

# A phase I study of a dual PI3-Kinase/mTOR inhibitor BEZ235 in adult patients with relapsed or refractory acute leukemia

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## Research article

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## Abstract

**Background** Combined inhibition of phosphatidylinositol 3-kinase (PI3K) and the mammalian target of rapamycin (mTOR) complexes may be an efficient treatment for acute leukemia. The primary objective of this phase I single centre open label study was to determine the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) of the dual pan-class I PI3K and mTOR inhibitor BEZ235 in patients with advanced leukemia.

**Methods** Herein patients > 18 years of age who had relapsed or showed refractory leukemia were treated with BEZ235 (orally at 300-400 mg BID (cohort -1/1)) to assess safety, tolerability, preliminary efficacy and pharmacokinetic (PK). Adverse events data and serious adverse events were analyzed and haematological and clinical biochemistry toxicities were assessed from laboratory test parameters. Response was assessed for the first time at the end of cycle 1 (day 29) and after every subsequent cycle. Pharmacokinetic and pharmacodynamic analyses of BEZ235 were also included (BEZ235 plasma levels, phosphorylation of AKT, S6 and 4EBP1). On statistics this trial is a multiple ascending dose study in which a following variant of the 3+3 rule ("Rolling Six"), a minimum of 6 and a maximum of 12 patients was recruited for the dose escalation and another 5 were planned for the expansion phase.

**Results** Twenty-four patients with ALL (n=11) or AML (n=12) or CML-BP (n=1) were enrolled. All patients had failed one (n=5) or more lines of therapy (n=5) and 14 patients were in refractory / refractory relapse. No formal MTD was defined, stomatitis and gastrointestinal toxicity at 400 mg BID dose was considered incompatible with prolonged treatment. The RP2D of BEZ235 was defined as 300 mg BID. Four of 24 patients showed clinical benefit. Twenty-two of 24 patients discontinued because of progression, (median time to progression 27 days (4d-112d)). There was no association between PK parameters and efficacy or tolerability.

**Conclusions** Combined inhibition of PI3K and mTOR inhibits a clinically meaningful driver pathway in a small subset of patients with ALL, with no benefit in patients with AML. Trial registration ClinicalTrials.gov, identifier NCT01756118. registered 19th Decembre 2012, <https://clinicaltrials.gov/ct2/show/NCT01756118>

## Background

The phosphatidylinositol 3-kinase (PI3K) / Akt / mammalian target of rapamycin (mTOR) signaling axis plays an important physiologic role in protein synthesis, gene transcription, cell growth and apoptosis.<sup>1,2</sup> Oncogenic activation of the PI3K pathway has been shown in a variety of solid tumors and hematologic malignancies and has been linked to treatment resistance and disease progression.<sup>3-9</sup> In acute myeloid leukemia (AML), constitutive activation of PI3K/Akt signaling has been observed in blast cells from 50% to 80% of patients, where it contributes to proliferation, survival and drug resistance, and may confer an inferior prognosis.<sup>3,10-12</sup> PI3K signaling is also a prominent feature in T-cell acute lymphoblastic leukemia (T-ALL)<sup>13</sup>, and has emerged as one of the essential signalling mechanisms in *BCR-ABL1* mediated leukemogenesis. In Philadelphia chromosome positive (Ph+) B-cell precursor acute lymphoblastic leukemia (BCP-ALL) PI3K signaling has been shown to be involved in mutation-independent resistance to ABL-directed tyrosine kinase inhibitors.<sup>14</sup> Recent preclinical data also suggest a role for dysregulated PI3K signalling in *BCR-ABL1* negative BCP-ALL.<sup>14,15</sup>

Dysregulation may occur at different levels of the PI3K/AKT/mTOR signalling cascade and involve distinct mechanisms, the prevalence of which differs depending on the disease entity. While mutations of the *PI3KCA* gene are rare in leukemia, events upstream of PI3K are commonly implicated in causing aberrant activation of this pathway, e.g. activating mutations of the receptor tyrosine kinases (RTKs), Fms-like tyrosine kinase 3 (*FLT3*) and *KIT* receptor tyrosine kinase, *BCR-ABL1* fusion gene, or activating mutations in *NRas* and *KRas*.<sup>16-19</sup> Inactivation of the phosphatase and tensin homolog deleted on chromosome 10 (*PTEN*), a negative regulator of the PI3K pathway, has been observed in AML but is particularly frequent in T-ALL, where it has been associated with treatment resistance and a poor prognosis.<sup>20</sup> AKT may also be activated as a consequence of *NOTCH1* activating mutations, which occur in more than 50% of T-ALL cases.<sup>7,21</sup> Notably, PI3K/Akt/mTOR network up-regulation has been detected in leukemia stem cells (LSCs) transplanted in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, where it exerted a powerful pro-survival effect.<sup>22,23</sup> Taken together, these observations lent compelling weight to the clinical exploration of PI3K inhibitors in the therapy of acute leukemia.

Pharmacologic inhibition of PI3K signalling exerted pronounced anti-proliferative effects and induced apoptosis in pre-clinical models using leukemia cell lines or primary leukemia samples, but identified limitations of selectively targeting single nodes within the PI3K/Akt/mTOR cascade. The mTOR serine/threonine kinase belongs to two separate complexes: mTORC1 and mTORC2. The mTORC1 pathway is rapamycin sensitive and controls protein translation through the phosphorylation of 4E-BP1 in most models. In AML, the translation process is frequently deregulated and rapamycin resistant. Furthermore, the activity of PI3K/Akt and mTOR is closely related,

as mTORC2 activates the oncogenic kinase Akt. As a consequence, specific mTORC1 inhibitors such as RAD001 acting downstream of AKT, have the disadvantage of counteracting their own effects by activating AKT via feed-back mechanisms.<sup>16,17,24-32</sup> The resulting rationale for dual inhibition of both pathways is supported by preclinical data demonstrating enhanced antileukemic activity when distal and proximal nodes of the PI3K signalling cascade were inhibited simultaneously.<sup>5,14,18,24,25,33</sup> Despite this encouraging preclinical data and the unmet clinical need in patients with acute leukemia who have relapsed or are refractory to induction treatment, no clinical trials of PI3K pathway inhibitors other than the mTORC1 inhibitor RAD001 have to our knowledge been conducted in patients with acute leukemia. BEZ235 is a potent dual pan-class I PI3K and mTOR inhibitor belonging to the class of imidazoquinoline derivatives. In addition to binding all four isoforms of PI3K, BEZ235 binds to the catalytic site of mTOR inhibiting both mTORC1 and mTORC2 complexes.

Others and we have previously shown that BEZ235 has potent anti-leukemic activity in preclinical models of ALL and AML.<sup>10,18,19,22</sup> This phase I study was conducted to determine the maximum tolerated dose (MTD) and recommended phase II dose of BEZ235, and to evaluate the safety, preliminary efficacy and pharmacokinetics of BEZ235 in patients with relapsed or refractory ALL or AML.

## Material And Methods

### Patient selection

Target population for the dose escalation phase encompassed patients > 18 years of age with a) AML who had relapsed after or were refractory to standard therapy and were considered inappropriate candidates for conventional salvage therapy, or were previously untreated but due to age, poor prognosis, or concurrent medical conditions were not considered suitable for standard induction therapy, b) T-ALL or Philadelphia chromosome negative BCP-ALL who had relapsed after at least induction and consolidation chemotherapy or had refractory disease and for whom no standard treatment was available, c) Philadelphia chromosome- or BCR-ABL1-positive BCP-ALL or CML-BP who had relapsed after or were refractory to first- and second-line therapy that included at least two ABL-kinase inhibitors, or were in hematologic remission at the time of enrollment but had evidence of minimal residual disease (MRD) and the presence of the T315I mutation or a high-resistance mutation shown to be unresponsive to approved tyrosine kinase inhibitors (TKIs). MRD analysis based on the detection of BCR-ABL1 transcripts by RT-PCR. These patients could not be eligible for allogeneic stem cell transplant (SCT) at the time of enrolment. In the presence of a T315I mutation, prior treatment with a second TKI was not required.

Additional eligibility criteria included serum bilirubin  $\leq 1.5$  x the institutional upper limit of normal (ULN) except with known Gilbert's Syndrome, alanine transaminase (ALT) and aspartate transaminase (AST)  $\leq 3$  x the ULN (or  $\leq 5.0$  x ULN in case of hepatic infiltration by leukemia), INR  $\leq 1.5$ , serum creatinine  $\leq 2$  mg/dl or creatinine clearance  $\geq 50$  ml/min, fasting plasma glucose (FPG)  $\leq 160$  mg/dL, HbA1c  $\leq 8$  % and WBC  $\leq 30 \times 10^9/L$ .

Cytoreductive therapy commonly used as maintenance or prephase therapy for ALL, i.e. vincristine, mercaptopurine, low-dose ( $\leq 15$  mg/m<sup>2</sup>) methotrexate and low-dose (cumulative dose  $\leq 1$ g/m<sup>2</sup>) cyclophosphamide were permitted up to one week prior to, glucocorticoids and hydroxyurea up to 1 day prior to the first dose of BEZ235.

Exclusion criteria included FAB M3 type AML, insulin dependent diabetes mellitus or a history of gestational diabetes mellitus, impaired cardiac function with a left ventricular ejection fraction < 45%, QTcF > 480 msec on screening ECG, active graft versus host disease (GVHD) if symptomatic  $\geq$  grade II, or requiring current medical treatment with the potential to interact with BEZ235 in terms of QT prolongation or p450 microsomal enzymes.

The BID schedule investigated in this trial was selected based on preliminary data obtained from the solid tumor phase 1 study CBEZ235ZUS07T.

### Study design

This was a phase 1, single center, open-label study designed to assess the safety, tolerability, preliminary efficacy and PK of BEZ235. During the initial dose escalation phase, successive patient cohorts were scheduled to receive BEZ235 orally twice a day during 28 day cycles. Dose escalation was based on a "rolling-six" design, a modification of the 3 + 3 design. The first cohort of subjects received a starting dose of 400 mg/BID. Dose escalation for subsequent patient cohort of subjects was guided by the incidence of BEZ235-related adverse events as graded by NCI Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (<http://evs.nci.nih.gov/ftp1/CTCAE>) in

the first four weeks of dosing (dose limiting toxicities (DLT) evaluation period). Up to 6 subjects could be enrolled to a dose level, although only 3 subjects were required to complete the DLT evaluation period prior to enrolling subjects in the next higher dose cohort. The patient population for the determination of MTD consisted of patients who fulfilled the minimum safety evaluation requirements of the study, which were met if in cycle 1, the patient has been treated with BEZ235 for  $\geq 21$  days, observed for  $\geq 28$  days following the first dose, and completed all relevant safety evaluations, or experienced a DLT during cycle 1. Patients who did not meet these minimum safety evaluation requirements were regarded as ineligible for the MTD determining population and were replaced.

### **Toxicity and safety assessment**

Adverse events data and serious adverse events were analyzed taking into account the NCI-CTCAE v4.03 and were presented in frequency tables by grade. Haematological and clinical biochemistry toxicities were assessed from laboratory test parameters. For patients with multiple occurrences of the same event the maximum grade (worst) was used. The safety analyses were performed in the safety population.

### **Response evaluation**

Assessment of response was first performed at the end of cycle 1 (day 29) and after every subsequent cycle until CR or CRi were achieved. After CR or CRi, ongoing response was evaluated on day 29 of every even cycle and at end of treatment.

### **Pharmacokinetic analysis**

Blood for PK analysis was collected on day 1 of cycle 1 prior to and 2h, 4h, and 8h after the first administration of BEZ235, after 24 hours immediately preceding the second administration of BEZ235 and on day 15 of cycle 1 immediately prior to administration of BEZ235. All blood samples were taken by either direct vein puncture or an indwelling cannula in accordance with the assessment schedule, (table 1). If a patient had a central line, blood sampling was also obtained from this source. The sample tubes were to be labelled with pre-printed labels which contain the following information: protocol number, centre number, patient number, patient initials, sample number, date sample taken and actual time of sample. All samples were given a unique sample number and the exact clock time of dosing, as well as actual sample collection date and time will be captured on the CRF page. Sampling problems were noted in the comments field of the CRF page. All pharmacokinetic specimens were stored frozen at least at  $-20^{\circ}\text{C}$  until shipment. Samples were packed carefully with suitable packing material and dry ice to keep them frozen during shipment. Laboratory analyses of specimens collected in this study for determination of drug or metabolite concentrations were conducted by the central laboratory at the University of Dresden.

Pharmacokinetic analyses were performed in 21 patients and in all over 235 plasma samples. BEZ235 plasma levels were determined by HPLC and fluorescence detection. For the HPLC assay zinc sulfate (5g/100ml) and acetone were added to plasma samples to precipitate protein. 300 $\mu\text{l}$  plasma were combined with 200 $\mu\text{l}$  ZnSo<sub>4</sub> and 300 $\mu\text{l}$  acetone, were vortexed for 5 min and subsequently centrifuged for 10min followed by HPLC analysis. HPLC analysis was performed using a ZirChrom PBD column, which was equilibrated with 95% water, 1ml TEMED, 5% methanol and adjusted to a pH of 4 with phosphoric acid (800 $\mu\text{l}$ ; pH3.6 / I Eluent). For elution an MN 125-4, 5 $\mu\text{m}$ , Rp SelectB column was used. The eluent was composed of 45% water, 1 ml TEMED and 55% methanol, adjusted to pH 2 with phosphoric acid (2250 $\mu\text{l}$  pH 2/I Eluent). Detection was performed with a fluorescence detector at 270nm extinction and 480nm emission. This methodology was validated to a detection limit of 1ng/ml BEZ235. The intra- und inter-assay variation for 10 samples each per measurement was below 10%. PK parameter analysis was performed for every patient individually based on a 2-compartment model using the TOPFIT PK computer program. For weighting the concentration was set to 1/y. All correlation coefficients were  $\geq 0,85$ .

## Pharmacodynamic parameters

Pharmacodynamic (PD) analysis included assessment of phosphorylation of AKT, S6 and 4EBP1 by WB and flow cytometry.

## Minimal residual disease

MRD assessment was performed after each cycle of BEZ235. Disease specific MRD markers were used for each patient individually.

## Mutation analysis

The presence of *PI3KCA*, *AKT* or *PTEN* mutations was evaluated by direct sequencing of exons with known mutation hotspots.

## Whole genome and RNA sequencing

Samples from representative time points during treatment with BEZ235 were used for whole genome and RNA sequencing in the best responding patient described below. Herein a skin punch biopsy was used as non-malignant tissue control and a bone marrow sample from relapse after allogeneic SCT before initiation of therapy with BEZ235 as tumor sample.

DNA from tumor (mononuclear bone marrow cells) and normal tissue (skin punch biopsy) were extracted using the DNeasy Blood & Tissue kit (Qiagen). Sequencing libraries were prepared using the TruSeq DNA Nano kit (Illumina) and sequenced on an Illumina HiSeq X sequencer in paired end mode with a read length of 150 bp. Whole genome sequencing data was analyzed using the One Touch Pipeline (OTP).<sup>34</sup> OTP works as a workflow management system that calls NGS pipelines. The used alignment, SNV- and InDel-calling are based on the established workflows in the International Cancer Genome Consortium (ICGC) and are basically described in<sup>35</sup>. Structural variation (SV) and copy number variation (CNV) methods have also been used.

Total RNA was extracted from mononuclear bone marrow cells using the RNeasy Mini kit (Qiagen). Sequencing libraries were prepared using the TruSeq Stranded Total RNA kit (Illumina) and sequenced on an Illumina HiSeq 4000 in paired end mode with a read length of 100 bp. RNA sequencing data was analyzed using STAR<sup>34</sup> and fusion transcripts were detected using STAR-Fusion (<https://www.biorxiv.org/content/early/2017/03/24/120295>).

## Statistical considerations

This was a Phase I multiple ascending dose study in which a following variant of the 3+3 rule ("Rolling Six"), a minimum of 6 and a maximum of 12 patients was recruited for the dose escalation and another 5 were planned for the expansion phase in ALL/AML. Demographics and Baseline Characteristics: standard descriptive statistics, such as the mean, median, range and proportion, were used to summarize the patient sample and to estimate parameters of interest. Ninety-five per cent confidence intervals were provided for estimates of interest where possible.

## Safety population

All patients who received at least one dose of study medication and had at least one post-baseline safety assessment (as evaluated by the existence of at least one Adverse Event CRF, including the case where no adverse event is reported).

# MTD determining population

All patients from the safety population who either received enough treatment and had sufficient safety evaluations or discontinued due to unacceptable toxicity. The minimum safety evaluation requirements will have been met if, in cycle 1, the patient has been treated with BEZ235 for  $\geq 21$  doses. In addition, the patient must have been observed for  $\geq 28$  days following the first dose, and must have completed sufficient safety evaluations or the patient experiences DLT during cycle 1. Patients who do not meet these minimum safety evaluation requirements will be regarded as ineligible for the MTD-determining population and were replaced.

## Trial registration

ClinicalTrials.gov, identifier NCT01756118. registered 19th Decembre 2012, <https://clinicaltrials.gov/ct2/show/NCT01756118>

## Results

Twenty-four patients with relapsed or refractory AML (n=12), BCP-ALL (n=10), T-ALL (n=1) and CML-BP (n=1) were enrolled between 21-Jun-2012 and 25-Nov-2013. 5 patients were in first and 5 patients in second or later relapse, 14 patients were refractory or in refractory relapse. Sixteen patients had recurrent disease after allogeneic SCT. Extramedullary leukemia was present in 5 patients. Baseline patient demographics and disease characteristics are provided in table 2.

## Treatment and adverse events

Six patients were evaluated at the starting dose of 400 mg BID. The most frequent non-hematologic adverse events (AEs) were gastrointestinal primarily of grade 1 and 2 with diarrhea (n=15), nausea/vomiting (n=19), stomatitis/mucositis (n=16), liver function test increase (n=11), Anorexia (n=8) and hyperglycemia (n=6). Grade 3/4 AEs included diarrhea (n=2), liver function test increase (n=2), hyperglycemia (n=2), lipase increase (n=1), amylase increase (n=1), gastroenteritis (n=1) and esophagitis (n=1). The hematologic side effects included neutropenia (n=5 with n=2 grade 3/4), anemia (n=4 with n=2 grade 3/4) and platelets count decrease (n=4 with all grade 3/4) (see table 3).

400 mg BID was considered not tolerable for prolonged administration and 18 pts. were subsequently treated at dose level -1 (300 mg BID). The most common non hematologic side effects especially the gastrointestinal related AEs showed a clear dose dependency as occurrence under 400 mg BEZ235 BID vs. 300 mg BID demonstrates (see table 3): nausea/vomiting, diarrhea and mucositis (all 3: 100% vs. 61%), Anorexia (83% vs. 17%), Dyspepsia and stomach and abdominal pain (33% vs. 17%), fatigue, bloating and flatulence (33% vs. 11%), anal pain (50% vs. 0%), constipation, weight loss and hypertriglyceridemia (33% vs. 0%) and erythema multiforme, skin infection muscle cramps, dizziness, dysgeusia and alopecia (17% vs. 0%). Other AEs especially in terms of hematologic toxicity showed an inverse or unclear dose dependency: liver function test increase (17% vs. 56%), Neutropenia (17% vs. 22%), Anemia (17% vs. 17%), platelet count decrease (0% vs. 22%), C-peptide increase (0% vs. 17%), Alkaline phosphatase increase, Lipase increase (0% vs. 11%), colitis, gastroenteritis, esophagitis, nail ridging, urea increase, serum amylase increase, dry skin and insomnia (0% vs. 6%). The same dose dependency also holds true in grade 3/4 AEs regarding gastrointestinal toxicity: diarrhea (33% vs. 0%), mucositis oral (17% vs. 0%), hyperglycemia (17% vs. 6%) but also in neutropenia (17% vs. 6%). Inverse or no correlation in grade 3/4 was given in liver function tests increase (0% vs. 6%), anemia (0% vs. 11%), platelet count decrease (0% vs. 22%) and lipase increase, serum amylase increase, gastroenteritis and esophagitis (0% vs. 6%) (see table 4). No patient started at dose level -1 was dose-reduced and none discontinued BEZ235 because of toxicity, 300 mg BID was selected as the RP2D.

## Dose-limiting toxicities and RP2D

In the starting cohort (dose level 400 BID), four patients completed cycle 1, i.e. the four week time period for assessment of DLT. While no formal DLT were observed, tolerability was poor, with stomatitis and a wide range of gastrointestinal toxicities which required treatment interruptions in 3 of 6 patients. Treatment at the 400 BID dose level would not be possible beyond four weeks and would be tolerated for

only less than four weeks in a substantial proportion of patients. At the 300 mg BID dose level tolerability was far better with no DLT and no adverse events that were considered incompatible with continued BEZ235 administration.

In conclusion, the RP2D for BEZ235 was determined to be 300 mg BID, without formal definition of DLTs and an MTD.

## Efficacy

Clear Responses were observed in 3 of 24 patients, all of them ALL (3/11). One patient with pro-B ALL reached sustained complete remission (CR) and two patients with *BCR-ABL1* positive BCP-ALL and with T-ALL showed reduction of blast counts. Notably one patient with AML harboring the *MLL (KMT2A)* aberration remained stable for 4 months but was not counted as a responder.

The patient who achieved a CR is particularly noteworthy in view of the depth and sustained nature of the response. This was a female patient first diagnosed with a pro-B ALL carrying the t(4;11) translocation and molecular analysis showing a *KMT2A/MLLT1* fusion transcript. The patient had received induction and consolidation therapy according to the GMALL protocol GMALL 07/03 and underwent allogeneic SCT in CR1 with lack of molecular remission with TBI-based MAC SCT and a matched unrelated donor. Relapse occurred six weeks after SCT, and the patient was enrolled in this study and allocated to the 300 mg BID cohort. Complete hematologic remission was documented 28 days after starting BEZ235. Assessment of MRD by RT-PCR for *KMT2A/MLLT1* transcripts and immunoglobulin gene rearrangement analysis demonstrated complete molecular response 6 years after starting BEZ235 and in parallel donor chimerism increased from 40-60% autologous signal to complete chimerism after 28 days and has remained complete to date. RT-PCR analysis, results of Immunoglobulin rearrangement and donor chimerism are depicted in figure 1A-C. Twenty-two of 24 pts. discontinued because of disease progression, median time to progression was 27 days (4d-112d).

Single-agent anti-leukemic efficacy was most pronounced in ALL, with an overall response rate of 27% and a sustained molecular remission in one patient. Results of PK analysis and assessment of PD markers associated with PI3K signaling did not correlate with response.

## Pharmacokinetic analysis

PK analysis revealed a remarkably high inter-individual variability of the AUC and Cmax, which covered a range from 5 to 1000 ng/ml. This variability is depicted in figure 2 for day 1 and persisted on day 15 at steady state (figure 3), with no dose-dependency. We observed non-linear PK when comparing day 1 and 15 data, with a proportionally too high AUC and Cmax at day 15. These PK data are in line with preliminary data generated in a separate study of BEZ235 for solid tumors using a once daily dosing schedule. Our PK data are consistent with a saturation effect in the elimination of BEZ235. Identification of 2 or 3 additional peaks in all plasma samples most likely representing metabolites of BEZ235 also suggests saturation of a metabolic pathway. This may limit elimination and could conceivably contribute to the PK variability, but it remains to be determined whether this saturation effect, which was observed in most patients, is linked with the occurrence of adverse events. Further analysis of these proposed metabolites has not been possible to date. Surprisingly, all three responders in whom PK data are available had low steady state trough levels below 100 ng/ml.

## Mutations and PD parameters

No activating mutations of *PIK3CA*, *AKT* or *PTEN* were identified in any of the 24 pts. Results of the PD analyses (phospho-flow cytometry and western blot (WB) analysis) showed no signs of activation in any patient in AKT, S6 and 4EBP1.

## Genetic characterization of the tumor from the best responding patient

To gain additional insights into the mechanisms leading to prolonged response in one ALL patients we performed whole genome sequencing of tumor and normal tissue at a coverage of 80x and 40x, respectively. Analysis of somatic mutations was hampered by the presence of donor DNA from the allogeneic bone marrow transplant in the samples and did not yield functionally relevant SNVs in genes

involved in PI3K/AKT/mTOR signalling pathway. Sequencing of RNA from tumor samples confirmed the presence of the *KMT2A/MLLT1* fusion gene.

## Discussion

This study represents the first step on a development path that aims at improving cure rates for advanced hematologic malignancies including ALL, AML and CML-BP by targeting the PI3K/Akt/mTOR signaling pathway. PI3K/AKT/mTOR signaling is known to play a central role in up regulating cell proliferation, survival, and drug resistance in numerous hematologic malignancies. In this trial clinical response was observed in 16% of patients with 30% response in ALL patients. Herein one patient with pro-B ALL achieved a remarkable long lasting complete hematologic and molecular remission with full donor chimerism and hematologic improvement was observed in two patients with BCP-ALL. In one AML patient stable disease of 4 months duration was induced, but in 91% of AML patients the study was discontinued because of disease progression. To summarize these findings single-agent anti-leukemic efficacy was most pronounced in ALL.

On further analysis using phospho-flow and western blotting yielded no evidence of PI3K pathway activation, even in the described responding patients was observed. The results of PK analysis and assessment of PD markers associated with PI3K signaling did also not correlate with response. This may be explained by the fact, that the PI3K/Akt/mTOR network shows a high complexity with extensive crosstalk with other signaling pathways. Furthermore, genetic analysis of the tumor sample from the best responding patient did not yield functionally relevant SNVs in genes involved in PI3K/AKT/mTOR signalling cascade. The mechanism underlying the prolonged response observed in this patient therefore remains unknown but it is possible that this patient belongs to a small subgroup of AML and ALL patients in which the PI3K pathway functions as oncogenic driver. Nevertheless, inhibition of one signaling component may not show a sufficient antitumor effect in most of the patients. In fact, one potential reason for the limited efficacy of single inhibitors in this pathway is the presence of signaling feedback loops operating through p70S6K and PI3K. The exposure to a dual catalytic PI3K/mTOR inhibitor such as BEZ235 should be sufficient to avoid PI3K/Akt pathway reactivation, but showed no satisfying effect in the majority of patients in this trial.

The mechanisms involved in activation of this pathway have been examined in AML and ALL and include activating mutations of the FLT3 and the KIT tyrosine kinase receptors, NRAS or KRAS mutations, PI3K overexpression<sup>37</sup>, low levels of PP2A phosphatase activity and autocrine/paracrine secretion of growth factors such as IGF-1 and VEGF.<sup>22</sup> The contribution of PTEN inactivation as a mechanism of PI3K pathway activation in AML is controversial; although PTEN is deleted in many solid cancers and T-cell acute lymphoblastic leukemia, PTEN deletion is extremely rare in AML.<sup>38-40</sup> In T-ALL pathogenesis it has been reported that in the absence of PTEN phosphatase tumor suppressor function, PI3Kgamma or PI3Kdelta alone can support leukemogenesis, whereas inactivation of both isoforms suppressed tumor formation. The reliance of PTEN null mutation T-ALL on the combined activities of PI3Kgamma/delta was further demonstrated by the ability of a dual inhibitor to reduce disease burden and prolong survival in mice as well as prevent proliferation and promote activation of proapoptotic pathways in human tumors. These results support combined inhibition of PI3Kgamma/delta as therapy for T-ALL.<sup>27,28</sup> The described mechanisms in the PI3K/Akt pathway may explain that response in AML as discussed was nearly not seen in this trial.

But according to preclinical data anti-tumor efficacy of BEZ is suggested in acute leukemia and in addition, preclinical and clinical data that demonstrate the relevance of mTOR and the anti-tumor effects of mTOR inhibition in numerous malignancies also suggest that compounds able to inhibit the PI3 kinase pathway at several levels simultaneously may possess greater efficacy. Therefore, dual inhibition of PI3 kinase and both mTOR complexes (C1 and C2) by BEZ235 is still an attractive agent for malignant diseases that have progressed despite standard therapy or for whom no standard therapy exists and therefore therapeutic targeting of the PI3K/Akt/mTOR network at multiple molecular levels may provide better antitumor effects than selective inhibition of only one component of the pathway. Moreover, this may prevent the development of drug resistance. The mechanisms by which the PI3K pathway is aberrantly activated, the relative contribution of different components of this pathway (PI3K, AKT, mTORC1 and mTORC2, S6 kinase etc.), positive and negative feedback mechanisms and the crosstalk with other signaling pathways appears to differ substantially between different tumor entities and potentially between patients. Along these lines, future treatment concepts involving BEZ235 may have to utilize different combination partners depending on the precise pathophysiological basis of PI3-K signaling in different diseases.

## Conclusions

The combined inhibition of PI3K and mTOR by BEZ235 interferes with a clinically meaningful driver pathway in a small subset of patients with ALL, with no benefit in patients with AML. Herein PK analysis and PD markers assessment associated with PI3K signaling did not correlate with response. The safety profile revealed a mainly gastrointestinal toxicity (apart from hematologic side effects) and the RP2D for BEZ235 was determined to be 300 mg BID, without formal definition of DLTs and an MTD. Taken these findings together we believe that the efficacy observed in the treated ALL patients warrants a further clinical exploration of dual PI3K/mTOR inhibition potentially as combination therapy with other small molecules or chemotherapeutics. Patients with Ph+ BCP-ALL or T-ALL may benefit from these combination therapies and correct dosing can likely circumvent the toxicities observed in this trial.

## Abbreviations

AEs	adverse events
ALL	acute lymphoblastic leukemia
ALT	alanine transaminase
AML	acute myeloid leukemia
AST	aspartate transaminase
BCP-ALL	B-cell precursor acute lymphoblastic leukemia
CML-BP	chronic myeloid leukemia in blast phase
CNV	copy number variation
DLT	dose limiting toxicities
FLT3	Fms-like tyrosine kinase 3
FPG	fasting plasma glucose
GVHD	graft versus host disease
ICGC	International Cancer Genome Consortium
LSCs	leukemia stem cells
MRD	minimal residual disease
MTD	maximum tolerated dose
mTOR	mammalian target of rapamycin
NCI-CTCAE	NCI Common Terminology Criteria for Adverse Events
NOD/SCID	non-obese diabetic/severe combined immunodeficiency mice
OTP	One Touch Pipeline
PD	pharmacodynamic
Ph+	Philadelphia chromosome positive
PI3K	phosphatidylinositol 3-kinase

PK	pharmakokinetic
PTEN	phosphatase and tensin homolog deleted on chromosome 10
RP2D	recommended phase 2 dose
RTKs	receptor tyrosine kinases
SCT	allogeneic stem cell transplant
SV	Structural variation
T-ALL	T-cell acute lymphoblastic leukemia
TKIs	tyrosine kinase inhibitors
ULN	upper limit of normal
WB	western blot

## Declarations

### Ethics approval and consent and to participate

Informed consent was obtained from all individual participants included in the study. The study has been approved by the responsible local ethics committee. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

### Consent for publication

Consent for publication was given by selected patient via institutional consent form.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

FL receives support from the Frankfurter Förderung "Nachwuchswissenschaftler" and the EUTOS funding program. FL and OO had advisory roles for Novartis, Ariad, Sanofi Aventis and Bristol-Myers Squibb. F. received funding of Novartis. OO was funded by Novartis, Bristol-Myers Squibb and the Deutsche José Carreras Leukämie Stiftung. All other authors declare no competing interests.

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### Author contributions

FL and OO treated patients, collected and analyzed data, wrote the manuscript. LW and GB treated patients. SB collected and analyzed data. ES, MB, SS, HP, SW and HS analyzed data. All authors read and approved the final manuscript.

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## Tables

Sample (Blood)	Volume (mL)	Cycle	Day	Sample No.	Scheduled time relative to BEZ235 dosing post-dose (hours)
	3	1	1	1	Pre-dose <sup>a</sup>
				2	1 hour post-dose
	3	1	1	3	2 hour post-dose
	3	1	1	4	3 hour post-dose
				5	5 hours post dose
	3	1	1	6	8 - 12 hours post-dose
(optional)	3	1	2	7	24 hours post-dose (trough)
	3	1	15	8	Pre-dose <sup>a</sup>
	3	1	15	9	1 hour post-dose
	3	1	15	10	3 hour post-dose
	3	1	15	11	5 hour post-dose
(optional)	3	1	15	12	8 - 12 hours post-dose

*Table 1: Blood collection plan*

Blood sample collection plan in the BEZ 235 phase I trial for PK and PD analysis is shown.

Patient characteristics	n (%)
<b>Age</b>	
Median	61
Range	29-82
<b>Sex</b>	
male/female	15 (63) / 9 (38)
<b>Disease</b>	
ALL	11 (46)
BCP-ALL	10
T-ALL	1
AML	12 (50)
CML-BP	1 (4)
Extramedullary disease	5 (21)
SCT prior to study therapy	16 (67)

*Table 2: Baseline patient characteristics*

Baseline patient characteristics reveal a median age of 61 and mainly patients with BCP-ALL and AML, who previously in most cases underwent an allogeneic SCT.

	400 mg (n=6)				300 mg (n=18)				All patients (N=24)			
	All grades		G 3/4		All grades		G 3/4		All grades		G 3/4	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Nausea,Vomiting	6	100	0	0	11	61	0	0	19	79	0	0
Diarrhea	6	100	2	33	11	61	0	0	17	71	2	8
Mucositis oral	6	100	1	17	11	61	0	0	17	71	1	4
Liver function tests increased	1	0	1	0	10	56	1	6	11	46	2	8
Anorexia	5	83	0	0	3	17	0	0	8	33	0	0
Hyperglycemia	1	17	1	17	7	39	1	6	8	33	2	8
Dyspepsia	2	33	0	0	3	17	0	0	5	21	0	0
Stomach or abdominal pain	2	33	0	0	3	17	0	0	5	21	0	0
Neutropenia	1	17	1	17	4	22	1	6	5	21	2	8
Anemia	1	17	0	0	3	17	2	11	4	17	2	8
Fatigue	2	33	0	0	2	11	0	0	4	17	0	0
Platelet count decreased	0	0	0	0	4	22	4	22	4	17	4	17
Bloating, Flatulence	2	33	0	0	2	11	0	0	4	17	0	0
Anal pain	3	50	0	0	0	0	0	0	3	13	0	0
C-peptide increase	0	0	0	0	3	17	0	0	3	13	0	0
Constipation	2	33	0	0	0	0	0	0	2	8	0	0
Alkaline phosphatase increased	0	0	0	0	2	11	0	0	2	8	0	0
Lipase increased	0	0	0	0	2	11	1	6	2	8	1	4
Weight loss	2	33	0	0	0	0	0	0	2	8	0	0
Hypertriglyceridemia	2	33	0	0	0	0	0	0	2	8	0	0
Hoarseness	2	33	0	0	0	0	0	0	2	8	0	0
Colitis	0	0	0	0	1	6	0	0	1	4	0	0
Gastroenteritis	0	0	0	0	1	6	1	6	1	4	1	4
Esophagitis	0	0	0	0	1	6	1	6	1	4	1	4
Erythema multiforme	1	17	0	0	0	0	0	0	1	4	0	0
Skin infection	1	17	0	0	0	0	0	0	1	4	0	0
Nail ridging	0	0	0	0	1	6	0	0	1	4	0	0
Urea increased	0	0	0	0	1	6	0	0	1	4	0	0
Serum amylase increased	0	0	0	0	1	6	1	6	1	4	1	4
Dry skin	0	0	0	0	1	6	0	0	1	4	0	0
Insomnia	0	0	0	0	1	6	0	0	1	4	0	0
Muscle cramps	1	17	0	0	0	0	0	0	1	4	0	0
Dizziness	1	17	0	0	0	0	0	0	1	4	0	0
Dysgeusia	1	17	0	0	0	0	0	0	1	4	0	0
Alopecia	1	17	0	0	0	0	0	0	1	4	0	0

Table 3: Treatment emergent adverse events

Treatment associated adverse events are listed according to frequency. Gastrointestinal AEs like vomiting, diarrhea and oral mucositis were most frequent but only in rare cases with higher grades (3/4). The most frequent AE with higher grade was lowering of thrombocyte count.

	400mg (n=6)		300mg (n=18)		All patients (N=24)	
	Grade 3/4		Grade 3/4		Grade 3/4	
	n	(%)	n	(%)	n	(%)
Diarrhea	2	33	0	0	2	8
Mucositis oral	1	17	0	0	1	4
Neutropenia (Neutrophil count decreased and/or WBC decreased)	1	17	1	6	2	8
Liver function test: values increased	1	17	1	6	2	8
Gastroenteritis	0	0	1	6	1	4
Esophagitis	0	0	1	6	1	4
Serum amylase increased	0	0	1	6	1	4
Lipase increased	0	0	1	6	1	4
Anemia	0	0	2	11	2	8
Platelet count decreased	0	0	4	22	4	17

Table 4: Adverse events grade 3/4 with

**dosage correlation**

Grade 3/4 AEs showed no clear dose correlation but predominance of gastrointestinal AEs in the 400mg BIS cohort and a higher hematotoxicity rate in the 300mg BID cohort.

## Figures

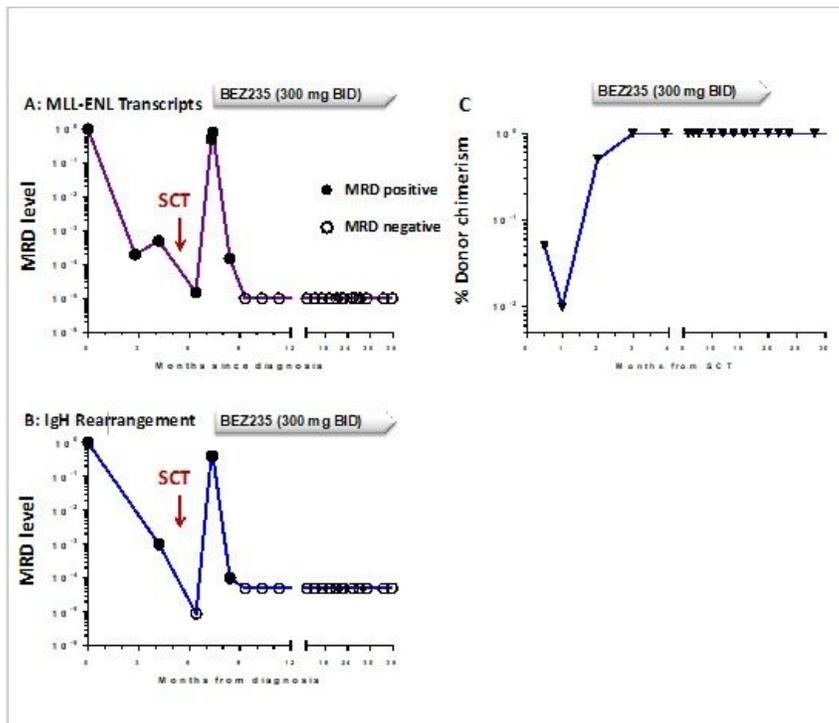
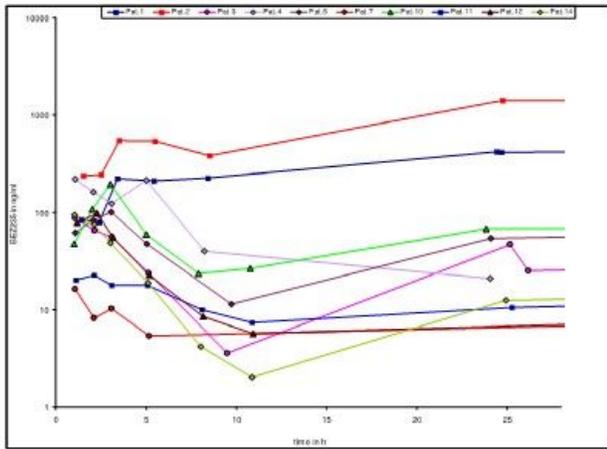


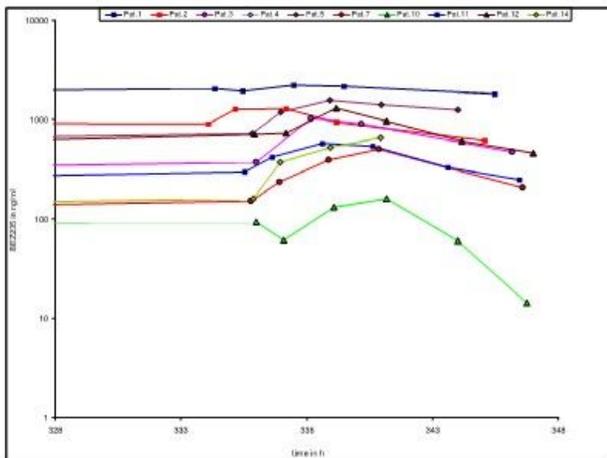
Figure 1

MRD markers and donor chimerism in best responding patient A/B MRD measurement via KMT2A/MLLT1 transcripts and IgH rearrangement in the best responding BPC-ALL patient revealed a response via SCT, but finally sustaining molecular response was reached via BEZ 235 treatment. C Donor chimerism in bone marrow was incomplete after allogeneic SCT with 1-5% autologous signal at day +29 after SCT and dropping at early relapse. Soon after start of treatment with BEZ complete chimerism was reached.



**Figure 2**

Plasma concentration BEZ235 day 1 Plasma concentrations of BEZ 235 at day 1 showing a broad interpatient variability. BEZ 235 concentrations did not correlate with response to treatment.



**Figure 3**

Plasma concentration BEZ235 day 5 Plasma concentrations of BEZ 235 at day 2 showing again a broad interpatient variability. BEZ 235 concentrations did not correlate with response to treatment.