Statistical Analysis Plan

Multicenter, open-label, Phase Ib/II trial to evaluate safety and efficacy for the combination of bosutinib plus atezolizumab in newly diagnosed chronic myeloid leukemia patients

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1. Administrative Information:

1.1. Title and trial registration:

Title:	Multicenter, open-label, Phase Ib/II trial to evaluate safety and efficacy for the combination of bosutinib plus atezolizumab in newly diagnosed chronic myeloid leukemia patients
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Study phase:	Ib/II
Product Name:	Bosutinib (Bosulif®, Pfizer) and Atezolizumab (Tecentriq®, Roche Registration GMBH)
EudraCT Number:	2020-000166-41
Sponsor:	Fundación ZeroLMC (Fundación Española para la curación de la Leucemia Mieloide Crónica) Plaza San Francisco, S/N, portal 13, planta 1, puerta 5 50006 Zaragoza

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1.5. Statistical Analysis Plan contributors:

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2. Abbreviations

Abbreviation	Definition
2GTKIs	2nd Generation Tyrosine Kinase Inhibitors
ABL	Abelson leukemia oncogene
AC	Accelerated Phase
ACTH	Adrenocorticotropic Hormone
AE	Adverse Event
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AESI	Adverse Event of Special Interest
ALT	Alanine aminotransferase/glutamic pyruvic transaminase/SGPT
ANC	Absolute Neutrophil Count
AP	Accelerated Phase
AST	Aspartate aminotransferase/glutamic oxaloacetic transaminase/SGOT
AUC	Area Under the Curve
BAL	Bronchoalveolar lavage
BC	Blast Crisis
BCR	Break point Cluster Region
BCR-ABL	BCR-ABL oncoprotein product of BCR-ABL fusion gene
BM	Bone Marrow
BP	Blast Phase
BUN	Blood Urea Nitrogen
CBC	Complete blood count
CCAA	Comunidades Autónomas (Autonomous Communities)
CCyR	Complete Cytogenetic Response
CEIm	Ethics committees for investigation with medicinal products.
CHF	Congestive Heart Failure
CHR	Complete Hematologic Response
CIB	Clinical Investigator's Brochure
CML	Chronic Myeloid Leukemia
CMR	Complete Molecular Response
CP	Chronic Phase
CP-CLM	Chronic phase Chronic Myeloid Leukemia
CRA	Clinical Research Associate
CRF	Case Report/Record Form; the term CRF can be applied to either EDC or paper
CRO	Clinical Research Organisation
CRP	C-Reactive Protein
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CT	Computed Tomography
CTA	Clinical Trial Assistant
CTCAE	NCI Common Terminology Criteria for Adverse Events
CV	Coefficient of Variation

Abbreviation	Definition
CYP3A4	Cytochrome 3A4
CyR	Cytogenetic Response
DLT	Dose-Limiting Toxicity
EC	European Commission
eCFR	Electronic Case Report Form
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report/record Form
EDC	Electronic Data Capture
ELN	European Leukemia Net
EMA	European Medicines Agency
EOS	End of Study
EOT	End of Treatment
ESR	Expedited Safety Report
FAS	Full Analysis Set
FDA	Food and Drug Administration (USA)
FISH	Fluorescent In-Situ Hybridization
FPFV	First Patient First Visit
FTH	Fundación Teófilo Hernando
GCP	Good Clinical Practice
GELMC	Grupo Español de Leucemia Mieloide Crónica
GGT	Gamma-glutamil transpeptidase
ICF	Informed Consent form
ICH	International Council of Harmonization
IEC	Independent Ethics Committee
IFN	Interferon
IL-2	Interleukin-2
IM	Imatinib
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IRR	Infusion-Related Reaction
IS	International Scale
IUD	Intra Uterine Device
IUS	Intra Uterine System
IV	Intravenous
IVIG	Intravenous Immunoglobulin
Kg	Kilogram
LFT	Liver Function Test
LN	Lymph Node
LPLV	Las Patient Last Visit
LVEF	Left Ventricular Ejection Fraction

Abbreviation	Definition
MCyR	Major Cytogenetic Response
MCyR	Minor Cytogenetic Response
MDSC	Myeloid Derived Suppressor Cells
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
MMR	Major Molecular Response
MR	Molecular Response
MR4	Molecular Response 4.0 log reduction from baseline
MR4.5	Molecular Response 4.5 log reduction from baseline
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
N/A	Not Applicable
NCCN	National Comprehensive Cancer Network
n-TEAE	No Treatment Emerged Adverse Event
NYHA	New York Heart Association
OD	Once Daily
OS	Overall Survival
PCR	Polymerase Chain Reaction
PCyR	Partial Cytogenetic Response
PD-L1	Programmed Death-ligand 1
PFS	Progression Free Survival
Ph+	Philadelphia chromosome
PHI	Protected Health Information
PPS	Per-Protocol Set
Pts	Patients
Q2WK	Every 2 weeks
QD	Once a Day
QT	QT interval; a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
QTcF	QT interval corrected (Fridericia)
REB	Research Ethics Board
REec	Registro Español de Estudios Clínicos
RP3D	Recommended Phase III Dose
RR	Relative Risk
RT-qPCR	Real Time-Quantitative Reverse Transcription PCR
SAE	Serious Adverse Event
SD	Standard Deviation
SOP	Standard Operating Procedure
SS	Safety Set
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBil	Total Bilirubin
TEAE	Treatment Emerged Adverse Event

Abbreviation	Definition
TFR	Treatment-Free Remission
TFS	Treatment-Free Survival
TKI	Tyrosine Kinase Inhibitor
Treg	Regulatory T cells
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
VAD	Ventricular Assist Device
VOE	Vascular Occlusive Event
WBC	White Blood Cell
WHO	World Health Organization

3. Introduction

3.1. Background:

Chronic Myeloid Leukemia (CML) is a haematological stem cell disorder associated with a specific chromosomal translocation known as the Philadelphia (Ph) chromosome; it is found in 95% of patients. The translocation consists in the fusion of the ABL proto-oncogene with the BCR gene, resulting in the production of an activated form of the ABL protein-tyrosine kinase. Expression of the BCR-ABL protein causes leukemia in mice, suggesting this protein is linked to disease pathogenesis.

Clinically, CML progresses through three distinct phases of increasing refractoriness to therapy: chronic phase (CP) (median duration 3-4 years; median survival up to 10 years with allogenic bone marrow transplant and 5-6 years with interferon (IFN), accelerated phase (AP) (median duration 3-9 months; median survival 8-18 months), and blast crisis (BC) (median survival 3-6 months). Most patients suffer the CP form, which is characterized by splenomegaly and leukocytosis with generally few symptoms.

National Comprehensive Cancer Network (NCCN) guideline on CML (NCCN guideline v1.2019) and European Leukemia Net (ELN) recommend continuing tyrosine kinase inhibitor (TKI) treatment (i.e., imatinib, nilotinib, dasatinib, ponatinib, bosutinib) indefinitely in all responding patients. Treatment discontinuation may be considered in individual patients, also outside studies, if proper, high-quality, and certified monitoring can be ensured at monthly intervals.

Imatinib (IM) rapidly became the gold standard therapy in CML that dramatically changed the prognosis of the disease. More potent second-generation tyrosine-kinase inhibitors (TKI) have been consecutively developed to counteract resistance to IM in a substantial proportion of patients such as dasatinib, nilotinib, bosutinib and ponatinib. Efficacy of second and third generation TKIs was first shown in second-line therapy for patients intolerant or resistant to IM. Superiority of dasatinib and nilotinib over IM was thereafter demonstrated in first-line therapy (DASISION and ENESTnd phase III trials), in terms of achievement of complete cytogenetic response (CCyR) at 12 months

and an increased rates of major molecular responses (MMR) and deep molecular responses.

Bosutinib is an oral, once-daily, second-generation tyrosine kinase inhibitor, with a manageable and distinct safety profile. Bosutinib exhibits dose-proportional increases in exposure between 200 and 600 mg, and its absorption increases with food. It has extensive tissue distribution, is highly bound to plasma proteins, and is primarily metabolized in the liver by cytochrome P450 (CYP) 3A4. Hepatic and renal impairment increases the exposure to bosutinib. Metabolic drug–drug interactions of bosutinib are limited in scope to CYP3A inhibitors and inducers but are significant in their extent. Co-administration of bosutinib with strong or moderate CYP3A inhibitors or inducers should be avoided.

Bosutinib is used for the treatment of chronic phase (CP), accelerated phase (AP), and blast phase (BP) Philadelphia chromosome-positive chronic myelogenous leukaemia (Ph+ CML) previously treated with one or more tyrosine kinase inhibitor(s) [TKI(s)] and for whom imatinib, nilotinib and dasatinib are not considered appropriate treatment options.

Atezolizumab is a monoclonal antibody targeting programmed death ligand 1 (PD-L1 or CD274 antigen). PD-L1 is thought to play an important role in suppressing the immune system triggered by disease, by reducing the proliferation of antigen specific CD8+ T-cells and controlling the accumulation of foreign antigen-specific T-cells. Up-regulation of PD-L1 has been suggested as a mechanism used by cancer cells to evade the host immune system.

Since the introduction of imatinib and subsequent second-generation tyrosine kinase inhibitors (2GTKIs), the prognosis of chronic-phase Chronic Myeloid Leukemia (CP-CML) has improved greatly. Initially considered to be a lethal disease with a median estimated overall survival (OS) of 3.2 years, CP-CML is now regarded as a chronic disease with a life expectancy similar to that of the healthy population. However, CML patients, despite their treatment response, cannot be considered cured and should take the treatment indefinitely.

Recently, data from different clinical trials have shown how a minority of patients who achieve a deep molecular response with imatinib and maintain the response, could stop treatment safety. Therefore, treatment free remission

(TFR) has been established as a new goal for CML patients. However, currently, TFR is only available for a small group of patients since deep molecular response is achieved by 5 years only in approximately 20% of patients treated with imatinib, and unfortunately, 50% of patients will eventually relapse. In addition, recent data have shown a time exposure to imatinib longer than 7 years as a prognostic factor for TFR. Starting treatment with 2GTKIS (dasatinib, nilotinib and bosutinib) have demonstrated higher probabilities of deep molecular responses.

We know how PD-1 is expressed at diagnosis in CML patients on T cells, and that its expression considerable decreases after achieving deep molecular response. The combination of TKI plus interferon has translated into higher probabilities of deep molecular responses compared to TKI monotherapy in several clinical trials. Atezolizumab is engineered to eliminate binding to Fc receptors and to prevent Fc-effector function. This modification eliminates antibody-dependent cell mediated cytotoxicity and thus avoids potential loss of PD-L1-expressing T-effector cells and reduced anticancer immunity.

The combination of bosutinib plus atezolizumab in first line CML chronic phase patients would increase molecular responses and therefore TFR probabilities in newly diagnosed CML patients.

3.2. Objectives:

Primary Objective:

The primary objective is to evaluate the safety profile of bosutinib 400mg/day in combination with atezolizumab as a first line treatment in participants with chronic phase-chronic myeloid leukemia.

Key Secondary Objectives:

- To evaluate the Major Molecular Response (MMR) and Molecular Response (MR) rates at 3, 6, 12 months and at the End of Treatment (EoT) visit. Based on:
  - MMR is defined as ≥ 3-log reduction in BCR-ABL transcripts or a ratio of ≤ 0.1% on the International Scale (IS)

- Molecular Response (MR): defined as
  - MMR – BCR-ABL ≤ 0.1% (IS).
  - MR4 – BCR-ABL ≤ 0.01% (IS).
  - MR4.5 ≤ 0.0032% (IS).
  - UNDETECTABLE.

- Percentage of participants alive at months 6 and 12 and at the EoT visit.
- Number of confirmed MR4 and MR4.5 at end of treatment.
- The rate of confirmed MR4 and MR4.5 at the end of treatment.
- Number of Complete Cytogenetic Responses (CCyR) at 1 year]
- The rate of Complete Cytogenetic Response (CCyR) at 1 year.
- Number of days to response (CCyR, MMR, MR4, MR4.5). [Time Frame: at the EoT visit]
- The median time to response and the overall estimated probability of response (CCyR, MMR, MR4, MR4.5).
- Number of overall surviving patients [Time Frame: at the EoT visit]
- Number of progression-free survival patients.[Time Frame: at the EoT visit]
- Number of failure-free survival patients. [Time Frame at the EoT visit]
- Number of event-free survival patients. [Time Frame: at the EoT visit]
- Immunological Studies

4. Study Methods

4.1. Trial design:

This is a phase Ib/II, two arms, open-label and dose-escalation study designed to determine the safety profile and the recommended phase III dose (RP3D) of bosutinib when administered in combination with atezolizumab in naïve patients with chronic myeloid leukemia in chronic phase. This study has two groups of patients.

First therapy

All patients included, both the first 10 patients and the next 26 patients, will receive bosutinib 400 mg/day in monotherapy during one cycle of treatment. All patients who tolerate this cycle of bosutinib treatment will continue to the second therapy (bosutinib plus atezolizumab).

Second Therapy

Group 1: The first 10 patients

Only for the first ten patients included in the study, atezolizumab will be introduced at a dose of 840 mg every two weeks combined with 400 mg/day of bosutinib for 2 cycles (8 weeks). These patients will be closely monitored for safety issues.

If dose-limiting toxicities (DLTs) appear in 2 or more patients the study will end.

A DLT would include either of the following adverse events that occurred during bosutinib therapy in combination with atezolizumab, if it is considered at least possibly related to bosutinib and/or atezolizumab:

- Grade 4 neutropenia (<500/μl) persisting for > 7 day duration or Grade 4 white cell count decrease for >7 day duration (<1000/μl).
- Febrile neutropenia (ANC<1000/ μl and fever ≥38.5°C).
- Grade 4 thrombocytopenia > 48 hour duration or with bleeding requiring platelet transfusion.
- Any Grade 3 or 4 clinically evident non-hematological toxicity.

- Any ≥Grade 3 toxicity that requires >14 days to resolve (to ≤Grade 1 despite optimal medical therapy).

DTLs will be graded according to the NCI CTCAE, v5.0.

If no relevant safety issue, regarding the combination is detected the study will continue.

These initial 10 patients will cross-over from bosutinib 400 mg/day plus atezolizumab 840 mg every 2 weeks to bosutinib 400 mg/day plus atezolizumab 1680 mg every 4 weeks for 10 cycles (40 weeks).

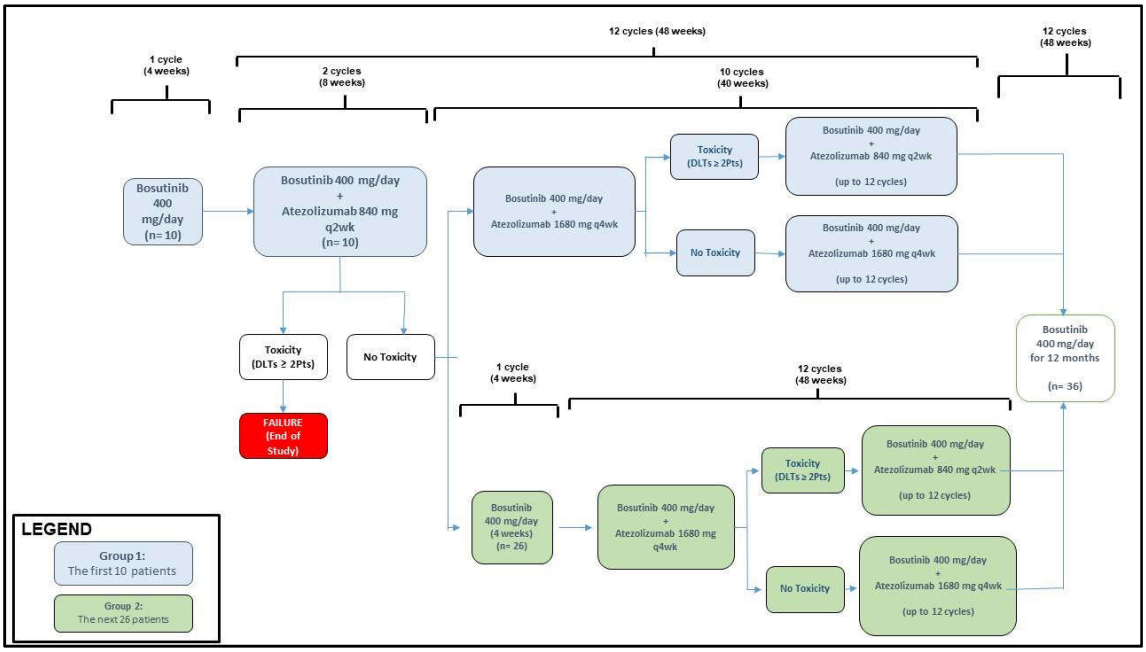
Group 2: The next 26 patients

After receiving bosutinib 400 mg/day in monotherapy for one cycle (1st therapy), the last 26 patients included will receive bosutinib 400 mg/day plus atezolizumab 1680 mg every 4 weeks for 12 cycles.

Patients will continue to be closely monitoring. If DLTs appear in 2 or more patients at a dose of 1680 mg of Atezolizumab every 4 weeks, the dose of atezolizumab will be reduced for all patients to an 840 mg Atezolizumab every 2 weeks dose.

Third Therapy

All patients completing 12 treatment cycles (48 weeks) will begin the last therapy of study.



4.2. Randomization:

Randomization does not apply.

4.3. Sample size:

There will be no formal calculation of the sample size. The sample size is selected to allow sufficient data collection for the DLT and safety assessment. It is an exploratory study. The minimum number of patients for the DLT evaluation will be 10 if no DLT is observed. After MTD is calculated, 26 more patients will be recruited for the dose variation phase. 36 patients (both, a first group of 10 patients and a second of 26 patients) will receive bosutinib 400 mg/day in monotherapy during one cycle of treatment. According to previous experience, we assume that, at most, 15% of the patients included will not enter the dose escalation phase after the first month of treatment with bosutinib, due to side effects. Consequently, a total of 30-31 patients will receive the combination of bosutinib plus atezolizumab.

4.4. Statistical interim analysis and stopping guidance:

There are no interim analyses planned.

4.5. Timing of final analysis:

Primary outcome, adverse events, will be recorded continuously throughout the study, starting on the date of the first administration of the study medication, and, at least, up to the 28-day follow-up visit after the last dose of treatment.

5. Trial Population

5.1. Screening data and recruitment

The target population of this study includes naïve adult patients of both genders with chronic myeloid leukemia in chronic phase.

Written informed consent must be obtained before performing any study specific medical procedures. The screening visit will take place at study days -28 to zero.

The screening RT-qPCR blood sample will be analyzed by a Sponsor designated central laboratory. In case the BCR-ABL1 status cannot be determined on this sample (e.g., due to technical or logistical issues) another blood sample for RT-qPCR is to be collected immediately and submitted to the central laboratory for determination of the BCR-ABL1 status.

Laboratory baseline assessments (including hematology and chemistry), physical examination including performance status, height, weight, ECG and vital signs must be performed within 28 days prior to Cycle 1/study Day 1, before the first IMP dose. As long as a patient that has signed the IC fulfil all inclusion criteria and none of the exclusion criteria, he/she could be included in this study.

5.2. Eligibility criteria

Inclusion Criteria -Patients eligible for inclusion in this study have fulfil the following criteria:

1. Male or female patient ≥ 18 years of age.
2. Evidence of a personally signed and dated informed consent document indicating that the patient (or a legal representative) has been informed of all pertinent aspects of the study.
3. Patients who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures
4. Newly Patient with Philadelphia chromosome positive chronic phase CML and BCR-ABL1 transcript detected at diagnosis.
5. ECOG Performance Status of 0, 1, or 2.
6. Adequate hepatic, renal and pancreatic function defined as:
    a. Total bilirubin within normal range or Direct bilirubin ≤ 1.5 x ULN,
    b. Aspartate aminotransferase (AST)/Alanine aminotransferase (ALT) ≤2.5 x upper limit of normal (ULN) or ≤5 x ULN if attributable to liver involvement of leukemia,
7. Have a negative pregnancy test documented prior to enrolment (for females of childbearing potential). A woman is considered of childbearing potential, i.e., fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy (CTFG, 21/09/2020). These women must have a negative serum or urine pregnancy test before initiation of study treatment and must also use highly effective methods of contraception while enrolled in the study. The use of highly effective contraception should continue for at least 14 days after the last dose of study treatment. Acceptable forms of highly effective contraception methods include (CTFG, 21/09/2020):
    a. Total abstinence [when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception]
    b. Male/female sterilization. These defined as:

i. Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed and documented by follow-up hormone level assessment.
ii. Male sterilization (at least 6 months prior to screening). For female patients on the study, study participation assumes the vasectomised male partner is the sole partner for that patient.

c. Contraceptive methods:
   i. Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, or transdermal.
   ii. Progestogen-only hormonal contraception associated with inhibition of ovulation: oral, injectable, or implantable.
   iii. Placement of an intrauterine device (IUD) or intrauterine system (IUS).

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

If a study patient gets pregnant or is suspected of being pregnant during the study or within 30 days as part of safety evaluations after the final dose of bosutinib, the study Doctor needs to be informed immediately and any ongoing study treatment with bosutinib has to be stopped immediately.

Exclusion Criteria - Patients eligible for this study must not fulfil the following criteria:

1. Pregnant or lactating women,
2. Participation in another clinical trial with any investigational drug within 30 days prior to study enrolment,
3. Any prior medical treatment for CML including tyrosine kinase inhibitors (TKIs) with the exception of hydroxyurea,
4. Period of time since CML diagnosis longer than 6 months,
5. Hypersensitivity to the active substances or to any of the excipients of the bosutinib and/or atezolizumab formulations,
6. Major surgery or radiotherapy within 14 days of enrolment,
7. Known clinically significant liver disease, including active viral, alcoholic, or other hepatitis, cirrhosis, and inherited liver disease,
8. Concomitant use of or need for medications known to prolong the QT interval,
9. Concomitant use with strong CYP3A inhibitors (ketoconazole, itraconazole, clarithromycin), moderate CYP3A inhibitors (erythromycin, fluconazole, diltiazem), or strong CYP3A inducers (rifampin, carbamazepine, phenytoin),
10. History of clinically significant or uncontrolled cardiac disease, including:
    a. Stage II to IV congestive heart failure (CHF) as determined by the New York Heart Association (NYHA) classification system for heart failure,
    b. Myocardial infarction within the previous 6 months,
    c. Symptomatic cardiac arrhythmia requiring treatment,
    d. Diagnosed or suspected congenital or acquired prolonged QT history or prolonged QTc. (QTcF should not exceed 500 msec),
11. Grade III or IV fluid retention,
12. Uncontrolled hypomagnesemia or uncorrected symptomatic hypokalemia, due to potential effects on the QT interval,
13. Uncontrolled or symptomatic hypercalcemia,

14. Recent or ongoing clinically significant gastrointestinal (GI) disorder e.g. Crohn's Disease, Ulcerative Colitis or prior total or partial gastrectomy,
15. Autoimmune or infectious active disease that require treatment,
16. CML patient not in chronic phase at diagnosis,
17. Patients with known atypical transcript. An atypical transcript is defined by the presence of any transcript in the absence of the major transcripts b3a2 (e14a2) and b2a2 (e13a2) or p210 protein,
18. Patients with known resistant mutation(s) (T315I, E255K/V, Y253H, F359C/V). It is not necessary to perform mutation tests on the patient to be included in the study if they were not previously performed,
19. Individuals with an active malignancy,
20. Known seropositivity to human immunodeficiency virus (HIV), current acute or chronic hepatitis B (hepatitis B surface-antigen positive) and/or hepatitis C,
21. Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that contraindicates the use of an investigational drug,
22. Patients with severe renal impairment.

5.3. Withdrawal/follow-up

Patients may suspend their participation in the study whenever they wish, based on his/her own judgement and opinion, without giving any explanation, although the investigator may investigate the reasons of a voluntary dropout.

The investigator may also decide to withdraw a patient from the trial if he/she does not comply with the protocol, due to AEs or treatment inefficacy or toxicity.

Patients who do not comply with at least 70% of the scheduled doses of bosutinib or that, in 4 or more times, the administration of atezolizumab is cancelled, will be withdrawn from the study.

6. Statistical Principles

6.1. General features

Principal features of this analysis have been specified in the protocol according to ICH E3 (“Structure and content of clinical study reports”) and ICH E9 (“Statistical Principles for Clinical Trials” and addendum Draft R1).

This is a phase Ib/II, single arm, open-label and dose-escalation study designed to determine the safety profile and the recommended phase III dose of bosutinib when administered in combination with atezolizumab in naïve patients with chronic myeloid leukemia in chronic phase.

The investigator should document and explain any deviation from the approved protocol. All significant protocol deviations will be recorded and reported in the Clinical Study Report.

6.2. Analysis Populations

All data will be received from the sponsor duly anonymized. All analyzes carried out and results of software applications will be duly dated and stored.

Full Analysis Set: The full analysis set (FAS) comprises all patients who complete the first three phases of treatment (combination of bosutinib and atezolizumab) successfully.

Safety Set: The safety set (SS) includes all patients who received at least one dose of study medication. Reporting and analysis of safety information for these patients will be split into subsets by study phases.

Per-Protocol Set: The per-protocol set (PPS) consists of a subset of the patients in the FAS who are compliant with requirements of the clinical study protocol (CSP).A compliance of 70% with bosutinib treatment will be necessary to be included in the PPS. Protocol deviations leading to exclusion from the PPS will be detailed in the Clinical Study Report.

6.3. Baseline patient characteristics

Demographic and other baseline data will be summarized descriptively for both the SS and the FAS. The list of baseline patient characteristics to be summarized is:

	Variables	Type of variables
Medical/Surgical History:	Diagnoses. Medical and surgical treatments.	Categorical nominal
Recent Medication Prior to First Dose of IMP	The relevant medication at the time of the IC signature will be obtained from each patient at the time of screening and before the first IMP dose.	Categorical nominal
Demographic information	Gender Age Race	Categorical nominal Continuous Categorical nominal
Leukemia Diagnosis and Prior Cancer Therapy	Initial leukemia diagnosis. Screening diagnosis	Categorical nominal
Sokal Risk Score	Sokal risk score	Continuous
ECOG	ECOG Performance Status(0, 1, 2, 3, 4, 5)	Categorical ordinal
Vital signs	Temperature. Pulse. Respiratory rate. Blood pressure	Continuous
Weight and Height	Height (cm). Body weight (Kg; 1 decimal)	Continuous
Physical Examination and Extra-medullary Leukemia Involvement	Examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, vascular and neurological. Findings on physical examination consistent with extra-medullary leukemic involvement will be recorded (e.g., lymph nodes, liver, and spleen size).	Categorical nominal
Blood Samples for BCR-ABL Molecular Response Assessment	Percent ratio of BCR-ABL transcripts versus control gene transcripts converted to IS	Continuous
Complete Blood Count with Differential	Peripheral total white blood cell count, hemoglobin, hematocrit, platelet count, red blood cell count, leucocytes, absolute neutrophil count (ANC), and WBC differential	Continuous
Serum chemistry	sodium, potassium, blood urea nitrogen (BUN) or urea, fasting glucose, albumin, creatinine, total cholesterol, triglycerides, total bilirubin (direct and indirect, only if total bilirubin is out normal range), GOT, GPT, GGT, alkaline phosphatase, LDH, magnesium, phosphorous and calcium. Amylase and lipase determinations will be added only in screening and during bosutinib plus atezolizumab combination treatment.	Continuous
Serology	Hepatitis B serology (Hepatitis B surface antigen, Hepatitis B core antibody, and Hepatitis B surface antibody) hepatitis C and HIV	Categorical nominal
Bone Marrow Analysis and Cytogenetics	Percentage of Ph+ metaphases in the bone marrow and is defined as complete, partial, minor, minimal and none	Continuous Categorical nominal
Pregnancy Test	hCG laboratory test	Categorical nominal
12-Lead Electrocardiogram	QTcF interval	Continuous

6.4. Outcome definitions

The primary objective is to evaluate the safety profile of bosutinib 400 mg daily in combination with atezolizumab as first line treatments in participants with chronic myeloid leukemia. The safety set population will be used for all safety analyses. For a detailed description of safety variables analysis see the 6.9 Safety Data Analysis section.

- Secondary variables:
  - Major Molecular Response (MMR) and Molecular Response (MR) rates at 3, 6, 12 months and at the End of Treatment (EoT) visit.
  - Percentage of participants alive at months 6 and 12 and at the EoT visit.
  - Number of confirmed MR4 and MR4.5 at end of treatment.
  - The rate of confirmed MR4 and MR4.5 at the end of treatment.
  - Number of Complete Cytogenetic Responses (CCyR) at 1 year]
  - The rate of Complete Cytogenetic Response (CCyR) at 1 year.
  - Number of days to response (CCyR, MMR, MR4, MR4.5). [Time Frame: at the EoT visit]
  - The median time to response and the overall estimated probability of response (CCyR, MMR, MR4, MR4.5).
  - Number of overall surviving patients [Time Frame: at the EoT visit]
  - Number of progression-free survival patients.[Time Frame: at the EoT visit]
  - Number of failure-free survival patients. [Time Frame at the EoT visit]
  - Number of event-free survival patients. [Time Frame: at the EoT visit]
  - Immunological Studies: phenotypic Assays:
    - Cell characterization: NK cells (CD3- CD56+; CD16+ CD56+; TNFα; IFNα; Granzyme b NK-LGL cells (CD56+ CD57+), T-LGL cells (CD3+ CD57+), CD8 TCRα/β, NK markers (NKG2D, KIR2DL2/DL3/DS2, KIR2DL5B).
    - b. Differentiation and maturation (NKG2A/CD16) and proliferation (NK67) markers of NK cells.

- c. CD4+ T cells activation markers: CD25 CD69 HLA-DR.
- d. Predictive markers of CML relapse: T regs (CD4+ CD25int-hi CD127low), CD8+ T cells (PD-1/PD-L1) and plasmacytoid dendritic cells (CD86+).

6.5. Analysis methods

Descriptive statistics are presented using mean and standard deviations for continuous variables and count and percentages for categorical variables. The 95% confidence interval (CI) for a proportion will be reported for the primary efficacy variable. The Shapiro-Wilks test will be used to test the normality of the distribution of continuous variables. Statistical differences between groups were analyzed using the chi-square test for categorical data and the Student's t test for quantitative data. Quantitative data showing non-normal distribution were analyzed using the Mann–Whitney U test. The Kaplan-Meier method is used to study survival. A p-value of <0.05 was considered statistically significant. All analyses were carried out with the free statistical program R (https://cran.r-project.org/).

Primary Objective:

Frequency and percentage of adverse events will be summarized by system organ class and/or preferred term, severity (based on CTCAE grades), type of adverse event and causality relation to study treatment by the study phases.95% confidence intervals of the proportion of adverse events will be computed using the exact binomial distribution.

Secondary Objectives: The rates of molecular responses at certain time points as well as the percentages will be presented together with an exact 95% binomial confidence interval.

All time to event endpoints will be analyzed using the Kaplan-Meier method and will be presented by Kaplan-Meier method plots.

6.6. Missing data

For time to event endpoints, a Last Observation Carried Forward & Next Observation Carried Backward statistical approach will be performed to the analysis of longitudinal repeated measures data where some follow-up observations may be missing.

6.7. Sensitivity analysis

A sensitivity analysis employing only participants with complete data will be performed in order to demonstrate that study conclusions are invariant to assumptions, the particular model, and methods of handling missing data.

6.8. Subgroup analysis

No subgroup analysis is planned

6.9. Safety data analysis

Reporting of safety information will be split into subsets by first phase, second phase, third phase, and fourth phase.

Adverse Events: Summary tables for adverse events (AEs) have to include only AEs that started or worsened during the on-treatment period, the treatment-emergent AEs (TEAEs). However, all safety data (including those from the pre-treatment period) will be listed and those collected during the pre-treatment period are to be flagged.

The incidence of treatment-emergent adverse events (new or worsening from baseline) will be summarized by system organ class and/or preferred term, severity (based on CTCAE grades), type of adverse event, causality relation to study treatment by the phases or subsets previously described.

Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by type of adverse event and safety subset.

Specific safety event categories will be considered. Such categories consist of one or more well-defined safety events which are similar in nature and for which there is a specific clinical interest in connection with the study treatment(s).

For each specified safety event category, number, and percentage of patients with at least one event part of the safety set will be reported. Also, AESI will be summarized and analyzed.

Laboratory abnormalities: For laboratory tests covered by the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 the study's biostatistician and reporting team will grade laboratory data accordingly. For laboratory tests covered by CTCAE, a Grade 0 will be assigned to all non-missing values not graded as 1 or higher. Grade 5 will not be used.

In some cases, (e.g., white blood cell differentials) the lower limits of normal ranges used in CTCAE definition have to be replaced by a clinically meaningful limit expressed in absolute counts.

For laboratory tests where grades are not defined by CTCAE, results will be graded by the low/normal/high classifications based on laboratory normal ranges.

The following by-safety subset summaries will be generated separately for hematology, and biochemistry tests:

- number and percentage of patients with clinically significant laboratory abnormalities, by parameter and worst post-baseline CTCAE grade. Each patient will be counted only for the worst grade observed post-baseline, regardless of the baseline status
- shift tables using CTCAE grades to compare baseline to the worst on-treatment value
- for laboratory tests where CTCAE grades are not defined, shift tables using the low/normal/high
- classification to compare baseline to the worst on-treatment value.
- listing of all laboratory data with values flagged to show the corresponding CTCAE grades and the classifications relative to the laboratory normal ranges.

Other Safety Data: other safety data collected (e.g., ECG, vital signs) will be listed and summarized using descriptive statistics as appropriate. Notable values will be flagged. Notable/Abnormal values for safety data will be further specified in the RAP.

The list of safety data will be summarized as:

Variables	Data
Laboratory (hematology, biochemistry, urinary and others), Vital signs, changes in physical exam findings and Electrocardiogram abnormalities	Number and percentage Description (mapped to MedDRA thesaurus terms) Time of appearance in relation to study's drug administration/data of onset (n-TEAE or TEAE, days/hours) End date or ongoing until recovery or stability Severity-CTCAE grades (from 0 to 5 or low/normal/high for laboratory tests or according to study protocol) Seriousness (according to definitions of study protocol) SAE (yes or not) Causality (Definitely Not Related, Probably Not Related, Possibly Related, Probably Related or Definitely Related) Expectedness (according to Clinical Investigator's Brochure) Outcome/evolution Any medication used to treat the AE or concomitant medication (yes or not, an specify active substance name, dose, and frequency) If it requires further exams/test(yes or not, an specify)
Other untoward medical events (such as injury, events that require surgery, accidents, or apparently unrelated illnesses)	
Hypersensitivity	
Adverse Event of Special Interests (AESI): 1. Myocardial infarction 2. Angina 3. Coronary artery disease 4. Cerebrovascular ischemic disease 5. New onset or worsening of peripheral artery occlusive disease 6. Retinal vascular thrombosis, both venous and arterial. 7. Venous thromboembolism that could result in significant compromise of organ function or other significant consequences 8. Any other event considered important by the investigator	
Pregnancy	

All variables will be recorded, ongoing or starting within 28 days after end of treatment/patient decision to discontinue treatment (pos-treatment).

6.10. References

- ICH Harmonised Tripartite Guideline: Structure and Content of Clinical Study Reports E3. Step 4, 30 November 1995. Available from: http://www.ich. org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E3/E3_Guideline.pd
- ICH topic E9: Note for guidance on statistical principles for clinical trials (CPMP/ICH/363/96). September 1998.Draft ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials (EMA/CHMP/ICH/436221/2017), step 2b – Revision 1. August 2017.
- Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, Williamson PR, Altman DG, Montgomery A, Lim P, Berlin J, Senn S, Day S, Barbachano Y, Loder E. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. JAMA. 2017 Dec 19; 318 (23):2337-2343. doi: 10.1001/jama.2017.18556.
- Introduction to Statistical Methods for Clinical Trials, by T.D. Cook and D.L. DeMets, Boca Raton: Chapman & Hall/CRC, 2008, ISBN: 1-58488-027-1, xxiii + 439 pp.