related effects on systolic blood pressure only. A single oral dose of AC220, at dose levels of 10, 30, 100 or 200 mg/kg elicited a marked, dose-related response in heart rate corrected QT interval characterized by sustained prolongation of QTc over a 24 hour monitoring period. Single dose administration of AC220 at dose levels of 100 or 200 mg/kg also produced significant dose-related effects on systemic blood pressure characterized by transient elevations in blood pressure over a 24 hour monitoring period. Administration of AC220 at 30 mg/kg produced significant dose-related effects on systolic blood pressure only.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies have been conducted with quizartinib (see discussion on non-clinical aspects).

2.3.3. Pharmacokinetics

The ADME profile of quizartinib has been evaluated in *in vitro* assays and *in vivo* model systems, in rat, mouse, dog and monkey.

Concentrations of quizartinib and its major and pharmacologically active metabolite AC886, in animal plasmas were determined by LC-MS/MS and analytical methods were validated or partially validated in all the species used in toxicology studies (rat: 5-1000ng/ml, dog: 5-1000 ng/ml and monkey: 1-1000 ng/ml). A full validation of measurement of quizartinib in rat plasma included selectivity, linearity, lower limit of quantitation (LLOQ), carry-over, intra-and inter-assay precision and accuracy, stock solution stability, short-term matrix stability, freeze-thaw and long-term matrix stability and dilution integrity. An LC-MS/MS method for the determination of AC220 and AC886 in adult and pup rat plasmas was also validated against the fully validated method (study 101626).

Regarding dog, a partial validation of LC-MS/MS was carried out for the determination of quizartinib in dog plasma, against the fully validated assay in rat plasma (study 101626). the same way, a partial validation was carried out for monkey plasma regarding quizartinib and AC886, this method has a calibration range from 1.00 to 1000 ng/mL, and this method is reproducible and rugged with no significant matrix interference in Cynomolgus monkey plasma tested.

The permeability of quizartinib was evaluated in Caco2 cells and was moderate. The determination of quizartinib as a substrate for P-gp as been determined on MDCKII-MDR1 and wild type cells and showed that quizartinib is a P-gp substrate.

The PK profile of quizartinib following intravenous (IV) or oral (PO) administration has been assessed in nu/nu mice, rats, dogs and monkeys. With regards to single dose, in mice, quizartinib results in dose proportional exposure increase from 0.1 to 300 mg/kg over a 24-hour period (AUC0-24). AUC increased approximately with dose. Maximum plasma concentration after a single dose (Cmax) was only proportional up to 30 mg/kg.
In male rats, pharmacokinetic parameters of AC220 were proportional to dose as measured by AUC upon oral dosing to 100 mg/kg. Dose proportionality was observed upon IV dosing. In male rats, average

clearance was low and volume of distribution was high, resulting in a long terminal half-life of 5.0 ± 0.4 hr. In females, clearance was even lower (4.71 mL/min/kg), but so was the volume of distribution (2.51 L/kg), resulting in greater exposure (1.5-fold) but a similar terminal half-life (6.1 h). The average plasma exposure of quizartinib was greater in female than in male rats. Oral dosing indicates that AC220 has good bioavailability (average male and female around 40%) in rats. Furthermore, in fed rats (male) the bioavailability increased to approximately 100%. Regarding dogs, PK of quizartinib seems to be relatively dose proportional over the dose range tested (in males). The results from IV dosing to male dogs showed that clearance was low at 6.6 mL/min/kg and volume of distribution was high (3.4 L/kg), resulting in a long terminal half-life of 5.9 h. Oral bioavailability was good at approximately 30% to 40%. Exposure is higher in females compared to males and clearance was lower in female dogs.

AC220 showed low exposure in monkeys, and the PK profiles in males and females were similar. The clearance is higher (about 30 mL/min/kg, on the order of hepatic plasma flow) and the bioavailability is low (8-14%).

For single dose absorption studies, a comparison between species and the different routes of administration (IV and VO) was performed in rat, dog, mice and monkey. Pharmacokinetic studies revealed an approximately dose-proportional increase in exposure in mice, rats and dogs. Quizartinib half-life ranged between 3 and 8h between IV and VO routes after single dose. Usually exposure in females was superior to males. PK in monkey showed low exposure.

Regarding repeat-dose, exposure of rats to AC220 was greater than proportional to the increase in dose level and increased with repeated administration. For AC886, exposure was proportional to the increase in the dose level of AC220 and exposure increased slightly with repeated administration of the parent compound. The exposure to the parent compound was generally higher than the exposure to the metabolite and the exposure of female rats to AC220 and AC886 higher than the exposure of male rats on both Days 1 and 28. Cmax was variable and was observed between 1 to 6 hours and 2 and 6 hours post dose and its main metabolite AC886 occurred between 1 and 6 h and post dose, respectively, indicating a rapid conversion of AC220. The t1/2 values ranged from 7.84 to 8.30 hours and were similar for both AC220 and AC886 and were unaffected by dose level or gender, but did tend to increase with repeated dosing. A moderate level of accumulation of both analytes with repeat dosing (accumulation ratio based on AUC 1.8-2.3) was observed.

As regards to biodistribution, quizartinib and AC886 appeared to be highly protein bound, in plasma with ≥99% in mouse, rat, dog, monkey and human plasma. Quizartinib binds HSA similarly to total plasma proteins. Both quizartinib and AC886 appear to bind or partition into red blood cells because plasma concentrations observed are approximately equal or lower than the blood spike concentration. Quizartinib is predominantly present in the plasma compartment (73%) and exhibits moderate binding to red blood cells (27%). 14[C]-quizartinib was widely distributed to tissues in a QWBA study in rats after oral gavage. Maximum concentration was reached within 2 and 4 hours in the majority of tissues. The highest concentration of drug-derived radioactivity at the time of maximal concentration was observed in multiple organs and organs associated with excretion and metabolism (liver and kidney) and the alimentary canal. The highest concentrations of radioactivity in tissues at tmax were found in stomach, small intestine, adrenal gland, liver, kidney medulla, pituitary gland, kidney cortex, pancreas, spleen, thyroid gland, brown adipose, mandibular salivary gland, stomach wall, myocardium, ovary, large intestine, lung, thymus, white adipose, bone marrow, lymph nodes, uterus wall, and prostate gland. A reversible association of [14C]-quizartinib-derived radioactivity with melanin is possible, phototoxicity testing of quizartinib was performed using standard 3T3 test and was negative.

Quizartinib was not detected in CSF in SD rats and poorly penetrates the brain following intravenous and oral administration (brain-to-plasma ratio approximately 10% to 25% in both cases).

No studies have been carried out regarding pregnant or nursing animals, placental transfer and excretion in milk.

In blood partitioning tests, quizartinib and AC886 were spiked to rat, dog, and monkey blood and each blood was processed on ice or at 37°C for 2 h. No time or temperature dependent partitioning of quizartinib and AC886 between the plasma and blood cell fractions were noted in rat and dog blood. On the other hand, AC886 concentrations in monkey blood were approximately 4-fold higher on ice than those at 37°C, whereas quizartinib concentrations was not affected by either on ice or at 37°C. The blood to plasma (B/P) ratios of quizartinib were not given but were calculated to be approximately 0.7 for rat and dog, i.e. 20% in RBC, and 1.0 for monkey and independent of concentration added (20 – 2000 ng/ml). The B/P ratio for AC886 was approximately 0.8 and 1.2 for rat and dog, respectively. Using ^{14}C-data from the ^{14}C-quizartinib rat distribution study 021390-1, a B/P value of 0.57 was found at Tmax.

The metabolic profile of quizartinib was evaluated in both *in vitro* and *in vivo* systems. Quizartinib showed low clearance and turn over on microsomes, hepatocytes and S9 for mouse (microsomes only) rat, dog, monkey species and humans. Reaction phenotyping revealed that both quizartinib and AC886 were substrates of CYP3A4 and CYP3A5, with little or no contribution of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6. Conversion of quizartinib to AC886 is mediated by CYP3A4 and CYP3A5 (Study PBC315-608).

Investigation of metabolism pathway for quizartinib was carried out in rat, mouse, dog and monkey. In dogs, AC886 was the major circulating metabolite at later time points, but another major metabolite at a fraction greater than 15%, identified as a morpholino oxidation product, was also found. The major route of elimination for quizartinib is faeces, less than 2% of total AC220 related radioactivity was recovered in the urine, while >87% of radioactivity was recovered in faeces. Major metabolic pathways of quizartinib identified AC886 as main metabolite in all species but 20 other metabolites were also identified in rat and dog. Two metabolic pathways are proposed: oxidation of the tert-butyl isoxazole and oxidation followed by subsequent ring-opening of the morpholino group. AC886, the major metabolite of quizartinib detected in human plasma has been shown to be present in urine in phase clinical trial and is pharmacologically active with similar potency and selectivity to AC220.

The [14C]-quizartinib non clinical absorption, metabolism, distribution, and excretion (ADME) studies showed that feces were the major route of elimination of quizartinib. ADME studies in rat and dog showed than less 2% of total quizartinib (radioactivity) was recovered in urine and more than 87% of radioactivity was recovered in faeces. In both species, quizartinib was mainly recovered and AC886 in a minor part (around 10%). In rats (study NR0052) showed that quizartinib and AC886 were eliminated directly into bile, supporting biliary excretion as a route of elimination.

2.3.4. Toxicology

Single dose toxicity

Single-dose toxicity in SD rats, Beagle dogs and Cynomolgus monkeys was assessed in an initial maximum tolerated dose (MTD) phases of dose range-finding studies in each species. The oral route was selected for all studies since this is the intended route of administration in Humans. A summary of single-dose toxicity studies with quizartinib is displayed in Table 4.

Table 4 Summary of single-dose toxicity studies with quizartinib

Study reference/GLP compliance	Species (number, sex)	Route/ Dose (mg/kg/d)/ Duration	NOAEL (mg/kg/week)	Major findings
AC010220: An escalating dose tolerance and a 7-day repeated dose oral toxicity study in Sprague-Dawley rats GLP ref 70039	SD rat 3-5 M 3-5 F	100, 150, 200, 250, 300 mg/kg oral (gavage), 22% HPβCD/Suspension 1 day and 7 consecutive days	Observed Maximum Non-Lethal dose (mg/kg): 100 mg/kg MTD 100 mg/kg	No abnormal findings at 100 mg/kg, except dark feces in 1 female. Deaths in females at ≥150 mg/kg (one each at 150 and 200 mg/kg and two each at 250 and 300 mg/kg). Oral administration of AC010220 at 125 mg/kg/day for 2 consecutive days and 100 mg/kg/day for 2 consecutive days produced mortality or preterminal sacrifice of all animals between Day 3 and Day 6. Female rats more susceptible to the toxicity of quizartinib than male rats. Kidney may be a target organ of AC010220 toxicity
AC010220: An oral maximum tolerated dose (MTD) and 7-day repeat dose toxicity study in Beagle dogs GLP ref 60023	Beagle dog 1M 1F	10, 20, 40, 80, 100, or 200 (Phase 1; escalating dose) 150 (Phase 2; single dose) 200, 250 (Phase 3) Oral (gavage), 10 or 22% HPβCD/Suspension	MTD 200 mg/kg	<u>Phase 1:</u> No mortality or abnormal clinical signs up to 200 mg/kg ≥40: Slight body weight loss in the female <u>Phase 2:</u> 150: Female dog loss body weight between Day −1 and Day 14. No macroscopic findings <u>Phase 3:</u> Loss of body weight associated with ↓ in food consumption and generalized icterus and firm liver lobes observed at necropsy.
AC010220: A single dose and 5-day repeat dose oral toxicity study in Cynomolgus monkeys No GLP ref 2008-1783	Cynomolgus monkey 1M 1F	30, 100, 200, 300, 400 mg/kg Oral (gavage), 22% HPβCD/Suspension	MTD 200 mg/kg	30: No abnormal clinical signs ≥100: Presence of white or brown froth or mucoid red material in the tray, decreased appetite and body weight loss (11 to 12%) soft, loose or liquid feces. ≥200 for 5 days: ↑ in liver enzymes, total bilirubin, creatinine, and blood urea and ↓ in reticulocytes, monocytes and lymphocytes.

Repeat dose toxicity

An overview of the pivotal repeat-dose toxicity studies with quizartinib is displayed in Table 9.

Table 5 Pivotal repeat-dose toxicity studies with quizartinib dihydrochloride

Study ID	Species/Sex/ Number/ Group	Dose/Route	Duration	NOAEL (mg /kg/day)	Major findings
70040	Rat Sprague-Dawley 10/sex/group	0, 5, 15, 60/30[a] mg/kg/day (22% HPβCD) Oral gavage	28 days	N.D.	**≥5:** ↓ bodyweight (F), ↓ Eos, ↓ RBC, ↑ MCV/MCH, ↓ ovary weight (F), ↓ testes weight, bone marrow (hypocellularity) **≥15:** ↓ Neut/Mono, ↓ Hb/Ht, ↑ AST/ALT/ALP, thymus (atrophy), kidney (tubular cell vacuolation; granular casts; acute tubular necrosis; tubular basophilia) **=60/30:** blood in urine, dark/loose/liquid feces, decreased activity, ↓ bodyweight (M), ↓ food intake, spleen (lymphoid atrophy), lymph nodes (lymphoid atrophy), adrenals (cortical hypertrophy; single cell necrosis; cystic degeneration), liver (single cell necrosis), heart (focal inflammation), testes (germ cell necrosis), epididymides (aspermia), GI tract (single cell necrosis; mucosal atrophy/erosion/ulcer) **Mortality:** All animals in 60/30 mg/kg/day were found dead or prematurely sacrificed up to d15
802411	Rat Sprague-Dawley 10/sex/group	0, 1, 3, 10 mg/kg/day (5% HPβCD) Oral gavage	13 weeks	3 mg/kg/day	**≥1:** ↓ bodyweight, ↓ WBC, ↓ Neut/Lymph/Eos/Mono/Baso, ↓ Retic. (M), kidney (tubular dilatation)(M), spleen (pigment dispositions), thymus (lymphoid atrophy/necrosis)(F), vagina (abnormal epithelial mucification) **≥3:** ↓ RBC (M), ↑ MCV/MCH/MPV, ↑ RDW, ↓ Retic. (F), ↑ ALP, ↑ Urea, bone marrow (hypocellularity)(F), kidney (tubular cell vacuolation)(M), thymus (lymphoid atrophy/necrosis)(M), ovary (cysts), **=10:** ↓ RBC (F), ↓ Hb/Ht, ↑ ALT, ↑ Creat., bone marrow (hypocellularity)(M), kidney (tubular basophilia; tubular crystal formation), spleen (extramedullary hematopoiesis, testis (degeneration/atrophy seminiferous epithelium), epididymides (aspermia)
60024	Dog Beagle 3/sex/group	0, 10/5, 50/25, 150/40[b] mg/kg/day (22% HPβCD) Oral gavage	28 days	10/5 mg/kg/day	**≥10/5:** thin body (F), ↓ Retic. **≥50/25:** ↓ bodyweight, ↓ RBC, ↓ Hb/Ht, ↓ WBC, ↓ Neut/Lymph/Mono, ↑ Bilir., ↑ ALT/AST/ALP, ↓ thymus weight, liver (crystal deposition; Kupffer cell activation; periportal hepatocellular vacuolation), thymus (atrophy), spleen (atrophy), bone marrow (hypocellularity), kidney (tubular basophilia; single cell necrosis), adrenals (cortical hypertrophy) **=150/40:** decreased activity **Mortality:** 1x 150mg/kg/day male euthanized on d8: ↓ bodyweight, decreased activity, liver (periportal hepatocellular vacuolation; kupfer cell activation), spleen (lymphoid atrophy), thymus (lymphoid atrophy), kidney (tubular vacuolation; tubular dilatation; single cell necrosis), adrenals (cortical hypertrophy)

802412	Dog Beagle 4/sex/group	0, 1, 5, 15 mg/kg/day (5% HPβCD) Oral gavage	13 weeks	5 mg/kg/day	**≥1:** ↓ thymus weight (F), kidney (tubular basophilia)(M), thymus (lymphoid atrophy/necrosis)(M) **≥5:** ↓ bodyweight, ↓ mean heart rate, ↓ Retic., liver (pigment deposits; reactive sinusoidal lining cells; bile duct hyperplasia), spleen (pigment deposits) **=15:** skin pallor, ↓ WBC, ↓ Neut/Lymp/Mono/Eos/Baso., ↓ RBC, ↓ Hb/Ht, ↑ AST/ALT/ALP, ↑ Bilir., ↑ liver weight, ↓ thymus weight (M), kidney (pigment deposits)(M), liver (single cell necrosis; fibrosis; inflammation; hepatocellular vacuolation; extramedullary hematopoiesis), bone marrow (hypocellularity)
1008-2493	Cynomolgus Monkey 3/sex/group	0, 10, 30, 100/60[c] mg/kg/day (22%/5% HPβCD[d]) Oral gavage	28 days	10 mg/kg/day	**≥10:** ↓ Mono/Baso (M), ↓ RBC, ↓ Hb/Ht, spleen (lymphoid atrophy), thymus (lymphoid atrophy), bone marrow (hypocellularity), lymph nodes (lymphoid atrophy), GALT (lymphoid atrophy) **≥30:** hunched posture (F), dehydration (F), ↓ WBC, ↓ Lymph/Baso, ↓ Retic., ↓ MCV (F), ↓ RDW (F), ↑ ALT, pancreas (acinar cell atrophy) **=100/60:** reduced activity, hunched posture (M), dehydration (M), ↑ AST, ↑ Creat/Urea, ↑ liver weight, adrenals (cortical hypertrophy), kidney (tubular dilatation/basophilia/single cell necrosis) **Mortality:** 1x 100/60mg/kg/day male found dead on d11, 1x 100/60mg/kg/day female found dead on d16, 1x 100/60mg/kg/day male euthanized on d33: ↓ bodyweight, decreased activity, , spleen (lymphoid atrophy), thymus (lymphoid atrophy), kidney (tubular vacuolation; tubular dilatation; single cell necrosis), adrenals (cortical hypertrophy)
803726	Cynomolgus Monkey 4/sex/group	0, 3, 10/6, 30/12[e] mg/kg/day (5% HPβCD) Oral gavage	13 weeks	3 mg/kg/day	**≥3:** ↑ Neut., ↓ Lymph/Eos, ↓ RBC, ↓ Hb/Ht, ↓ Retic., ↓ TProt., ↑ MCV, ↑ RDW, ↑ Plat., ↑ AST/ALT, ↑ Bilir., ↑ Urea/Creat., bone marrow (hypocellularity)(F), spleen (lymphoid atrophy)(F) **≥10/6:** decreased activity, emesis, ↓ bodyweight, bone marrow (hypocellularity)(M), thymus (atrophy), lymph nodes (lymphoid atrophy), testes (germ cell depletion), uterus/ovary/vagina (atrophy), esophagus/tongue (epithelial degeneration/atrophy), adrenals (cortical hyperplasia), liver (cytoplasmic rarefaction, single cell necrosis, hepatocellular vacuolation), GI tract (inflammation)(M) **=30/12:** hunched posture, dehydration, soft/liquid feces, spleen (lymphoid atrophy)(M), epididymides (aspermia) **Mortality:** 2x 10/6mg/kg/day and 3x 30/12 mg/kg/day animals found dead between d6 and d36: 2x anemia, 1x secondary to bone marrow hypocellularity, 2x unknown

[a] Dosed reduced to 30 mg/kg/day from day 5-7 (2 day dosing-free period)
[b] Dose reduced to 5, 25, 40 mg/kg/day from day 9 (2 day dosing-free period)
[c] Dose reduced to 60 mg/kg/day from day day 15 (5 day dosing-free period)
[d] Concentration vehicle was lowered to 5%
[e] Dose reduced to 6 and 12 mg/kg/day from day 42-48 (10 day dosing-free period)
N.D. = not determined

Genotoxicity

Table 6 Genotoxicity studies with quizartinib dihydrochloride

Type of test/study ID/GLP	Test system	Concentrations/ Concentration range/ Metabolising system	Results Positive/negative/equivocal
Gene mutations in bacteria 961163 GLP	*Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537), *Escherichia coli* WP2*uvrA*	48-72hrs +/- S9 0, 1.58, 5, 15.8, 50, 158, 500, 1581, 5000 µg/plate (22% HPβCD)	Positive: *Salmonella typhimurium* strain TA98 +/-S9 and TA100 +S9.
Gene mutations in mammalian cells 962099 GLP	L5178Y $TK^{+/-}$ mouse lymphoma cells	3hrs –S9: 1.84-28.8µg/ml 3hrs +S9: 3.32-20.2µg/ml 24hrs –S9: 0.00894-0.143µg/ml (22% HPβCD)	Negative
Chromosomal aberrations *in vitro* 961164 GLP	Human peripheral blood lymphocytes	4hrs –S9: 40-320µg/ml 4hrs +S9: 160-1280µg/ml 21hrs –S9: 5-20µg/ml (22% HPβCD)	Negative
Chromosomal aberrations *in vivo* 961901 GLP	Sprague-Dawley rats 5/sex/group Bone marrow micronucleus	1 day: 0, 15, 50, 100 mg/kg (5% HPβCD)	Negative
Chromosomal aberrations *in vivo* 961165 GLP	Sprague-Dawley rats Bone marrow micronucleus	28 days: 0, 5, 15, 60/30 mg/kg/day (5% HPβCD)	Equivocal: Significant increases in micronucleated immature erythrocytes were observed in animals treated with 15 mg/kg/day for 28 days, however within historical control range. All animals in 60/30 mg/kg/day were found dead or prematurely sacrificed up to d15

Carcinogenicity

No carcinogenicity studies have been conducted with quizartinib (see discussion on non-clinical aspects).

Reproduction Toxicity

An overview of the embryo-foetal development toxicity studies with quizartinib is displayed in Table 11.

Table 7 Embryo-foetal development toxicity studies with quizartinib dihydrochloride

Study ID	Species/Sex/ Number/ Group	Dose/Route	Dosing period	NOAEL (mg /kg/day)	Major findings
901971 Non-GLP DRF	Rat Sprague-Dawley 5F/group	0, 1, 3, 10, 20 mg/kg/day (5% HPβCD) Oral gavage	GD 6-17	3 mg/kg/day	**Dams:≥10:** ↓ bodyweight, ↓ food intake, ↓ gravid uterus weight **=20:** dehydration **Foetuses:** **≥10:** ↑ late resorption, ↓ bodyweight, 4/4 litters (36 pups) subcutaneous oedema (anasarca), 1/4 (10 pups) shortened jaw (micrognathia) **=20:** total early resorption
901985	Rat Sprague-Dawley 25F/group	0, 0.6, 2, 6 mg/kg/day (5% HPβCD) Oral gavage	GD 6-17	Dams: 6 mg/kg/day Foetuses: 2 mg/kg/day	**Dams:** **=6:** ↓ gravid uterus weight **Foetuses:** **=6:** ↓ bodyweight, 11/25 litters (58 pups) anasarca, 9/25 litters (35 pups) oedema ventral cervical region, 23/25 (217 pups) oedema top of hindpaw, 7/25 (9 pups) incomplete ossification frontal/interparietal bone, ↑ unossified/incomplete/semi-bipartite/bipartite thoracic centrum variants

The juvenile toxicity studies were conducted in dose range-finding and definitive studies in juvenile male and female SD rats (Table 12).

Table 8 Juvenile toxicity studies with quizartinib dihydrochloride

Study ID	Species/Sex /Number/ Group	Dose/Route	Dosing period	NOAEL (mg /kg/day)	Major findings
20083444 Non-GLP DRF	Rat Sprague-Dawley 8/sex/group	0, 0.3, 1, 3 mg/kg/day (5% HPβCD) Oral gavage	PND 10-28	1 mg/kg/day	≥0.3: ↓ kidney weight, ↓ liver weight, ↓ testes weight, , ↓ ovaries, ↓ thymus weight ≥1: ↓ spleen weight =3: ↓ bodyweight, ↓ food intake (F), ↓ WBC, ↓ RBC, ↓ Hb/Ht, ↑ MCV/MCH
20083208	Rat Sprague-Dawley 20/sex/group	0, 0.3, 3, 10 mg/kg/day (5% HPβCD) Oral gavage	PND 10-70	0.3 mg/kg/day	≥3: ↓ Neut/lymph., ↓ RBC. ↓ Retic., ↑ MCV/MCH, ↑ Urea/Creat., ↓ kidney weight, ↓ spleen weight, ↓ thymus weight, bone marrow (hypocellularity), testes (atrophy), epididymides (aspermia) =10: All animals euthanized/dead up to PND 36 (severe bone marrow hypocellularity)

Toxicokinetic data

Table 9: Toxicokinetic data of AC220 in pregnant rats

Study ID	Daily Dose (mg/kg)	Study day	Animal AUC (ng.h/ml)		Animal:Human Exposure Multiple*	
			♂	♀	♂	♀
901985 2 weeks	0.6	GD6 GD17	- -	726 993	- -	0.09 0.12
	2	GD6 GD17	- -	3290 4390	- -	0.40 0.53
	6	GD6 GD17	- -	17200 28000	- -	2.08 3.38

* Based on human AUC_{0-24} (60mg/day quizartinib dihydrochloride (53 mg free base)) of 8276 ng·h/ml

Table 10 Toxicokinetic data of AC220 in juvenile rats

Study ID	Daily Dose (mg/kg)	Study day	Animal AUC (ng.h/ml)		Animal:Human Exposure Multiple*	
			♂	♀	♂	♀
20083208 8 weeks	0.3	PND10 PND 21 PND 56 PND 70	509 605 434 566	554 501 1600 607	0.06 0.07 0.05 0.07	0.07 0.06 0.19 0.07
	3	PND10 PND 21 PND 56 PND 70	6970 8390 5620 7910	7230 7870 6700 8990	0.84 1.01 0.68 0.96	0.87 0.95 0.81 1.09
	10	PND10 PND 21 PND 56 PND 70	30300 79800 - -	29400 66800 - -	3.66 9.64 - -	3.55 8.07 - -

* Based on human AUC_{0-24} (60mg/day quizartinib dihydrochloride (53 mg free base)) of 8276 ng·h/ml

Local Tolerance

No specific studies have been conducted with quizartinib to evaluate local tolerance (see discussion on non-clinical aspects).

Other toxicity studies

Antigenicity

Hartley-derived albino guinea pigs (10/sex) were topically treated with 100% (0.3 g) of quizartinib dihydrochloride, once per week, for 3 consecutive weeks (Study 20079155). Following a 2-week rest period, a challenge was performed by topically treating with 100% quizartinib dihydrochloride. Challenge responses in the test animals were compared with those of the concurrent challenge control animals (5/sex). Following challenge with 100% quizartinib dihydrochloride, no dermal reactions in the test and challenge control animals occurred. The dermal scores were limited to 0.

Impurities

The potential toxicities of 5 impurities were evaluated in a 28-day repeat dose oral toxicity study in rats (Study No. 1258-023). In the study, quizartinib spiked with impurities induced some changes including decreased thymus, spleen, and testes weights and increased ALT/AST values. Although it was considered to be related to the addition of impurities, they were concluded to be non-adverse.

Phototoxicity

The phototoxic potential of quizartinib was examined in Balb/c 3T3 mouse fibroblasts (Study 20009465). Quizartinib dihydrochloride dissolved in 1% DMSO/Dulbecco's phosphate buffered saline supplemented with Ca2+ and Mg2+ (DPBS) at concentrations up to 10.5 mg/mL was tested in the range-finding or definitive assay. For evaluation of phototoxicity, the cells were exposed to 5 J/cm2 of ultraviolet A light (UVA) from a 6500W xenon arc solar simulator equipped with a 1 mm thick Schott WG 320 filter. Based on historical data, this exposure also included approximately 18 mJ/cm^2 of ultraviolet B light (UVB). In both assays, quizartinib at the highest achievable concentration of 10.5 mg/L in the range-finding study and 10.05 mg/L in the definitive study in 1% DMSO in DPBS did not reach the IC50 for cytotoxicity. Because quizartinib only showed cytotoxicity in the presence of ultraviolet A, no exact photo irritation factor (PIF) could be calculated (the range-finding PIF of >3.313 and the definitive assay mean PIF of >1.576). On the other hand, the mean photo effects were calculated to be less than 0.15.

Ocular and dermal irritation

Female NZW rabbits (3/group) received a 0.0354 g of quizartinib dihydrochloride in the conjunctival sac of the right eye (Study 20079153). Exposure to quizartinib produced conjunctivitis (redness, swelling, and/or discharge) in 3/3 test eyes at the 1-h scoring interval. Complete resolution of the conjunctivitis occurred in 2/3 test eyes by the 48-h scoring interval and in the remaining test eye by the 72-h scoring interval. An additional ocular finding of quizartinib remaining in the eye was noted in all test animals. Male NZW rabbits (3/group) received a 0.57 g of quizartinib dihydrochloride as a single dermal application (Study 20079156). Exposure to quizartinib produced well-defined erythema at 3/3 test sites at the 1-h scoring interval. The dermal irritation resolved completely at 2/3 test sites by the 48-h scoring interval and at the remaining test site by the 72-h scoring interval.

2.3.5. Ecotoxicity/environmental risk assessment

Table 11 Summary of main study results

Substance (INN/Invented Name): Quizartinib					
CAS-number (if available): 950769-58-1					
PBT screening		Result			**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107 or …	P.M.			Potential PBT (Y/N)
PBT-assessment					
Parameter	**Result relevant for conclusion**				**Conclusion**
Bioaccumulation	log K_{ow}	P.M.			B/not B
	BCF	P.M.			B/not B
Persistence	ready biodegradability	P.M.			P/not P
	DegT50	P.M. $DT_{50,\ water}$ = / d (/) $DT_{50,\ sediment}$ = / d (/) $DT_{50,\ system}$ = / d (/)			l=lake; r=river; p =pond; DT_{50} values corrected to 12°C. Conclusion: P/ not P
Toxicity	NOEC algae NOEC crustacea NOEC fish	P.M.			T/not T
	CMR	not investigated			potentially T
PBT-statement :	The compound is not considered as PBT nor vPvB The compound is considered as vPvB The compound is considered as PBT				
Phase I					
Calculation	**Value**	**Unit**			**Conclusion**
PEC $_{surface\ water}$, default prevalence	0.265 (default)	µg/L			> 0.01 threshold (Y/N)
Other concerns (e.g. chemical class)					(Y/N)
Phase II Physical-chemical properties and fate					
Study type	**Test protocol**	**Results**			**Remarks**
Adsorption-Desorption	OECD 106 or …	K_{oc} = P.M.			List all values
Ready Biodegradability Test	OECD 301LETTER	P.M.			
Aerobic and Anaerobic Transformation in Aquatic Sediment systems	OECD 308	P.M. $DT_{50,\ water}$ = / d (/) $DT_{50,\ sediment}$ = / d (/) $DT_{50,\ system}$ = / d (/) % shifting to sediment =			Not required if readily biodegradable l=lake; r=river; p =pond; DT_{50} values at 20°C; Significant shifting to sediment observed.
Phase IIa Effect studies					
Study type	**Test protocol**	**Endpoint**	**value**	**Unit**	**Remarks**
Algae, Growth Inhibition Test/*Species*	OECD 201	NOEC	P.M.	µg/L	endpoint
Daphnia sp. Reproduction Test	OECD 211	NOEC	P.M.	µg/L	endpoint
Fish, Early Life Stage Toxicity Test/*Species*	OECD 210	NOEC	P.M.	µg/L	endpoint
Activated Sludge, Respiration Inhibition Test	OECD 209	EC	P.M.	µg/L	respiration
Phase IIb Studies					
Bioaccumulation/*Species*	OECD 305	BCF	P.M.	L/kg	%lipids:
Aerobic and anaerobic transformation in soil	OECD 307	DT50 %CO_2	P.M.		for all 4 soils
Soil Microorganisms: Nitrogen Transformation Test	OECD 216	%effect	P.M.	mg/kg	
Terrestrial Plants, Growth Test/*Species*	OECD 208	NOEC	P.M.	mg/kg	
Earthworm, Acute Toxicity Tests/*Species*	OECD 207	NOEC	P.M.	mg/kg	
Collembola, Reproduction Test/*Species*	ISO 11267	NOEC	P.M.	mg/kg	
Sediment dwelling organism/*Species*		NOEC	P.M.	mg/kg	normalised to 10% o.c.

2.3.6. Discussion on non-clinical aspects

The inhibition of FLT3-ITD kinase activity (i.e. phosphorylation) by quizartinib was demonstrated on MV4-11 cell line *in vitro*, this cell line derived from AML patient and express FLT3 with ITD mutation. The inhibition of tumour cell growth was demonstrated on this MV4-11 AML cell line, after treatment with quizartinib compared the RS4;11 cell line (full FLT3). The effects were more marked in the MV4-11 cell line compared to RS4;11 and the IC50 of quizartinib was 0.3 nM. Three *in vivo* studies have been provided on MV4-11 model in mice, 2 with a localised model and 1 on a disseminated one. A time–dependent reduction of phosphorylated FLT3 was retrieved after administration of quizartinib as a single dose, and the maximal effect was reached at 6h post dose (7% of p-FLT3). A rebund in p-FLT3 was observed starting at 48h post dose. Dose-dependent inhibition of quizartinib of tumor growth was demonstrated from 1 to 10 mg/kg, on a 28-day treatment and no regrowth until 32 days after discontinuation of quizartinib dihydrochloride was observed. In the disseminated MV4-11 model, quizartinib demonstrated efficacy by prolonged survival rate correlated with delayed disease onset, as measured by clinical signs and detection of circulating MV4-11 cells in peripheral blood and bone marrow. The efficacy of quizartinib was superior by continuous treatment compared to 31-day cycle.

Absorption, distribution, metabolism and excretion of quizartinib and AC886 have been thoroughly evaluated following intravenous and oral administration in mice, rats, dogs, and Cynomolgus monkeys, species used for pharmacology and toxicology studies. Quizartinib showed approximate dose proportional exposure in rats, mice, and dogs following oral administration. The lower exposure (AUC and Cmax) to quizartinib and AC886 was observed in monkeys. Quizartinib is metabolized to the major active metabolite, AC886 by CYP3A4/5. At the clinically relevant plasma concentrations, the blood to plasma (B/P) ratio is approximately 1.3 for quizartinib and approximately 3 for AC886 in humans. Therefore, it is concluded that quizartinib at clinically relevant concentrations is predominantly present in the red blood cell (RBC) compartment (50%-70%), while for AC886 this is more than approximately 80%. In animal species tested, the B/P ratios of quizartinib were calculated to be approximately 0.7 for rat and dog, i.e. 20% in RBC, and 1.0 for monkey. The B/P ratio for AC886 was approximately 0.8 and 1.2 for rat and dog, respectively. Using 14C-data from the 14C-quizartinib rat distribution study 021390-1, a B/P value of 0.57 was found at Tmax, meaning hardly any compound in RBC. These non-clinical PK values are lower than what was found for human blood, which may be related to the large difference in elimination half-life but seem to be more in line with the very high plasma protein binding.

The toxicological profile of quizartinib has been evaluated during single and repeat-dose toxicity studies in rats, dogs, and cynomolgus monkeys (the choice of the species has to be better justified), genotoxicity studies, reproductive and developmental toxicity studies in rats, juvenile toxicity and some special toxicity studies such as phototoxicity, skin and ocular irritation studies. The main target organs are bone marrow, liver and kidney with less severe effects on the thymus, and reproductive organs (ovary, vagina and testes). The main safety concerns identified during non-clinical studies include QTc prolongation, myelosuppression, lymphoid depletion, gastrointestinal toxicity, and liver and kidney function abnormalities. Those effects are usually retrieved for oncolytic agents; more specifically, bone marrow and lymphoid tissues are the main targets in AML therapy. Birefringent crystal depositions in the dog liver and rat kidney were observed in the pivotal repeat-dose toxicity studies.

Quizartinib underwent a complete genotoxicity tests battery *in vitro* and *in vivo,* with respect to gene mutations in bacteria and mammalian cells. The Ames test was positive, when quizartinib was tested at dose level up to the maximum recommended dose (5000 µg/plate) with or without metabolic activation, in contrast to its negative result mammalian cell mutation (mouse lymphoma thymidine kinase) assay. Quizartinib was negative in a chromosome aberration assay or in a single dose rat bone marrow micronucleus assay. In human lymphocytes, no increases in chromosomal aberration were observed in

absence or presence of metabolic activation at all concentrations tested. No increases in micronucleus frequency were found in micronucleus assays in rat as a single dose, PO at dosage levels up to 100 mg/kg (next to the MTD). Micronucleus assay conducted in conjunction with the 28-day toxicity study in rats showed a slight but statistically significant increase in the incidence of micronucleated immature erythrocytes. However, none of the individual values or the group means fell outside the historical control range. It appears that no exposure data are available for micronucleus study (single dose), but exposure was assessed during 28-day micronucleus study and some margin of exposure exist in rats. In the view of results, the genotoxicity characteristic of quizartinib could be considered as equivoqual. Therefore, the CHMP recommended that a toxicological transgenic rodent (TGR) mutation assay should be conducted to further investigate the genotoxicity potential.

Quizartinib is intended to be administered in patients with advanced cancers; therefore, carcinogenicity studies are not deemed necessary consistently with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Harmonised Tripartite Guideline S9, "Nonclinical Evaluation for Anticancer Pharmaceuticals" (ICH S9, 2009). The lack of dedicated carcinogenicity studies is acceptable.

Fertility studies in animals have not been conducted with quizartinib. However, adverse findings in male and female reproductive systems were observed in repeat dose toxicity studies in rats and monkeys. In female rats, ovarian cysts and vaginal mucosal modifications were observed at doses approximately 10 times the RHD based on AUC. Findings in female monkeys included atrophy of the uterus, ovary, and vagina; observed at doses approximately 0.3 times the RHD based on AUC. In male rats, testicular seminiferous tubular degeneration and failure of sperm release were observed at approximately 8 times the RHD based on AUC. Findings in male monkeys included germ cell depletion in the testes; observed at approximately 0.5 times the RHD based on AUC. After a four-week recovery period, all these findings except the vaginal mucosal modifications in the female rats were reversible.

In embryo foetal reproductive toxicity studies, embryo foetal lethality and increased post-implantation loss were observed at maternally toxic doses. Foetotoxicity (lower foetal weights, effects on skeletal ossification) and teratogenicity (foetal abnormalities including oedema) were observed at doses approximately 3 times the RHD based on AUC. Quizartinib is considered to be potentially teratogenic.

Concerning phototoxicity, while it is possible that quizartinib accumulates in melanin-containing tissues after repeated administration of quizartinib at the dosage and administration for which the application is being filed, quizartinib was concluded not to have phototoxic potential; no treatment-related changes were seen either in ophthalmology or histopathology of the eye or skin in rats, dogs, or cynomolgus monkeys; and the frequency of Grades $\geq$3 Skin and subcutaneous tissue disorders SOC, eye disorders SOC, and potentially relevant clinical findings was low. Therefore, the risk of quizartinib-induced phototoxicity in patients is considered low.

Regarding the ERA for quizartinib, the PBT assessment and adverse effects on reproductive organs cannot be determined with certainty. Further tests to determine log Kow and effects on reproduction and fish development, including a study to determine log Kow for quizartinib using the slow stirring method (OECD test guideline 123) should be conducted by the applicant. In the meantime, and as a precautionary measure quizartinib remains consider as a PBT and has a potential risk to the environment.

2.3.7. Conclusion on the non-clinical aspects

Overall, the non-clinical documentation submitted was considered adequate. The CHMP recommended additional studies to further elucidate the genotoxicity potential of quizartinib as well as the potential risk to the environment.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 12 Summary of completed quizartinib monotherapy clinical studies in subjects with relapsed or refractory AML

Study ID	Design	Study Posology	Study Obj.	Subjs by arm entered/ compl.	Gender M/F Median Age	Diagnosis Incl. criteria	Primary End point
Pivotal study							
AC220-007 Phase 3 Trial conduct Period: 07 May 2014 to 22 Feb 2018	Open-label randomized, 2-arm study (quizartinib vs salvage chemoth.); HSCT allowed	30 mg/day for 2 weeks; escalation to 60 mg/day if QTc ≤450 ms. Continuous for 28-day cycles Post-HSCT Quizartinib per Investigator choice	Efficacy, safety, PK	Planned: 363 subjects; 242 for quizartinib 121 for salvage chemotherapy Actual: 367 subjects: 245 subjects quizartinib; 122 subjects salvage chemotherapy	Quizartinib M: 113/245 (46.1%) F: 132/245 (53.9%) 55.0 yrs Salvage: M: 64/122 (52.5%) F: 58/122 (47.5%) 57.5 yrs	AML; In first relapse (duration of remission ≤ 6 months) +/- HSCT; or refractory after first line therapy, FLT3-ITD (+) (allelic ratio ≥3%)	OS
Supportive studies							
2689-CL-2004 Phase 2 Trial conduct period: 21 May 2012 to 9 Mar 2015	Open-label, randomized, multiple dose (quizartinib of either 30 mg/day or 60 mg/day); HSCT allowed during the study	Starting dose of 30 mg/day or 60 mg/day (solution or tablet); escalation to 60 mg/day or 90 mg/day, respectively, for lack or loss of response.	Efficacy. Secon d: safety, other efficacy end-points	76 randomised: 38 in the 30 mg/day and 38 in the 60 mg/day	30 mg: M: 22/38 (57.9%) F: 16/38 (42.1%) 56.5 yrs 60 mg: M: 22/38 (57.9%) F: 16/38 (42.1%) 53.0 yrs	Relapsed or refractory AML after 1 salvage regimen or HSCT; FLT3-ITD (+) (allelic ratio >10%)	CRc rate
AC220-002 Phase 2 Trial conduct Period: 16 Nov 2009 to 28 Sep 2012	Open-label, non-randomized multiple dose; no active control; HSCT allowed during the study	28-day cycles of 200 mg/day quizartinib (oral solution) under Protocol Amendment 3 and earlier versions; 28-day cycles of a starting dose of 135 mg/day (males, n=166) or 90 mg/day (females, n=150) quizartinib	Efifcacy, second: safety, PK, PD	Planned: 333 treated N=333; Cohort 1: N=157 Cohort 2: N=176	Cohort 1: M: 77/157 (49.0%) F: 80/157 (51.0%) 69.0 yrs Cohort 2: M: 93/176 (52.8%) F: 83/176 (47.2%) 51.0 yrs	Cohort 1: ≥ 60 yrs AML relapsed or refractory <1 year after 1 first-line chemotherapy regimen Prior HSCT excluded. Cohort 2: ≥ 18 yrs AML relapsed or refractory after 1 second-line (salvage) regimen or after HSCT FLT3-ITD (+) (allelic ratio>10%) or FLT3-ITD (-)	CRc rate CR rate
CP0001	Phase 1, First-in-human, open-label, 3+3 dose-escalation study	Initially dosed 14 days followed by a 14-day rest period (N=51); range 12 mg/day up to 450 mg/day. Continuous dosing (N=25) at 200 mg and 300 mg daily. The MTD was 200 mg continuous dosing	Safety, tolerability and PK. Secon d.:PD and efficacy	N=76 enrolled	M: 46/76 (60.5%) F: 30/76 (39.5%) 59.5 yrs	Relapsed or refractory AML or previously untreated not eligible for induction chemotherapy FLT3-ITD (+) (allelic ratio >10%) or FLT3-ITD (-)	

2.4.2. Pharmacokinetics

Absorption

After oral administration under fasted conditions, peak concentration (median T_{max}) of quizartinib and AC886 measured post-dose was 4 hours (range 2 to 8 hours) and 5 hours (range 4 to 120 hours), respectively.

Table 13 Geometric mean (%CV) PK parameters[a] by quizartinib dose from QuANTUM-R

	Quizartinib dose (mg)	Cmax (ng/mL)	AUC (ng·h/mL)
Quizartinib	17.7[b]	194 (63.5)	4030 (73.5)
	26.5[b]	264 (72.5)	5250 (87.3)
	53.0	376 (71.1)	7060 (91.8)
AC886	17.7[b]	81 (56.9)	1830 (58.1)
	26.5[b]	125 (62.0)	2740 (59.3)
	53.0	210 (61.6)	4550 (60.1)

[a] Cycle 1 Day 28
[b] Includes subjects receiving concomitant CYP3A inhibitors

In study AC220-019, the effect of a high-fat and high-calorie meal intake on the pharmacokinetics of quizartinib and its active metabolite AC886 was investigated in healthy male and female volunteers following a 26.5 mg dose (commercial tablet) (Table 19).

Table 14 Pharmacokinetic parameters and ratio of quizartinib and AC886 after 26.5 mg quizartinib (commercial tablet) under fasted and fed conditions (study AC220-019)

Treatment	AUClast (ng/mL/h)	AUCinf (ng/mL/h)	Cmax (ng/mL)	tmax (h)
quizartinib				
fasted (n=34)	8340 (CV%=33.0)	8730 (CV%=36.6)	99.3 (CV%=25.5)	4 (2-8)
fed (n=29)	8790 (CV%=40.3)	9460 (CV%=42.5)	90.9 (CV%=26.9)	6 (4-12)
ratio* (90% CI)	105.39 (90.79-122.35)	108.39 (91.54-128.34)	91.58 (82.15-102.08)	-
AC886				
fasted (n=34)	1750 (CV%=54.3)	1970 (CV%=52.1)	13.0 (CV%=60.9)	5 (4-120)
fed (n=29)	1820 (CV%=75.1)	2450 (CV%=43.6)	10.2 (CV%=87.9)	36 (6-144)
quizartinib + AC886				
fasted (n=34)	10400 (CV%=22.0)	10700 (CV%=22.7)	114 (CV%=22.5)	-
fed (n=29)	11000 (CV%=34.8)	11900 (CV%=27.0)	100 (CV%=27.8)	-

Distribution

In study AM16-H0044-R01, the permeability of quizartinib through Caco-2 cell monolayers was investigated at a concentration of 6336 ng/mL. The P_{app} from apical to basal direction was 0.981×10^{-6} cm/s. Propranolol and mannitol were used as reference compounds as high permeable compound and as membrane-impermeant control, respectively. The P_{app} of propranolol was 23.8×10^{-6} cm/s and the P_{app} of mannitol was 0.682×10^{-6} cm/s. Quizartinib plasma protein binding was determined by ultracentrifugation at 44.2, 442, and 2208 ng/mL using pooled frozen plasma (study PBC315-607).

Table 15 Plasma protein binding of quizartinib and AC866 at different concentrations

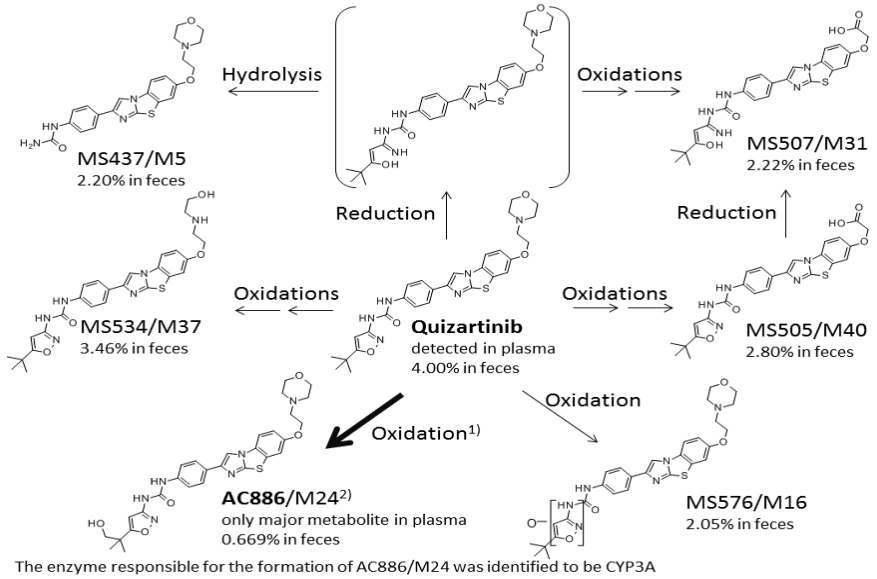
	concentration		
	44.2 ng/mL	**442 ng/mL**	**2208 ng/mL**
quizartinib	99.32 ± 0.17	98.96 ± 0.09	99.08 ± 0.16
AC886	99.68 ± 0.05	99.72 ± 0.04	99.66 ± 0.11

In study NR0025, quizartinib was added to whole blood at a final concentration of 633.6 ng/mL and incubated for 45 minutes at 37°C while mixing gently. The plasma-to-blood ratio for quizartinib in a human whole blood sample was 0.92. In studies MS-2012-007 and AM16-H0036-R01, samples containing quizartinib (6.75, 152.63, 1457.7 and 4133.82 ng/mL) and AC886 (10, 200, 1600 and 4000 ng/mL) were prepared in blood and allowed to reach equilibrium at 37°C and concentrations of quizartinib and AC886 in plasma samples immediately prepared at room temperature and at equilibrium were measured. The blood-to-plasma ratios were calculated to be 1.48, 1.31, 1.10, and 0.97 for quizartinib and 3.40, 2.79, 1.62, and 1.30 for AC886, respectively.

The apparent volume of distribution (Vd/F) was 453 L in healthy volunteers and 276 L in subjects with AML.

Elimination

In vitro studies showed that quizartinib is slowly metabolised to AC886 by CYP3A4 and CYP3A5. AC886 is further metabolised to several metabolites by CYP3A4 (main contributor) and CYP3A5. The biotransformation pathway is shown in Figure 8.



1) The enzyme responsible for the formation of AC886/M24 was identified to be CYP3A
2) AC886 undergoes further metabolism (putative secondary metabolites: M14, M18, M19, M20, M26).
3) Most of other metabolites (not shown in this pathway) were minor (<2% in feces) .

Figure 6 Postulated metabolic pathway of quizartinib

In humans, the metabolism profile was investigated in plasma (2-6 h period after dosing), urine (0-72 h) and faeces (0-264 h). Quizartinib is primarily metabolised by CYP3A via oxidative pathways which produces the active metabolite AC886, which is then further metabolised by the same isozyme. The AC886 to quizartinib ratio is 0.6. Quizartinib has a half-life of approximately 75 hours. In plasma, parent compound is the major component and AC886 is the major metabolite, with large intra-individual variability. In urine, radioactivity is only excreted as metabolite. In faeces, radioactivity is mainly excreted as metabolite (4% is excreted as parent). In total 41 metabolites were observed in faeces.

The median (%CV) effective half-life (t1/2) for quizartinib and AC886 is 73 hours and 119 hours, respectively. The median accumulation ratio (AUC0-24) for quizartinib and AC886 was 5 and 8, respectively. The time for quizartinib and AC886 to achieve steady state exposure was 15 days and 25 days, respectively.

Quizartinib is primarily eliminated by the hepatobiliary route with excretion mainly via faeces. Renal excretion is a minor route of elimination of quizartinib.

The apparent systemic clearance (CL/F) estimated for the central compartment from the population pharmacokinetic analysis in AML subjects was 6 L/hour. The half-life of quizartinib in healthy volunteers ranged from 63 to 103 h and is independent of the administered dose. The elimination half-life of AC886 is 53 to 100 h (independent of the dose).

Dose proportionality and time dependencies

The dose proportionality over a dose range of 26.5 to 79.5 mg was investigated in study AC220-014 (Table 21).

Table 16 Statistical analysis (power model) of dose proportionality for PK parameters for quizartinib in healthy volunteers (study AC220-014)

PK parameter	parameter	estimation	95% CI
quizartinib			
Cmax	intercept slope	0.909 1.07	-0.0213, 1.84 0.842, 1.30
AUClast	intercept slope	4.70 1.12	2.71, 6.69 0.634, 1.61
AUCinf	intercept slope	4.96 1.08	3.06, 6.85 0.610, 1.54
AC886			
Cmax	intercept slope	0.642 0.839	-1.11, 2.39 0.408, 1.27
AUClast	intercept slope	4.62 1.01	3.71, 5.54 0.785, 1.23
AUCinf	intercept slope	4.87 0.961	4.06, 5.68 0.762, 1.16

In healthy volunteers only single dose was investigated. However, based on the half-lives of quizartinib and AC886 accumulation is expected with once daily dosing. In the patient population multiple dosing was investigated. The accumulation ratios were 4.9 and 7.6 for quizartinib and AC886, respectively. Steady state was achieved by Day 15 following once daily dosing.

Special populations

The effect of intrinsic factors including age, body weight, body surface area (BSA), gender, race, patient status, renal impairment, and hepatic function on the pharmacokinetics of quizartinib and AC886 were evaluated in the Population PK analysis of pooled data. In addition, a dedicated hepatic impairment study was conducted.

A population pharmacokinetic analysis in AML patients with mild to moderate renal impairment (eGFR 30 to 89 mL/min/1.73 m^2) showed that renal function did not affect the pharmacokinetics of quizartinib and its active metabolite.

The effect of mild and moderate hepatic impairment on quizartinib and AC886 PK were evaluated in a dedicated hepatic impairment study (study AC220-016). Male and female subjects (53.7 ± 7.4 years of age) with mild hepatic impairment (n=8), moderate hepatic impairment (n=8) and normal hepatic function (n=16 in two separate control groups) were treated with 26.5 mg quizartinib. Quizartinib exposure increased in both mild hepatic impairment and moderate hepatic impairment; therefore, hepatic impairment decreased the clearance of quizartinib. The aggregate exposure of quizartinib and AC886 increased in subjects with mild hepatic impairment (by 13% and 17% for C_{max} and AUC, respectively), which was not considered clinically meaningful.

Table 17 PK parameters in subjects with normal hepatic function or mild or moderate hepatic impairment-study AC220-016

subjects	AUClast (ng×h/mL)	AUCinf (ng×h/mL)	Cmax (ng/mL)	tmax (h)	t½ (h)
normal (n=8)	7088 (CV%=53.9)	7304 (CV%=54.5)	97.1 (CV%=37.3)	3.5 (2.0-4.0)	85.8 (CV%=25.2)

mild (n=7)	7824 (CV%=47.1)	9462 (CV%=25.9)	109 (CV%=37.3)	3.0 (2.0-4.0)	116 (CV%=28.0)
normal (n=8)	5976 (CV%=42.6)	6132 (CV%=42.7)	90.2 (CV%=37.9)	3.0 (2.0-5.0)	86.2 (CV%=26.8)
moderate (n=7)	8500 (CV%=33.1)	9044 (CV%=34.2)	104 (CV%=40.7)	2 (1.0-4.0)	123 (CV%=18.8)

The correlation of liver function test with PK was also assessed in the Population PK analysis. The effect of liver function test was not identified as a significant covariate of quizartinib and AC886 clearance. There are no data to evaluate the effect of severe hepatic impairment on quizartinib and AC886 PK.

Gender, race, body weight and BSA and age did not have a significant effect on either quizartinib or AC886 clearance (data not shown).

A summary of elderly age groups for AML subjects treated with quizartinib is displayed in Table 23.

Table 18. Summary of elderly age groups for AML subjects treated with quizartinib (monotherapy AML subjects) (Safety Analysis Set)

Type of Study[a]	Age 65-74 n (%)[b]	Age 75-84 n (%)[b]	Age 85+ n (%)[b]
Controlled Trial	52 (7.1)	12 (1.6)	0
Non Controlled Trials	160 (21.7)	35 (4.7)	3 (0.4)

[a] Controlled trial includes AC220-007 and non-controlled trials include AC220-CP0001, AC220-002, 2689-CL-2004, and 2689-CL-0011.
[b] Denominator for the percentage is 737, which is the total exposed patients in the pooling group of "Monotherapy AML Subjects".

Pharmacokinetic interaction studies

CYP inhibition

The *in vitro* inhibition potential of quizartinib (0.27-35.4 µM) for CYP isozymes was investigated in studies NR0021 (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) and NR0035 (CYP2B6 and CYP2C8) using pooled human liver microsomes. Quizartinib did not inhibit any of the CYPs isoforms at the maximum concentration tested. The results are summarised in Table 24.

Table 19 *Direct inhibition of quizartinib for different CYP enzymes*

CYP isozyme	concentration range (µM)	substrate	positive inhibitor	IC_{50} value (µM)	Study
1A2	0.31-40	phenacetin	α-naphthoflavone	>40	NR0021
1A2	1	phenacetin	α-naphthoflavone	>1	PBC315-333
2B6	0.31-40	bupropion	ticlopidine	>40	NR0035
2B6	1	bupropion	sertraline	>1	PBC315-333
2C8	0.31-40	taxol	quercetin	>40	NR0035
2C8	1	paclitaxel	trimethorprim	>1	PBC315-333
2C9	0.31-40	diclofenac	sulphenazole	>40	NR0021
2C9	1	diclofenac	sulphenazole	>1	PBC315-333
2C19	0.31-40	mephenytoin	omeprazole	>40	NR0021
2C19	1	S-mephenytoin	benzylnirvanol	>1	PBC315-333
2D6	0.31-40	dextromethorphan	quinidine	>40	NR0021
2D6	1	bufuralol	quinidine	>1	PBC315-333
3A	0.31-40	testosterone	ketoconazole	>40	NR0021
3A	10	testosterone	ketoconazole	>10	PBC315-333
3A	10	midazolam	ketoconazole	>10	PBC315-333

In study PBC315-333, the time-dependent inhibition of quizartinib (0.88 µM for CYP1A2, 2B6, 2C8, 2C9, 2C19 and 2D6 and 8.8 µM for CYP3A) was investigated in pooled human liver microsomes with no pre-incubation as control. Quizartinib did inhibit the different CYPs with and without pre-incubation.

The potential of AC886 to inhibit CYP isozymes forms was examined in human liver microsomes in study NR0033 (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) and in study NR0036 (CYP2B6 and CYP2C8) (Table 25).

Table 20 Direct inhibition of AC886 for different CYP enzymes

CYP isozyme	concentration range (μM)	substrate	positive inhibitor	IC_{50} value (μM)	study
1A2	0.31-40	phenacetin	furafylline	>40	NR0033
1A2	1	phenacetin	α-naphthoflavone	>1	PBC315-333
2B6	0.31-40	bupropion	ticlopidine	>40	NR0036
2B6	1	bupropion	sertraline	>1	PBC315-333
2C8	0.31-40	taxol	quercetin	11.3	NR0036
2C8	1	paclitaxel	trimethorprim	>1	PBC315-333
2C9	0.31-40	diclofenac	sulphenazole	>40	NR0033
2C9	1	diclofenac	sulphenazole	>1	PBC315-333
2C19	0.31-40	mephenytoin	ticlopidine	24.7	NR0033
2C19	1	S-mephenytoin	benzylnirvanol	>1	PBC315-333
2D6	0.31-40	dextromethorphan	quinidine	>40	NR0033
2D6	1	bufuralol	quinidine	>1	PBC315-333
3A	0.31-40	testosterone	ketoconazole	>40	NR0033
3A	1	testosterone	ketoconazole	>1	PBC315-333
3A	1	midazolam	ketoconazole	>1	PBC315-333

Time-dependent inhibition was investigated in study PBC315-333. The time-dependent inhibition of AC886 (1 μM) was investigated in pooled human liver microsomes with no pre-incubation as control. AC886 did inhibit the different CYPs with and without pre-incubation.

Furthermore, a study was performed to investigate the Ki of AC886 for CYP2C8, 2C9 and 3A4 using human liver microsomes (study NR0054). AC886 is a weak non-competitive inhibitor of CYP2C19 (Ki = 10.4 μM), but is effectively not an inhibitor of CYP3A4 (Ki = 48.9 μM) and CYP2C8 (Ki = 60.8 μM).

In vitro studies showed that quizartinib was not a reversible inhibitor, time-dependent inhibitor of major human CYP enzymes. AC886 is not a CYP inhibitor at therapeutic concentrations.

Drug transporter inhibition

The inhibition potential of quizartinib towards drug transporters was investigated in several *in vitro* studies (studies 2689-me-003, OPT-215-005 (includes OPT-2015-039) and AM16-H0064-R01. The results are summarised in Table 26. Quizartinib has a potential to inhibit P-glycoprotein, primarily on P-glycoprotein-mediated gastrointestinal transport. Quizartinib has minimal potential to affect other transporters at therapeutic concentrations.

Table 21 Inhibition of quizartinib for different transporters

transporter	concentration range (μM)	substrate	positive inhibitor	IC_{50} value (μM)	study
P-gp	1-100 10 10	digoxin quinidine quinidine	verapamil elacridar elacridar	9.6 >10 >10	2689-ME-0003 OPT-2015-005 AM16-H0064-R01
Breast Cancer Resistance Protein (BCRP)	10 10	prazosin prazosin	Ko143 Ko143	>10 >10	OPT-2015-005 AM16-H0064-R01
organic anion transporting polypeptide 1B1 (OATP1B1)	10	estradiol-17β -d-glucuronide	rifampicin	>10	OPT-2015-005
OATP1B3	10	CCK-8	rifampicin	>10	OPT-2015-005

OCT1	10	MPP+	quinidine	>10	OPT-2015-005
OCT2	10	metformin	quinidine	>10	OPT-2015-005
OAT1	10	p-aminohippurate	probenecid	>10	OPT-2015-005
OAT3	10	p-aminohippurate	probenecid	>10	OPT-2015-005
multi-anion and toxin extrusion protein 1 (MATE1)	10	metformin	cimetidine	>10	OPT-2015-005
MATE2-K	10	metformin	cimetidine	>10	OPT-2015-005
BSEP	0.032-10	taurocholate	rifampicin	4.9	OPT-2015-005

The inhibition potential of AC886 towards drug transporters was investigated in several *in vitro* studies (studies 2689-me-0005, OPT-215-005, OPT-2015-039 and AM16-H0064-R01). The results are summarized in Table 27. AC886 was not an inhibitor of drug transporters at therapeutic concentrations based on the *in vitro* studies.

Table 22 Inhibition of AC886 for different transporters

transporter	concentration range (µM)	substrate	positive inhibitor	IC_{50} value (µM)	study
P-gp	1-30 10 10	digoxin quinidine quinidine	verapamil elacridar elacridar	>30 >10 >10	2689-me-0005 OPT-2015-005 AM16-H0064-R01
BCRP	10 10	prazosin prazosin	Ko143 Ko143	>10 >10	OPT-2015-005 AM16-H0064-R01
OATP1B1	10	estradiol-17β -d-glucuronide	rifampicin	>10	OPT-2015-005
OATP1B3	10	CCK-8	rifampicin	>10	OPT-2015-005
OCT1	10	MPP+	quinidine	>10	OPT-2015-005
OCT2	10	metformin	quinidine	>10	OPT-2015-005
OAT1	10	p-aminohippurate	probenecid	>10	OPT-2015-005
OAT3	10	p-aminohippurate	probenecid	>10	OPT-2015-005
MATE1	10	metformin	cimetidine	~10	OPT-2015-005
MATE2-K	10	metformin	cimetidine	~10	OPT-2015-005
BSEP	10	taurocholate	rifampicin	>10	OPT-2015-005

Also an *in vitro* Drug-Drug Interaction study was performed with quizartinib and AC886 on the metabolism of cytrabine using pooled liver S9 (study AM15-H0085-R01). Neither quizartinib nor AC886 inhibited the formation of uracil 1-β-D-arabinofuranoside from cytarabine up to 26.5 and 30 µM, respectively. Gemcitabine was used as a positive control inhibitor and inhibited the formation of uracil 1-β-D-arabinofuranoside from cytarabine.

Induction via AhR, PXR and CAR

The induction potential of quizartinib was investigated using cultured hepatocytes (n=3) in study XT133087. The induction potential towards AhR (via CYP1A2), CAR (via CYP2B6) and PXR (via CYP3A4) was investigated. Similar mRNA expression as negative control was observed and thus no induction was observed.

Table 23 Induction of quizartinib via AhR (CYP1A2), CAR (CYP2B6) and PXR (CYP3A4)

CYP isozyme	concentration range (µM)	positive control	mRNA increase
1A2	1-10	omeprazole	≤2-fold
2B6	1-10	phenobarbital	<2-fold
3A4	1-10	rifampin	<2-fold

The induction of quizartinib was also investigated in freshly isolated human hepatocytes (one donor) in study NR0021. No induction was observed for CYP1A2 and 3A4 up to a concentration of 10 µM quizartinib. The positive controls omeprazole (CYP1A2) and rifampicin (CYP3A4) led to induction.

Effects of other drugs on the pharmacokinetics of quizartinib and AC886

In vitro, it was elucidated that quizartinib and AC886 are mainly metabolised by CYP3A4 and 3A5. Furthermore, quizartinib is a substrate of P-glycoprotein.

The effect of a gastric reducing agent (lansoprazole) was investigated in study AC220-018. The proton pump inhibitor lansoprazole decreased quizartinib C_{max} by 14% and AUCinf by 5%.

Furthermore, the effect of strong CYP3A/P-glycoprotein inhibition (ketoconazole) and fluconazole was investigated in study AC220-015. The effect of induction on the PK of quizartinib was investigated using PBPK modelling (report PBPK modelling). Co administration of ketoconazole (200 mg twice daily for 28 days) with single dose administration of Vanflyta resulted in increased Cmax by 17%, and AUCinf by 94%. At steady state, exposure (Cmax and AUC0-24) was estimated to be increased by 86% and 96%, respectively. Concomitant administration of fluconazole increased both predicted quizartinib AUCtau and Cmax,ss by approximately 20%.

Population PK results showed an approximately 1.9-fold increase in exposure (predicted AUCtau and $C_{max,ss}$ from Study AC220-015) relative to the reference subject with AML for quizartinib co-administered with strong CYP3A inhibitors. Doses were reduced from 30 mg/day to 20 mg/day or 60 mg/day to 30 mg/day when quizartinib was co-administered with a strong CYP3A inhibitor in the Phase 3 Study AC220-007.

In the Phase 3 Study AC220-007, concomitant use of strong or moderate CYP3A inducers was prohibited, but weak CYP3A inducers were allowed. Of the subjects randomized to quizartinib, 41 (17.0%) received CYP3A inducers, predominantly weak CYP3A inducers such as corticosteroids. The PBPK results indicated that co-administration of rifampin, a strong CYP3A inducer, with quizartinib resulted in an approximately 72% decrease in quizartinib exposure and an approximately 66% decrease in AC886 exposure (AUCinf).

The effect of moderate CYP3A inducers was not assessed.

2.4.3. Pharmacodynamics

Mechanism of action

Quizartinib is an inhibitor of the receptor tyrosine kinase FLT3. Quizartinib and its major metabolite AC886 competitively bind to the adenosine triphosphate (ATP) binding pocket of FLT3 with high affinity (Kd=1.3 nM and 0.54 nM, respectively). Quizartinib and AC886 inhibit FLT3 kinase activity, preventing autophosphorylation of the receptor, thereby inhibiting further downstream FLT3 receptor signalling and blocking FLT3 ITD dependent cell proliferation.

Primary and Secondary pharmacology

- A Phase 2 open-label, quizartinib monotherapy efficacy (ACE) study in patients with acute myeloid leukemia with and without FLT3-ITD activating mutations (Study AC220-002)

AML-patients received daily quizartinib doses on a 28-day schedule. The first 17 subjects were given a quizartinib dose of 200 mg/day (regardless of sex), but that dose was reduced to 90 mg/day for females, and 135 mg/day for males for subsequent subjects. Subjects received quizartinib at the clinic on Days 1, 2, 8, and 15 of Cycle 1, Days 1 and 15 of Cycle 2, Day 1 of Cycle 3, and subsequently every 14 days. Heparinized blood was drawn predose on day 1 before initial treatment, as well as predose at various days after treatment, and plasma was isolated from duplicate draws. Phospho- and total-FLT3, STAT5, and KIT levels were measured by MSD assay (Meso Scale Discovery electro-chemiluminescence platform) in the blood lysates from days 1, 2, and 8 of treatment. FLT3 levels in the plasma were determined in samples from day 1, 15, and 29. FLT3 genotyping of the 292 subjects whose blood was tested for phosphoproteins revealed that 215 were positive for the FLT3 internal tandem duplication (ITD) mutation that results in constitutively activated/phosphorylated FLT3 and 76 were ITD negative. FLT3 genotyping of the 264

subjects whose plasma was tested for FLT3 levels revealed that 199 were positive for the FLT3-ITD mutation and 64 were ITD negative.

FLT3

Median phospho FLT3 (pFLT3) levels decreased from 3312 RLU at day 1 to 1759 RLU and 1235 RLU at days 2 and 8, respectively. These median values are significantly lower than predose levels at both time points tested, as well as significantly lower at day 8 compared to day 2 ($p < 0.0001$, Wilcoxon matched pairs signed rank test).

Median total (t)FLT3 levels decreased from 5639 RLU at day 1 to 4584 RLU and 142 RLU at days 2 and 8, respectively. As for pFLT3, these median values are significantly lower than predose levels at both time points tested, as well as significantly lower at day 8 compared to day 2 ($p < 0.0001$).

The degree of FLT3 phosphorylation relative to levels of total FLT3, pFLT3 levels for each subject were normalized to the corresponding tFLT3 levels and expressed as a percentage. Starting from a median p/tFLT3 pre-treatment level of 70%, levels decreased significantly to 20% on day 2 ($p < 0.0001$).

Another indicator of the effects of quizartinib on FLT3 phosphorylation is to examine the changes in p/tFLT3 ratio compared to the pre-treatment level. Overall, there was a median reduction to 31% of pre-treatment day 1 levels on day 2 of treatment.

Changes in tFLT3 levels are indicative of the quizartinib treatment, since the majority of FLT3 in the peripheral blood is largely due to presence of circulating AML blasts. At day 2, the median change in tFLT3 levels was an increase to 131% of pre-treatment levels. This been previously observed in the quizartinib CP0001 phase I trial, as well as in *in vitro* cell-based experiments that cells dependent on FLT3 signaling increase the expression of this receptor in the presence of FLT3 inhibitors, including quizartinib, likely as a compensatory mechanism when phosphorylation of the kinase and subsequent downstream survival signals are blocked (7, 8). In sharp contrast, the median change in tFLT3 levels at day 8 was reduced to 3.0% of pre-treatment levels.

For the 193 subjects with measured changes in p/tFLT3, at day 2 of treatment, 66% of subjects had a greater than 50% reduction, while 42% had a greater than 75% reduction. Of the 196 subjects at day 2, and the 200 subjects at day 8 with measured tFLT3 changes, a large majority of subjects (63%) had negative inhibition values at day 2 (increases in tFLT3), but over three quarters of the subjects (79%) had a greater than 75% reduction in tFLT3 by day 8, with 69% having a greater than 90% inhibition in tFLT3 levels.

Effect of quizartinib dose on FLT3

Initially, all subjects enrolled in the study were started at 200 mg quizartinib per day, but the doses were subsequently reduced to 135 mg/day for male subjects, and 90 mg/day for female. To see if the differences in doses translated to differences in FLT3 responses, pFLT3, tFLT3, p/tFLT3, changes in p/tFLT3 from day 1, and changes in tFLT3 from day 1 separated by the doses subjects received were analyzed. There were no statistically significant differences in p/tFLT3 responses at day 2 between the doses given. This was also applicable for absolute pFLT3 and tFLT3 levels, p/tFLT3 signal ratios, and changes in tFLT3 levels from day 1.

Effect of FLT3-ITD Status on the FLT3 Levels

Of the 292 subjects tested, 26% were negative for the FLT3-ITD mutation. As expected, pFLT3 levels are statistically significantly higher ($p < 0.0001$, Mann Whitney test) for the ITD+ subjects at day 1 before treatment since this mutation results in constitutive activation/phosphorylation of FLT3. After treatment at days 2 and 8, pFLT3 levels had been sufficiently reduced in subjects with and without the mutation such that median values were not significantly different.

Median tFLT3 levels from ITD positive subjects were higher both before treatment on day 1 ($p = 0.0005$) and after treatment on day 2 ($p < 0.0001$). By day 8 of treatment, tFLT3 levels were reduced in all subjects such that there was no significant difference in the median levels, irrespective of ITD status.

While the median percentage of phosphorylated FLT3 in relation to total FLT3 were similar before treatment (65% for ITD+ vs. 86% for ITD-), the median percentage of phosphorylated FLT3 was only 18% for ITD+ subjects, vs. 90% for ITD- subjects ($p < 0.0001$) at day 2 of treatment.

Changes in tFLT3 levels from pre-treatment also showed differences between ITD+ and ITD- subjects. Similar to data for all subjects mentioned earlier, ITD+ subjects had an increase in tFLT3 levels to a median of 133% of pre-treatment levels on day 2, while ITD- subjects maintained tFLT3 levels (98%) found before treatment ($p = 0.044$). Importantly, tFLT3 levels were drastically reduced to 1.8% of day 1 pre-treatment levels for ITD+ subjects, while ITD- subjects were only reduced to 44% of pre-treatment levels ($p < 0.0001$).

STAT5

Constitutively activated/phosphorylated STAT5 is a marker closely associated with activated FLT3 in the context of the ITD mutation in AML patients (9). These authors demonstrated that inhibition of FLT3 activity in FLT3-ITD AML cells with a selective small molecule inhibitor (SU5614) concomitantly reduced STAT5 phosphorylation, an effect also seen with quizartinib (CR0002, CR0008, (10)). In this study, the effects of quizartinib on STAT5 levels were also examined. Median phosphor STAT5 levels decreased from 684 RLU at day 1 to 402 RLU and 234 RLU at days 2 and 8, respectively. These median values are significantly lower than pre-dose levels at both time points tested, as well as significantly lower at day 8 compared to day 2 ($p < 0.0001$, Wilcoxon matched pairs signed rank test).

The overall median tSTAT5 levels was decreased from 153100 RLU before treatment to 153043 RLU on day 2 after treatment, this reduction was significantly different ($p = 0.0045$, Wilcoxon matched-pairs signed rank test), likely due to the changes in paired samples, i.e., samples from the same subject at the different time points. Wen analyzed using an unpaired t test, significance of the difference in medians is lost ($p = 0.7774$, Mann Whitney test). By day 8, median tSTAT5 levels were reduced from day 1 and 2 levels to 126087 RLU ($p < 0.0001$, Wilcoxon matched pairs signed rank test). Many normal leukocytes in the blood harbor STAT5, so the measurable increased levels contributed by the AML blasts and their subsequent reduction caused by quizartinib treatment are particularly notable.

The median percent p/tSTAT5 levels were significantly reduced from 0.49% before treatment to 0.25% and 0.17% on days 2 and 8, respectively, after treatment ($p < 0.0001$). At day 2, p/tSTAT5 levels were reduced to a median of 56% of pretreatment levels and were reduced further to a median of 45% by day 8 ($p < 0.0001$).

Of the 237 subjects at day 2 and the 248 subjects at day 8 with measured changes in p/tSTAT5 from pretreatment levels, 47% of subjects had greater than 50% inhibition in p/tSTAT5 levels at day 2, which increased to 53% by day 8. The percentage of subjects with >90% inhibition increased from 8.9% at day 2 to 17% of subjects at day 8.

There were no statistically significant differences in p/tSTAT5 responses between the doses given. This was also applicable for absolute pSTAT5 and tSTAT5 levels, as well as for p/tSTAT5 signal ratios before and after treatment.

The effects of FLT3 ITD status on pSTAT5 levels showed that, as with pFLT3 levels, median pSTAT5 levels are significantly higher for ITD+ subjects (912 RLU vs. 352 RLU; $p < 0.0001$). At day 2 after treatment, median pSTAT5 levels are still significantly higher for ITD+ subjects (431 RLU vs. 305 RLU; $p = 0.0096$). By day 8, median pSTAT5 levels for ITD+ subjects (229 RLU) were not different from ITD- subjects (245 RLU). Median tSTAT5 levels were significantly higher for ITD+ subjects before treatment (156747 RLU vs.

138320 RLU; p = 0.0185) and on day 2 after treatment (157067 RLU vs. 133333 RLU; p = 0.0314) compared to ITD- subjects. By day 8, median tSTAT5 were not different. The median percentage of STAT5 that was phosphorylated before treatment (0.67%) was significantly higher for ITD+ subjects vs. ITD- subjects (0.22%; p < 0.0001). At day 2, ITD+ subjects had a median p/tSTAT5 signal ratio higher than ITD- subjects (0.26% vs. 0.21%; p = 0.0314). By day 8, median p/tSTAT5 signal ratios were the same, at 0.17%.

FLT3-ITD status had a large effect on changes in p/tSTAT5 signal ratios after treatment. On day 2, ITD+ subjects had a median p/tSTAT5 level of 41% of pretreatment levels, compared to 104% of pre-treatment levels for ITD- subjects (p < 0.0001). On day 8, ITD+ subjects had a median p/tSTAT5 level of 27% of pre-treatment levels, compared to 87% of pretreatment levels for ITD- subjects (p < 0.0001).

KIT

Activating mutations in the receptor tyrosine kinase c-KIT (KIT) have been frequently associated with AML (11). Quizartinib has some kinase inhibitory activity on KIT, but with approximately 4-fold reduced binding and 17-fold less kinase inhibitory activity in cells (data not shown). By day 8, the median pKIT level was reduced to 348 RLU, a significant reduction from both days 1 and 2 (p < 0.0001, Wilcoxon matched pairs signed rank test). Levels for tKIT showed that, as with tFLT3 in ITD+ subjects above, there was a significant increase in median tKIT levels for all subjects from 149747 RLU before treatment to 153137 RLU at day 2 after treatment (p = 0.0010). By day 8, median tKIT level was 138825 RLU, significantly lower compared to day 1 pretreatment and day 2 after treatment (p < 0.0001).

Median p/tKIT signal ratios were 0.42% before treatment on day 1, 0.39% after treatment on day 2, and 0.24% on day 8. All three comparisons (day 1 vs. 2, 1 vs. 8, and 2 vs. 8) were significant reductions in the paired data (p < 0.0001). On day 2, median p/tKIT level was 93% of day 1 pretreatment level, but by day 8, it was significantly reduced to 75% of pretreatment level (p < 0.0001).

There were 235 subjects that had a measured change in p/tKIT levels from day 1 at day 2, and 247 subjects at day 8. The percentages of subjects at the various levels of p/tKIT inhibition after quizartinib treatment on days 2 and 8 showed that, while only 4.7% of subjects had >50% inhibition in p/tKIT levels at day 2, 28% had that level of inhibition by day 8.

As with FLT3 and STAT5, there were no statistically significant differences in p/tKIT responses between the doses given.

Median pKIT levels were higher in ITD+ subjects (704 RLU vs. 429 RLU, p = 0.0036) at day 1 before quizartinib treatment, and after treatment on day 2 (706 RLU vs. 297 RLU, p = 0.0003), but not at day 8 of treatment, when compared to ITD- subjects. For tKIT, ITD+ subjects had significantly higher median levels at all three time points. Median tKIT levels were 156274 RLU vs. 133682 RLU at day 1 (p < 0.0001), 155795 RLU vs. 133884 RLU at day 2 (p < 0.0001), and 141862 RLU vs. 129828 RLU at day 8 (p = 0.0060) for ITD+ vs. ITD- subjects, respectively. The differences in pKIT and tKIT levels translated into differences in p/tKIT signal ratios. Median levels of p/tKIT before treatment were 0.45% for ITD+ subjects and 0.30% for ITD- subjects (p = 0.0249). On day 2 after treatment, median p/tKIT levels were 0.44% vs. 0.23% for ITD+ vs. ITD- subjects, respectively (p = 0.0023). On day 8, there was no difference in median p/tKIT levels.

While there was no difference in the median change at day 2 of treatment between ITD+ and ITD- subjects (95% of predose vs. 92%, respectively), ITD+ subjects had a significantly greater reduction in p/tKIT signal ratios from pretreatment (71% of day 1) compared to ITD- subjects (85% of day 1) at day 8 (p = 0.0200).

Plasma human FLT3 Ligand

Regarding the levels of human FLT3 ligand (hFL) in the plasma before (day 1) and after quizartinib treatment on days 15 and 29 there were significant increases from the median pretreatment level of 8.8 pg/mL to 34 pg/mL on day 15, and to 49 pg/mL on day 29 for all paired comparisons of the data sets ($p < 0.0001$).

Some subjects had very large-fold changes in plasma FL levels from day 1. At day 15, the median increase in FL was 3.2-fold over pretreatment levels that was further significantly increased to 5.0-fold by day 29 ($p < 0.0001$). Of the 208 subjects with measured changes in plasma hFL levels from pretreatment at day 15, 36% of subjects had increases ≥5-fold in plasma hFL levels. Of the 158 subjects with measured changes at day 29, 50% had ≥5-fold increases in plasma hFL levels. There was a decrease in the percentage of subjects with <5-fold increases in hFL from 64% of subjects at day 15 to 50% at day 29. In addition, here was an increase in the percentage of subjects with ≥7.5-fold increases by day 29, including an 18% increase in subjects with ≥10-fold increases in plasma hFL levels. As with the phosphoproteins, the three different doses of quizartinib did not have significant effects on the measured levels of plasma hFL before or after treatment.

Before treatment, FLT3-ITD+ subjects had a median plasma hFL level of 6.3 pg/mL, while the median levels in ITD- subjects was significantly higher at 21 pg/mL ($p < 0.0001$). At day 15, median levels were not significantly different at 33 and 35 pg/mL for ITD+ and ITD- subjects, respectively. But by day 29, ITD+ subjects had a significantly higher median hFL level of 55 pg/mL, compared to 41 pg/mL for ITD- subjects ($p = 0.0244$). The differences in measured levels translated to significant differences in changes in hFL levels from pre-treatment. For FLT3-ITD+ subjects, there was a median 4.4- fold increase in plasma hFL at day 15, compared to only a 1.2-fold increase in ITD- subjects ($p < 0.0001$). Similarly, at day 29, median increase in hFL levels from day 1 was 9.5-fold for ITD+ subjects, compared to only 1.6-fold for ITD- subjects.

- *A phase 2, randomized, open label study of the safety and efficacy of two doses of quizartinib in subjects with FLT3-ITD positive relapsed or refractory AML (Study 2689-CL-2004):*

A total of 76 relapsed/refractory FLT3-ITD AML subjects were enrolled in a Phase 2b study. Of those, 56 subjects (30 at 30 mg/day; 26 at 60 mg/day) had sufficient day 1 and either day 8 (n = 47) or day 15 (n = 9) pre-dose plasma samples available for evaluation. Pharmacokinetic analysis to determine levels of quizartinib and its active metabolite AC886 in each plasma sample were performed in-house.

The objective of this study was to determine if the plasma levels of quizartinib achieved in human subjects receiving 30 or 60 mg of daily dosing were sufficient to inhibit the cellular kinase activities of FLT3 with an ITD mutation, wild type (WT) FLT3 following FLT3 ligand stimulation, and c-KIT following stem cell factor stimulation in an ex vivo PIA cell-based assay. The concentrations of AC220+ AC886 that result in the inhibition of kinase activity (50% and 90% inhibition [IC50 and IC90, respectively] values, for wild type FLT3, FLT3-ITD and KIT were calculated.

Inhibition of FLT3-ITD, FLT3-WT, and KIT by MSD assay PIA

Examination of the PIA assay data in the ITD cells with just undiluted plasma between subjects receiving 30 mg vs. 60 mg quizartinib showed a mean inhibition of 98.0 ± 3.3% (n = 30) for 30 mg subjects, and 100.4 ± 4.0% (n = 26) for 60 mg subjects. These data include combined results from both day 8 and day 15 samples, as the levels of quizartinib + AC886 were not significantly different between days 8 and 15 for either dose.

The median values at the 1:4 and 1:16 dilutions were significantly higher for subjects receiving 60 mg ($p <0.0001$). As the data for the 1:64 and 1:256 dilutions had normal, Gaussian distributions, they were analyzed using an unpaired t test for the mean values. Subjects receiving 60 mg quizartinib had higher mean inhibitory activity ($p < 0.0001$).

Regarding the PIA results in the THP-1 cells for FLT3-WT inhibition medians and means were significantly higher in the subjects receiving 60 mg in the undiluted, 1:4, and 1:16 groups ($p < 0.0001$). In the 1:64 dilution, there was still a significant difference, $p = 0.0021$. In the 1:256 dilution groups, there was no significant difference between the doses, as very low levels of inhibitory activity remained in the plasma at that dilution.

Another method to visualize and compare the differences between quizartinib activity on the three different kinases tested, as well as the differences in drug activity between the two doses, was to plot the mean inhibition versus the inverse plasma dilution, or titer, to determine the IT50, which is the plasma titer that results in 50% inhibition of kinase activity. For subjects receiving 30 mg, the median IT50 in the ITD cells was 23.2 (range 7.3-45.9, n = 30) (inverse dilution). For subjects receiving 60 mg, the median IT50 in the ITD cells was significantly higher ($p < 0.0001$, unpaired t test) at 52.9 (range 19.1-135.5, n = 26), demonstrating the expected 2-fold difference in inhibitory activity in subjects receiving 2-fold higher amounts of drug. For FLT3-WT in THP-1 cells, the mean IT50s were 4.8 (range 3.0-8.0, n = 26) and 7.4 (range 3.7-13.8, n = 26) for the 30 and 60 mg doses, respectively. KIT inhibitory activity was weaker at these doses of quizartinib. Undiluted plasmas had a mean inhibition of 43.3 ± 16.1% and 63.9 ± 14.1% at 30 and 60 mg, respectively, so IT50s could only be calculated for one subject at 30 mg and 10 subjects at 60 mg. Of note, for subjects receiving 60 mg of quizartinib, a nearly 53-fold average dilution of plasma is required to reach 50% inhibitory activity of FLT3-ITD cells, while only a 7.4-fold average dilution, and 3.4-fold average dilution are required to reach the same level of inhibition in the FLT3-WT and KIT cells.

As the PIA assay is a functional PK assay, it was verified that the resulting IT50s correlated with the measured PK levels in each plasma. Linear regression analysis shows statistically significant correlation for all assays, with Spearman correlation $p < 0.001$ for FLT3-ITD and FLT3-WT assays, and $p < 0.048$ for the KIT assay.

The data analysis included an examination of the IC90 (plasma concentration of quizartinib + AC886 that resulted in a 90% inhibition of kinase activity) compared to the measured plasma concentrations. The IC90s were 182 nM in the FLT3-ITD cells, 1160 nM in the FLT3-WT cells, and 3977 nM in the KIT cells. For subjects receiving 30 mg of quizartinib, median plasma concentrations were 382 nM at day 8, and 441 nM at day 15, well above the 182 nM FLT3-ITD IC90. For subjects receiving 60 mg of quizartinib, PK values were 808 nM and 1097 nM at days 8 and 15, respectively, which are equivalent to 4-fold and 6-fold above the FLT3-ITD IC90, respectively. Subjects receiving 30 or 60 mg of quizartinib had high levels of drug in their plasma at days 8 and 15 to inhibit cellular activity of FLT3-ITD by at least 90%. In contrast, average levels of drug in the plasma from subjects receiving 30 mg were below the 1160 nM IC90 for inhibition of FLT3-WT, while subjects receiving 60 mg were at or just below the IC90. In both dose groups, plasma drug levels were well below the IC90 for inhibition of KIT activity.

- *Exposure effect relationships*

Exposure-response/effect analyses for quizartinib in RR AML consisted of description of the relationship between quizartinib and AC886 (active metabolite) exposure and OS and QT interval corrected using Fridericia's formula (QTcF).

The Kaplan-Meier curves of the survival, by quartiles of quizartinib, AC886, and quizartinib + AC886 exposure (Cmax, AUC or Ctrough) based on 238 patients from Study AC220-007 suggested a lower probability of survival (higher risk of death) for subjects in the lowest quartile of exposure to quizartinib compared to the other quartiles of exposure which exhibited considerable overlap in the survival probability over time. This effect was not present for the active metabolite for which the four quartiles exhibited considerable overlap in the survival probability over time (Figure 7).

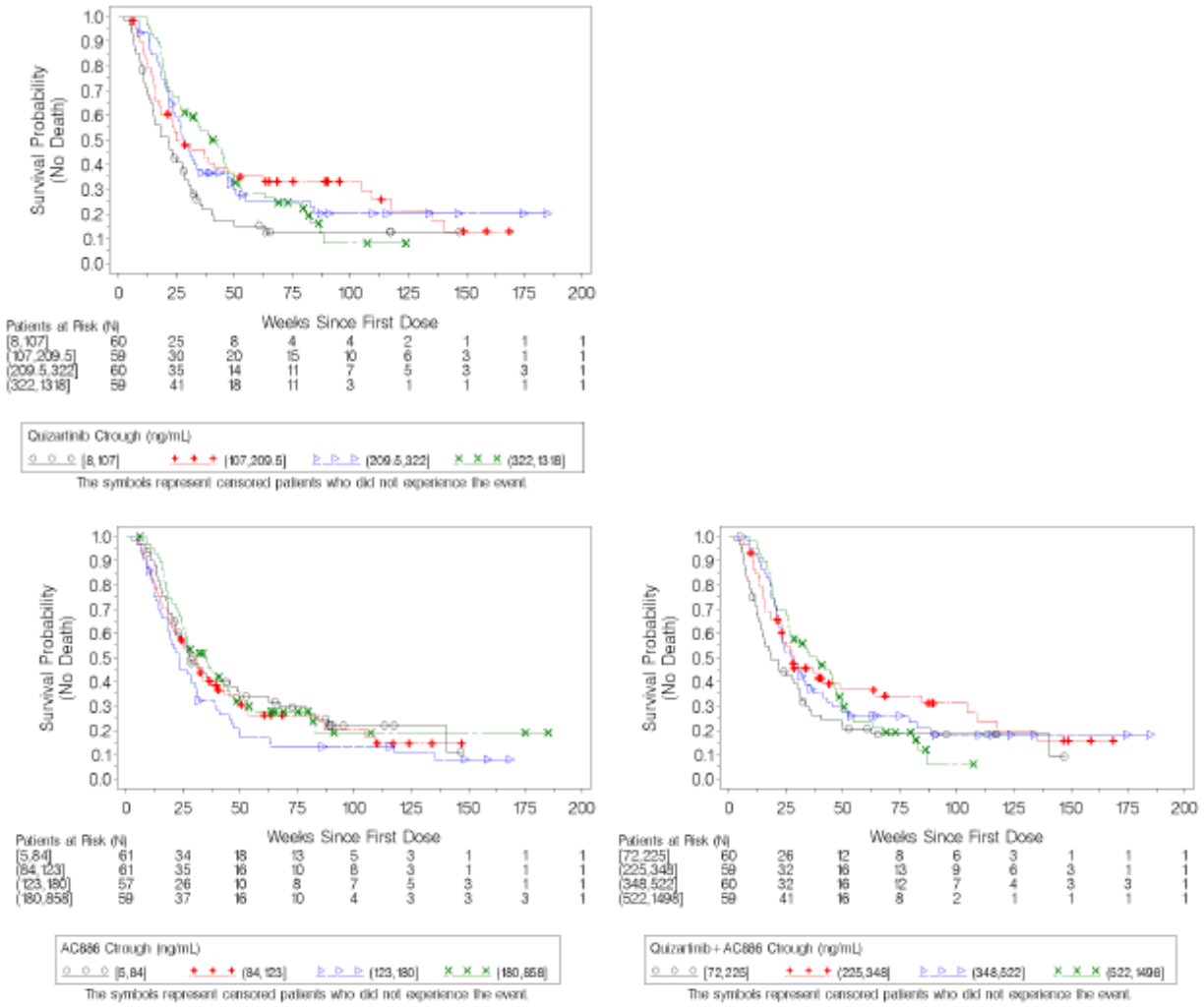
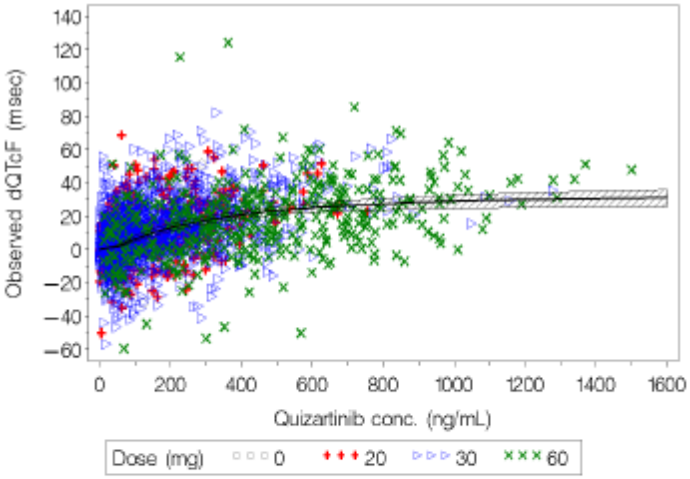
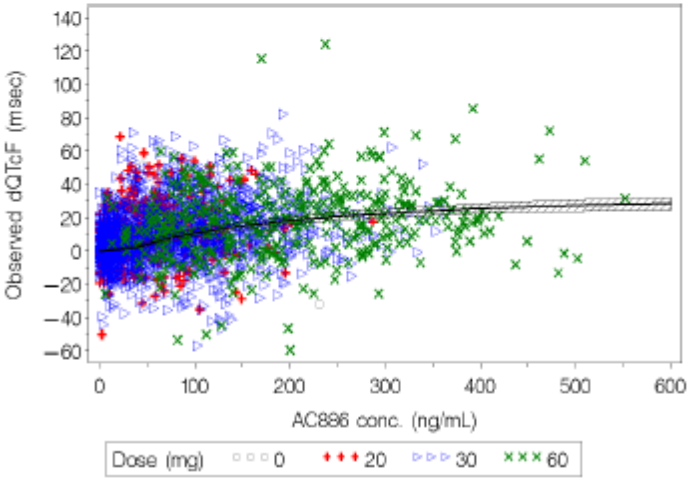


Figure 7 Kaplan-Meier plots of survival probability versus weeks since first dose, by quartiles of exposure for overall survival analysis

Regarding the effect of exposure on QTcF prolongation the relationship between plasma exposure to quizartinib and the active metabolite (AC886) and ΔQTcF was analysed (Figure 8). In this model, hypokalaemia was identified as a significant covariate on baseline QTcF, but not on ΔQTcF. Quizartinib had a more dominant effect on QTcF when compared to AC886. Based on the exposure- ΔQTcF relationship and the popPK model it was calculated that changing the QTcF cut-off values for dose escalation from ≤ 450 ms to ≤ 470 ms would result in a 0.36% increase in patients who get a dose interruption (from 1.14 % at ≤ 450 ms to 1.50 % ≤ 470 ms), while it would allow for a dose escalation in 91.3% of subjects when compared to 67.7% when the cut off would remain at ≤ 450 ms.



The solid line represents the model–predicted median drug effect. Shaded area represents the 90% uncertainty around median drug effect predictions. Predicted dQTcF represents contributions of Quizartinib and AC886.



The solid line represents the model–predicted median drug effect. Shaded area represents the 90% uncertainty around median drug effect predictions. Predicted dQTcF represents contributions of Quizartinib and AC886.

Figure 8. Scatterplots of ΔQTcF versus quizartinib and AC886 concentrations, overlaid with model-predicted mean and 90% confidence interval of the mean based on final QTcF model (AC220-007)

No clinical secondary pharmacology studies have been conducted with quizartinib (see discussion on clinical pharmacology).

2.4.4. Discussion on clinical pharmacology

The administration of quizartinib with food decreased quizartinib Cmax by 8% and increased AUCinf by 8%. The Tmax was delayed by two hours. These changes in exposure are not considered clinically meaningful. *In vitro* binding of quizartinib and AC886 to human plasma proteins is greater than or equal to 99%. Quizartinib and AC886 partition into red blood cells showed a blood to plasma ratio of 1.3 and 2.8, respectively. The apparent volume of distribution estimated for the central compartment (Vc/F) from the population pharmacokinetic analysis in AML subjects was 276 L.

Statistical analyses of the PK parameters Cmax, AUClast, and AUCinf of quizartinib and AC886 using the power model approach showed that the slopes were all close to unity and the 95% CIs for the estimations

included unity, indicating that the exposure to quizartinib and AC886 was dose proportional for the tested dose range.

Age (18 to 81 years), race, sex, body weight, or renal impairment (eGFR 30 to 89 mL/min/1.73 m2) did not have a clinically meaningful effect on quizartinib and AC886 exposure based on a population pharmacokinetic analysis.

Concomitant use of quizartinib with a strong CYP3A inhibitor increased the exposure of quizartinib and its active metabolite AC886 compared to the use of quizartinib alone in healthy volunteers. Increased quizartinib exposure may increase the risk of toxicity. There were no clinically meaningful changes to quizartinib exposure with co administration of a moderate CYP3A inhibitor in healthy subjects (200 mg fluconazole twice daily for 28 days co administered with a single dose of quizartinib). Concomitant use of quizartinib Vanflyta with strong CYP3A inducers decreased the exposure of quizartinib and its active metabolite AC886 compared to the use of Vanflyta alone based on a physiologically based pharmacokinetic (PBPK) analysis. Decreased quizartinib exposure may lead to reduced efficacy. The effect of moderate CYP3A inducers was not assessed. No clinical study with CYP3A inducers was conducted.

In vitro studies showed that quizartinib is a potential P gp inhibitor at maximal intestinal concentrations. Co-administration of quizartinib with a P gp substrate (e.g., digoxin, dabigatran) may increase the concentration of the P gp substrate. No clinical interaction study with a P gp substrate was conducted. There were no clinically meaningful changes to quizartinib exposure with co administration of a gastric acid reducing agent. However the CHMP agreed that the clinical relevancy of P-glycoprotein inhibition by quizartinib needs to be further studied. The potential increased toxicity due to drug-drug interaction with is reflected in the RMP and a study to evaluate the effect of quizartinib on the PK of dabigatran should be conctucted.

According to the results from study AC220-002, the inhibitory effect of quizartinib on the FTL3 RTK has been confirmed. Quizartinib has shown a decrease in the concentration of phosphorylated FTL3 and intermediate compounds generated in the pathway downstream, as STAT5. Higher inhibition has been observed in FLT3-ITD mutated population compared to FLT3-ITD non-mutated population. In pharmacodynamic studies, quizartinib has demonstrated an inhibition of FLT3 at high doses (200mg, 135mg & 90mg). The pharmacodynamic studies have been performed to show the inhibition of FLT3 at low doses, which were the selected doses for the Phase III study. These doses have shown a clinical effect that is comparable to the results obtained in the exploratory studies. Nevertheless, this effect might not be only attributable to the FLT3 inhibition but also to additional RTK inhibition.

Exposure-response analysis suggested a lower probability of survival for subjects in the lowest quartile of exposure to quizartinib compared to the other quartiles of exposure which exhibited considerable overlap in the survival probability over time. This effect was not present for the active metabolite. Regarding the effect of exposure on QTcF prolongation there is a clear relationship between quizartinib and AC886 exposure and prolongation of QT interval corrected by Fridericia's formula (QTcF). This relationship is best described as a (non-linear) sigmoid model. Quizartinib had a more predominant effect on QTcF when compared to AC886. However, due to uncertainties in the exposure modelling and the large inter-individual variability the interpretation of the exposure-efficacy relationship is difficult.

No clinical secondary pharmacology studies have been performed. However, non-clinical *in vitro* studies showed that also the kinases CSF1R/FMS, PDGFRα, PDGFRβ and RET are strongly inhibited by quizartinib and its metabolite AC866. At a 60 mg/day quizartinib dose, the Ctrough at day 15 is 0.48 µM, which gives at 99% protein binding an effective concentration of about 5 nM. This is well above the IC50 of quizartinib and also in the range of the Kd of KIT-inhibition and of these other kinases by quizartinib. KIT has been investigated clinically and found to be inhibited slightly but significantly by the intended dose of quizartinib. Therefore, these kinases may also be affected clinically by quizartinib.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology aspects of quizartinib have been reasonably well investigated.

2.5. Clinical efficacy

2.5.1. Dose response studies

The dosing regimen of quizartinib selected for the phase 3 study was based on data from one phase 1 and two phase 2 clinical studies in relapsed or refractory subjects with AML. In these studies, quizartinib was not resumed post-HSCT.

A phase 1dose-escalation study (CP0001) was conducted in patients with relapsed or refractory AML (median of ≥3 lines of therapy) or previously untreated AML who were unsuitable for induction chemotherapy. Patients were included regardless of FTL3-ITD status. A total of 76 subjects received quizartinib monotherapy in the study; 51 were treated on an intermittent dosing (ID) schedule (14 days on treatment followed by 14 days off treatment) of quizartinib starting at 12 mg/day up to a maximum of 450 mg/day, and the remaining 25 subjects received quizartinib 200 or 300 mg/day according to a continuous dosing (CD) schedule for 28 days (1 cycle). The MTD was 200 mg/day CD, and the DLT at this dose was Grade 3 QT interval prolongation. Best disease response of any type of CR (2 CR, 3 CRp, 5 CRi) was observed in 13.2%, and PR in 17.1%. Complete responses of any type were observed at doses as low as 40 mg ID. At least 1 PR was reported for each dose cohort except at the lowest dose of 12 mg. Response rates were higher in FLT3-ITD (+) (allelic ratio >10%) patients compared to FLT3-ITD (-) patients (52.9% vs 13.5%).

A phase 2 study (AC220-002) in patients with relapsed or refractory AML investigated quizartinib monotherapy in two cohorts. Cohort 1 included subjects 60 years of age or older who relapsed within 1 year after first line chemotherapy or were refractory to first-line chemotherapy (subjects with prior HSCT excluded). Cohort 2 included subjects 18 years of age or older who were relapsed or refractory after 1 second line (salvage) regimen or after HSCT. Patients were included regardless of FLT3-ITD status. Based on the results of study CP0001 subjects were initially dosed at 200 mg/day starting dose. The protocol was subsequently amended to starting quizartinib doses of 135 mg/day (males) and 90 mg/day (females) as the 200 mg/day dose was associated with a high rate of QTcF prolongation (35.3%). The differential dose between females and males was based upon exploratory PK analysis. The primary endpoint of CRc (CR+CRp+CRi) was achieved by 49.2% FLT3-ITD (+) subjects: 54.5% subjects in Cohort 1 and 44.9% subjects in Cohort 2. CRc was achieved by 28.6% FLT3-ITD (-) subjects: 29.5% in Cohort 1 and 27.5% in Cohort 2. Median OS for FLT3-ITD (+) subjects was approximately 25 weeks for both cohorts. For the FLT3-ITD (-) subjects in Cohort 1, the median OS was 19.1 weeks and in Cohort 2, the median OS was 25.1 weeks. The frequency of QTcF >500 ms was 15.1% to 17.3% with 90 mg/day to 135 mg/day. There was 1 event of Grade 4 QTcF prolongation/Torsade de pointes in a female subject with atrial fibrillation receiving 90 mg of quizartinib, which resolved after treatment discontinuation.

Study 2689-CL-2004 was a Phase 2, open-label, randomized study of quizartinib monotherapy in adult subjects (aged ≥18 years) with morphologically documented primary AML or AML secondary to MDS. Subjects were required to be refractory to or have relapsed after second-line AML therapy or after HSCT and were required to have FLT3-ITD positive AML (allelic ratio >10%). Subjects were randomly assigned to receive a quizartinib starting dose of either 30 mg/day or 60 mg/day. Dose escalation from 30 mg/day to 60 mg/day or from 60 mg/day to 90 mg/day was permitted in subjects who did not achieve CR, CRp, or CRi by the end of Cycle 1 (ie, Day 28), or in those who achieved a response (CR, CRp, CRi, or PR) and subsequently relapsed. Subjects received quizartinib oral solution or tablets daily in 28-day cycles.

Treatment with quizartinib continued until the subject no longer received clinical benefit from therapy or until unacceptable toxicity occurred. Subjects who underwent HSCT did not resume quizartinib treatment after the transplant.

The co-primary objectives were to evaluate the CRc rate (CR + CRp + CRi) and the rate of Grade ≥2 QTcF prolongation (>480 ms). Seventy-six subjects were enrolled. Median age was 54.5 years (range: 19 years to 77 years). Baseline characteristics in the 2 treatment groups were balanced except that 18.4% of subjects in the 30 mg arm had an allelic ratio of >50% compared with 44.7% in the 60 mg arm. Seventy-four subjects (30 mg arm, N = 38; 60 mg arm, N = 36) received at least 1 dose of quizartinib. Twenty-four (63.2%) subjects in the 30 mg arm had their dose of quizartinib escalated to 60 mg/day, while 7 (19.4%) subjects in the 60 mg arm had their dose of quizartinib escalated to 90 mg/day. The CRc rate was 47.4% in each treatment arm. Trends toward longer OS (median: 27.3 weeks versus 20.9 weeks) and duration of CRc (median: 9.1 weeks versus 4.2 weeks) were observed in the 60 mg arm versus the 30 mg arm. The transplant rate was 42.1% in the 60 mg arm compared with 31.6% in the 30 mg arm. In addition, fewer subjects in the 60 mg arm required dose escalation. A post-hoc analysis showed that 5 of 23 subjects in the 30 mg arm achieved CRc following escalation to 60 mg/day compared with 0 of 5 subjects in the 60 mg arm who had their dose escalated to 90 mg.

Treatment post-HSCT

Limited data is available on treatment post-HSCT. One phase 1 study (2689-CL-0011) studied quizartinib as post-HSCT therapy for subjects with FLT3-ITD mutated AML in first remission following HSCT enrolled 13 subjects in 2 cohorts that received 40 or 60 mg quizartinib as a starting dose. A total of 10 of the 13 subjects received more than 1 year of maintenance and were alive at the end of the study. OS ranged from approximately 13 weeks to 142 weeks. Literature data are available on maintenance treatment with a tyrosine kinase inhibitor (TKI) following HSCT with FLT3 inhibitors (sorafenib, midostaurin, and crenolanib) in AML (12).

2.5.2. Main study

Study AC220-007

Methods

Study AC220-007 was a phase 3 open-label randomized study of quizartinib monotherapy versus salvage chemotherapy in subjects with FLT3-ITD positive acute myeloid leukaemia refractory to or relapsed after first-line treatment with or without haematopoietic stem cell transplantation (HSCT) consolidation.

Study Participants

Inclusion Criteria

The main inclusion criteria were:

1. Provision of written informed consent approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) with privacy language in accordance with national regulations (e.g., HIPAA authorization for US sites) prior to any study-related procedures, including withdrawal of prohibited medications if applicable.

2. Age ≥18 years at the time of informed consent.

3. Morphologically documented primary AML or AML secondary to myelodysplastic syndrome (MDS), as defined by World Health Organization criteria, as determined by pathology review at the study site.

4. Refractory or relapsed AML after first-line therapy, with or without HSCT. First-line therapy can consist of 1 or 2 induction blocks, and must have included at least 1 cycle of an anthracycline/mitoxantrone-containing induction block at a standard dose.

• Refractory to first-line therapy is defined as: o After 1 cycle, lack of achievement of CR, CRp, or CRi and a reduction in bone marrow blasts of less than 50%.

o After 2 cycles, lack of achievement of CR, CRp, or CRi

• Relapse within 6 months or less after first-line therapy is defined as (all criteria must be met):

o Achievement of CR, CRi, or CRp, as defined by 2003 International Working Group criteria after initial AML therapy with or without consolidation or maintenance, and with or without HSCT as consolidation
o Duration of CR, CRi or CRp is measured from the date of the bone marrow assessment which confirmed response to the date of the bone marrow assessment that identified relapse or the appearance of peripheral blasts.

5. Presence of the FLT3-ITD activating mutation in bone marrow or peripheral blood (allelic ratio as determined by a central laboratory with a cutoff of >3% FLT3-ITD/total FLT3).

6. Eligibility for pre-selected salvage chemotherapy, according to the Investigator's assessment.

7. ECOG performance score 0-2.

8. Discontinuation of prior AML treatment before the start of study treatment (except hydroxyurea, which is permitted for blast control up to the day of starting study treatment) for at least 2 weeks for cytotoxic agents, or for at least 5 half-lives for non-cytotoxic agents.

9. Serum creatinine $\leq 1.5 \times$ upper limit of normal (ULN), or glomerular filtration rate >25 mL/min/1.73m2, as calculated with the modified Cockcroft-Gault formula.

10. Serum potassium, magnesium, and calcium (serum calcium corrected for hypoalbuminemia) within institutional normal limits. Subjects with electrolytes outside the normal range will be eligible if these values are corrected upon retesting following any necessary supplementation.

11. Total serum bilirubin $\leq 1.5 \times$ ULN.

12. Serum aspartate transaminase (AST) and/or alanine transaminase (ALT) $\leq 2.5 \times$ ULN.

Exclusion Criteria

The main exclusion criteria were:

1. Acute promyelocytic leukemia (AML subtype M3).

2. AML secondary to prior chemotherapy for other neoplasms, except AML secondary to prior MDS.

3. History of another malignancy, unless the candidate had been disease-free for at least 5 years.

- Subjects with treated nonmelanoma skin cancer, carcinoma in situ, or cervical intraepithelial neoplasia were eligible regardless of the time spent disease-free, if they had completed definitive treatment.
- Subjects with organ-confined prostate cancer, with no evidence of recurrent or PD, were eligible if hormonal therapy had begun, or if the tumor had been surgically removed or treated with definitive radiotherapy.

4. Persistent, clinically significant >Grade 1 nonhematologic toxicity from prior AML therapy.

5. Clinically significant GVHD or GVHD requiring initiation of treatment or treatment escalation within 21 days, and/or greater than Grade 1 persistent or clinically significant non-hematologic toxicity related to HSCT.

6. History of or current central nervous system involvement with AML.

7. Clinically significant coagulation abnormality, such as disseminated intravascular coagulation.

8. Prior treatment with quizartinib or participated in a prior quizartinib study.

9. Prior treatment with a FLT3 targeted therapy including sorafenib or investigational FLT3 inhibitors (not including the multikinase inhibitor, midostaurin).

12. Uncontrolled or significant cardiovascular disease, including the following:

-QTcF interval >450 ms (average of triplicate determinations).

-Subject had bradycardia of less than 50 beats per minute (bpm; as determined by central reading) unless the subject had a pacemaker.

-Diagnosed or suspected long interval between the start of the Q wave and the end of the T wave (Long QT syndrome or LQTS) or known family history of LQTS.

-History of clinically relevant ventricular arrhythmias, such as ventricular tachycardia, ventricular fibrillation, or Torsade de pointes.

-History of second- or third-degree heart block. Candidates with a history of heart block were eligible if they had pacemakers and had no history of fainting or clinically relevant arrhythmia with pacemakers.

-Myocardial infarction within 6 months prior to screening.

-Uncontrolled angina pectoris within 6 months prior to screening.

-New York Heart Association class 3 or 4 congestive heart failure.

-Left ventricular ejection fraction ≤45% or institutional lower limit of normal.

-Uncontrolled hypertension.

-Complete left or right bundle branch block.

Treatments

Subjects were randomized to receive either quizartinib (20 or 30 mg tablets administered orally once daily) or salvage chemotherapy (administered subcutaneously [LoDac] or intravenously [MEC and FLAG-IDA]).

Quizartinib treatment

The starting dose was 30 mg/day unless the subject was receiving concurrent therapy with a strong CYP3A inhibitor, in which case the starting dose was 20 mg/day. The dose of quizartinib was increased from 30 mg/day to 60 mg/day or from 20 mg/day to 30 mg/day (CYP3A inhibitor) starting on Day 16 (± 1 day) if the subject's average QTcF, based on triplicate reading, was ≤450 ms on and before Day 15 (±1 day).

Dose escalations were allowed for subjects who fail to achieve a CR, CRp or CRi after at least one 28-day cycle of therapy and not receiving the maximum dose may undergo dose escalation providing the following criteria are met: The subject has not had a Grade 3 or higher, non-hematologic, and related

adverse event (AE); No increase in QTcF more than 60 msec above baseline; The subject must not have aplastic bone marrow at the time of the proposed dose escalation.

In addition, subjects who achieved a response (CR, CRi, CRp, or PR) at any time and who subsequently relapsed may undergo dose escalation provided they meet the same criteria as above.

Dose reductions were based on the following criteria: Initiation of treatment with strong CYP3A inhibitor, QTcF prolongation, non-haematologic toxicity, or myelosuppression.

If a subject undergoes HSCT, quizartinib should be discontinued 7 days before the start of a conditioning regimen. Treatment with quizartinib may be resumed at 30 to 100 (+ 7) days after the transplant. Quizartinib may be restarted if:

- Subject has an absolute neutrophil count (ANC) >1 x 10^9/L and platelet count >50 $\times$ 10^9/L without platelet transfusion support within 1 week, or a platelet count >25 $\times$ 10^9/L without platelet transfusion support within 2 weeks prior to first dose.
- Subject does not have (1) active acute, or ≥Grade 3 graft versus host disease (GVHD) or (2) active GVHD therapy (not prophylaxis) initiation within 21 days.

The starting dose of quizartinib post-HSCT should be 30 mg daily or 20 mg daily in case of treatment with a strong CYP3A inhibitor. Subjects will dose escalate starting on Day 16 if the QTcF interval is less or equal to 450 msec prior to or at the day 15 ECG evaluations.

Salvage treatment

The investigator pre-selected the specific salvage chemotherapy regimen before randomization of each subject. All salvage chemotherapy was administered during 28-day cycles. The start of Cycle 2 (MEC, FLAG-IDA, or LoDAC) and subsequent cycles (LoDAC) could be delayed for up to 14 days to allow for recovery from toxicity. Dose reductions were allowed for toxicity and were documented in the source record. Subjects on salvage chemotherapy, including those that underwent HSCT, were followed on study; no cross-over to investigational study drug was planned or allowed.

Low Dose Cytarabine (LoDAC) Cytarabine (20 mg) was administered twice daily by subcutaneous injection for 10 days (Days 1 through 10) over continuous 28-day cycles. A delay of up to 14 days between cycles is allowed for recovery from toxicity.

MEC Chemotherapy Mitoxantrone (8 mg/m2/day) was administered by 5 minute intravenous (IV) injection for 5 days (Days 1 through 5); Etoposide (100 mg/m^2/day) was administered by 1 hour IV infusion immediately after mitoxantrone for 5 days (Days 1 through 5); Cytarabine (1000 mg/m^2/day) was administered by 1 hour IV infusion immediately after etoposide for 5 days (Days 1 through 5).

FLAG-IDA Chemotherapy G-CSF (300 μg/m2/day) was administered by 2-hour IV infusion for 5 days (Days 1 through 5) or alternatively, G-CSF (5 μg/kg/day) was administered SC for 5 days (Days 1 through 5). Additional G-CSF is recommended 7 days after the completion of chemotherapy, until ANC is >0.5$\times$$10^9$/L; Fludarabine (30 mg/$m^2$/day) was administered by 30 minute IV infusion for 5 days (Days 2 through 6); Cytarabine (2000 mg/m^2/day) was administered by 4 hour IV infusion, beginning 4 hours after the fludarabine infusion, for 5 days (Days 2 through 6); Idarubicin (10 mg/m^2/day) was administered over 5 to 10 minutes in a fast-running saline drip for 3 days (Days 2 through 4).

Duration of treatment

In subjects receiving quizartinib or LoDAC, treatment should continue until there was no longer clinical benefit from therapy, or until unacceptable toxicity occurs.

Subjects receiving MEC or FLAG-IDA were received 1 cycle of therapy and were assessed for response on Day 15. If bone marrow cellularity was 20% or greater with at least a 50% reduction in blasts, subjects could receive a second cycle of the same therapy. If marrow cellularity was 5% or less, subjects should

be observed for recovery. Subjects achieving complete remission (CR), complete remission with incomplete hematologic recovery (CRi), or complete remission with incomplete platelet recovery (CRp) (per Investigator assessment) could receive a second cycle of the same therapy at the Investigator's discretion. Treatment should be discontinued if there is no response (NR) or progressive disease (PD).

Objectives

The primary objective of the study was to determine whether quizartinib monotherapy prolonged OS compared to salvage chemotherapy in subjects with FLT3-ITD mutation-positive (FLT3-ITD [+]) AML who were refractory to or had relapsed within 6 months after first-line AML therapy.

The secondary objective was to determine event-free survival (EFS) following treatment with quizartinib vs salvage chemotherapy.

Key exploratory objectives included:

- To compare the composite CR (CRc = CR + CR with incomplete platelet recovery [CRp] + CR with incomplete hematologic recovery [CRi]) rate
- To compare the CR rate
- To compare the duration of CRc
- To compare the duration of CR
- To determine leukemia-free survival (LFS)
- To compare the transplant rate of quizartinib to salvage chemotherapy
- To determine the corrected interval between the start of the Q wave and the end of the T wave (QT) [QTc] prolonging effects of quizartinib in relation to plasma drug concentrations

Outcomes/endpoints

The primary efficacy endpoint was overall survival (OS), defined as the time between the date of randomization and the date of death from any cause. OS will be censored at the last date when subjects were known to be alive.

The secondary efficacy endpoint was event free survival (EFS), defined as the time from randomization until documented refractory disease, relapse after CRc, or death from any cause, whichever occurred first. Subjects alive without treatment failure or relapse or lost to follow-up at the time of analysis were censored at the date of their last response assessment.

Exploratory efficacy variables included the following:

- Leukemia-free survival defines as the time from the first documented best response of CRc (CR, CRp, or CRi) until documented relapse or death from any cause. Subjects alive without relapse or lost to follow-up at the time of analysis were censored at the date of their last response assessment.
- Composite complete remission (CRc) rate defines as the percent of subjects achieving a best response of CR, CRp, or CRi.
- Complete remission (CR) rate defined as the percent of subjects achieving CR.
- Duration of CRc defined as the time from the first documented CRc (CR + CRp + CRi) until documented relapse. Subjects alive without relapse, lost to follow-up, or who have died without report of relapse as of the time of analysis will be censored at the date of their last response assessment.

- Duration of CR defined as the time from the first documented CR until documented relapse. Subjects alive without relapse, lost to follow-up, or who have died without report of relapse as of the time of analysis will be censored at the date of their last response assessment.
- Transplantation rate (bridge to transplant) defined as the percent of subjects undergoing HSCT directly following protocol specified treatment with no intervening AML therapy.

Sample size

Calculation of sample size was based on comparison of OS, the primary efficacy endpoint, in the 2 treatment arms (quizartinib and salvage chemotherapy) at a 2-sided significance level of 0.05 with log-rank test, assuming that median survival was 3.9 months in the salvage chemotherapy arm with an increase to 6 months, in the quizartinib arm (hazard ratio 0.65). A total of 280 events (deaths) were necessary to meet a power requirement of 90%, given an interim analysis planned at 140 events (deaths) with O'Brian-Fleming boundary for superior efficacy and a conditional power of 10% for futility. For the purpose of sample size calculation, subjects were assumed to be accrued at a rate of 19.2 per month. "Dropouts" were considered to be subjects for whom no primary outcome data is available. The dropout rate was assumed to be 10%. The target accrual was a total of approximately 363 subjects randomized in a 2:1 ratio (242 subjects in the quizartinib monotherapy group and 121 subjects in the salvage chemotherapy arm) over 17 months.

Randomisation

Subjects were randomized via the interactive response technology (IRT) system into the 2 treatment arms in a 2:1 ratio (245 quizartinib and 122 salvage chemotherapy) using a permuted block size of 6. Randomization was stratified according to 2 factors (6 total strata). Randomization was stratified by prior therapy and response and pre-selected salvage chemotherapy, without regard to investigative site (randomization schedules were centralized): The randomization implemented stratifications to minimize the impact of the following 2 potential prognostic factors on efficacy results:

-Prior therapy and response:

- Relapsed in ≤6 months (not post-HSCT)
- Refractory
- Relapsed in ≤6 months post-allogeneic HSCT

-Pre-selected chemotherapy, even for subjects subsequently randomized to quizartinib:

- High-intensity chemotherapy (MEC; FLAG-IDA)
- Low-intensity chemotherapy (LoDAC)

Blinding (masking)

This was an open-label study.

Statistical methods

The Intent-to-treat (ITT) analysis set included all subjects who are randomized and will be classified according to the treatment to which they were randomized. The per-protocol analysis set (PPS) included all subjects in the ITT analysis set who have no major protocol violations that would affect assessment of efficacy endpoints. The PPS was used for supportive analysis of efficacy in this study. Subjects will be analyzed based on the treatment assigned from the randomization. Major protocol violations were

defined and documented prior to the unblinding of long-term survival data in the database to the Daiichi Sankyo clinical team. In addition, subjects who were randomized but did not receive any dose of study treatment and subjects who had negative FLT3-ITD test result were excluded from PPS.

The safety analysis set included all subjects who received at least 1 dose of the study drug. Subjects were classified according to their actual treatment received (as treated).

The ITT analysis set was used for the analysis of all efficacy endpoints. Supporting analyses were conducted with the PPS Analysis Set, as specified.

There were 2 planned cut-off dates for efficacy analyses: 1 interim analysis, when approximately 50% [140 of 280] of the expected number of events [deaths] had occurred, and a final analysis at 280 OS events. The stopping criteria for efficacy are based on the α- spending function methodology of Lan-DeMets family with O'Brien-Fleming parameters. At the interim analysis of OS, the trial would be stopped to reject null hypothesis if an observed z-statistic is less than -2.9626 (or 1-sided p value is less than 0.001525) if the interim analysis is based on exactly 140 events. The actual interim analysis was performed when 153 (55%) OS events were reported. The interim stopping boundary for OS was not crossed at that time and the DMC recommended for the study to continue without modification.

The primary analytical approach for the primary efficacy endpoint was a stratified logrank test performed at the overall 1-sided α=0.025 level, with 2 stratification factors used for randomization (total of 6 strata). The results from an unstratified log-rank analysis also were provided.

Sensitivity analyses included:

- OS censored at the start date of HSCT for subjects who undergo HSCT.
- OS censored at the start date of FLT3 inhibitors (Sorafenib, Gilteritinib, and Crenolinib) administered after discontinuation of study treatment.
- OS analyzed for the PPS analysis set.
- A landmark analysis of 13-week, 26-week, 52-week and 104-week survival after randomization was performed.
- The stratified and unstratified Cox models for OS were performed.
- If there are more than 5% of the patients untreated in either treatment group, a sensitivity analysis by re-sampling OS data for those untreated patients from the remaining patients in the same treatment group and re-estimating hazard ratio based on the complete set of re-sampled + remaining treated patients will be conducted for OS analysis after DBL.

Median OS was estimated for each treatment group from the 50th percentile of the corresponding Kaplan-Meier estimates, and the 95% confidence intervals (CI) for the median of each treatment group will be calculated using the method of Brookmeyer and Crowley.

Subgroup analyses

Analysis of OS and EFS were conducted for the subgroups defined according to the following: Age (<65, and ≥ 65 years); Sex at birth (male, and female); Race (White, Black or African American, Asian, and Other); Geographical region (North America, Europe and Australia, and Asia); Stratification factors from randomization (response to prior therapy, and preselected salvage therapy (low or high intensity), separately); FLT3-ITD allelic ratio (using central testing) at randomization (>50%, 25< and ≤50%, 3≤ and ≤25%, <3%); De novo AML, and Secondary AML; Prior allogeneic HSCT (yes vs. no); AML cytogenetic risk score (favourable, intermediate, unfavorable, and unknown risk); Blast count at baseline (< median, and ≥ median)

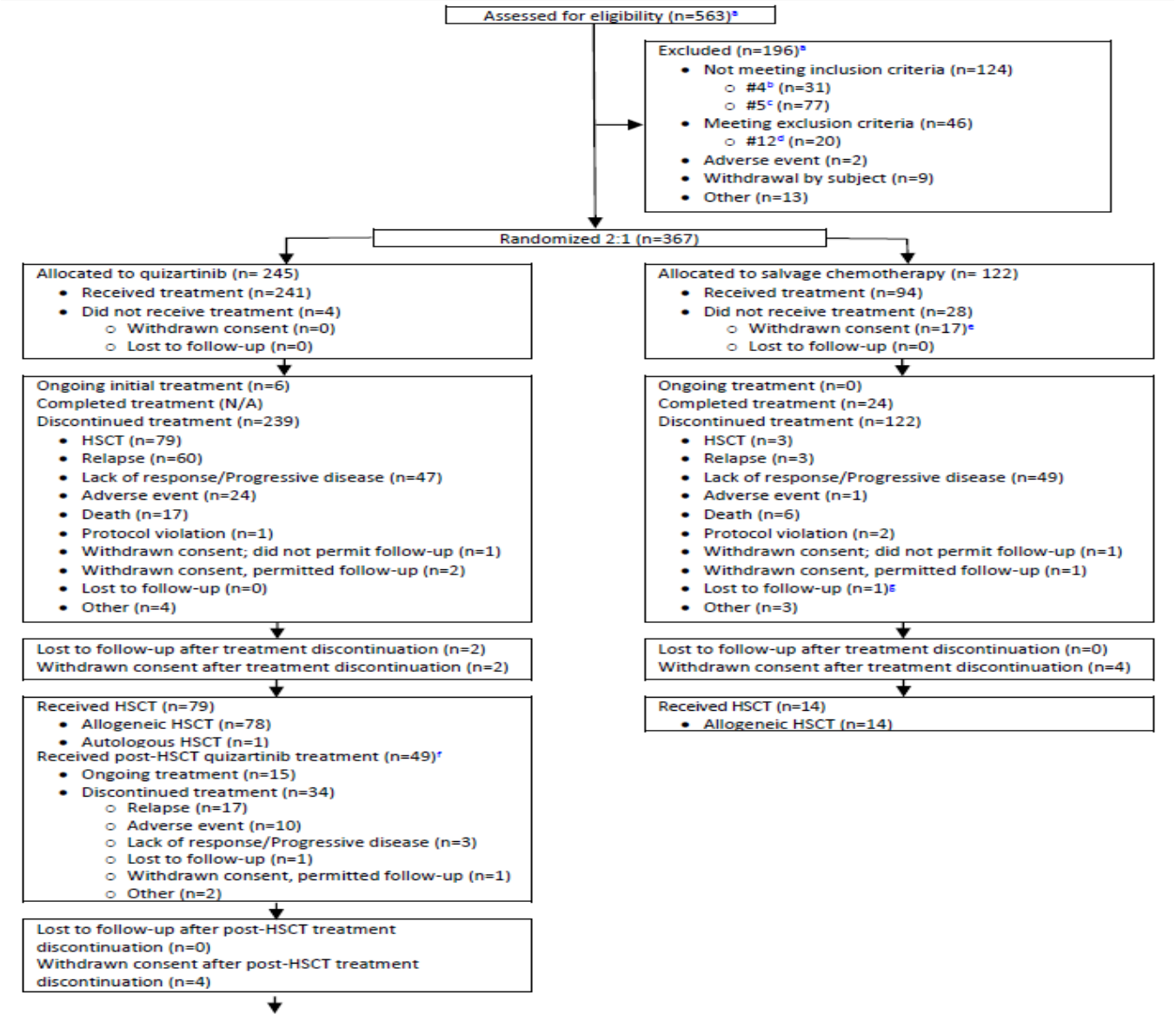
Multiplicity

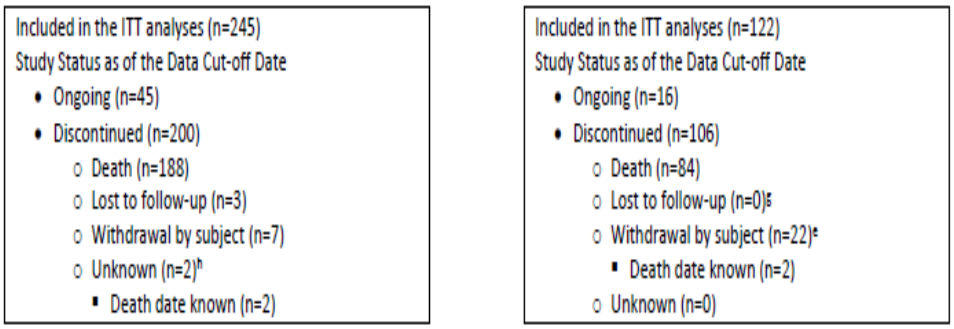
Per the protocol, the study utilizes a group sequential design with one interim analysis performed after 140 events. The Lan-DeMets spending function with the O'Brien-Fleming boundary was employed to control the overall Type I error of 1-sided 0.025 for the primary efficacy analysis of OS. Therefore, the nominal Type I error was 0.001525 at interim analysis and 0.0245 at the final analysis. The type I error spent at interim and final analysis were made using the pre-specified alpha spending function.

To ensure a study-wise Type 1 error rate of 0.025 (one-sided) accounting for the statistical test of the secondary efficacy endpoint, EFS, a hierarchical ("gatekeeping") testing procedure was employed. Thus, if the primary analysis of OS was statistically significant at interim or final analysis, the secondary endpoint of EFS was planned to be analyzed using the same boundaries and same nominal Type I error as those for the primary analysis at interim or final analysis.

Results

Participant flow





HSCT = hematopoietic stem cell transplantation; ITT = Intent-to-Treat; n = number of subjects in the category; N/A = not applicable.

[a] Two subjects were screened and randomized in error. They were randomized into the study using different subject IDs. They are counted twice in "Assessed for eligibility" category, and counted in both "Excluded" and "Randomized" categories.

[b] Inclusion criteria # 4. In first relapse (with duration of remission of 6 months or less) or refractory after prior therapy, with or without HSCT

[c] Inclusion criteria # 5. Presence of the FLT3-ITD activating mutation in bone marrow or peripheral blood

[d] Exclusion criteria #12. Uncontrolled or significant cardiovascular disease

[e] Two subjects on salvage chemotherapy withdrew consent but the date of death was known.

[f] Of the 49 subjects who received post-HSCT quizartinib treatment, one subject had undergone autologous HSCT and 48 subjects had HSCT.

[g] One subject on salvage chemotherapy was marked as lost to follow-up as the primary reason for discontinuing treatment, but date of death was known.

[h] Two subjects on quizartinib did not have an end of study CRF completed (referred to as "discontinued/unknown" in the figure), but date of death was known.

Recruitment

The study was conducted at 48 sites in Europe, 30 sites in North America, 11 sites in Asia, and 5 sites in Australia. The number of subjects enrolled at each site was as follows: Europe and Australia (195 subjects), North America (141 subjects), and Asia (31 subjects). The first subject first visit date was 7 May 2014; the data cut-off date was 22 February 2018.

Conduct of the study

The protocol (Original Version) was dated 18 November 2013. There were 7 protocol amendments: Amendment 1 dated 24 December 2013; Amendment 2 dated 26 May 2015; Amendment 3 dated 6 October 2015, Amendment 3.1 dated 19 November 2015; Amendment 4 dated 4 May 2016; Amendment 5 dated 15 August 2016; and Amendment 6 dated 30 June 2017. Protocol Amendment 1 dated 24 December 2013 was the first protocol disseminated to the IRBs and investigators. In Protocol Amendment 2, dated 26 May 2015, the FTL3-ITD allelic ratio cut-off was changed from 3% to ≥3%. Operationally, the definition of duration of remission was broadened to allow calculation in two ways: either from the date of a bone marrow assessment documenting CRc OR from the date of allogeneic transplant, depending on an individual subject's circumstances, and whichever was the later of the two.

For Protocol Amendment 3, dated 6 October 2015, sponsorship was changed from Ambit Biosciences to DSI. Following Protocol Amendment 4, dated 4 May 2016, sponsorship in Europe was transferred from Daiichi Sankyo Development Limited to DSI, in preparation of the closing of the UK Daiichi Sankyo Development Limited office in November 2016.

Major changes prior to the interim analysis included the following (Protocol Amendment 5, 15 August 2016):

- The adaptive design feature was removed. This adaptive design optionally would have increased the total number of events required for the final analysis from 280 to 406 (sample size from 326 to 473) if the interim analysis result fell within the so-called "promising zone". The sponsor changed this to

a traditional GSD with an interim analysis planned at 140 events (deaths) using O' Brian-Fleming boundary for superior efficacy and a conditional power of 10% for futility boundary.

- Planned enrollment was increased from 326 to 363 without changing the total number of planned events at 280 to allow for the number of events to be reached in a more reasonable timeframe.

Baseline data

Table 29 displays the demographic characteristics for all enrolled subjects.

Table 24 Demographics (ITT Analysis Set) - Study AC220-007

	Quizartinib Monotherapy	**Salvage Chemotherapy**	**Total**
Baseline characteristics	**N=245**	**N=122**	**N=367**
Age (Years)			
N	245	122	367
Mean (SD)	53.8 (14.19)	54.2 (14.87)	53.9 (14.40)
Median (Min-Max)	55.0 (19-81)	57.5 (18-78)	56.0 (18-81)
< 60 years	150 (61.2)	67 (54.9)	217 (59.1)
≥ 60 years	95 (38.8)	55 (45.1)	150 (40.9)
< 65 years	180 (73.5)	89 (73.0)	269 (73.3)
≥65 years to < 75 years	53 (21.6)	30 (24.6)	83 (22.6)
≥75 years	12 (4.9)	3 (2.5)	15 (4.1)
Gender - n (%)			
Male	113 (46.1)	64 (52.5)	177 (48.2)
Female	132 (53.9)	58 (47.5)	190 (51.8)
Race - n (%)			
White	184 (75.1)	93 (76.2)	277 (75.5)
Black or African American	9 (3.7)	3 (2.5)	12 (3.3)
Asian	24 (9.8)	16 (13.1)	40 (10.9)
Other	9 (3.7)	2 (1.6)	11 (3.0)
Unknown	19 (7.8)	8 (6.6)	27 (7.4)
BMI at baseline (kg/m^2)			
n	241	122	363
Mean (SD)	25.8 (5.99)	26.1 (6.63)	25.9 (6.21)
Median (Min-Max)	24.4 (15, 54)	24.5 (17, 65)	24.4 (15, 65)
Region - n (%)			
North America	100 (40.8)	41 (33.6)	141 (38.4)
Europe + Australia	127 (51.8)	68 (55.7)	195 (53.1)
Asia	18 (7.3)	13 (10.7)	31 (8.4)
ECOG performance status – n (%)			
0	87 (35.5)	47 (38.5)	134 (36.5)
1	131 (53.5)	54 (44.3)	185 (50.4)
2	27 (11.0)	21 (17.2)	48 (13.1)

The baseline disease characterises are displayed in **Table 30**.

Table 25. Baseline AML characteristics (ITT analysis set) - Study AC220-007

Parameter	Quizartinib Monotherapy (N = 245)	Salvage Chemotherapy (N = 122)	Total (N = 367)
Time from Diagnosis to Randomization (weeks)			
n	245	122	367
Mean (SD)	24.50 (14.224)	29.67 (33.662)	26.22 (22.703)
Median	23.43	23.21	23.29
Min, Max	3.7, 73.1	3.3, 312.3	3.3, 312.3
Antecedent Hematologic Disorders, n (%)			
No	229 (93.5)	113 (92.6)	342 (93.2)
Yes:	16 (6.5)	9 (7.4)	25 (6.8)
Type: MDS	15 (6.1)	7 (5.7)	22 (6.0)
Other	1 (0.4)	2 (1.6)	3 (0.8)
Blast Count (Bone Marrow) at Baseline			
n	243	120	363
Mean (SD)	62 (26.12)	63.9 (25.63)	62.7 (25.94)
Median	66.4	70.0	69.0
Min, Max	5, 99	6, 100	5, 100
<Median, n (%)	124 (50.6)	52 (42.6)	176 (48.0)
≥Median, n (%)	119 (48.6)	68 (55.7)	187 (51.0)
WHO Classification of AML with Recurrent Genetic Abnormalities, n (%)			
AML with mutated NPM1	115 (46.9)	57 (46.7)	172 (46.9)
AML with myelodysplasia-related changes	21 (8.6)	15 (12.3)	36 (9.8)
AML with t(6;9)(p23;q34); DEK-NUP214	6 (2.4)	4 (3.3)	10 (2.7)
AML with mutated CEBPA	5 (2.0)	5 (4.1)	10 (2.7)
AML with t(8;21)(q21;q22); RUNX1-RUNX1T1	3 (1.2)	2 (1.6)	5 (1.4)
AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1	3 (1.2)	0 (0.0)	3 (0.8)
Therapy-related myeloid neoplasms	1 (0.4)	1 (0.8)	2 (0.5)
AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1	0 (0.0)	1 (0.8)	1 (0.3)
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11	0 (0.0)	1 (0.8)	1 (0.3)
AML with t(9;11)(p22;q23); MLLT3-MLL	1 (0.4)	0 (0.0)	1 (0.3)
APL with t(15;17)(q22;q12); PML-RARA	0 (0.0)	0 (0.0)	0 (0.0)
AML, Not Otherwise Characterized, n (%)			
Acute myelomonocytic leukemia	58 (23.7)	16 (13.1)	74 (20.2)
AML minimally differentiated	42 (17.1)	16 (13.1)	58 (15.8)
AML with maturation	38 (15.5)	18 (14.8)	56 (15.3)
Acute monoblastic/acute monocytic leukemia	33 (13.5)	22 (18.0)	55 (15.0)
AML without maturation	30 (12.2)	10 (8.2)	40 (10.9)
Acute Erythroid Leukemia, n (%)			
Erythroleukemia, erythroid/myeloid	2 (0.8)	3 (2.5)	5 (1.4)
Acute megakaryoblastic leukemia	1 (0.4)	1 (0.8)	2 (0.5)
Myeloid sarcoma	1 (0.4)	1 (0.8)	2 (0.5)
Pure erythroid leukemia	0 (0.0)	0 (0.0)	0 (0.0)
Acute basophilic leukemia	0 (0.0)	0 (0.0)	0 (0.0)
Acute panmyelosis with myelofibrosis	0 (0.0)	0 (0.0)	0 (0.0)
Myeloid Proliferations Related to Down Syndrome, n (%)			
Blastic plasmacytoid dendritic cell neoplasm	1 (0.4)	0 (0.0)	1 (0.3)
Transient abnormal myelopoiesis	0 (0.0)	0 (0.0)	0 (0.0)
Myeloid leukemia associated with Down Syndrome	0 (0.0)	0 (0.0)	0 (0.0)
FAB Classification: Subtype, n (%)			
M1: AML with Minimal Differentiation	64 (26.1)	23 (18.9)	87 (23.7)
M4: Acute Myelomonocytic Leukemia	58 (23.7)	29 (23.8)	87 (23.7)

Parameter	Quizartinib Monotherapy (N = 245)	Salvage Chemotherapy (N = 122)	Total (N = 367)
M2: AML with Differentiation	54 (22.0)	27 (22.1)	81 (22.1)
M5: Acute Monoblastic Leukemia	37 (15.1)	18 (14.8)	55 (15.0)
M0: Acute Undifferentiated Leukemia	16 (6.5)	12 (9.8)	28 (7.6)
M6: Acute Erythroid Leukemia	2 (0.8)	1 (0.8)	3 (0.8)
M7: Acute Megakaryocytic Leukemia	1 (0.4)	1 (0.8)	2 (0.5)
M3: Acute Promyelocytic Leukemia	0 (0.0)	0 (0.0)	0 (0.0)
Risk Status with Specific Cytogenetic Patterns[a], n (%)			
Intermediate: normal, +8, +6, −y	191 (78.0)	81 (66.4)	272 (74.1)
Unknown risk	19 (7.8)	19 (15.6)	38 (10.4)
Unfavorable: del5q, −5, or del7q, −7, complex	23 (9.4)	14 (11.5)	37 (10.1)
Favorable: inv(16), t(16;16), or t(8;21), t(15;17)	12 (4.9)	8 (6.6)	20 (5.4)

AML = acute myeloid leukemia; APL = Acute promyelocytic leukemia; CEBPA = CCAAT enhancer-binding protein alpha; FAB = French-American-British; ITT = Intent-to-Treat; max = maximum; MDS = myelodysplastic syndrome; min = minimum; n = number of subjects in the category; N = population size; NPM1 = nucleophosmin 1; SD = standard deviation; WHO = World Health Organization.

[a] The predictive value of hierarchical cytogenetic classification in older adults with AML: analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 Trial. Grimwade D et al. Blood, 01 Sep 2001: 98:5. 1312-1320

Note: Denominator for percentages is the number of subjects in the ITTAnalysis Set.

NPM1 and CEBPA status was determined on the basis of local testing.

Numbers analysed

Table 26 Analysis sets (enrolled subjects)-Study AC220-007

Analysis Set	Quizartinib Monotherapy N = 245 n (%)	Salvage Chemotherapy (N = 122) n (%)	Total (N = 367) n (%)
ITT Analysis Set	245 (100.0)	122 (100.0)	367 (100.0)
Safety Analysis Set	241 (98.4)	94 (77.0)	335 (91.3)
PPS	231 (94.3)	88 (72.1)	319 (86.9)

Outcomes and estimation

- *Primary efficacy endpoint– Overall Survival*

At the time of the analysis, 75% of the randomised patients had died.

Table 27. Analysis of overall survival (ITT)-Study AC220-007

Parameter	Quizartinib Monotherapy (N = 245)	Salvage Chemotherapy (N = 122)
Subjects (%) with Events	190 (77.6)	86 (70.5)
Subjects (%) Without Events (Censored)	55 (22.4)	36 (29.5)
Time to Events (Weeks)[a]		
Median[a] (95% CI for Median)	27.0 (23.1, 31.3)	20.4 (17.3, 23.7)
1st , 3rd Quartile	15.4, 62.6	8.3, 39.6
OS Probability (95% CI)[b]		
13 Weeks	0.81 (0.76, 0.85)	0.65 (0.55, 0.73)
26 Weeks	0.52 (0.45, 0.58)	0.39 (0.30, 0.48)
52 Weeks	0.27 (0.21, 0.32)	0.20 (0.12, 0.28)
104 Weeks	0.18 (0.13, 0.24)	0.15 (0.09, 0.24)
Stratified Log-Rank Test[c]		
p-value (1-Sided)	--	0.0177
Hazard Ratio (Relative to Salvage Chemotherapy) [Stratified]	--	0.758
95% CI	--	(0.584, 0.983)
p-value for HR=1 (1-Sided)	--	0.0185
Hazard Ratio (Relative to Salvage Chemotherapy)[d] **(Unstratified)**	--	0.761
95% CI	--	(0.589, 0.982)
p-value for HR=1 (1-Sided)	--	0.0178

CI = confidence interval; FLAG-IDA = fludarabine, cytarabine, and G-CSF with idarubicin; G-CSF = granulocyte-colony stimulating factor; HR = hazard ratio; HSCT = hematopoietic stem cell transplantation; ITT = Intent-to-Treat; LoDAC = low-dose cytarabine; MEC = mitoxantrone, etoposide, and intermediate-dose cytarabine; N = population size; OS = overall survival.

a Median and quartiles are calculated using Kaplan-Meier method and CI for median is calculated using the Brookmeyer-Crowley method.

b Survival probability and CI are calculated based on Kaplan-Meier product-limit method and Greenwood's formulae.

c Stratification factors include prior therapy and response (Relapsed in ≤6 months (not post-HSCT), Refractory, or relapsed in ≤6 months post allogeneic HSCT), and pre-selected chemotherapy (High intensity chemotherapy [MEC or FLAG-IDA], or low intensity chemotherapy [LoDAC]).

d Unstratified analysis includes only treatment in the model.

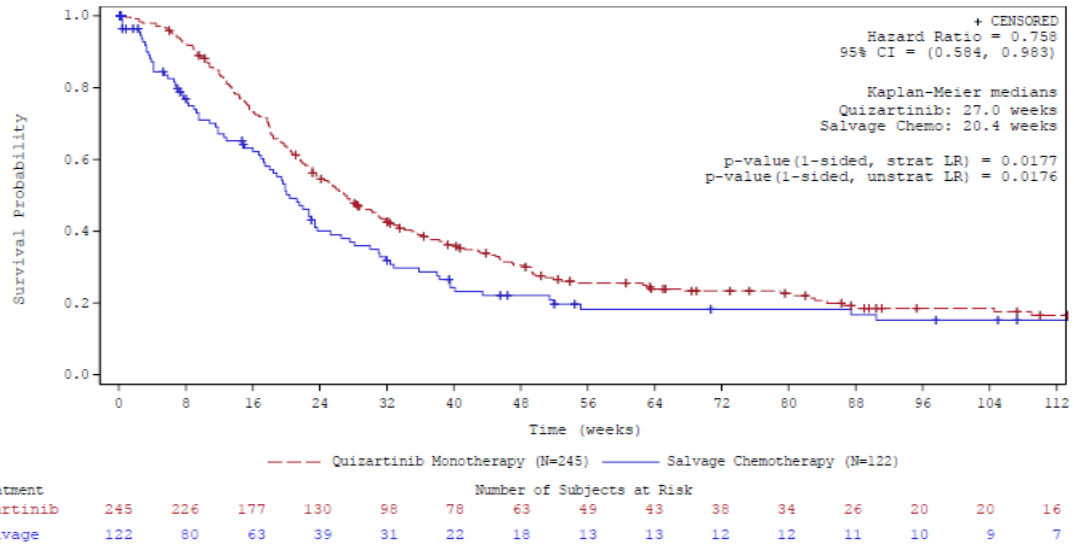
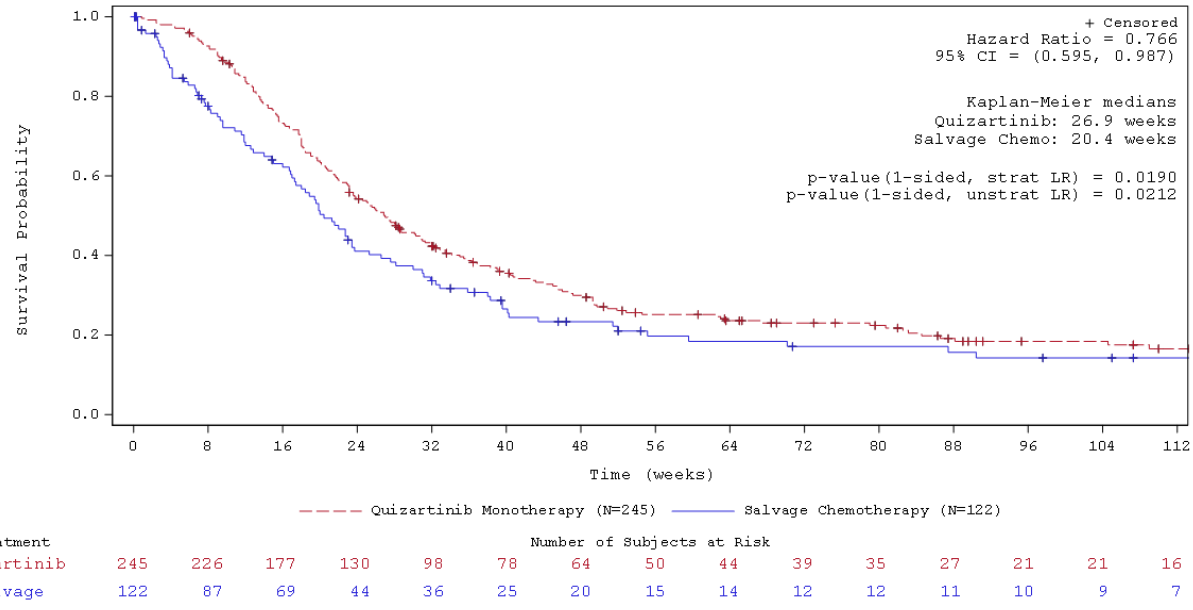


Figure 9. Kaplan-Meier plot of overall survival by treatment arm (ITT analysis set)- Study AC220-007

Table 28 Updated Analysis of Overall Survival with New Data for 13 Subjects (ITT Analysis Set)-Study AC220-007

Parameter	Quizartinib Monotherapy (N = 245)	Salvage Chemotherapy (N = 122)
Subjects (%) with Events	192 (78.4)	93 (76.2)
Subjects (%) Without Events (Censored)	53 (21.6)	29 (23.8)
Time to Events (Weeks)[a]		
Median[a] (95% CI for Median)	26.9 (23.1, 31.0)	20.4 (17.0, 25.3)
1st , 3rd Quartile	15.4, 62.6	9.0, 40.3
OS Probability (95% CI)[b]		
13 Weeks	0.81 (0.76, 0.85)	0.66 (0.56, 0.74)
26 Weeks	0.51 (0.45, 0.57)	0.40 (0.31, 0.49)
52 Weeks	0.26 (0.21, 0.32)	0.21 (0.14, 0.29)
104 Weeks	0.18 (0.13, 0.24)	0.14 (0.08, 0.22)
Stratified Log-Rank Test[c]		
p-value (1-Sided)	--	0.0190
Hazard Ratio (Relative to Salvage Chemotherapy) [Stratified]	--	0.766
95% CI	--	(0.595, 0.987)
p-value for HR=1 (1-Sided)	--	0.0198

a. Median and quartiles are calculated using Kaplan-Meier method and CI for median is calculated using the Brookmeyer-Crowley method.
b. Survival probability and CI are calculated based on Kaplan-Meier product-limit method and Greenwood's formulae.
c. Stratification factors include prior therapy and response (Relapsed in ≤6 months (not post-HSCT), Refractory, or relapsed in ≤6 months post allogeneic HSCT), and pre-selected chemotherapy (High intensity chemotherapy [MEC or FLAG-IDA], or low intensity chemotherapy [LoDAC]).Notes: The denominator for percentages is the number of subjects in the ITT Analysis Set. OS is defined as the time from the date of randomization to the date of death from any cause.



Note: P-values are 1-sided and based on LR test. Hazard ratio is obtained using an unadjusted stratified Cox PH model. Stratification factors at randomization were 1) Prior therapy and response (Relapsed in ≤6 months not post-HSCT, Refractory, or Relapsed in ≤6 months post allogeneic HSCT), and 2) Pre-selected chemotherapy (High intensity chemotherapy (MEC or FLAG-IDA), or low intensity chemotherapy (LoDAC).

Figure 10 Updated Kaplan-Meier Plot of Overall Survival by Treatment Arm with New Data for 13 Subjects with Missing Overall Survival Status (ITT Analysis Set)

- *Secondary efficacy endpoint– Event-Free Survival*

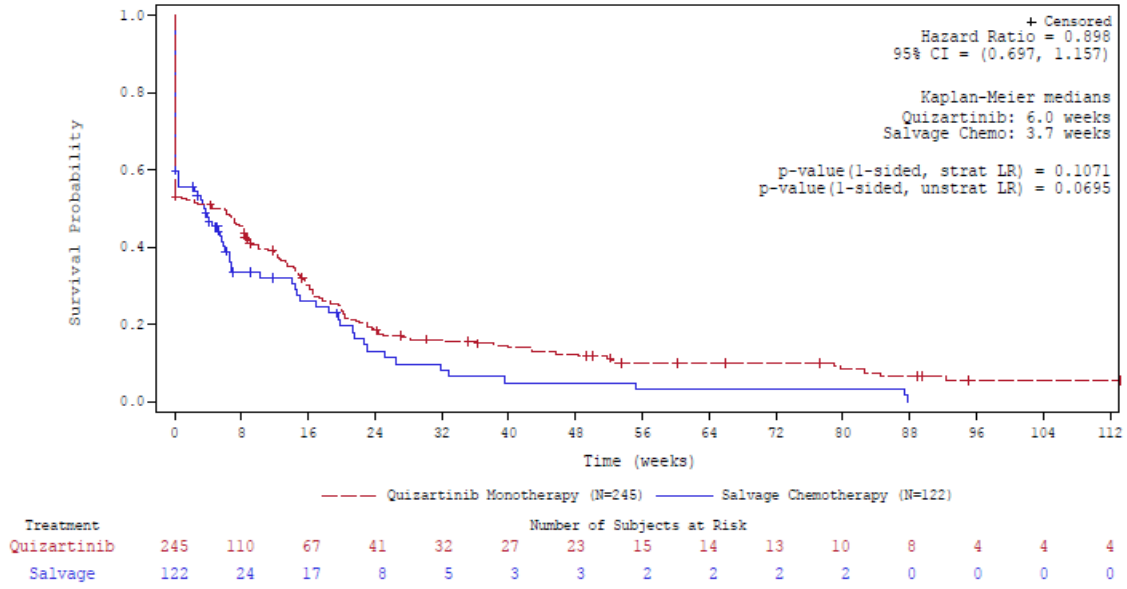


Figure 11. Kaplan-Meier plot of event-free survival using sponsor-derived assessment criteria (ITT analysis set)-Study AC220-007

Table 29. Analysis of EFS using sponsor-derived response criteria (ITT analysis set-Study AC220-007)

Parameter	Quizartinib Monotherapy (N = 245)	Salvage Chemotherapy (N = 122)
Subjects (%) with Events	216 (88.2)	92 (75.4)
Subjects (%) without Events (Censored)	29 (11.8)	30 (24.6)
Time to Events (Weeks)[a]		
Median	6.0	3.7
95% CI for Median	(0.1, 8.3)	(0.4, 5.9)
1st Quartile	0.1	0.1
3rd Quartile	19.7	17.0
EFS Probability (95% CI)[b]		
13 Weeks	0.37 (0.30, 0.43)	0.32 (0.23, 0.42)
26 Weeks	0.17 (0.13, 0.22)	0.11 (0.05, 0.20)
52 Weeks	0.11 (0.07, 0.16)	0.05 (0.01, 0.12)
104 Weeks	0.06 (0.03, 0.10)	0.00 (--, --)
Stratified Log-Rank Test[c]		
p-value (1-Sided)	--	0.1071
Unstratified log-rank test[d]		
p-value (1-Sided)	--	0.0695
Hazard Ratio (Relative to Salvage Chemotherapy) (Stratified)	--	0.898
95% CI	--	(0.697, 1.157)
p-value for HR=1 (1-sided)	--	0.2034
Hazard Ratio (Relative to Salvage Chemotherapy) (Unstratified)	--	0.879
95% CI	--	(0.685, 1.127)
p-value for HR=1 (1-sided)	--	0.1541

CI = confidence interval; CR = complete remission; CRc = composite complete remission; CRi = complete remission with incomplete hematologic recovery; CRp = complete remission with incomplete platelet recovery; EFS = event-free survival; FLAG-IDA = fludarabine, cytarabine, and G-CSF with idarubicin; G-CSF = granulocyte-colony stimulating factor; HR = hazard ratio; HSCT = hematopoietic stem cell transplantation; ITT = Intent-to-Treat; LoDAC = low-dose cytarabine; MEC = mitoxantrone, etoposide, and intermediate-dose cytarabine; N = population size.

[a] Median and quartiles are calculated using Kaplan-Meier method and confidence interval for median is calculated using the Brookmeyer-Crowley method.

[b] Survival probability and confidence interval are calculated based on Kaplan-Meier product-limit method and Greenwood's formulae.

[c] Stratification factors include prior therapy and response (Relapsed in ≤6 months not post-HSCT, Refractory, or relapsed in ≤6 months post allogeneic HSCT), and pre-selected chemotherapy (high intensity chemotherapy [MEC or FLAG-IDA], or low intensity chemotherapy [LoDAC]).

[d] Unstratified analysis includes only treatment in the model.

- *Exploratory efficacy endpoint- Response rate*

Table 30.Best overall response to therapy as assessed using sponsor-derived response criteria (ITT analysis set)-Study AC220-007

Response Assessment Category	Quizartinib Monotherapy (N = 245) n (%)	Salvage Chemotherapy (N = 122) n (%)
Evaluable (Subjects with at least 1 post-baseline assessment)	**232 (94.7)**	**82 (67.2)**
Non-Evaluable (Subjects without a post-baseline assessment)	**13 (5.3)**	**40 (32.8)**
CRc	**118 (48.2)**	**33 (27.0)**
95% CI	(41.8, 54.6)	(19.4, 35.8)
CR	**10 (4.1)**	**1 (0.8)**
95% CI	(2.0, 7.4)	(0.0, 4.5)
CRp	**9 (3.7)**	**0 (0.0)**
95% CI	(1.7, 6.9)	(0.0, 3.0)
CRi	**99 (40.4)**	**32 (26.2)**
95% CI	(34.2, 46.8)	(18.7, 35.0)
CRia	**74 (30.2)**	**23 (18.9)**
95% CI	(24.5, 36.4)	(12.3, 26.9)
CRib	**25 (10.2)**	**9 (7.4)**
95% CI	(6.7, 14.7)	(3.4, 13.5)
Partial Response	**52 (21.2)**	**4 (3.3)**
95% CI	(16.3, 26.9)	(0.9, 8.2)
No Response	**62 (25.3)**	**45 (36.9)**
95% CI	(20.0, 31.2)	(28.3, 46.1)

AML = acute myeloid leukemia; ANC = absolute neutrophil count; CI = confidence interval; CR = complete remission; CRc = composite complete remission; CRi = complete remission with incomplete hematologic recovery; CRp = complete remission with incomplete platelet recovery; HSCT = hematopoietic stem cell transplantation; ITT = intent-to-treat; n = number of subjects in the category; N = population size; NR = no response; RBC = red blood cell.

Notes: Denominator for percentages is number of subjects in Intent-to-Treat Analysis Set. Best overall response is defined as the best measured response (CR, CRp, CRi, PR, NR or Unknown) during study drug period but prior to HSCT or any other AML therapy. CRc is comprised of CR, CRp and CRi. Response was derived from objective criteria per modified Cheson criteria. Rate and 95% CI are calculated from binomial distribution.

CRia: CRis that satisfy all other criteria for CR, but ANC<1 $\times 10^9$/L with or without complete platelet recovery, with or without RBC/platelet transfusion

CRib: Rest of CRis that satisfy all other criteria for CR or CRp, but with RBC/platelet transfusion

Table 31 Summary of composite complete remission (CRc) rate using objective response criteria (per-protocol analysis set)

	Quizartinib Monotherapy (N=231) n (%)	Salvage Chemotherapy (N=88) n (%)
Composite Complete Remission (CRc)		
n	114	31
Rate (%)	49.4	35.2
(95% CI)[a]	(42.7, 56.0)	(25.3, 46.1)
Randomization Strata (Factor 1 x Factor 2)		
High-Intensity Chemotherapy		
Relapsed, no prior HSCT	40/81 (49.4)	16/34 (47.1)
Refractory	26/59 (44.1)	7/20 (35.0)
Relapsed, post-HSCT	19/37 (51.4)	8/14 (57.1)
Low-Intensity Chemotherapy		
Relapsed, no prior HSCT	12/21 (57.1)	0/7 (0.0)
Refractory	8/18 (44.4)	0/6 (0.0)
Relapsed, post-HSCT	9/15 (60.0)	0/7 (0.0)
Odds Ratio[b]	1.802	--
(95% CI)	(1.086, 2.989)	--
p-value for treatment[b]	0.0212	--
p-value for homogeneity across strata[c]	0.0120	--

CI = confidence interval; CMH = Cochran-Mantel-Haenszel; CR = complete remission; CRc = composite complete remission; CRi = complete remission with incomplete hematologic recovery; CRp = complete remission with incomplete platelet recovery; HSCT = hematopoetic stem cell transplant.
Notes: Denominator for percentages is number of subjects in Per-Protocol Analysis Set.
CRc = Any occurence of CR, CRp, or CRi.
[a] Rate and confidence interval are calculated based on binomial distribution.
[b] Odds ratio and confidence interval are calculated based on the Cochran-Mantel-Haenszel (CMH) method, and 2-sided p-value is based on CMH test, using the 6 randomization strata.
defined by 1. Prior therapy and response (3 levels), and 2. High vs. low-intensity chemotherapy (2 levels).
[c] 2-sided p-value from Breslow-Day test for homogeneity of odds ratio across the strata.

- *Exploratory efficacy endpoint- Time to first/best composite complete remission*

The median (range) time to first CRc [CR, CRp, CRi] was 4.86 (3.7, 19.7) weeks in the quizartinib arm and 4.00 (2.0, 14.9) weeks in the salvage chemotherapy arm. The median (range) time to best CRc (where CR>CRp>CRi) was 7.50 (3.7, 45.0) weeks in the quizartinib arm and 4.00 (2.0, 19.4) weeks in the salvage chemotherapy arm.

- *Exploratory efficacy endpoint-Duration of response*

A median duration of CR could not be evaluated because only 11 subjects (10 subjects in the quizartinib arm and one subject in the salvage chemotherapy arm) met the criteria for CR. The median (95% CI) duration of response for CRc was 12.1 (10.4, 27.1) weeks in the quizartinib arm and 5.0 (3.3, 12.6) weeks in the salvage chemotherapy arm. Results were similar in the analysis when subjects who received HSCT were censored (11.7 and 5.0 weeks, respectively) in the quizartinib and salvage chemotherapy arms.

- *Exploratory efficacy endpoint- Leukaemia-Free Survival*

Among the subjects in the ITT analysis set who had the best response of CRc, 76.3% subjects in the quizartinib arm and 60.6% of subjects in the salvage chemotherapy arm had events, which were defined as documented relapse or death from any cause (Table 37).

Table 32. Analysis of leukemia-free survival using sponsor-derived response criteria (ITT analysis set)-Study AC220-007

	Quizartinib Monotherapy (N = 245)	Salvage Chemotherapy (N = 122)
Subjects with CRc	**118**	**33**
Subjects (%) with Events	**90 (76.3)**	**20 (60.6)**
Subjects (%) without Events (censored)	**28 (23.7)**	**13 (39.4)**
Leukemia-Free Survival (Weeks)[a]		
Median[a]	12.0	11.1
95% CI for Median	(9.3, 14.7)	(3.9, 19.9)
1st Quartile	4.7	3.9
3rd Quartile	41.3	24.9
Kaplan-Meier estimates (95% CI)[b]		
13 Weeks	0.43 (0.34, 0.52)	0.44 (0.23, 0.63)
26 Weeks	0.34 (0.25, 0.43)	0.22 (0.07, 0.42)
52 Weeks	0.21 (0.13, 0.29)	0.11 (0.02, 0.29)
104 Weeks	0.12 (0.06, 0.21)	0.00 (--, --)

CI = confidence interval; CR = complete remission; CRc = composite complete remission; CRi = complete remission with incomplete hematological recovery; CRp = complete remission with incomplete platelet recovery; ITT = Intent-to-Treat; LFS = leukemia-free survival; N = population size.
Notes: Denominator for percentages are based on number of subjects with CRc.
LFS is defined as the time from the first documented response of CRc (CR, CRp, or CRi) until documented relapse or death from any cause. Subjects without a documented response of CRc are excluded from the analysis of LFS.
[a] Median and quartiles are calculated using Kaplan-Meier method and CI for median is calculated using the Brookmeyer-Crowley method.
[b] Estimate and CI are calculated based on Kaplan-Meier product-limit method and Greenwood's formulae.

- *Exploratory efficacy endpoint -HSCT rate post-treatment*

Of the patients in the quizartinib arm who had an allogeneic transplant, 48/78 (61.5%) continued/maintained treatment with quizartinib post-transplant. The median duration of quizartinib therapy post HSCT was 129 days (range 1 to 1037 days). In the quizartinib arm, 44.9% of patients who achieved CRc and 19.7% of patients who failed to achieve CRc went on to transplant. In the salvage chemotherapy arm, 33.3% of patients who achieved CRc and 3.4% of patients who failed to achieve CRc went on to transplant.

Table 33. Summary of transplantation post-randomization (ITT analysis set)-Study AC220-007

Parameter	Quizartinib Monotherapy (N=245)	Salvage Chemotherapy (N=122)
HSCT		
n	78	14
Rate (%)[a]	31.8	11.5
95% CI[b]	(26.1, 38.1)	(6.4, 18.5)
Randomization Strata		
Relapsed, no HSCT/ Low-intensity chemo	5/23 (21.7)	0/12 (0.0)
Relapsed, no HSCT/ High-intensity chemo	35/86 (40.7)	6/42 (14.3)
Refractory/ Low-intensity chemo	5/18 (27.8)	0/ 9 (0.0)
Refractory/ High-intensity chemo	27/62 (43.5)	6/32 (18.8)
Relapsed, post-HSCT /Low-intensity chemo	3/16 (18.8)	0/ 8 (0.0)
Relapsed, post-HSCT/ High-intensity chemo	3/40 (7.5)	2/19 (10.5)
Odds Ratio[c]	--	3.8
95% CI[c]	--	(2.0, 7.2)
p-value for treatment[c]	--	<0.0001
p-value for homogeneity across strata[d]	--	0.2830

AML = acute myeloid leukemia; CI = confidence interval; CMH = Cochran-Mantel-Haenszel; HSCT = hematopoietic stem cell transplantation; ITT = Intent-to-Treat; n = number of subjects in the category; N = population size.
[a] HSCT rate is the percent of subjects undergoing allogeneic HSCT directly following the protocol treatment with no intervening AML therapy.
[b] Rate and confidence interval are calculated based on binomial distribution.
[c] Odds ratio and confidence interval are calculated based on Mantel-Haenszel method, and 2-sided p-value is based on CMH test, using the 6 randomization strata defined by 1) High vs low-intensity chemotherapy (2 levels), and 2) Prior therapy and response (3 levels).
[d] 2-sided p-value from Breslow-Day test for homogeneity of odds ratio across the strata.
Note: Denominator for percentages is the number of subjects in the Intent-to-Treat Analysis Set.
The high intensity chemo and low-intensity chemo were stratification factors and refer to the pre-selected choices made at the time of randomization. Similarly, response to prior AML therapy was also a prespecified stratification factor.

Ancillary analyses

Sensitivity analyses of OS are shown in Table 39.

Table 34. Comparison of overall survival using different analysis methods-Study AC220-007

Population	Median (95% CI for Median)		Hazard Ratio Relative to Salvage Chemotherapy (95% CI)	Stratified p value
	Quizartinib Monotherapy	Salvage Chemotherapy		
ITT	27.0 (23.1, 31.3)	20.4 (17.3, 23.7)	0.758 (0.584, 0.983)	0.0177
Sensitivity Analysis Populations				
PPS	26.9 (23.1, 31.0)	20.0 (16.7, 25.3)	0.754 (0.567, 1.001)	0.0246
Censored for HSCT	24.6 (20.9, 27.3)	19.9 (16.7, 22.7)	0.787 (0.587, 1.054)	0.0519
Censoring at FLT3 Inhibitors	28.6 (23.7, 33.4)	21.6 (17.0, 26.6)	0.740 (0.553, 0.989)	0.0203

CI = confidence interval; FLT3 = Feline McDonough Sarcoma-like tyrosine kinase 3; HSCT = hematopoietic stem cell transplantation; ITT = Intent-to-Treat; OS = overall survival; PPS = Per Protocol Analysis Set.

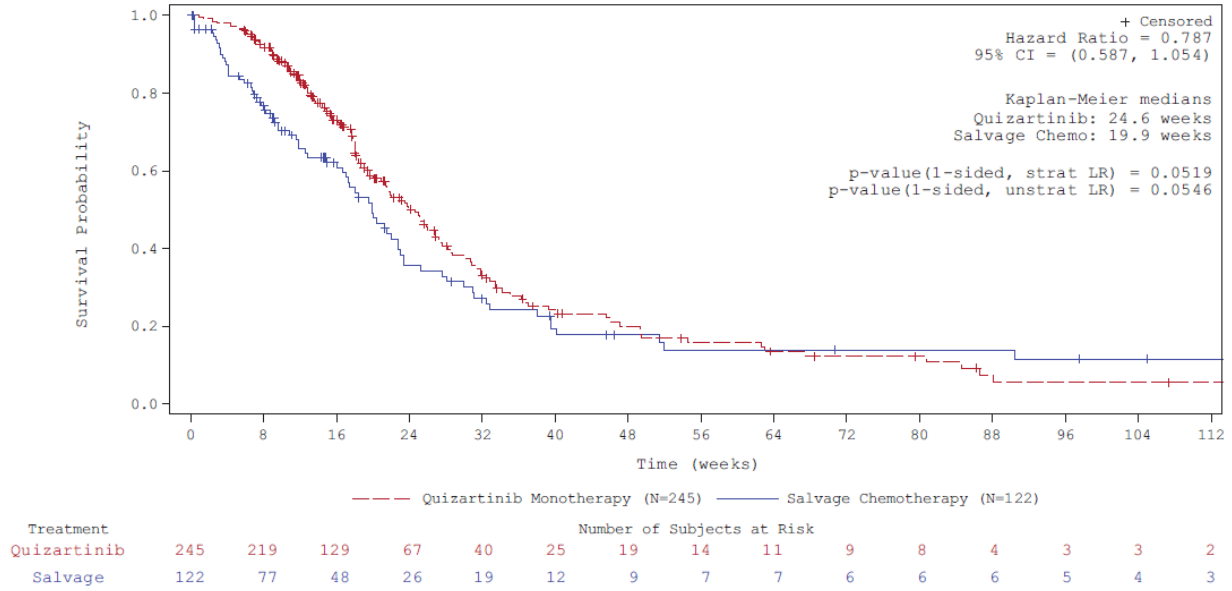


Figure 12 KM-Plot-Supportive analysis of overall survival (censoring at HSCT) ITT analysis set-Study AC220-007

Subgroup analyses of OS

Pre-defined subgroup analyses for OS were performed based on demographics (age, sex, race, and geographical region) and baseline disease characteristics (prior response to therapy, FLT3-ITD allelic ratio, AML type and risk score, transplant history, and blast count) (Figure 13).

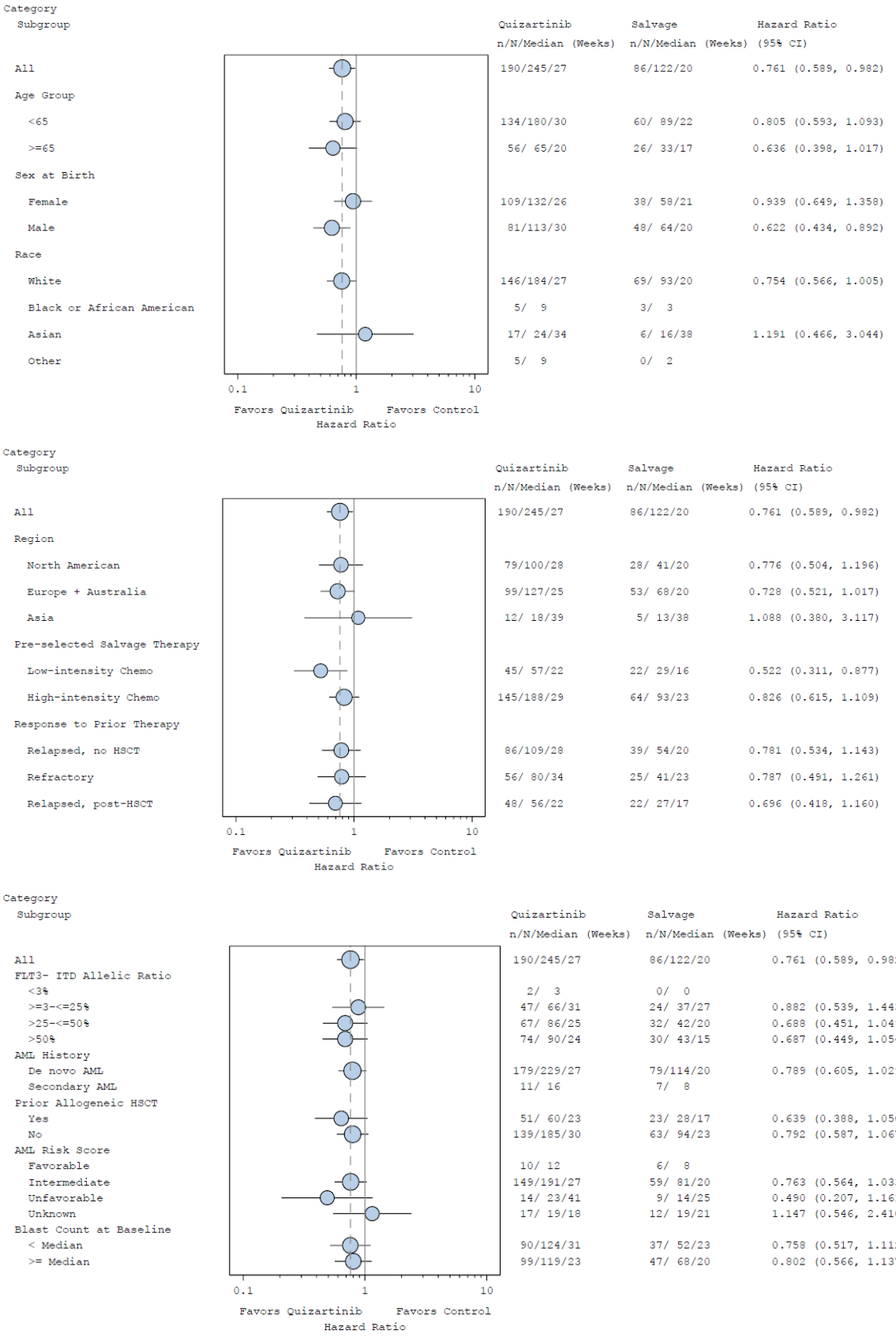


Figure 13. Forest plot of overall survival by subgroups (ITT analysis set) -Study AC220-007

OS in treated vs randomised non-treated subjects salvage chemotherapy arm

An additional analysis was performed to compare OS in the treated (n=94) vs randomized non-treated subjects (n=28) in the salvage chemotherapy arm (ITT analysis set). Median OS was 21.3 (95% CI: 3.6, 32.9) and 20.0 (95% CI: 16.7, 26.6), respectively.

Table 35 Analysis of Overall Survival of Treated versus Randomized and Not Treated Subjects in the Salvage Chemotherapy Arm (ITT Analysis Set) Study AC220-007

Parameter	R-N-T Patients in Salvage Chemotherapy (N = 28)	Treated Patients in Salvage Chemotherapy (N = 94)
Patients (%) with Events	10 (35.7)	76 (80.9)
Patients (%) without Events (Censored)	18 (64.3)	18 (19.1)
Time to Events (Weeks)[a]		
Median[a] (95% CI for Median)	21.3 (3.6, 32.9)	20.0 (16.7, 26.6)
1st , 3rd Quartile	3.6, 32.9	9.0, 39.6

CI = confidence interval; ITT = Intent-to-Treat; N = population size.

[a] Median and quartiles are calculated using Kaplan-Meier method, and confidence interval for median is calculated using the Brookmeyer-Crowley method.

Tipping Point Analysis of OS

Table 36 Summary of Tipping Point Analysis Based on Updated OS Data-Study AC220-007

Percentage Cut for Resampling Subset[a]	Median[b] OS (Weeks) For Resampling Subset	Mean of Median OS Estimates (Chemo Arm)	Patients in Resampling Subset	Hazard Ratio		1-Sided Stratified Log-Rank P-Value		Difference Of Median OS (Weeks)	
				Mean	2.5, 97.5 Percentile	Mean	2.5, 97.5 Percentile	Mean	2.5, 97.5 Percentile
100%	20.4	20.5	116	0.76	0.74, 0.80	0.0191	0.0074, 0.0443	6.33	5.29, 7.00
90%	22.7	20.7	106	0.77	0.74, 0.81	0.0209	0.0082, 0.0481	6.20	5.29, 7.00
80%	25.3	20.9	93	0.77	0.74, 0.81	0.0243	0.0097, 0.0528	5.99	4.86, 7.00
75%	27.6	21.0	87	0.78	0.75, 0.82	0.0256	0.0103, 0.0553	5.87	4.86, 6.86
50%	40.1	21.8	58	0.80	0.76, 0.83	0.0383	0.0172, 0.0742	5.10	4.86, 5.57
25%	90.4	22.0	29	0.82	0.79, 0.86	0.0662	0.0335, 0.1120	4.86	4.86, 4.86

OS = overall survival.

a.The percentage cut for a resampling subset corresponds to the percent longest survivors among the 116 patients

b. Median OS (weeks) for the resampling pool, i.e., the top xx% longest-surviving patients of the salvage chemotherapy arm

Summary in each row is based on N=5000 iterations of resampling.

Resampling of OS data for subjects randomized but not treated

A sensitivity analysis was performed in which survival times for patients who were randomized but not treated were imputed using a method of sampling from the distribution of survival times from patients within the same strata who were treated. This process was performed 5000 times and the distribution of the estimated Hazard Ratios presented, along with relevant descriptive statistics. Results are presented below.

Table 37 Summary Statistics of the Hazard Ratio for OS From the Resampling Method -Study AC220-007

Hazard Ratio	
N (simulation)	5000
Mean	0.74
SD	0.035
Median	0.73
Minimum	0.62
Maximum	0.90
2.5th Percentile	0.67
97.5th Percentile	0.81
Hazard Ratio category	
<0.758 (Hazard Ratio from Primary Analysis)	3728 (74.6%)
≥0.758	1272 (25.4%)

FLAG-IDA = fludarabine, cytarabine, and G-CSF with idarubicin; G-CSF = granulocyte-colony stimulating factor; HSCT = hematopoietic stem cell transplantation; MEC = mitoxantrone, etoposide, and intermediate-dose cytarabine; N = population size; OS = overall survival; SD = standard deviation.
Note: Resampling is done by randomly replacing 28 untreated subjects without death events in the salvage chemo group using samples from the remaining 94 salvage chemo subjects stratified by response to prior therapy (relapsed in ≤6 months (not post-HSCT), refractory, or relapsed in ≤6 months post-allogeneic HSCT) and pre-selected chemotherapy use (High intensity chemotherapy [MEC or FLAG-IDA]), so that the proportion of each stratum is the same as that in 28 subjects. Hazard ratio is obtained from the stratified Cox model.

Table 38.Stratification factors used in the resampling approach-Study AC220-007

Stratum	Response to Prior Therapy	Pre-Selected Chemotherapy Use	28 Untreated Subjects	Rest of Subjects in Salvage Chemotherapy Arm
1	Relapsed in ≤6 Months (Not Post-HSCT)	Low	3	9
2	Refractory	Low	3	6
3	Relapsed in ≤6 Months (Postallogeneic HSCT)	Low	1	7
4	Relapsed in ≤6 Months (Not Post-HSCT)	High	5	37
5	Refractory	High	12	20
6	Relapsed in ≤6 Months (Postallogeneic HSCT)	High	4	15
Total			28	94

HSCT = hematopoietic stem cell transplantation

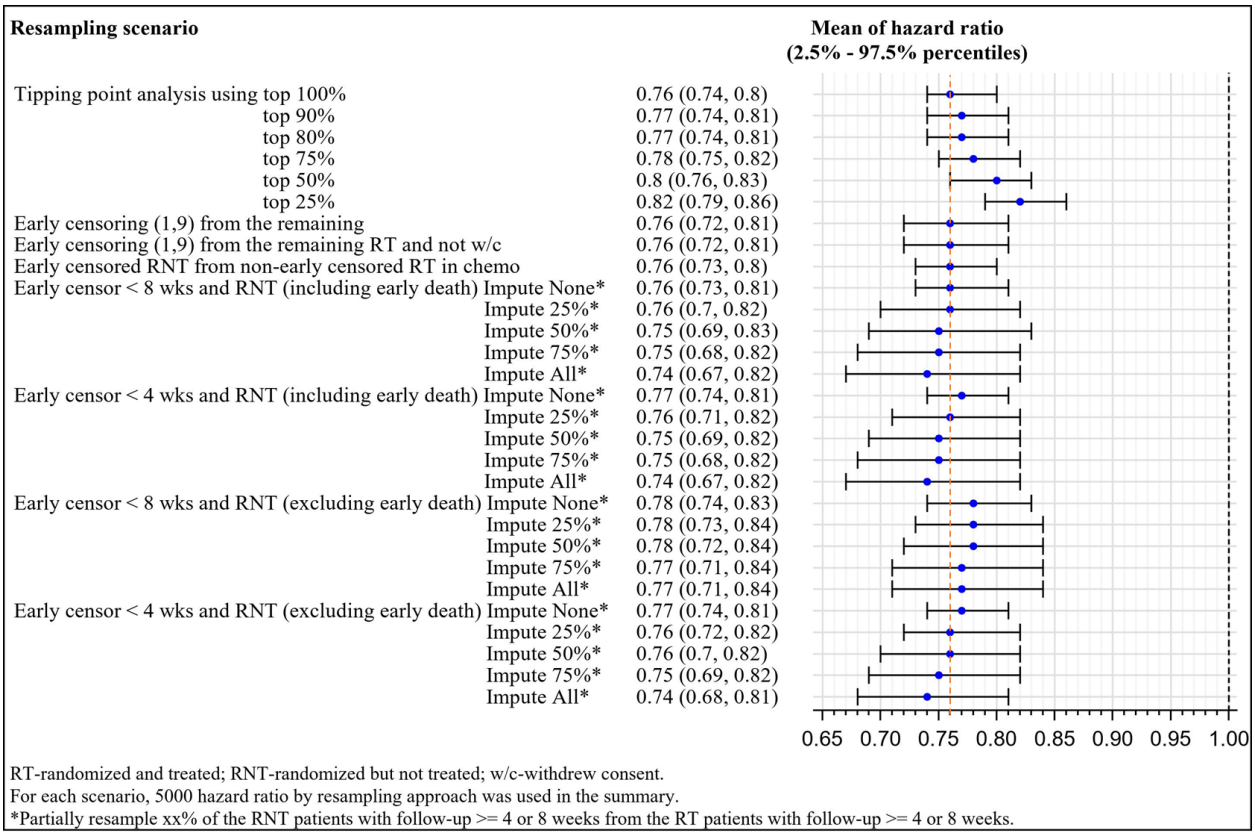


Figure 14 Summary of Sensitivity Analysis for Overall Survival Study AC220-007

Comparison of event-free survival using different analysis methods

Table 39 Comparison of event-free survival using different analysis methods-Study AC220-007

Population	Median (95 % CI for Median)		Hazard Ratio Relative to Salvage Chemotherapy[a] (95% CI)	p-value (Stratified LR Test)
	Quizartinib Monotherapy	Salvage Chemotherapy		
ITT	6.0 (0.1, 8.3)	3.7(0.4, 5.9)	0.898 (0.697, 1.157)	0.1071
PPS	6.1 (0.1, 8.4)	0.1 (0.1, 4.4)	0.719 (0.545, 0.948)	0.0064
ITT Censored at HSCT	6.0 (0.1, 8.3)	3.7 (0.4, 5.9)	0.984 (0.757, 1.279)	0.3351
Using Investigator Response Assessment	6.4 (0.1, 12.0)	3.7 (0.4, 7.3)	0.914 (0.708, 1.180)	0.1263

CI = confidence interval; CR = complete remission; CRc = composite complete remission; CRi = complete remission with incomplete hematologic recovery; CRp = complete remission with incomplete platelet recovery; EFS = event-free survival; HSCT = hematopoietic stem cell transplantation; ITT = intent-to-treat; LR = log-rank; PPS = Per Protocol Analysis Set

[a] Stratification factors include prior therapy and response (relapsed in ≤6 months [not post-HSCT], refractory, or relapsed in ≤6 months post-allogeneic HSCT) and pre-selected chemotherapy (high intensity chemotherapy [MEC or FLAG-IDA] or low intensity chemotherapy [loDAC]).

Post-randomization transplant for subjects who had allogenic HSCT with no intervening AML therapy

Table 40. Summary of post-randomization transplant for subjects who had allogenic HSCT with no intervening AML therapy (ITT analysis set) -Study AC220-007

	Quizartinib Monotherapy) (N = 245) n (%)	Salvage Chemotherapy (N = 122) n (%)	Total (N = 367) n (%)
Number of Transplants*			
1	78 (31.8)	14 (11.5)	92 (25.1)
Graft Type			
Allogeneic	78 (100.0)	14 (100.0)	92 (100.0)
Related Donor	38 (48.7)	8 (57.1)	46 (50.0)
Matched	29 (37.2)	7 (50.0)	36 (39.1)
Not Matched	9 (11.5)	1 (7.1)	10 (10.9)
Unrelated Donor	40 (51.3)	6 (42.9)	46 (50.0)
Matched	30 (38.5)	6 (42.9)	36 (39.1)
Not Matched	10 (12.8)	0 (0.0)	10 (10.9)
Cord Transplant			
No	70 (89.7)	14 (100.0)	84 (91.3)
Yes	8 (10.3)	0 (0.0)	8 (8.7)
Autologous	0 (0.0)	0 (0.0)	0 (0.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)
Outcome of Transplant			
Successful	41 (52.6)	5 (35.7)	46 (50.0)
Subjects with Post-HSCT Quizartinib Treatment	30 (38.5)	0 (0.0)	30 (32.6)
Engraftment Failure	7 (9.0)	1 (7.1)	8 (8.7)
Subjects with Post-HSCT Quizartinib Treatment	1 (1.3)	0 (0.0)	1 (1.1)
Rejection	3 (3.8)	1 (7.1)	4 (4.3)
Subjects with Post-HSCT Quizartinib Treatment	1 (1.3)	0 (0.0)	1 (1.1)
Relapse	24 (30.8)	6 (42.9)	30 (32.6)
Subjects with Post-HSCT Quizartinib Treatment	14 (17.9)	0 (0.0)	14 (15.2)
Unknown	3 (3.8)	1 (7.1)	4 (4.3)
Subjects with Post-HSCT Quizartinib Treatment	2 (2.6)	0 (0.0)	2 (2.2)
Total Subjects with Post-HSCT Quizartinib Treatment	48 (61.5)	0 (0.0)	48 (52.2)
Reduced Intensity Conditioning			
No	42 (53.8)	6 (42.9)	48 (52.2)
Yes	26 (33.3)	7 (50.0)	33 (35.9)
Unknown	9 (11.5)	1 (7.1)	10 (10.9)
Missing	1 (1.3)	0 (0.0)	1 (1.1)

AML = acute myeloid leukemia; eCRF = electronic case report form; ITT = Intent-to-Treat; HSCT = hematopoietic stem cell transplantation; n = number of subjects in the category; N = population size.
Notes: Subjects are considered if they had post randomization allogeneic transplantation with no intervening AML therapy after discontinuation of quizartinib.
Denominator for percentages in * is the number of subjects in the Intent-to-Treat Analysis Set. For the rest of the table, number of subjects who had at least one transplant in * is used as denominator when calculating percentages.
Outcome of transplant was provided by the investigator from the choices presented on the eCRF.

Summary of main study

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit-risk assessment (see later sections).

Table 41. Summary of efficacy for trial AC220-007

Title: A Phase 3 Open-Label Randomized Study of Quizartinib Monotherapy Versus Salvage chemotherapy in Subjects with FLT3-ITD Positive Acute Myeloid Leukemia (AML) Refractory to or Relapsed after First-Line Treatment with or without Hematopoietic Stem Cell Transplantation (HSCT) Consolidation			
Study identifier	AC220-007		
Design	phase 3, randomized, open-label, 2-arm study.		
	Duration of main phase: Duration of Run-in phase:		MEC or FLAG-IDA were to receive 1 cycle of therapy and may have received a second cycle of the same therapy at the investigator's discretion. LoDAC or quizartinib continuously until no clinical benefit, unacceptable toxicity or HSCT. Quizartinib could be resumed at 30 to 100 days post transplant. N/A
Hypothesis	Superiority		
Treatments groups	Quizartinib (N=245)		Quizartinib starting dose 30 mg/day, increased to 60 mg/day on day 16 if QTcF ≤450 ms. In case of strong CYP3A inhibitor, start dose was 20 mg/day with increase to 30 mg/day. Continuous. Possible to resume after HSCT. 245 randomised (ITT), n=49 resumed therapy post HSCT.
	Salvage chemotherapy (N=122)		Low intensity chemotherapy: LoDAC, Continuous Randomised n=29 High intensity chemotherapy: MEC (N=40) or FLAG-IDA (n=53) 1 cycle of 28 days of therapy. Potential for second cycle based on response.
Endpoints and definitions	Primary endpoint	Overall survival (OS)	Time between the date of randomization and the date of death from any cause.
	Secondary endpoint	Event free survival (EFS)	Time from randomization until documented refractory disease, relapse after CRc, or death from any cause, whichever occurred first.
	Exploratory endpoints	Leukemia-Free Survival (LFS)	Time from the first documented response (CR, CRp, or CRi) until documented relapse or death from any cause.
		Composite complete remission (CRc)	The percent of subjects achieving a best response of CR, CRp, or CRi. CRi was determined using sponsor-modified criteria.
		Duration of CRc	Time from the first documented CRc (CR + CRp + CRi) until documented relapse.
		HSCT rate	Percent of subjects undergoing allogenic HSCT directly following protocol specified treatment with no intervening AML therapy.
Database lock	22 February 2018		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	ITT population, defined as all patients who were randomised		
Descriptive statistics and estimate variability	Treatment group	Quizartinib	Salvage Chemotherapy
	Number of subjects	N=245	N=122

	Evaluable (Subjects with at least 1 postbaseline assessment)	N=232 (94.7)	N=82 (67.2)
	OS, median (weeks)	27.0	20.4
	95% CI	23.1, 31.3	17.3, 23.7
	EFS, median (weeks)	6.0	3.7
	95% CI	0.1, 8.3	0.4, 5.9
	CRc n (%)	118 (48.2)	33 (27.0)
	95% CI	41.8, 54.6	19.4, 35.8
	Duration of CRc, median (weeks)	12.1	5.0
	95% CI	10.4, 27.1	3.3, 12.6
	LFS, median (weeks)	12.0	11.1
	95% CI	9.3, 14.7	3.9, 19.9
	HSCT, n (%)	78 (31.8)	14 (11.5)
	95% CI	26.1, 38.1	6.4, 18.5
Effect estimate per comparison	Primary endpoint OS	Comparison groups	Quizartinib vs Salvage chemotherapy
		Hazard Ratio(stratified)	0.758
		95% CI	0.584, 0.983
		P-value (1-sided)	0.0185
	Secondary Endpoint EFS	Hazard Ratio (Stratified)	0.898
		95% CI	0.697, 1.157
		P-value	0.2034
	Exploratory endpoint CRc	OR	2.467
		95% CI	1.541, 3.950
		P-value	0.0001
	Exploratory endpoint HSCT rate	OR	3.8
		95% CI	2.0, 7.2
		P-value	<0.0001
Notes	Stratification factors for this study were relapsed or refractory prior therapy, with or without prior HSCT and preselected for low- or high-intensity chemotherapy.		
Analysis description	**Pre-specified sensitivity analyses primary endpoint**		
Effect estimate per comparison	OS censored for HSCT	Comparison groups	Quizartinib vs Salvage chemotherapy
		Hazard Ratio (stratified)	0.787
		95% CI	0.587, 1.054
		P-value	0.0519
	OS PPS analysis	Comparison groups	Quizartinib vs Salvage chemotherapy
		Hazard Ratio (stratified)	0.754
		95% CI	0.567, 1.001
		P-value	0.0246

Clinical studies in special populations

Table 42 Summary of elderly age groups for AML subjects treated with quizartinib (monotherapy AML Subjects) (Safety Analysis Set)

Type of Study[a]	Age 65-74 n (%)[b]	Age 75-84 n (%)[b]	Age 85+ n (%)[b]
Controlled Trial	52 (7.1)	12 (1.6)	0
Non Controlled Trials	160 (21.7)	35 (4.7)	3 (0.4)

[a] Controlled trial includes AC220-007 and non-controlled trials include AC220-CP0001, AC220-002, 2689-CL-2004, and 2689-CL-0011.
[b] Denominator for the percentage is 737, which is the total exposed patients in the pooling group of "Monotherapy AML Subjects".

Supportive studies

Study 2689-CL-2004

Study 2689-CL-2004 is described under section "dose-response studies". An overview of key efficacy data from Study 2689-CL-2004 is provided in Table 33.

Table 33: Key Efficacy Results from Study 2689-CL-2004

	Quizartinib 30 mg/day[a] (N = 38)	Quizartinib 60 mg/day[a] (N = 38)
CRc Rate		
n (%)	18 (47.4)	18 (47.4)
90% CI[b]	(33.3, 61.8)	(33.3, 61.8)
CR[c] Rate		
n (%)	2 (5.3)	1 (2.6)
95% CI[b]	(0.6, 17.7)	(0.1, 13.8)
CRp[c] Rate		
n (%)	0	2 (5.3)
95% CI[b]	--	(0.6, 17.7)
CRi[c] Rate		
n (%)	16 (42.1)	15 (39.5)
95% CI[b]	(26.3, 59.2)	(24.0, 56.6)
CRia		
n (%)	16 (42.1)	12 (31.6)
95% CI[b]	(26.3, 59.2)	(17.5, 48.7)
CRib		
n (%)	0	3 (7.9)
95% CI[b]	(0, 9.3)	(1.7. 21.4)
Median Duration of CRc, wk (95% CI)	4.2 (2.1, 9.7)	9.1 (4.1, 22.3)
Median OS, wk (95% CI)	20.9 (17.7, 25.3)	27.3 (17.3, 34.9)
Median EFS, wk (95% CI)	12.0 (8.3, 16.1)	13.7 (9.7, 26.1)
Transplant Rate, n (%)	12 (31.6)	16 (42.1)

CI = confidence interval; CR = complete remission; CRc = composite complete remission; CRi = complete remission with incomplete hematological recovery; CRp = complete remission with incomplete platelet recovery; EFS = event-free survival; FLT3-ITD = Feline McDonough sarcoma-like tyrosine kinase- internal tandem duplication; OS = overall survival; wk = week
[a] The denominators for percentages are based on number of subjects in each treatment arm.
[b] Exact CI was estimated using binomial distribution. For Study 2689-CL-2004, there was no adjustment for multiplicity for the co-primary endpoint of CRc.
[c] Best overall response at the end of study. Best response was defined as the best measured response (CR > CRp > CRi > PR) post-baseline.

Study AC220-002

Study AC220-002 is described under section ''dose-response studies''.

In Cohort 1, the CRc rate was 56.3% and the median duration of CRc was 12.1 weeks. The transplantation rate was 9.8%. Median OS was 25.4 weeks. The median OS was 32.7 weeks for subjects who underwent transplant and 24.9 weeks for subjects who did not.

In Cohort 2, the CRc rate was 45.6% and the median duration of CRc was 11.3 weeks (Table 34). In this cohort, the transplantation rate was 34.5%. Median OS was 24.0 weeks. The median OS was 34.1 weeks for subjects who underwent transplant and 18.4 weeks for subjects who did not.

An overview of key efficacy data for FLT3-ITD positive subjects from Study AC220-002 is provided in Table 34.

Table 34: Key Efficacy Results in FLT3-ITD (+) Subjects in Study AC220-002

	Cohort 1 (N = 112) n (%)	Cohort 2 (N = 136) n (%)
CRc Rate		
n (%)	63 (56.3)	62 (45.6)
95% CI	(46.6, 65.6)	(37.0, 54.3)
CR Rate		
n (%)	3 (2.7)	5 (3.7)
95% CI	(0.6, 7.6)	(1.2, 8.4)
CRp Rate		
n (%)	4 (3.6)	2 (1.5)
95% CI	--	--
CRi Rate		
n (%)	56 (50.0)	55 (40.4)
95% CI	--	--
CRia	43 (38.4)	44 (32.4)
CRib	13 (11.6)	11(8.1)
Median Duration of CRc, wk (95% CI)	12.1 (6.3, 15.7)	11.3 (8.1, 16.3)
Median OS, wk (95% CI)	25.4 (21.3, 29.7)	24.0 (21.1, 27.1)

CI = confidence interval; CRc = composite complete remission; CRi = complete remission with incomplete hematological recovery, includes subjects who met CRia criteria plus subjects who met CRib criteria; CRia = all criteria specified for CR are met except for incomplete hematological recovery with residual neutropenia <1 × 10^9/L with or without complete platelet recovery. RBC and platelet transfusion independence is not required; CRib = All criteria for CR or CRp are met, except for recent RBC or platelet transfusion; CRp = complete remission with incomplete platelet recovery; FLT3-ITD = Feline McDonough sarcoma-like tyrosine kinase-internal tandem duplication; OS = overall survival; RBC = red blood cell; wk = week

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The main evidence of efficacy submitted is a single pivotal phase III study for the efficacy and safety of quizartinib in patients with R/R AML FLT3-ITD positive (study AC220-007).

Treatment alternatives for the refractory/relapsed AML population include salvage chemotherapy (NCCN GL 2.2018); therefore, the comparison with salvage chemotherapy is endorsed. The optional maintenance therapy post allo HSCT can be understood considering the disease biology of FLT3-ITD positive AML and the molecular mechanism of quizartinib. However, the study design is not considered

adequate to disentangle the benefit of quizartinib in the post-HSCT setting as there was no re-randomisation post-HSCT.

The criteria for allocation of patients to low or high intensity treatment as well as criteria to decide upon allo HSCT and/or to resume treatment after allo HSCT were not pre-specified but baseline characteristics were balanced and appear to be as expected considering the treatment strata.

The proposed analysis methods are considered to be appropriate. The type I error is adequately controlled for multiple primary analyses (OSx2) and multiple endpoints (OS and EFS) through the O'Brien-Fleming sequential procedure and gatekeeping tests. Sensitivity analyses included analyses based on censoring rules around the use of subsequent therapy. In general, populations of analysis are well described.

Efficacy data and additional analyses

The starting dose of 30 mg/day before HSCT appears reasonably justified by the dose-finding studies (CP0001, AC220-002 and 2689-CL-2004). The maximum dose is limited by the risk of QTc prolongation. Support for an increase of the dose to 60 mg (if QTcF ≤450 ms after two weeks) comes from the phase 2 study 2689-CL-2004 showing a trend for increased survival for 60 mg compared to 30 mg. However, this is based on a limited number of patients and the exposure-effect relationship is not considered robust. So given the enhanced risk of QTc-prolongation, the rationale for the 60 mg dose is not completely justified.

In the pivotal study, demographic and baseline disease characteristics were generally well balanced between the two arms. Median age was 56 years for both arms and only a 26.7% of the patients were ≥ 65 years old. Patients included were relatively young (22.6% ≥65 yrs and 4.1% ≥75 yrs), whereas most patients with AML will be above 60 years of age at the time of diagnosis. Most patients were ECOG performance status 1 (50.4%) and 13.1% of them were ECOG PS=2. Most patients (75.7%) had no prior transplant history.

Overall, the study population reflects a heterogeneous target population with R/R FLT3-ITD (+) AML in a good to reasonable condition with the majority eligible for high intensity treatment. Uncertainties remain on the efficacy in patient groups not included in the study and the CHMP was therefore of the view that the indication should be restricted to patients fit for intensive first line treatment and with early relapse, reflecting the population included in the pivotal trial.

Results for the primary endpoint in the ITT population showed a statistically significant effect on OS with a HR of 0.758 (95% CI: 0.584 to 0.983; p=0.0185, median 27.0 weeks for quizartinib and 20.4 weeks for salvage chemotherapy), corresponding to a relative risk reduction of 24% in favour of quizartinib. This effect could be considered clinically pertinent in the target population with a dismal prognosis and an unmet medical need. However, these results are considered not compelling taking into account the issues with the internal and external validity of the results in the context of a single pivotal open label trial. Further, there was an early steep drop in the estimated survival probability for the control group. The analysis of the updated OS showed similar difference in medians and similar HR as the original analysis.

The results from the sensitivity analyses on different populations (PPS, censored for HSCT, censoring at FLT3 inhibitors) seem comparable to those obtained from the ITT analysis in terms of relative benefit and the median times gained by quizartinib-treated patients. Subgroup analysis of OS across various particular subgroups did not reveal an obvious differential benefit across pre-defined subgroups compared with the overall population. Nevertheless, patients eligible for low intensity chemotherapy seem to retrieve a larger benefit from quizartinib as compared to what is observed in the overall population.

In study AC220-007, almost 23% (28/122) of the patients assigned to the comparator arm never received study drug compared to 1.6% (4/245) in the quizartinib arm. Three additional sensitivity analyses were conducted to address the imbalance in the number patients randomized but not treated in the 2 arms: PPS OS analyses where untreated patients were excluded; re-sampling OS analysis, where the 28 untreated subjects of the chemo arm were replaced by random treated subjects from this very arm; untreated vs treated chemo OS analysis. These 3 analyses were consistent and apparently support an absence of impact of the non-treated imbalance in the study outcome. Nevertheless, patients who escaped the randomized treatment could share some characteristics not accessible to the presented analyses. Their outcomes possibly would have differed from what has been observed in patients kept in the study under allocated treatment. This adds some uncertainty to the observed results.

Based on resampling analyses, the estimated difference in median OS seems at least ~ 5 weeks. However, uncertainties remain on the reliability of the effect estimate due to the wide 95%-confidence intervals around these estimates. In addition, statistical significance of OS in the updated analysis is not much stronger than usual (0.0190 against a critical p-value of 0.0231 (one-sided)). This statistical significance is also lost quite early (already if between 10-20% of lowest/shortest OS are removed from the control arm for imputing only 6 patients with early censoring). Based on these data statistical robustness of OS results is therefore not established and uncertainties on the effect size remain.

The primary endpoint was not supported by a statistical significant effect for the secondary endpoint EFS, although a trend for an improvement of median EFS with quizartinib was observed (6.0 weeks for quizartinib vs 3.7 weeks for chemotherapy arm; HR =0.898, 95% CI: 0.697 to 1.157; p=0.2034). The PPS analysis showed significant results with a HR of 0.719 (6.1 weeks for quizartinib vs 0.1 weeks for chemotherapy arm; 95% CI: 0.545 – 0.948; p=0.0096). Because subjects in the salvage chemotherapy arm who were randomised but not treated were removed from the PPS analysis population, the number of patients at the start of follow-up in the PPS analysis was smaller, which meant that the relative percentage of events occurring directly after start of follow up was greater than 50%. It is therefore considered that the median EFS time of 0.1 in the control group is unlikely to represent the true median EFS in the control population. The EFS results are uninterpretable and uninformative, with the key issues being that the data collected did not allow applying an objective criterion of relapse across both arms given the difference in treatment duration.

In relation to CRc rate by sponsor-modified 2003 IWG response (Cheson) criteria for AML 94.7% of the quizartinib-treated patients and 67.2% salvage chemotherapy-treated patients were evaluable for the best overall response rate, in the ITT population. Results showed a higher percentage of CRc for quizartinib arm compared to salvage chemotherapy arm (48.2% vs 27.0%); the majority of the responses were CRi [99/245 (40.4%) vs 32/122 (26.2%)]. CR and PR was observed in 4.1% vs 0.8% and 21.2% vs 3.3% of the patients, respectively. A median duration of CR could not be evaluated because only 11 subjects (10 subjects in the quizartinib arm and one subject in the salvage chemotherapy arm) met the criteria for CR. Even if an important difference in CRc is observed comparing the two arms, a difference of 7.1 weeks in the duration of the response could not be considered as clinically pertinent. In addition, despite longer duration of CRc in the quizartinib arm, there was no difference in LFS (12.0% vs 11.1%).

It was noted that a higher rate of patients on quizartinib proceeded to HSCT for those in CRc, that more patients proceeded to HSCT in the quizartinib arm with partial response or no response and in particular and unexpectedly in the low intensity group, while these patients are at baseline unlikely to proceed to HSCT. It can therefore not be excluded that this is partly influenced by investigator's knowledge of treatment and thus may have influenced the OS results in favour of quizartinib.

The magnitude of the treatment effect on CRc and HSCT rate is likely to be overestimated based on the ITT analysis given that patients who withdrew before treatment appear to have been counted as

"non-responders". Based on the PPS, CRc rates were 49.4% vs 35.2% for patients treated with quizartinib and salvage chemotherapy, respectively. Numerical higher response rates were seen for quizartinib independent of relapse (+/- prior HSCT) /refractory status of the patient. However, when stratified for intensity of chemotherapy, no difference was seen in patients eligible for high intensity chemotherapy, which are the majority of patients. This suggests that the observed increase in best overall response rate is driven by the subgroup of patients not eligible for high intensity chemotherapy. This was also seen when the results of CRc were presented for the six randomization strata based on the PPS population (data not shown).

Overall, 78 patients (31.8%) in the quizartinib arm and 14 patients (11.5%) in the salvage chemotherapy arm underwent an allogenic HSCT post protocol treatment. Comparable results were observed based on the PPS analysis (31.6% vs 13.6%, respectively). From these, a higher percentage of successful transplant (52.6% vs 35.7%) and a lower percentage of relapse were shown in quizartinib arm compared to salvage chemotherapy arm (30.8% vs 42.9%). The increase in HSCT rate could potentially be of clinical relevance as HSCT offers the only potentially curative treatment option in this patient population and offers thus a potential increase in OS. However, based on the OS data the quizartinib curve approaches that of the salvage chemotherapy arm and it is uncertain whether an OS benefit remains in the long-term.

A total of 48 (62%) out of 78 patients resumed quizartinib post-HSCT, one patient received quizartinib post-autologous HSCT. Quizartinib could be resumed as early as day 30 post HSCT, however reasons for the restart were unknown and in the absence of re-randomisation, it cannot be excluded that patients starting treatment were those with a better prognosis. Further, at the end of the study 15 patients were still on quizartinib whereas 34 discontinued treatment, mainly due to relapse (n=17) or an adverse event (n=10). Though the data do not allow assessing the efficacy of quizartinib post-HSCT and limited number of patients was treated, use of post-HSCT was part of the overall treatment strategy of the study.

2.5.4. Conclusions on the clinical efficacy

The single pivotal trial was borderline positive on the primary endpoint, overall survival. Notably, there was extensive missing data and non-administrative censoring, predominantly in the control arm of this open-label study, and statistical significance was not robust to sensitivity analyses. Moreover, results of the primary endpoint are not supported by established effects on the secondary endpoint event free survival and the exploratory endpoint complete remission. Consequently, the efficacy of quizartinib in the claimed indication is not considered established.

2.6. Clinical safety

The pivotal active-controlled, open-label, randomised phase 3 study AC220-007 provides the primary support for the safety profile for the indication of quizartinib monotherapy in subjects with FLT3-ITD-positive RR AML. Additional support is provided by the RR AML pooled group, which includes a total of 673 subjects with RR AML from 4 studies, including 241 subjects from the pivotal study, all of which enrolled subjects with RR AML who received quizartinib dihydrochloride QD, at doses ranging from 30 mg to 300 mg QD, depending on the study. The RR AML pool included subjects with RR AML that was FLT3-ITD positive (studies AC220-007 and 2689-CL-2004) and regardless of FLT3-ITD status (studies CP0001 and ACC220-002).

Patient exposure

Exposure in the RR AML and AML pooled groups

As of 22 Feb 2018, 737 subjects with AML, 13 subjects with solid tumours, and 307 healthy volunteers, and a further 370 subjects in the 6 investigator-initiated studies (estimated as of 16 Jul 2018) have been exposed to quizartinib. The number of subjects exposed to quizartinib is provided for each of the 5 sponsored studies comprising the pooled analyses and the number of those subjects who are included in each of the RR AML and AML pooled groups are shown in **Table 48**. The AML pooled group consists of all subjects exposed to quizartinib in the 5 studies (737 subjects). The RR AML pooled group consists of subjects exposed to quizartinib in 4 studies (673 subjects). In the RR AML pooled group, there were 64 fewer subjects because 51 of 76 subjects from Study CP0001 who were not in the target dose group (as they received intermittent therapy) and all 13 subjects from Study 2689-CL-0011 who were not in the target indication (as they received treatment in the post-allogeneic HSCT period) were not included.

For the clinical safety results, the data will focus on the target population, i.e. the RR AML Pooled Group and the pivotal AC220-007 study.

Table 43. Quizartinib doses studied and number of subjects exposed in RR AML and AML pooled groups

Study	Quizartinib Dose Studied (mg/day)	Subjects Exposed	RR AML Pooled Group	AML Pooled Group
AC220-007	60	241	241	241
AC220-002	90, 135, and 200	333	333	333
2689-CL-2004	30 or 60	74	74	74
CP0001				
Continuous dosing	200 or 300	25	25	25
Intermittent dosing	12, 18, 27, 40, 60, 90, 135, 200, 300, and 450	51	NA	51
All subjects		-	-	76
2689-CL-0011 (post-HSCT subjects)	40 and 60	13	NA	13
Total		737	673	737

AML = acute myeloid leukemia; HSCT = hematopoietic stem cell transplantation; NA = not applicable; RR = relapsed/refractory.

Drug exposure

A summary of quizartinib exposure by treatment duration, total patient-years, grouped exposure duration, cumulative dose, and average daily dose for Study AC220-007 and the RR AML pooled group is shown in Table 49.

Table 44.Summary of study drug exposure to quizartinib for study AC220-007 and RML AML pooled group (safety analysis set)

	AC220-007 Quizartinib Monotherapy N = 241	RR AML Pooled Group N = 673
Treatment Duration, days		
Mean (SD)	154.0 (182.50)	126.4 (154.83)
Median	97.0	79.0
Minimum, Maximum	1, 1182.0	1, 1296.0
Total Patient-Years of Exposure	101.6[a]	232.9
Exposure Duration Group, n (%)		
1 days to 15 days	NA	673 (100.0)
16 days to 30 days	NA	640 (95.1)
31 days to 90 days	NA	587 (87.2)
91 days to 180 days	NA	297 (44.1)
181 days to 365 days	NA	114 (16.9)
366 days to 730 days	NA	43 (6.4)
>730 days	NA	10 (1.5)
Cumulative Dose, mg		
Mean (SD)	5518.5 (5701.70)	8358.5 (12092.76)
Median	3710.0	5265.0
Minimum, Maximum	30, 31200	30, 208800
Average Daily Dose, mg[b]		
Mean (SD)	38.1 (12.37)	78.8 (51.25)
Median	35.7	61.4
Minimum, Maximum	15.2, 58.2	2.0, 314.0
RDI[c]		
Mean (SD)	0.9 (0.29)	NA
Median	0.9	NA
Minimum, Maximum	0.3, 2.0	NA

AML = acute myeloid leukemia; N = total number of subjects; n = number of subjects in the category; NA = not applicable; RDI = relative dose intensity; RR = relapsed/refractory; SAP = Statistical Analysis Plan; SD = standard deviation.

[a] Total Patient-Years of Exposure = Mean treatment duration (days) multiplied by total N divided by 365.25.

[b] Average daily dose is calculated as cumulative dose divided by the treatment duration.

[c] Relative dose intensity is defined as the actual average daily dose divided by the planned average daily dose for the quizartinib arm. See Study AC220-007 SAP for further details.

Note: Cumulative Dose = cumulative amount of drug administered.

Denominator for percentages is the number of subjects in the Safety Analysis Set.

Treatment Duration (days) = date of last dose - date of first dose + 1.

In Study AC220-007, subjects randomized to quizartinib received therapy over a median of 97 days (four 28-day cycles), while subjects randomized to salvage chemotherapy received a median of one 4-week cycle.

Adverse events

In study AC220-007, TEAEs are defined as AEs that first occurred or worsened in severity after the first dose of study drug.

Table 45 Overview of Treatment-Emergent Adverse Events in Study AC220-007 and the RR AML Pooled Group (Safety Analysis Set)

	Study AC220-007		RR AML Pooled Group N =673 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
TEAEs	238 (98.8)	93 (98.9)	667 (99.1)
TEAEs Grade ≥3	211 (87.6)	74 (78.7)	597 (88.7)
Treatment-emergent SAEs	168 (69.7)	37 (39.4)	502 (74.6)
TEAEs associated with outcome of death	36 (14.9)	11 (11.7)	193 (28.7)
TEAEs associated with discontinuation of study drug	44 (18.3)	1 (1.1)	173 (25.7)
Treatment-related TEAEs	205 (85.1)	66 (70.2)	585 (86.9)
Treatment-related TEAEs Grade ≥3	154 (63.9)	48 (51.1)	428 (63.6)
Any treatment-related treatment-emergent SAE	64 (26.6)	15 (16.0)	263 (39.1)
Treatment-related TEAEs associated with outcome of death	9 (3.7)	4 (4.3)	28 (4.2)
Treatment-related TEAEs associated with discontinuation of study drug	17 (7.1)	0 (0.0)	67 (10.0)

AE = adverse event; AML = acute myeloid leukemia; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; RR = relapsed/refractory; SAE = serious adverse event; TEAE = treatment-emergent adverse event.

Treatment-related TEAEs

Treatment-related TEAEs were defined as TEAEs that were assessed by the investigator as definitely, possibly, or not related to study drug.

Table 46 Most frequent (≥5% in RR AML Pool) treatment-related TEAEs by PT in study AC220-007 and the RR AML Pooled Group (safety analysis set)

Preferred Term	Study AC220-007		RR AML Pooled Group N = 673 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
Subjects with Any Treatment-Related TEAE	**205 (85.1)**	**66 (70.2)**	**585 (86.9)**
Nausea	80 (33.2)	32 (34.0)	225 (33.4)
Electrocardiogram QT prolonged	60 (24.9)	1 (1.1)	171 (25.4)
Anaemia	60 (24.9)	20 (21.3)	159 (23.6)
Vomiting	48 (19.9)	14 (14.9)	142 (21.1)
Fatigue	39 (16.2)	14 (14.9)	137 (20.4)
Diarrhoea	30 (12.4)	24 (25.5)	116 (17.2)
Febrile neutropenia	32 (13.3)	16 (17.0)	118 (17.5)
Thrombocytopenia	51 (21.2)	15 (16.0)	99 (14.7)
Decreased appetite	25 (10.4)	9 (9.6)	93 (13.8)
Dysgeusia	14 (5.8)	1 (1.1)	91 (13.5)
Neutropenia	34 (14.1)	9 (9.6)	72 (10.7)
Leukopenia	12 (5.0)	2 (2.1)	39 (5.8)
Pyrexia	19 (7.9)	15 (16.0)	60 (8.9)
Oedema peripheral	12 (5.0)	10 (10.6)	57 (8.5)
Hypokalaemia	23 (9.5)	8 (8.5)	57 (8.5)
Dyspepsia	9 (3.7)	4 (4.3)	55 (8.2)
Platelet count decreased	27 (11.2)	9 (9.6)	53 (7.9)
Headache	20 (8.3)	5 (5.3)	44 (6.5)
Alanine aminotransferase increased	25 (10.4)	1 (1.1)	48 (7.1)
Abdominal pain	13 (5.4)	6 (6.4)	43 (6.4)
Constipation	10 (4.1)	9 (9.6)	43 (6.4)
White blood cell count decreased	30 (12.4)	12 (12.8)	42 (6.2)
Asthenia	9 (3.7)	5 (5.3)	41 (6.1)
Dizziness	9 (3.7)	4 (4.3)	39 (5.8)
Neutrophil count decreased	28 (11.6)	10 (10.6)	39 (5.8)
Petechiae	5 (2.1)	2 (2.1)	34 (5.1)

AE = adverse event; AML = acute myeloid leukemia; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; QT = interval between the start of the Q wave and the end of the T wave; RR = relapsed/refractory; TEAE = treatment-emergent- adverse event.

Adverse Events of Special Interest (AESIs)

Table 47 Treatment-emergent AESIs in Study AC220-007 and the RR AML Pooled Group (Safety Analysis Set)

AESI Category	Study AC220-007		RR AML Pooled Group N = 673 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
Subjects with Any AESI	**220 (91.3)**	**81 (86.2)**	**614 (91.2)**
Infection	185 (76.8)	68 (72.3)	510 (75.8)
Haemorrhages	119 (49.4)	36 (38.3)	368 (54.7)
Torsade de Pointes/QT prolongation	74 (30.7)	7 (7.4)	199 (29.6)
Hepatic disorders	77 (32.0)	15 (16.0)	165 (24.5)
Cardiac arrhythmias	29 (12.0)	10 (10.6)	85 (12.6)
Cardiac failure	4 (1.7)	2 (2.1)	28 (4.2)

AE = adverse event; AESI = adverse event of special interest; AML = acute myeloid leukaemia;
MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; PT = preferred term; QT = interval between the start of the Q wave and the end of the T wave;
RR = relapsed/refractory; SAP = Statistical Analysis Plan; SMQ = Standardised MedDRA Queries; SOC = system organ class; TEAE = treatment-emergent adverse event.

- *Torsade de pointes/QT prolongation*

Subjects exposed to quizartinib presented more AESIs in the Torsade de Pointes/QT prolongation category than subjects in salvage chemotherapy arm of Study AC220-007 (74 [30.7%] subjects in quizartinib arm of Study AC220-007 and 199 [29.6%] subjects in the RR AML pool vs 7 [7.4%] subjects in salvage chemotherapy arm). The majority of subjects with AESI in the Torsade de Pointes/QT prolongation category were ECG QT prolonged.

Table 48 AESI – Torsade de pointes/QT prolongation in Study AC220-007 and the RR AML pooled group (Safety analysis set)

Preferred Term	Study AC220-007		RR AML Pooled Group (N = 673) n (%)
	Quizartinib Monotherapy (N = 241) n (%)	Salvage Chemotherapy (N = 94) n (%)	
Subjects with Any TEAE of Special Interest	**74 (30.7)**	**7 (7.4)**	**199 (29.6)**
Electrocardiogram QT prolonged	64 (26.6)	2 (2.1)	178 (26.4)
Syncope	12 (5.0)	2 (2.1)	22 (3.3)
Ventricular tachycardia	1 (0.4)	2 (2.1)	4 (0.6)
Cardiac arrest	0 (0.0)	1 (1.1)	3 (0.4)
Loss of consciousness	0 (0.0)	0 (0.0)	2 (0.3)
Cardio-respiratory arrest	0 (0.0)	0 (0.0)	0 (0.0)
Torsade de Pointes	0 (0.0)	0 (0.0)	1 (0.1)

AE = adverse event; AESI = adverse event of special interest; AML = acute myeloid leukemia; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; QT = interval between the start of the Q wave and the end of the T wave; PT = preferred term; RR = relapsed/refractory; SAP = Statistical Analysis Plan; SMQ = Standardised MedDRA Queries; SOC = system organ class; TEAE = treatment-emergent adverse event.

Eight (3.3%) subjects in the quizartinib arm had ECG QTcF values >500 ms (Grade 3 QTcF) by central ECG reading.

Table 49 Summary of QTcF Intervals in Study AC220-007 and the RR AML Pooled Group (Safety Analysis Set)

QTcF Interval, ms	Study AC220-007[a]		RR AML Pooled Group[b] (N = 673) n (%)
	Quizartinib Monotherapy (N = 241) n (%)	Salvage Chemotherapy (N = 94) n (%)	
n	241	94	667
New >450	114 (47.3)	6 (6.4)	425 (63.7)
New >480	38 (15.8)	0 (0.0)	193 (28.9)
New >500	8 (3.3)	0 (0.0)	75 (11.2)
Increase >30 from baseline	151 (62.7)	14 (14.9)	509 (76.3)
Increase >60 from baseline	30 (12.4)	1 (1.1)	183 (27.4)

AML = acute myeloid leukemia; bpm = beats per minute; ECG = electrocardiogram; N = total number of subjects; n = total number of subjects with baseline and at least 1 post-baseline value; QTcF = corrected QT interval by Fredericia; RR = relapsed/refractory.

a Baseline for the ECG parameter is defined as the average of the last 3 ECG measurements taken prior to the firstdose of study drug. Post-baseline is defined as occurring after the first administration of study drug up to 30 days after the last administration of study drug, including unscheduled visits.

b The baseline value is defined as the average of all non-missing values on the last date before initial administration of study drug. Worst post-baseline value is summarized. Assessments performed more than 30 days after the discontinuation of study drug are not summarized.

Note: Post-baseline is defined as occurring after the first administration of study treatment up to 30 days after the last administration of study treatment, including unscheduled visits.

"New" is the category of the QTc abnormality that was not present prior to the first dose and became present in at least 1 post-baseline ECG assessment.

- *Hepatic disorders*

TEAEs in the hepatic disorders AESI category were reported in 32.0% of subjects in the quizartinib arm of Study AC220-007 and 24.5% of subjects in the RR AML pool.

Table 50 AESI – Hepatic disorders in Study AC220-007 and the RR AML Pooled group (≥1% of Subjects in RR AML Pool) (Safety Analysis Set)

Preferred Term	Study AC220-007		RR AML Pooled Group N = 673 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
Subjects with Any TEAE in the Hepatic Disorders AESI Category	**77 (32.0)**	**15 (16.0)**	**165 (24.5)**
Alanine aminotransferase increased	32 (13.3)	4 (4.3)	66 (9.8)
Aspartate aminotransferase increased	26 (10.8)	1 (1.1)	51 (7.6)
Blood bilirubin increased	24 (10.0)	3 (3.2)	44 (6.5)
Hyperbilirubinaemia	2 (0.8)	0 (0.0)	12 (1.8)
International normalised ratio increased	6 (2.5)	2 (2.1)	10 (1.5)
Liver function test abnormal	4 (1.7)	1 (1.1)	10 (1.5)
Gamma-glutamyltransferase increased	7 (2.9)	3 (3.2)	8 (1.2)
Hepatocellular injury	1 (0.4)	0 (0.0)	7 (1.0)

AE = adverse event; AESI = adverse event of special interest; AML = acute myeloid leukemia; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; RR = relapsed/refractory; SAP = Statistical Analysis Plan; SMQ = Standardised MedDRA Queries; TEAE = treatment-emergent adverse event.

- *Intracranial/cerebral haemorrhage*

TEAEs within the haemorrhages AESI category were less frequent in the quizartinib arm of Study AC220-007 (119 [49.4%] subjects) than in the RR AML pool (368 [54.7%] subjects). The most frequent TEAEs overall in this AESI category were epistaxis (28 [11.6%] and 104 [15.5%] subjects in the quizartinib arm of Study AC220-007 and the RR AML pool, respectively) and petechiae (27 [11.2%] and 96 [14.3%] subjects, respectively).

In Study AC220-007, TEAEs in the haemorrhage AESI category were reported for 49.4% of subjects in the quizartinib arm and 38.3% of subjects in the salvage chemotherapy arm. The most frequent haemorrhage AESIs in either group were epistaxis and petechiae (Table 56).

Table 51. TEAESI – Haemorrhages occurring in ≥5% of subjects in the quizartinib arm or considered medically significant in study AC220-007 (safety analysis set)

	Quizartinib Monotherapy (N = 241)					Salvage Chemotherapy (N = 94)			
TEAESI Category	TEAE n (%)	Grade ≥3 n (%)	SAE n (%)	Study Drug Discontinuation n (%)	Death n (%)	TEAE n (%)	Grade ≥3 n (%)	SAE n (%)	Death n (%)
Subjects with any haemorrhage AESI	119 (49.4)	24 (10.0)	19 (7.9)	8 (3.3)	7 (2.9)	36 (38.3)	8 (8.5)	3 (3.2)	2 (2.1)
Epistaxis	28 (11.6)	4 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	8 (8.5)	1 (1.1)	0 (0.0)	0 (0.0)
Petechiae	27 (11.2)	2 (0.8)	1 (0.4)	0 (0.0)	0 (0.0)	6 (6.4)	0 (0.0)	0 (0.0)	0 (0.0)
Gingival Bleeding	16 (6.6)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.2)	1 (1.1)	0 (0.0)	0 (0.0)
Contusion	15 (6.2)	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
Haematuria	12 (5.0)	1 (0.4)	2 (0.8)	0 (0.0)	0 (0.0)	2 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
Haemorrhage intracranial	5 (2.1)	5 (2.1)	5 (2.1)	3 (1.2)	4 (1.7)	2 (2.1)	2 (2.1)	2 (2.1)	1 (1.1)
Subdural haemorrhage	1 (0.4)	1 (0.4)	1 (0.4)	1 (0.4)	0 (0.0)	1 (1.1)	1 (1.1)	1 (1.1)	1 (1.1)
Cerebral haemorrhage	2 (0.8)	2 (0.8)	2 (0.8)	1 (0.4)	2 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

AESI = adverse event of special interest; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; SAE = serious adverse event; TEAE = treatment-emergent adverse event; TEAESI = treatment-emergent adverse event of special interest.

Serious adverse event/deaths/other significant events

Serious adverse events (SAEs)

Treatment-emergent SAEs were reported in 168 (69.7%) subjects of the quizartinib arm of Study AC220-007 and in 502 (74.6%) subjects in the RR AML pooled group ().

Table 57).

Table 52. Summary of TESAEs (≥1% of subjects in RR AML Pool) by PT in study AC220-00 and the RR AML Pooled Group (safety analysis set)

	Study AC220-007		
Preferred Term	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	RR AML Pooled Group N = 673 n (%)
Subjects with Any Treatment-Emergent SAE	**168 (69.7)**	**37 (39.4)**	**502 (74.6)**
Febrile neutropenia	50 (20.7)	9 (9.6)	190 (28.2)
Acute myeloid leukaemia[a]	NA	NA	83 (12.3)
Pneumonia	22 (9.1)	3 (3.2)	72 (10.7)
Sepsis	16 (6.6)	4 (4.3)	44 (6.5)
Electrocardiogram QT prolonged	5 (2.1)	0 (0.0)	43 (6.4)
Pyrexia	8 (3.3)	2 (2.1)	35 (5.2)
Anaemia	6 (2.5)	0 (0.0)	20 (3.0)
Urinary tract infection	6 (2.5)	0 (0.0)	19 (2.8)
Cellulitis	6 (2.5)	0 (0.0)	17 (2.5)
Thrombocytopenia	3 (1.2)	0 (0.0)	17 (2.5)
Bacteraemia	4 (1.7)	1 (1.1)	15 (2.2)
Gastrointestinal haemorrhage	1 (0.4)	0 (0.0)	15 (2.2)
Atrial fibrillation	2 (0.8)	0 (0.0)	14 (2.1)
Septic shock	5 (2.1)	1 (1.1)	14 (2.1)
Nausea	5 (2.1)	0 (0.0)	13 (1.9)
Vomiting	5 (2.1)	0 (0.0)	13 (1.9)
Pneumonia fungal	3 (1.2)	2 (2.1)	12 (1.8)
Renal failure acute	6 (2.5)	0 (0.0)	12 (1.8)
Lung infection	3 (1.2)	0 (0.0)	11 (1.6)
Device related infection	3 (1.2)	1 (1.1)	10 (1.5)
Diarrhoea	2 (0.8)	0 (0.0)	10 (1.5)
Haemorrhage intracranial	5 (2.1)	2 (2.1)	10 (1.5)
Disease progression[a]	NA	NA	9 (1.3)
Febrile bone marrow aplasia	1 (0.4)	0 (0.0)	9 (1.3)
Neutropenic sepsis	7 (2.9)	2 (2.1)	9 (1.3)
Neutropenia	4 (1.7)	0 (0.0)	8 (1.2)
Pancytopenia	3 (1.2)	0 (0.0)	8 (1.2)
Syncope	5 (2.1)	0 (0.0)	8 (1.2)
Dehydration	0 (0.0)	0 (0.0)	7 (1.0)
Respiratory failure	2 (0.8)	0 (0.0)	7 (1.0)
Upper gastrointestinal haemorrhage	1 (0.4)	0 (0.0)	7 (1.0)

Deaths

On-treatment deaths due to any cause

The majority of treatment-emergent deaths in the quizartinib arm of Study AC220-007 and the RR AML pool were attributed to AML disease progression (Table 58).

Table 53. Summary of on-treatment deaths by primary cause of death in study AC220-007 and the RR AML Pooled Group (safety analysis set)

Primary Cause of Death	Study AC220-007		RR AML Pooled Group N = 599 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
Subjects with On-Treatment Deaths	**80 (33.2)**	**16 (17.0)**	**215 (35.9)**
AML disease progression	49 (20.3)	7 (7.4)	130 (21.7)
Adverse event	31 (12.9)	9 (9.6)	83 (13.9)
Other/unknown	0 (0.0)	0 (0.0)	2 (0.3)

AML = acute myeloid leukemia; N = total number of subjects; n = number of subjects in the category; RR = relapsed/refractory.
Note: On-treatment death is defined as death that occurs between first dose date and ≤30 days after the last dose of study drug.
Primary cause of death was not collected for Studies 2689-CL-0011 and 2689-CL-2004.

TEAEs associated with outcome of death

A summary of the proportion of subjects having TEAEs with death is displayed in Table 59.

Table 54. TEAEs associated with outcome of death in ≥2 subjects in study AC220-007 and the RR AML Pooled Group (safety analysis set)

Preferred Term	Study AC220-007		RR AML Pooled Group N = 673 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
Subjects with Any TEAEs Associated with Outcome of Death	**36 (14.9)**	**11 (11.7)**	**193 (28.7)**
Acute myeloid leukaemia[a]	NA	NA	77 (11.4)
Pneumonia	7 (2.9)	1 (1.1)	15 (2.2)
Sepsis	2 (0.8)	0 (0.0)	12 (1.8)
Haemorrhage intracranial	4 (1.7)	1 (1.1)	8 (1.2)
Disease progression[a]	NA	NA	7 (1.0)
Septic shock	2 (0.8)	1 (1.1)	6 (0.9)
Cerebral haemorrhage	2 (0.8)	0 (0.0)	4 (0.6)
Lung infection	2 (0.8)	0 (0.0)	4 (0.6)
Multi-organ failure	1 (0.4)	1 (1.1)	4 (0.6)
Respiratory failure	1 (0.4)	0 (0.0)	4 (0.6)
Cardiac failure	1 (0.4)	0 (0.0)	3 (0.4)
Disseminated intravascular coagulation	1 (0.4)	0 (0.0)	3 (0.4)
Pneumonia fungal	0 (0.0)	1 (1.1)	3 (0.4)
Renal failure acute	1 (0.4)	0 (0.0)	3 (0.4)
Bacterial sepsis	0 (0.0)	0 (0.0)	2 (0.3)
Bronchopulmonary aspergillosis	1 (0.4)	0 (0.0)	2 (0.3)
Cardiac arrest	0 (0.0)	0 (0.0)	2 (0.3)
General physical health deterioration	0 (0.0)	0 (0.0)	2 (0.3)
Graft versus host disease in intestine	2 (0.8)	0 (0.0)	2 (0.3)
Leukocytosis	1 (0.4)	0 (0.0)	2 (0.3)
Myocardial infarction	1 (0.4)	0 (0.0)	2 (0.3)
Respiratory distress	1 (0.4)	0 (0.0)	2 (0.3)
Subdural haematoma	0 (0.0)	0 (0.0)	2 (0.3)

AE = adverse event; AML = acute myeloid leukemia; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; RR = relapsed/refractory; TEAE = treatment-emergent adverse event.
[a] In Study AC220-007, death due to disease progression or worsening of AML was not recorded as a TEAE.

Laboratory findings

- *Haematology*

In both treatment arms, the worst post-baseline hemoglobin values (anaemia) were Grade 2 or Grade 3. The worst post-baseline leukopenia values in both treatment arms were Grade 3 or Grade 4; a greater proportion of subjects in the salvage chemotherapy arm had Grade 4 values than subjects in the quizartinib arm. For both the quizartinib and salvage chemotherapy arms, the worst post-baseline lymphocyte values (lymphopenia) were Grades 2, 3, and 4; the proportion of subjects with Grade 4 values was greater in the salvage chemotherapy arm than in the quizartinib arm. The majority of subjects in both treatment arms had Grade 4 neutrophil and platelet values; these values generally occurred in a similar proportion of subjects in both groups.

- *Hepatic function*

Table 55 Summary of Liver Enzymes and Total Bilirubin Elevation in Study AC220-007 and the RR AML Pooled Group (Safety Analysis Set)

Category	Study AC220-007		RR AML
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	Pooled Group N = 673 n (%)
Liver Enzymes			
AST ≥3 × ULN or ALT ≥3 × ULN	30 (12.4)	2 (2.1)	110 (16.3)
AST ≥5 × ULN or ALT ≥5 × ULN	9 (3.7)	1 (1.1)	38 (5.6)
AST ≥8 × ULN or ALT ≥8 × ULN	4 (1.7)	1 (1.1)	10 (1.5)
AST ≥10 × ULN or ALT ≥10 × ULN	2 (0.8)	1 (1.1)	6 (0.9)
AST ≥20 × ULN or ALT ≥20 × ULN	1 (0.4)	1 (1.1)	2 (0.3)
ALP ≥2 × ULN	45 (18.7)	4 (4.3)	128 (19.0)
Total Bilirubin Elevation			
TBL ≥1.5 × ULN	35 (14.5)	10 (10.6)	92 (13.7)
TBL ≥2 × ULN	19 (7.9)	5 (5.3)	53 (7.9)
TBL ≥2 × ULN and ALT ≥3 × ULN[a]	8 (3.3)	1 (1.1)	17 (2.5)
TBL ≥2 × ULN and (AST ≥3 × ULN or ALT ≥3 × ULN)[a]	8 (3.3)	1 (1.1)	18 (2.7)
TBL ≥2 × ULN and (AST ≥3 × ULN or ALT ≥3 × ULN) and ALP <2 × ULN[a]	8 (3.3)	1 (1.1)	9 (1.3)

AML = acute myeloid leukemia; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; N = total number of subjects; n = number of subjects in the category; RR = relapsed/refractory; TBL = total bilirubin; ULN = upper limit of normal.
a Elevations of TBL and ALT/AST do not have to be concurrent.
Note: Assessments performed more than 30 days after the discontinuation of study drug are not summarized.
Percentage is calculated using the number of subjects in the column heading as denominator.
Only includes laboratory collected after the first dose of study drug and less than 30 days after the last dose of study drug.

- *ECG*

Table 56 Summary of Notable ECG Values in Study AC220-007 and the RR AML Pooled Group (Safety Analysis Set)

ECG Value	Study AC220-007[a]		RR AML Pooled Group[b] N = 673 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
QTcF Interval, ms			
n	241	94	667
New >450	114 (47.3)	6 (6.4)	425 (63.7)
New >480	38 (15.8)	0 (0.0)	193 (28.9)
New >500	8 (3.3)	0 (0.0)	75 (11.2)
Increase >30 from baseline	151 (62.7)	14 (14.9)	509 (76.3)
Increase >60 from baseline	30 (12.4)	1 (1.1)	183 (27.4)
PR Interval, ms			
n	241	94	664
Increase >25% from baseline and >200 ms post-baseline	2 (0.8)	0 (0.0)	11 (1.7)
QRS Interval, ms			
n	241	94	667
Increase >25% from baseline and >100 ms post-baseline	2 (0.8)	0 (0.0)	15 (2.2)
Heart Rate, bpm			
n	241	94	667
Decrease >25% from baseline and <50 bpm post-baseline	4 (1.7)	0 (0.0)	17 (2.5)
Increase >25% from baseline and >100 bpm post-baseline	32 (13.3)	7 (7.4)	76 (11.4)

AML = acute myeloid leukemia; bpm = beats per minute; ECG = electrocardiogram; HR = heart rate; N = total number of subjects; ms = millisecond; n = total number of subjects with baseline and at least 1 post-baseline value;
QTcF = corrected QT interval by Fredericia; RR = relapsed/refractory.
a Baseline for the ECG parameter is defined as the average of the last 3 ECG measurements taken prior to the first dose of study drug. Post-baseline is defined as occurring after the first administration of study drug up to 30 days after the last administration of study drug, including unscheduled visits.
b The baseline value is defined as the average of all non-missing values on the last date before initial administration of study drug. Worst post-baseline value is summarized. Assessments performed more than 30 days after the discontinuation of study drug are not summarized.
Note: "New" is the category of the QTc abnormality that was not present prior to the first dose and became present in at least 1 post-baseline ECG assessment.

Safety in special populations

Age

Table 57 Overview of Treatment-Emergent Adverse Events by Age Group (RR AML Subjects 60 mg Dose Group)

MedDRA Terms	Age <65 N=205 n (%)	Age 65 to 75 N=60 n (%)	Age ≥75 N=12 n (%)
Total AEs	202 (98.5)	60 (100.0)	12 (100.0)
Serious AEs – Total	140 (68.3)	44 (73.3)	7 (58.3)
- Fatal	36 (17.6)	13 (21.7)	0
- Hospitalization/prolong existing hospitalization	130 (63.4)	42 (70.0)	7 (58.3)
- Life-threatening	28 (13.7)	10 (16.7)	1 (8.3)
- Disability/incapacity	4 (2.0)	1 (1.7)	0
- Other (medically significant)	23 (11.2)	8 (13.3)	0
AE leading to drop-out	36 (17.6)	15 (25.0)	1 (8.3)
Psychiatric disorders (SOC)	49 (23.9)	14 (23.3)	2 (16.7)
Nervous system disorders (SOC)	99 (48.3)	25 (41.7)	7 (58.3)
Accidents and injuries (SOC)	50 (24.4)	13 (21.7)	6 (50.0)
Cardiac disorders (SOC)	38 (18.5)	17 (28.3)	2 (16.7)
Vascular disorders (SOC)	57 (27.8)	18 (30.0)	3 (25.0)
Cerebrovascular disorders (HLGT)[a]	7 (3.4)	3 (5.0)	0
Infections and infestations (SOC)	137 (66.8)	42 (70.0)	10 (83.3)
Anticholinergic syndrome (PT)	0	0	0
Quality of life decreased (PT)	0	0	0
Sum of Orthostatic hypotension [b]	1 (0.5)	0	0
Fall (PT)	5 (2.4)	5 (8.3)	3 (25.0)
Loss of consciousness (PT)	0	0	0
Syncope (PT)	8 (3.9)	3 (5.0)	2 (16.7)
Dizziness (PT)	28 (13.7)	10 (16.7)	2 (16.7)
Ataxia (PT)	0	0	0
Coordination abnormal (PT)	0	0	0
Balance disorder (PT)	0	0	0
Fractures and dislocations NEC (HLT)	0	0	0
Limb fractures and dislocations (HLT)	0	2 (3.3)	0
Pelvic fractures and dislocations (HLT)	1 (0.5)	0	0
Skull fractures, facial bone fractures and dislocations (HLT)	0	0	0
Spinal fractures and dislocations (HLT)	2 (1.0)	0	0
Thoracic cage fractures and dislocations (HLT)	0	0	0

AE = adverse event; AML = acute myeloid leukemia; HLGT = high level group term; HLT = high level term; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; PT = preferred term; RR = relapsed/refractory; SOC = system organ class; TEAE = treatment-emergent adverse event.
Notes: Percentage is calculated using number of subjects in the column heading as denominator.
A TEAE is defined as an AE that occurs, having been absent before the first dose of study drug or worsened in severity after first dose of study drug, on or after first dose of study drug and up to 30 days after last dose of study drug. AEs collected after 30 days after the last dose of study drug were not considered TEAEs unless they were treatment-related.
Subjects may have more than one event per system organ class and preferred term. At each level of subject summarization, a subject was counted once if he/she reported one or more adverse events.
Adverse events were coded using MedDRA Version 16.1.
[a] HLGT CNS Vascular Disorders
[b] Sum of the following preferred terms: Blood pressure orthostatic, Blood pressure orthostatic abnormal, Blood pressure orthostatic decreased, Orthostatic hypotension

Gender

In the RR AML pool, the most frequent TEAEs (≥35%) reported in male subjects were nausea (44.1%), pyrexia (35.9%), and febrile neutropenia (35.9%). In female subjects, the most frequent TEAEs reported were nausea (53.8%), vomiting (38.7%), febrile neutropenia (38.1%), and diarrhoea (37.8%). TEAEs of Grade ≥3 were reported in similar proportions of female and male subjects (89.7% and 85.6%, respectively) in the 60-mg dose group of the RR AML pool. Proportionally more female subjects than male subjects were reported with Grade ≥3 ECG QT prolonged (6.9% and 1.5%, subjects, respectively), thrombocytopenia (27.6% and 18.9%, subjects, respectively), and anaemia (30.3% and 25.8%, subjects, respectively). Proportionally more male subjects than female subjects were reported with Grade ≥3 neutrophil count decreased (12.9% and 9.0%, respectively).

In Study AC220-007, the events of vomiting, diarrhoea, ECG QT prolonged, thrombocytopenia, headache, oedema peripheral, and dyspnoea occurred more frequently (≥5% difference) in females than in males.

A categorical summary of QT/QTcF elevations by sex in the RR AML pool showed that treatment-emergent QTcF interval increase >450 ms was reported for 70.4% of females versus 57.1% of males. QTcF interval increases of >30 ms and >60 ms from baseline were generally similar between male and female subjects.

Race

The majority of subjects in the RR AML pool were white (551 [81.9%] subjects). Small sample sizes for races other than White limit the analyses, but in general, the type and frequencies of TEAEs reported were similar across subgroups.

Baseline Body Mass Index

The majority of subjects on quizartinib in Study AC220-007 and the RR AML pooled group had a baseline BMI of 18.5 kg/m2 to <25 kg/m^2. Although patients with BMI below 18 seemed to have more AEs, this is difficult to interpret with the small numbers.

Prior allogeneic HSCT

In general, the most frequent TEAEs were the same for both groups, though there was a trend toward a higher frequency of TEAEs in the subjects with a prior allogeneic HSCT compared to those without a prior allogeneic HSCT. The TEAEs with the largest difference (≥10%) in frequency between the prior allogeneic HSCT subjects and those without were febrile neutropenia (30 [42.9%]) and 64 [30.9%] subjects, respectively), oedema peripheral (23 [32.9%] and 32 [15.5%] subjects, respectively), and GVHD in skin (11 [15.7%] and 5 [2.4%] subjects, respectively). These events were generally of low grade in both groups, except for febrile neutropenia, where the frequency of Grade ≥3 was higher in the subjects with a prior allogeneic HSCT compared to those without (28 [40.0%] and 59 [28.5%] subjects, respectively). For the other frequent TEAEs of Grade ≥3, the events with the largest difference in frequency between the prior allogeneic HSCT subjects and those without were pneumonia (12 [17.1%]) and 16 [7.7%] subjects, respectively), fatigue (7 [10.0%] and 7 [3.4%] subjects, respectively), and GVHD in skin (6 [8.6%] and 0 subjects, respectively). Among frequent TEAEs (>10% incidence), liver chemistry events were reported in similar proportions of subjects with and without prior allogeneic HSCT: ALT increased (9 [12.9%] and 28 [13.5%] subjects, respectively), AST increased (7 [10.0%] and 23 [11.1%] subjects, respectively), and blood bilirubin increased (5 [7.1%] and 23 [11.1%] subjects, respectively).

Geographic Regions

The safety profile seemed similar in North America versus Europe/Australia.

Safety related to drug-drug interactions and other interactions

Concomitant use of strong CYP3A inhibitor medications

In Study AC220-007, subjects on strong CYP3A inhibitors received a reduced dose of quizartinib.

Table 58. Overview treatment-related TEAEs by concomitant use of strong CYP3A inhibitors RR AML Pool (safety analysis set)

Total Quizartinib	Yes N=280 n (%)	No N=393 n (%)	Total N=673 n (%)
Any Treatment-Related TEAE	235(83.9)	350(89.1)	585(86.9)
Treatment-Related TEAE Associated with Discontinuation of Study Treatment	26(9.3)	41(10.4)	67(10.0)
Treatment-Related TEAE Associated with Dose Interruption	60(21.4)	82(20.9)	142(21.1)
Treatment-Related TEAE Associated with Death as Outcome	13(4.6)	15(3.8)	28(4.2)
Treatment-Related TEAE ≥ Grade 3	180(64.3)	248(63.1)	428(63.6)
Treatment-Related TEAE ≥ Grade 4	83(29.6)	143(36.4)	226(33.6)
Any Treatment-Related TESAE	119(42.5)	144(36.6)	263(39.1)
Treatment-Related TESAE Associated with Discontinuation of Study Treatment	19(6.8)	30(7.6)	49(7.3)
Treatment-Related TESAE Associated with Dose Interruption	36(12.9)	47(12.0)	83(12.3)
Treatment-Related TESAE Associated with Death as Outcome	13(4.6)	15(3.8)	28(4.2)
Treatment-Related TESAE ≥ Grade 3	113(40.4)	133(33.8)	246(36.6)
Treatment-Related TESAE ≥ Grade 4	41(14.6)	42(10.7)	83(12.3)
Any Treatment-Related AESI	174(62.1)	231(58.8)	405(60.2)
Treatment-Related AESI Associated with Discontinuation of Study Treatment	16(5.7)	27(6.9)	43(6.4)
Treatment-Related AESI Associated with Dose Interruption	37(13.2)	54(13.7)	91(13.5)
Treatment-Related AESI Associated with Death as Outcome	9(3.2)	9(2.3)	18(2.7)
Treatment-Related AESI ≥ Grade 3	113(40.4)	132(33.6)	245(36.4)
Treatment-Related AESI ≥ Grade 4	27(9.6)	20(5.1)	47(7.0)

ECG QT abnormalities in subjects with and without concomitant use of strong CYP3A inhibitors are presented in Table 64.

Table 59. ECG QT abnormalities by concomitant use of strong CYP3A inhibitor medications (RR AML Pool)

	Yes N = 280 n (%)	**No N = 393 n (%)**	**Total N = 673 n (%)**
Subjects with TEAEs of ECG QT prolonged	77 (27.5)	101 (25.7)	178 (26.4)
QTcF Interval			
n	278	389	667
New >450 ms	182 (65.5)	243 (62.5)	425 (63.7)
New >480 ms	84 (30.2)	109 (28.0)	193 (28.9)
New >500 ms	31 (11.2)	44 (11.3)	75 (11.2)
Increase >30 ms from baseline	217 (78.1)	292 (75.1)	509 (76.3)
Increase >60 ms from baseline	85 (30.6)	98 (25.2)	183 (27.4)

AML = acute myeloid leukemia; CYP = cytochrome P450; ECG = electrocardiogram; ms = millisecond; N = total number of subjects; n = number of subjects in the category; QTcF = corrected QT interval by Fredericia; RR = relapsed/refractory; TEAE = treatment-emergent adverse event.

Discontinuation due to adverse events

Table 60 Summary of Discontinuation Due to Adverse Events (in ≥2 Subjects in the RR AML Pool) by Preferred Term in Study AC220-007 and the RR AML Pooled Group (Safety Analysis Set)

Preferred Term	Study AC220-007		RR AML Pooled Group N = 673 n (%)
	Quizartinib Monotherapy N = 241 n (%)	Salvage Chemotherapy N = 94 n (%)	
Subjects With any TEAE Associated With Discontinuation of Study Drug	**44 (18.3)**	**1 (1.1)**	**173 (25.7)**
Acute myeloid leukaemia[a]	NA	NA	44 (6.5)
Electrocardiogram QT prolonged	2 (0.8)	0 (0.0)	11 (1.6)
Febrile neutropenia	2 (0.8)	0 (0.0)	11 (1.6)
Pneumonia	6 (2.5)	0 (0.0)	11 (1.6)
Gastrointestinal haemorrhage	0 (0.0)	0 (0.0)	6 (0.9)
Sepsis	2 (0.8)	0 (0.0)	6 (0.9)
Renal failure acute	2 (0.8)	0 (0.0)	5 (0.7)
Diarrhoea	0 (0.0)	0 (0.0)	4 (0.6)
General physical health deterioration	0 (0.0)	0 (0.0)	4 (0.6)
Leukocytosis	2 (0.8)	0 (0.0)	4 (0.6)
Neutropenia	1 (0.4)	0 (0.0)	4 (0.6)
Pyrexia	2 (0.8)	0 (0.0)	4 (0.6)
Abdominal pain	1 (0.4)	0 (0.0)	3 (0.4)
Asthenia	0 (0.0)	0 (0.0)	3 (0.4)
Blood bilirubin increased	2 (0.8)	0 (0.0)	3 (0.4)
Disease progression[a]	NA	NA	3 (0.4)
Graft versus host disease in intestine	3 (1.2)	0 (0.0)	3 (0.4)
Haemorrhage intracranial	3 (1.2)	1 (1.1)	3 (0.4)
Multi-organ failure	1 (0.4)	0 (0.0)	3 (0.4)
Pancytopenia	1 (0.4)	0 (0.0)	3 (0.4)
Septic shock	1 (0.4)	0 (0.0)	3 (0.4)
Thrombocytopenia	1 (0.4)	0 (0.0)	3 (0.4)
Acute febrile neutrophilic dermatosis	1 (0.4)	0 (0.0)	2 (0.3)
Bone marrow failure	0 (0.0)	0 (0.0)	2 (0.3)
Cellulitis	1 (0.4)	0 (0.0)	2 (0.3)
Disseminated intravascular coagulation	1 (0.4)	0 (0.0)	2 (0.3)
Fatigue	0 (0.0)	0 (0.0)	2 (0.3)
Hepatic failure	1 (0.4)	0 (0.0)	2 (0.3)
Hyperbilirubinaemia	0 (0.0)	0 (0.0)	2 (0.3)
Influenza	1 (0.4)	0 (0.0)	2 (0.3)
Pleural effusion	0 (0.0)	0 (0.0)	2 (0.3)
Skin infection	0 (0.0)	0 (0.0)	2 (0.3)

AE = adverse event; AML = acute myeloid leukemia; MedDRA = Medical Dictionary for Regulatory Activities; N = total number of subjects; n = number of subjects in the category; QT = interval between the start of the Q wave and the end of the T wave; RR = relapsed/refractory; SAE = serious adverse event; TEAE = treatment emergent adverse event.
[a] In Study AC220-007, disease progression or worsening of AML was not recorded as a TEAE or SAE.

2.6.1. Discussion on clinical safety

Safety results with quizartinib 30 and 60 mg qd are mainly coming from the pivotal phase III AC220-007. The study AC220-007 data have been pooled with the data from patients receiving quizartinib from 30 mg to 300 mg in additional 3 studies providing a pooled safety database about 673 patients with RR AML.

In the study AC220-007, patients were supposed to receive 30 mg/day during the first 2 weeks and the dose was increased to 60 mg/day on Day 16 depending on QTcF (≤450 ms). For subjects taking a strong CYP3A inhibitor, the starting dose was 20 mg/day and the dose of quizartinib was increased to 30 mg/day provided that they met the above QTcF requirements. Half of the patients in the quizartinib arm were exposed to the drug for 97 days (approximately 3.2 months). Salvage therapy was administered over a median period of 10 days for LoDAC, 5 days for MEC, and 6 days for FLAG-IDA. Due to the differences in treatment duration per protocol between quizartinib and salvage therapy, the comparison between treatment arms is hampered.

Within the RR AML pool, 113 (64 60 mg+ 49 >60 mg) patients received a dose of ≥60 mg for > 6 months and 43 (27 60 mg +16 >60 mg) patients for >12 months. Although it is acknowledged that this patient population has a dismal prognosis, the safety data available are considered limited in terms of number of patients and long-term follow-up and therefore do not allow to comprehensively determine long-term toxicities. Study AC220-A-U302 (an ongoing Phase 3 study of quizartinib in combination with chemotherapy for the treatment of newly diagnosed FLT3-ITD mutation-positive acute myeloid leukaemia) is needed as an additional pharmacovigilance activity in order to obtain more data and further characterise the long-term safety.

In study AC220-007, a higher percentage of patients in the quizartinib arm compared to the salvage chemotherapy arm reported TEAEs was considered to be drug-related and in the same way a higher percentage of patients in the quizartinib arm reported Grade ≥3 TEAEs, treatment-emergent SAEs, TEAEs associated with discontinuation of study drug both overall and specifically drug-related. The higher percentage of death observed in the quizartinib arm compared to salvage chemotherapy arm is likely attributable to the asymmetric early censoring caused by patients withdrawing consent in the salvage chemotherapy arm. Excluding patients with early consent withdrawal resulted in numerically higher percentage of death in the salvage chemotherapy arm compared to the quizartinib arm (77.6% quizartinib arm vs 80% salvage chemotherapy arm).

Proportions of patients reporting TEAEs were generally similar to that reported in the RR AML Pooled Group, except for the TEAEs associated with outcome of death that were more than twice as many in RR AML Pooled Group (28.7%) compared with quizartinib arm (14.9%) in the study AC220-007 and treatment-related treatment-emergent SAE that were a third more in RR AML Pooled Group (39.1%) than in quizartinib arm (26.6%) in the study AC220-007.

TEAEs with a higher incidence (reported at ≥ 5% frequency) in the quizartinib arm compared with the salvage chemotherapy arm were nausea, vomiting, decreased appetite, anaemia, febrile neutropenia, urinary tract infection, cellulitis, fatigue, electrocardiogram QT prolonged, hypokalaemia, cough, thrombocytopenia, dyspnoea, dysgeusia, neutropenia and hypomagnesaemia, while pyrexia and diarrhoea were more observed (reported at ≥ 5% frequency) in the salvage chemotherapy arm than in quizartinib arm. Hypokalaemia, thrombocytopenia and neutropenia had a higher incidence (reported at ≥ 5% frequency) in the quizartinib arm than in RR AML Pooled Group.

The most frequently reported treatment-related AEs (>15%) in patients treated with quizartinib were nausea, anaemia, ECG QT prolonged, thrombocytopenia, vomiting, and fatigue. Especially ECG QT prolonged (24.8% vs 1.1%), vomiting (19.9% vs 14.9%), and ALT increased (10.4% vs 1.1%) occurred more often in quizartinib treated patients. Diarrhoea (25.5% vs 12.4%) and pyrexia (16.0% vs 7.9%) had higher frequencies in the control group.

Grade ≥ 3 TEAEs occurred more often in the quizartinib arm (87.6%) compared with salvage chemotherapy arm (78.7%). The most frequent TEAEs of Grade ≥3 in the quizartinib arm of Study AC220-007 were febrile neutropenia, anaemia, thrombocytopenia, and neutropenia. Pneumonia and sepsis were also relatively frequent TEAEs of Grade ≥3. Febrile neutropenia and neutropenia of Grade ≥ 3 occurred more frequently in the quizartinib arm than in the salvage chemotherapy arm while Grade ≥ 3 anaemia and thrombocytopenia occurred with similar frequency in both treatment arms. Grade ≥3 pneumonia was also similar for both treatment arms.

Due to the differences in treatment duration per protocol between quizartinib and salvage therapy, the comparison between treatment arms is hampered. When adjusted for exposure, the rate of AEs was lower for quizartinib compared to the salvage chemotherapy group. Although this is reassuring, the assessment of exposure-adjusted AEs is confounded, because patients having benefit and tolerating quizartinib well will be treated for a longer period than patients with poor tolerance for the treatment. When looking at the first cycle only for both treatment arms, most incidences of AEs were higher or similar in the chemotherapy arm, except for QT prolonged and AST increased which occurred more often in the quizartinib arm. Further, AEs occurred more frequently in the quizartinib arm compared to LoDAC, but less compared to the medium/high intensity chemotherapy during the first cycle. Treatment-related AEs show a similar pattern. This suggests that the safety profile of quizartinib might be beneficial compared to medium/high intensity chemotherapy in the first cycle. Of importance, QT prolongation occurred significantly more often in the quizartinib arm during cycle 1 (18.7%) compared to both LoDAC (0.0%) and medium/high intensity chemotherapy (2.8%).

The safety profile per age group showed that quizartinib is more toxic in patients of 65-75 years versus patients <65 years. In addition, the data in patients older than 75 years is very limited therefore the safety profile in patients of 75 years and older is considered as missing information. The overall safety profile of quizartinib (AEs, grade ≥3 AEs, SAEs, AEs with outcome death, AEs associated with study drug discontinuation) seems to be worse in females compared to males, especially when looking at treatment-related AEs. Also, ECG QT prolongation occurred more often in females (in the pivotal study 30.2% vs 22.3%). Small differences were found in baseline QTcF values in females compared to males that are not considered to fully explain the differences seen in QT prolongation between females and males.

AEs were in general comparable within the other subgroups analysed, although some subgroups were small and difficult to interpret. It is reassuring that TEAEs of ECG QT prolonged and incidences of treatment emergent QTcF abnormalities and significant increases from baseline occurred in similar proportions in patients with or without concomitant use of strong CYP3A inhibitors. The average daily dose of quizartinib in patients in the RR AML pool receiving 60 mg was 39.7 mg in patients not taking concomitant strong CYP3A4 inhibitors (n=178) and 34.9 mg in patients taking concomitant strong CYP3A4 inhibitors (n=99). It is difficult to assess with these numbers if the study protocol of AC220-007 regarding reduction for concomitant use of strong CYP3A inhibitors is adhered to, but at least in the most relevant dose group (60 mg) the average dose is lower in patients with concomitant use.

Infections: In general infections occurred with a similar frequency in study AC220-007 in the quizartinib arm (76.8%, n=185) and in the salvage chemotherapy arm (72.3%, n=68). This was due to febrile neutropenia and pneumonia in most cases. The events in the infections were more often higher grade and serious in the quizartinib arm versus the salvage chemotherapy arm.

QTc interval prolongation/cardiac arrest: Both quizartinib and AC886 induced blockade of hERG current and IKs, and thereby caused QT prolongation by a decrease in the net repolarisation currents in non-clinical studies. The effect on IKs was more dominant than that on hERG current. Drug-induced interference with the fast component of the delayed rectifier current IKr is the most common cause of acquired long QT syndrome/TdP, but blockade of the slow delayed rectifier current IKs also contributes

importantly to drug-induced long QT syndrome (Cubbedu, *Current Cardiology Reviews*, 2016; Veerman et al., *Circ Arrhythm Electrphysiol*, 2013).

Of the 241 patients treated with quizartinib in the phase 3 clinical study, 3.3% were found to have a QTcF interval greater than 500 ms, 12.4% had an increase from baseline QTcF greater than 60 ms, based on central review of ECG data. QT prolongation was reported as Grade 3 in 4.1%, Grade 2 in 12.9% and Grade 1 in 9.5%. There were no cases of torsade de pointes (Grade 4), sudden death or cardiac arrest reported at the recommended doses (i.e., 26.5 mg or 53 mg). One patient in a phase 2 clinical study developed torsade de pointes while receiving 79.5 mg. The event resolved following discontinuation of quizartinib. One patient in a phase 2 study experienced a fatal cardiac arrest in the setting of staphylococcal sepsis while receiving 135 mg of quizartinib (a higher dose than the recommended). The patient had some evidence of QT prolongation prior to the cardiac arrest, and quizartinib-induced arrhythmia cannot be excluded for this patient. Furthermore, the role of QT prolongation cannot be ruled out in a report of fatal myocardial infarction in the pivotal study, cardiac arrest in the RR AML pool, and intracerebral haemorrhage due to falls in both the pivotal study and RR AML pool. Additionally, quizartinib-related cardiac deaths were identified in the ongoing AC220-A-302 study in patients with newly diagnosed FLT3-ITD+ AML.

Routine risk minimisation measures providing relevant information to healthcare professionals about ECG monitoring and management of QT prolongation with dose adjustments, co-medication, treatment of electrolyte abnormalities and additional risk minimization with HCP guides and PACs were considered necessary. Furthermore, quizartinib should be contraindicated in patients with long QT syndrome.

The uncertainties about the optimal dosing (60 vs 30 mg) and extrapolation of the study results in terms of safety to the clinical practice however remain.

Cardiac arrhythmias and cardiac failure were reported at the same proportion in both arms (12.0% vs 10.6%; 1.7% vs 2.1%, respectively). One patient in the quizartinib arm of the pivotal study died of cardiac failure and 2 more in the RR AML Pool and a causal relationship cannot be ruled out.

Hepatic disorders: TEAEs within the hepatic disorders AESI category were more frequent in the quizartinib arm (32.0% vs 16.0% control arm). The most frequent AESIs overall in this category were ALT increased (13.3%), AST increased (10.8%) and blood bilirubin increased (10.0%). Discontinuation due to hepatic disorders occurred in 2.1% and in 2.1% a SAE was reported. Patients treated with quizartinib can develop serious hepatic events (laboratory abnormalities or hepatic events), also in the 60 mg dosing group and a causal relationship with quizartinib cannot be ruled out in all cases.

Haemorrhages: In study AC220-007 the AE of haemorrhages was reported in 49.4% in the quizartinib arm and 38.3% in the control arm. The most frequent AEs were epistaxis and petechiae. In the RR AML Pool haemorrhages occurred in 54.7%. Most events were low grade, however 6 patients died in the quizartinib arm due to intracranial or cerebral haemorrhage. Serious intracranial/cerebral haemorrhages were observed in patients treated with quizartinib, which was in the majority of cases associated with thrombocytopaenia. A causal relation with quizartinib cannot be ruled out. Furthermore, it is noted that intracerebral haemorrhage was preceded by falls in some cases, including a case in the pivotal study. Falls could be caused by loss of conciousness/syncope and cardiac arrhythmias and a possible causal role of QT prolongation cannot be excluded.

Differentiation syndrome/acute febrile neutrophilic dermatosis (AFND): There were 19 patients treated with quizartinib in the clinical development program with AFND, including 8 patients in the pivotal trial. Because FLT3 inhibition has been associated with AFND this was considered as an adverse drug reaction for quizartinib. DS is also reported for the FLT3-inhibitor gilteritinib in 3%. A proposal for the management and monitoring of DS post-marketing and a warning for the possible occurrence of DS is needed.

Serious TEAEs were reported in a higher proportion in the quizartinib arm (69.7%, n=168) than in the salvage chemotherapy arm (32.4%, n=37). The most common treatment-emergent SAEs in quizartinib arm were febrile neutropenia (20.7%, n=50), pneumonia (9.1%, n=22), and sepsis (6.6%, n=16). Overall, 26.6% of subjects in the quizartinib arm and 16.0% of subjects in the salvage chemotherapy arm had at least 1 treatment-related SAE. The most frequently reported (incidence ≥2%) events in the quizartinib arm were febrile neutropenia (7.5%), sepsis (2.5%), ECG QT prolonged (2.1%), and nausea (2.1%). The most frequently reported treatment-related SAE in the salvage chemotherapy arm was febrile neutropenia (5.3%).

AML was the primary cause of on-treatment death for 49 patients (20.3%) in the quizartinib arm and 7 patients (7.4%) in the salvage chemotherapy arm. Proportions are similar between quizartinib arm and RR AML pool. Most frequently TEAEs associated with outcome of death were pneumonia (in 7 patients [2.9%]) and haemorrhage intracranial (in 4 patients [1.7%]) followed by sepsis, septic shock, cerebral haemorrhage, lung infection, graft versus host disease in intestine in 2 patients (0.8%) each. In 9 fatal cases in study AC220-007 the relationship was reported as treatment-related. A causal relationship with quizartinib cannot be ruled out for these fatal cases and especially the case with myocardial infarction raises concern. QT intervals were not available during the event of myocardial infarction. In addition, related cardiac deaths were identified in the ongoing AC220-A-302 study in patients with newly diagnosed FLT3-ITD+ AML. This implicates that quizartinib can lead to fatal events even in patients receiving lower doses than 60 mg per day.

TEAEs leading to discontinuation, dose interruptions and dose reductions occurred in 18.3%, 34.9% and 21.6% of quizartinib-treated patients compared with 1.1% each in the salvage chemotherapy arm.

The safety profile of quizartinib pre- and post-HSCT was largely comparable with no new safety signals. However, the number of patients is limited (n=49). The lack of sufficient safety data in the post-HSCT is considered as missing information.

2.6.2. Conclusions on the clinical safety

The main serious safety signal is the association of quizartinib with QT prolongation via blockade of IKs current with occurrence of TdP and fatal cardiac deaths in the clinical development program. However, the safety profile, with the proposed risk minimisation measures, could have been considered acceptable.

2.7. Risk Management Plan

Safety concerns

Table 61: Summary of safety concerns

Important identified risks	QTc interval prolongation/torsade de pointes Increased exposure to quizartinib due to drug-drug interactions with strong CYP3A inhibitors Decreased efficacy of quizartinib due to drug-drug interactions with strong or moderate CYP3A inducers
Important potential risks	Embryo-foetal and reproductive toxicity Drug-drug interactions due to P-gp inhibition by quizartinib
Missing information	Long-term safety of quizartinib (including safety post-HSCT) Safety of quizartinib in subjects ≥75 years old

Pharmacovigilance plan

Table 62: Summary of On-Going and Planned Additional Pharmacovigilance Activities

Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 1 - Imposed mandatory additional pharmacovigilance activities, which are conditions of the marketing authorisation				
AC220-A-U302 (QuANTUM-First) Ongoing	Primary: To compare the effect of quizartinib vs placebo (administered with standard induction and consolidation chemotherapy, then administered as continuation therapy for up to 36 cycles) on event-free survival (EFS) in subjects with newly diagnosed AML with FLT3-ITD mutations. Secondary: To further characterize the safety profile of quizartinib administered with standard induction and consolidation chemotherapy, then administered as continuation therapy for up to 36 cycles.	Long-term safety of quizartinib (including safety post-HSCT)	Final report	31 Dec 2021
Category 2 – Imposed mandatory additional pharmacovigilance activities, which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
Study AC220-A-U104 Planned	Primary: The primary objective of the study is to evaluate the effect of quizartinib on the PK of dabigatran.	Effect of quizartinib on P-gp substrates at clinically relevant intestinal concentrations	Final report	31 Aug 2020

Risk minimisation measures

Table 63: Summary Table of Risk Minimisation Activities by Safety Concern

Safety Concern	Risk Minimisation Measures
QTc interval prolongation/ torsade de pointes	Routine risk minimisation measures: Contraindication in SmPC Section 4.3 for subjects with Long QT syndrome. Inclusion in the list of ADRs in Section 4.8 of the SmPC. Warning in Section 4.4 of the SmPC with specific information on ECG monitoring, discontinuation, and/or reversibility. Dose adjustment guidelines in Section 4.2 of the SmPC. Guidance on correction of electrolyte imbalance is described in Section 4.4 of the SmPC. Additional risk minimisation measures: Healthcare Professional Guide to reinforce prescriber's awareness about the risk of QTc prolongation/torsade de pointes and the risk minimisation measures. Patient Alert Card to ensure that special information regarding Vanflyta and the risk of QTc interval prolongation/torsade de pointes is held by the patient at all times and reaches the relevant healthcare professional as appropriate.
Increased exposure to quizartinib due to drug-drug interactions with strong CYP3A inhibitors	Routine risk minimisation measures: Recommendations for quizartinib dose adjustment if concomitant use of strong CYP3A inhibitors is described in Section 4.2 of the SmPC. Information on drug-drug interactions in Section 4.5 of the SmPC. No additional risk minimisation measures.
Decreased efficacy of quizartinib due to drug-drug interactions with strong or moderate CYP3A inducers	Routine risk minimisation measures: Recommendation that concomitant use of strong CYP3A inducers with quizartinib should be avoided is included in Section 4.5 of the SmPC. Information on drug-drug interactions in Section 4.5 of the SmPC. No additional risk minimisation measures.
Embryo-foetal and reproductive toxicity	Routine risk minimisation measures: Warning in Section 4.4 of the SmPC Information on risk of embryo-foetal and reproductive toxicity in Section 4.6 of the SmPC. No additional risk minimisation measures.
Drug-drug interactions due to P-gp inhibition by quizartinib	Routine risk minimisation measures: Recommendation that caution should be used when co-administration of a P-gp substrate is required is included in Section 4.5 of the SmPC. Information on drug-drug interactions in Section 4.5 of the SmPC. No additional risk minimisation measures.
Long-term safety of quizartinib (including safety post-HSCT)	No risk minimisation measures.
Safety of quizartinib in subjects ≥75 years old	Routine risk minimisation measures: Information about the limited experience of quizartinib in elderly included in Section 4.8 of the EU SmPC. No additional risk minimisation measures.

Abbreviations: ADR = adverse drug reaction; CYP = cytochrome P450; DSUR = Drug Safety Update Report; HSCT = hematopoietic stem cell transplantation; PBRER = periodic benefit-risk evaluation report; P-gp = P-glycoprotein; QT = interval between the start of the Q wave and the end of the T wave; QTc = corrected QT interval; SmPC = Summary of Product Characteristics

Conclusion on the RMP

The CHMP and PRAC, having considered the data submitted in the application were of the opinion that due to the concerns identified with this application, the risk management plan cannot be agreed at this stage.

2.8. Pharmacovigilance

Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

Periodic Safety Update Reports submission requirements

Not applicable.

2.9. New Active Substance

Based on the CHMP review of the available data, the CHMP considers that quizartinib is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

2.10. Product information

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Vanflyta (quizartinib) is proposed as monotherapy for the treatment of adult patients with early relapsed or refractory FLT3 ITD positive AML suitable for intensive first line treatment, and for continuation of treatment post-transplant.

3.1.2. Available therapies and unmet medical need

There is no universally accepted standard of care for patients with R/R FLT3-ITD mutation positive AML; patients should be enrolled in a clinical trial whenever possible. Possible general salvage regimens are

IDAC (intermediate dose cytarabine) with or without anthracycline, MEC or FLAG-IDA but no specific therapies are approved in the EU for the intended population.

In view of the inherent poor prognosis of R/R FLT3-ITD mutation positive AML and as no therapies are approved in the EU, there is an unmet medical need.

3.1.3. Main clinical studies

The main evidence of efficacy is based on study AC220-007; a phase 3, open-label, randomized study of quizartinib monotherapy versus salvage chemotherapy in subjects with FLT3-ITD positive acute myeloid leukemia refractory to or relapsed after first-line treatment with or without HSCT consolidation.

3.2. Favourable effects

Results of OS in the ITT population showed a median of 27.0 weeks for quizartinib vs 20.4 weeks for salvage chemotherapy (HR of 0.758; 95% CI: 0.584-0.983, logrank 1-sided p=0.0177) and in PPS population showed a median 26.7 weeks vs 20.0 weeks respectively (HR of 0.754, 95% CI: 0.567-1.001, p=0.0246). Subgroup analyses showed a beneficial effect (HR<1) for most prognostic factors such as age, randomisation factors, AML risk score, FLT3-ITD allelic ratio, and for patients eligible for low and high intensity treatment.

A numerical increase was seen in EFS in the ITT: 3.7 weeks in the salvage therapy arm vs 6.0 weeks in quizartinib arm, which was not statistically significant (HR: 0.898; 95% CI: 0.697, 1.157, log rank p=0.1071).

In relation to CRc rate by sponsor-modified 2003 IWG response (Cheson) criteria for AML, results showed a higher percentage of CRc for quizartinib arm compared to salvage chemotherapy arm (48.2% vs 27.0%); the majority of the responses were CRi [99/245 (40.4%) vs 32/122 (26.2%)]. CR and PR were observed in 4.1% vs 0.8% and 21.2% vs 3.3% of the patients, respectively. Median duration of CRc was 12.1 weeks for quizartinb arm vs 5.0 weeks for salvage chemotherapy arm by sponsor-derived response criteria.

Overall, there were 78 patients (31.8%) in the quizartinib arm and 14 patients (11.5%) in the salvage chemotherapy arm that underwent an allogenic HSCT post protocol treatment. From these, a higher percentage of successful transplant (52.6% vs 35.7%) and a lower percentage of relapse (30.8% vs 42.9%) were shown in quizartinib arm compared to salvage chemotherapy arm.

3.3. Uncertainties and limitations about favourable effects

In the pivotal study, 23% (28/122) of the patients assigned to the comparator arm were not treated compared to a 1.6% (4/245) of non-treated patients in the quizartinib arm. Three additional sensitivity analyses were conducted to address the imbalance in the number patients randomized but not treated in the 2 arms: PPS OS analyses where untreated patients were excluded; re-sampling OS analysis, where the 28 untreated subjects of the chemo arm were replaced by random treated subjects from this very arm; untreated vs treated chemo OS analysis. These 3 analyses were consistent and apparently support an absence of impact of the non-treated imbalance in the study outcome. Nevertheless, patients who escaped the randomized treatment could share some characteristics not accessible to the presented analyses. Their outcomes possibly would have differed from what has been observed in patients kept in the study under allocated treatment. This adds uncertainty to the observed results. Based on resampling analyses, the estimated difference in median OS seems at least ~ 5 weeks. However, uncertainties remain on the reliability of the effect estimate due to the wide 95%-confidence intervals around these estimates. In addition, statistical significance of OS in the updated analysis is not much stronger than

usual (0.0190 against a critical p-value of 0.0231 (one-sided)). This statistical significance is also lost quite early (already if between 10-20% of lowest/shortest OS are removed from the control arm for imputing only 6 patients with early censoring). Based on these data statistical robustness of OS results is therefore not established and uncertainties on the effect size remain.

The benefit of quizartinb in OS is not supported by the secondary endpoint EFS and the exploratory endpoint CRs. The EFS results are uninterpretable and uninformative, with the key issue being that the data collected did not allow applying an objective criterion of relapse across both arms given the difference in treatment duration. The effect on OS and CRc rate appears to be driven by the subgroup of patients eligible for low intensity chemotherapy.

Criteria for allocation of patients to low or high intensity treatment as well as criteria to decide upon allo HSCT and/or to resume treatment after allo HSCT were not pre-specified but baseline characteristics were balanced and appear to be as expected considering the treatment strata. It is of concern that a higher rate of patients on quizartinib proceeded to HSCT for those in CRc, that more patients proceeded to HSCT in the quizartinib arm with partial response or no response and in particular and unexpectedly in the low intensity group, while these patients are at baseline unlikely to proceed to HSCT. It can therefore not be excluded that this is partly influenced by investigator's knowledge of treatment and thus may have influenced the OS results in favour of quizartinib.

The rationale for an increase in dose from 30 mg/day to 60 mg/day is uncertain from an efficacy point of view, whereas the risk of QT prolongation is increased (see section 3.5 ''Uncertainties and limitations about unfavourable effects'').

3.4. Unfavourable effects

The most common adverse reactions with quizartinib were decreased white blood cell count (90.5%), decreased lymphocyte count (80.1%), infections (69.3%), decreased haemoglobin (67.6%), decreased neutrophil count (61.8%), decreased platelet count (61.4%), bleeding (49.4%), nausea (48.1%), asthenic conditions (39.4%), pyrexia (38.2%), febrile neutropenia (33.6%), vomiting (33.2%), diarrhoea (29.0%,) QT prolongation (26.6%), rash (22.8%), abdominal pain (22.4%), peripheral oedema (21.6%) and decreased appetite (20.3%).

The most common Grade $\geq$ 3 adverse reactions were infection (44.8%), febrile neutropenia (30.7%), bleeding (10.0%), asthenic conditions (7.9%), QT prolongation (4.1%), pancytopenia (3.7%), and vomiting (3.3%).

Most frequent caused for SAEs were febrile neutropenia, pneumonia and sepsis. The most frequent reason for on-treatment deaths was AML disease progression (~20% in both AC220-007 and RR AML pool). The number was lower in the control arm (7.4%, cave shorter treatment duration). In 12.9% the primary cause of death was an AE in the quizartinib arm vs. 9.6% in the control arm, mostly due to respiratory infections, sepsis, and haemorrhages. Most frequently reported reasons for discontinuation were pneumonia, febrile neutropenia, GVDH, intracranial haemorrhage, and ECG QT prolonged.

Incidence of adverse events in the Torsade de Pointes/QT prolongation category was as high in AC220-007 as in the RR AML Pool (30.7% vs 29.6). The main event was ECG QT prolongation in 26.6% in the AC220-007 study and 26.4% in the RR AML pool. The incidence of the ECG QT prolongation was lower in the control arm (7.4%). In the RR AML pool cardiac arrest occurred in 3 patients and there was one case of Torsade de Pointes in a patient receiving 90 mg quizartinib. A total of 3.3% had QTcF >500 ms and 12.4% had an >60 ms increase from baseline.

3.5. Uncertainties and limitations about unfavourable effects

In the pivotal study less than half of the patients received the intended dose of 60 mg at day 16, and it is unknown if this can be explained by dose modifications according to protocol or by intolerability, in particular due to QT prolongation. It is unclear what the minimum effective concentration is and whether effective concentration can be achieved by 30 mg/day dosing, given that 60 mg/day dosing is associated with substantial cardiac toxicity.

It is uncertain whether the reported safety profile from the clinical development can be extrapolated to clinical practice. Indeed, the safety database in several subgroups is limited. The study population was relatively healthy with median age of 59.0 years with a poorly represented elderly population (especially ≥ 75 years), mostly ECOG PS=0 or 1 (>80%), normal hepatic function (about 80%), normal renal function (about 90% creatinine clearance >30 ml/min) and lack of significant comorbidities like uncontrolled or significant cardiovascular disease (including QTcF interval <450 ms).

Cardiac deaths for which a relation with quizartinib could not be ruled out were observed in the pivotal study and related cardiac deaths were reported in an ongoing study with newly diagnosed patients. In addition to the uncertainty on the appropriateness of the step-up dosing, the uncertainties in the exposure modelling and the large inter-individual variability make it difficult to predict if the serious cardiac events will not occur with the 60 mg dosing.

3.6. Effects Table

Table 64 Effects Table for quizartinib in adult patients with early relapsed or refractory FLT3 ITD positive AML (data cut-off: 22 Feb 2018)

Effect	Short Description	Unit	Quizartinib	Salvage Chemo	Uncertainties/ Strength of evidence	References
Favourable Effects						
OS (ITT)	Time from randomization until death from any cause.	weeks	27.0 (23.1, 31.3)	20.4 (17.3, 23.7)	HR =0.758; (95% CI: 0.584-0.983, p=0.0177). Uncertainties on (criteria for) allocation of patients to low/high intensity treatment, allo HSCT and post-HSCT treatment might impact OS	CSR
EFS	Time from randomization until documented refractory disease, relapse after CRc, or death from any cause, whichever occurred first.	months	1.4	0.9	HR: 0.898; 95% CI: 0.697, 1.157, log rank p=0.1071).	
CRc rate	The percent of subjects achieving a best response of CR, CRp, or CRi.	%	48.2 (41.8, 54.6)	27.0 (19.4, 35.8)	By sponsor-modified 2003 IWG response (Cheson) criteria for AML. OR=2.467; 95% CI: 1.541, 3.950 P-value=0.0001 CR: 4.1 vs 0.8 CRi: 40.4 vs 26.2 CRp: 3.7 vs 0	

Effect	Short Description	Unit	Quizartinib	Salvage Chemo	Uncertainties/ Strength of evidence	References
Unfavourable Effects						
TEAEs	All grades Grade ≥ 3	%	98.8 87.6	98.9 78.7	Difference in duration of treatment, limited data long-term exposure and limited follow-up	CSR
TEAEs associated with death	Overall	%	14.9	11.7	Treatment related: 3.7 vs 4.3	
White blood cell count decreased	All grades Grade ≥ 3	%	90.5 83.0	79.8 74.5		
Infection	All grades Grade ≥ 3	%	69.3 44.8	64.9 34.0		
Nausea	All grades Grade ≥ 3	%	48.1 2.5	41.5 1.1		
Bleeding	All grades Grade ≥ 3	%	39.8 6.6	38.3 8.5		
Febrile neutropenia	All grades Grade ≥ 3	%	33.6 30.7	27.7 21.3		
Vomiting	All grades Grade ≥ 3	%	33.2 3.3	21.3 1.1		
Diarrhoea	All grades Grade ≥ 3	%	29.0 1.7	36.2 3.2		
QT prolongation	All grades Grade ≥ 3	%	26.6 4.1	2.1 0.0		

Abbreviations: AML: acute myeloid leukemia; CI: confidence interval; CR: complete remission; CRc: composite complete remission; CRi: complete remission with incomplete hematologic recovery; CRp: complete remission with incomplete platelet recovery; CSR: clinical study report; HR: Hazard ratio; ITD: internal tandem duplication; IWG: International working group; OS: overall survival; QT: interval between the start of the Q wave and the end of the T wave; TEAE: treatment emergent adverse event.

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The reported effect of quizartinib on the primary endpoint OS is unreliable. The combination of a higher percentage of patients assigned to the comparator arm that withdrew the study without being treated, and more informative censoring in the comparator arm aggravated by the smaller number of patients randomised to this arm, does not allow a robust estimate of the difference in OS between the quizartinib and comparator arm. Moreover, the effect is driven by the small subgroup eligible for low-intensity chemotherapy which reduces the internal consistency and sample size.

Furthermore, the results of the primary endpoint are not supported by the secondary endpoint EFS. The EFS results are uninterpretable and uninformative, with the key issues being that the data collected did not allow applying an objective criterion of relapse across both arms given the difference in treatment duration. Further, despite that a higher percentage of patients underwent HSCT in the quizartinib arm, the (long-term) magnitude of the benefit of HSCT is uncertain and it cannot be excluded that HSCT transplant rate was partly influenced by the investigator's knowledge on type of treatment and thus may have influenced the OS results in favour of quizartinib.

The main serious safety signal is the association of quizartinib with QT prolongation via blockade of IKs current with occurrence of TdP and fatal cardiac deaths in the clinical development program. However, the safety profile, with the proposed risk minimisation measures, could have been considered acceptable.

3.7.2. Balance of benefits and risks

Given the uncertainties on the reliability of the effect size of the overall survival it is not possible to

conclude that the benefits outweigh the risks.

3.7.3. Additional considerations on the benefit-risk balance

N/A.

3.8. Conclusions

The overall B/R of Vanflyta is negative.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Vanflyta is not similar to Rydapt, Vyxeos, Mylotarg and Dacogen within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy for Vanflyta in the treatment of adult patients with early relapsed or refractory FLT3 ITD positive AML suitable for intensive first line treatment, and for continuation of treatment post-transplant, the CHMP considers by consensus that the efficacy of the above mentioned medicinal product is not sufficiently demonstrated and therefore recommends the refusal of the granting of marketing authorisation for the above mentioned medicinal product on the following grounds:

- The single pivotal trial was borderline positive on the primary endpoint, overall survival. Notably, there was extensive missing data and non-administrative censoring, predominantly in the control arm of this open-label study, and statistical significance was not robust to sensitivity analyses. Moreover, results of the primary endpoint are not supported by established effects on the secondary endpoint event free survival and the exploratory endpoints related to complete remission. Consequently, efficacy has not been established.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and follow-up measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

Furthermore, following review of the available data in the context of the applicant's claim of new active substance status, the CHMP position at the time of this report is reflected in section 2.9 (new active substance. However, in light of the negative recommendation, the CHMP is of the opinion that it is not appropriate to conclude on the new active substance status at this time.

5. References

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