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Liposomal Grb2 antisense oligodeoxynucleotide (BP1001) in patients with refractory or relapsed haematological malignancies: a single-centre, open-label, dose-escalation, phase 1/1b trial

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Summary

Background—Activating mutations of tyrosine kinases are common in leukaemias. Oncogenic tyrosine kinases use the growth factor receptor-bound protein 2 (Grb2) for signal transduction, leading to activation of mitogen-activated protein kinase (MAPK) 1 and MAPK3 (ERK2 and ERK1). We hypothesised that inhibition of Grb2 would suppress ERK1 and ERK2 activation and inhibit leukaemia progression. To inhibit Grb2, a liposome-incorporated antisense oligodeoxynucleotide that blocks Grb2 protein expression, BP1001, was developed. We report the first phase 1 findings of BP1001.

Methods—In this single-centre, open-label, dose-escalation phase 1/1b trial, we enrolled participants (aged ≥18 years) with refractory or relapsed acute myeloid leukaemia, Philadelphia-chromosome-positive chronic myeloid leukaemia (in chronic, accelerated, or blast phase), acute lymphoblastic leukaemia, or myelodysplastic syndrome, at MD Anderson Cancer Center (Houston, TX, USA). We used a 3 + 3 dose escalation strategy, with at least three patients enrolled at each dose level. We administered BP1001 intravenously, twice weekly, for 28 days, with a starting dose of 5 mg/m^2. If two or more patients developed toxic effects of grade 3 or higher, that dose level was deemed toxic. The dose was escalated if it did not produce dose-limiting toxic effects, and patients would be sequentially enrolled into cohort 2 (10 mg/m^2), cohort 3 (20 mg/m^2), cohort 4 (40 mg/m^2), cohort 5 (60 mg/m^2), or cohort 6 (90 mg/m^2). After completion of monotherapy, we assessed the safety and toxicity of BP1001 (60 or 90 mg/m^2) in combination with 20 mg low-dose cytarabine (twice-daily subcutaneous injections) in a phase 1b study in patients with refractory or relapsed acute myeloid leukaemia (ie, those who were refractory to at least one previous therapy regimen and no more than one previous salvage regimen). The objectives of this study were to establish the toxicity and tolerance of escalating doses of BP1001 monotherapy in patients with refractory or relapsed leukaemia, to assess the maximum tolerated dose of BP1001, and to determine the optimal biologically active dose of BP1001, defined as a 50% reduction in Grb2 expression in circulating leukaemia cells. We also aimed to assess the in-vivo pharmacokinetics of BP1001 and tumour response. The study is completed and is registered with ClinicalTrials.gov, number NCT01159028.

Findings—Between July 23, 2010, and Feb 23, 2016, we enrolled and treated 39 patients, of whom 27 were assessable for dose-limiting toxicity. The first patient treated had mucositis and hand–foot syndrome, which were assessed as possibly related to BP1001 and counted as a dose-limiting toxicity. We noted no other dose-limiting toxicities, and a maximum tolerated dose was not identified. The highest tested dose of BP1001 was 90 mg/m^2. The most common grade 3–4 adverse events were cardiopulmonary disorders (25 [64%] of 39 patients), and fever (including neutropenic fever) and infections (17 [44%] patients). Grade 5 adverse events were cardiopulmonary disorders (two [5%] of 39 patients), fever (including neutropenic fever) and infections (two [5%] of 39 patients), and multi-organ failure (one [3%] of 39 patients). Nine (33%) of 27 patients who had peripheral blood blasts at the start of therapy had a reduction of 50% or more in peripheral blood blasts while receiving BP1001 montherapy. Three (10%) of 29 patients who had bone marrow blasts at the start of therapy had a reduction in bone marrow blasts of 50% or more while receiving BP1001 monotherapy. Per investigator's assessment, seven (22%) of 32 patients benefited from BP1001 monotherapy and had extended cycles of treatment. Of seven patients receiving BP1001 plus low-dose cytarabine combination therapy, two had complete remission, one had complete remission with incomplete haematological recovery, and two had stable disease with no dose-limiting toxicity; one patient died and one withdrew, both because of disease progression. There were eight deaths; none were treatment related.

Interpretation—BP1001 is well tolerated, with early evidence of anti-leukaemic activity in combination with low-dose cytarabine. To further explore this anti-leukaemic activity, the efficacy of BP1001 plus low-dose cytarabine combination is being investigated in an ongoing phase 2 study in patients with previously untreated acute myeloid leukaemia who are ineligible for intensive induction therapy.

Introduction

The growth factor receptor-bound protein 2 (Grb2) is an evolutionally conserved, ubiquitously expressed protein. *GRB2* is mapped to the human chromosome region 17q24-q25, which is often duplicated in leukaemias.[1,2] Grb2 is a 25–26 kDa adaptor protein composed of one Src homology 2 (SH2) domain flanked by two Src homology 3 (SH3) domains.[3,4] Grb2 uses its SH2 domain to bind to phosphotyrosine residues found in BCR-ABL, KIT, FLT3, and JAK2 tyrosine kinases,[5–7] which are often mutated or overexpressed in leukaemias. Grb2 also uses its SH3 domains to bind to proline-rich motifs, such as those found in the guanine nucleotide exchange factor Son of Sevenless (SOS).[8,9] By binding to Grb2, oncogenic tyrosine kinases stimulate SOS guanine nucleotide exchange activity and activate the Ras–mitogen-activated protein kinase (MAPK) 1 and MAPK3 pathway (also known as ERK1 and ERK2).[3,4,10] This activation results in transformation of fibroblasts,[10] proliferation of haemopoietic cells,[11] and induction of leukaemia-like disease in mice.[12] Grb2 is crucial in transducing the signals of oncogenic tyrosine kinases, and inhibition of Grb2 suppresses fibroblast transformation,[10] haemopoietic cell proliferation,[11,13,14] and induction of leukaemia-like disease in mice.[12,15] We hypothesise that Grb2 might be a novel therapeutic target against leukaemia.

BP1001 is an anti-sense oligodeoxynucleotide developed to block Grb2 expression and function. BP1001 is made with DNAbilize® technology (BioPath Holdings Inc, Bellaire,

TX, USA), which is a combination of a P-ethoxy nucleic acid backbone and a neutral dioleoylphosphatidylcholine (DOPC) lipid delivery vehicle. The P-ethoxy backbone is made by adding an ethyl group to the non-bridging oxygen atom of the phosphate bonds, which results in an un charged, nuclease-resistant DNA antisense oligodeoxynucleotide.

BP1001 is comprised of an antisense oligodeoxynucleotide targeted against the translation initiation site of the GRB2 transcript incorporated in a DOPC liposome. Results from preclinical studies[13,15,16] showed that BP1001 was effective in decreasing the proliferation of BCR-ABL-positive leukaemic cell lines, and in increasing the survival of mice bearing the BCR-ABL-positive leukaemia xenografts.[15,16] Results from tissue distribution studies showed that intravenous administration of BP1001 was widely distributed throughout the body.[15,16] The tissue half-life of BP1001 was between 2 and 3 days, with the highest accumulation in spleen and liver, which are the major organs of leukaemia manifestation.[15] BP1001 was also detected in the bone marrow, where the concentration remained relatively constant for 3 days.[15] Furthermore, BP1001 did not impair hepatic or renal biochemical functions, or affect haematological or coagulation parameters.[15,16] Since BP1001 decreased leukaemia cell growth and prolonged survival in a leukaemia mouse model, we studied the clinical activity of BP1001 in patients with leukaemia. To our knowledge, this is the first description of findings from a phase 1 study of a Grb2 inhibitor. The aims of this study were to assess safety, pharmacokinetics, and efficacy of escalating doses of BP1001 in adult patients with refractory or relapsed acute myeloid leukaemia, Philadelphia-chromosome-positive chronic myelogenous leukaemia, acute lymphoid leukaemia, or myelodysplastic syndrome. After completion of dose-escalation monotherapy, we assessed the safety and toxicity of BP1001 in combination with low-dose cytarabine in a phase 1b study in patients with refractory or relapsed acute myeloid leukaemia.

Methods

Study design and participants

This was a single-centre, open-label, dose-escalation phase 1/1b trial, with all patients enrolled at MD Anderson Cancer Center (Houston, TX, USA).

Eligible patients were aged 18 years or older, with a life expectancy of 3 months or more, and had refractory or relapsed acute myeloid leukaemia, Philadelphia-chromosome-positive chronic myeloid leukaemia (chronic, accelerated, or blast crisis), acute lymphoblastic leukaemia, or myelodysplastic syndrome. Patients with chronic myeloid leukaemia had to have shown resistance or intolerance to at least two tyrosine kinase inhibitors. Patients with acute myeloid leukaemia and acute lymphoblastic leukaemia had to have received at least one previous treatment and either not achieved a response or relapsed on treatment. Patients with myelodysplastic syndrome had to have failed previous therapy with a hypomethylating agent or, if disease was associated with a del(5q) chromosomal abnormality, lenalidomide. Patients with del(5q) who were unable to receive or were intolerant to lenalidomide were also eligible. Ade quate Eastern Cooperative Oncology Group performance status (0–2), hepatic function, and renal function (alanine aminotransferase, creatinine concentrations, and bilirubin concentrations less than two times the upper limit of normal) were required for enrolment. Women of childbearing age and childbearing potential were required to use

barrier contraception throughout the trial and have a negative serum or urine pregnancy test before initiating the protocol. Eligible patients should not have received anti-cancer therapy for at least 2 weeks before study entry, with the exception of low-dose cytarabine given as subcutaneous injections (no less than 15 days before study entry), hydroxyurea, anagrelide (no less than 24 h before study entry), tyrosine kinase inhibitor (no less than 5 days before study entry), and interferon (no less than 2 weeks before study entry).

Patients were excluded if they were breastfeeding or pregnant, had an uncontrolled active infection, had serious intercurrent medical illnesses, had a history of adverse reactions or hypersensitivity to low-dose cytarabine, or had previously received any investigational therapy within 14 days or 5 half-lives. Enrolment in the phase 1b portion of the study (BP1001 plus low-dose cytarabine) was limited to patients with a diagnosis of refractory or relapsed acute myeloid leukaemia (except acute promyelocytic leukaemia) or those who were refractory to at least one previous therapy regimen and no more than one previous salvage regimen. Patients who were considered for phase 1b had to have met all other eligibility criteria.

All patients provided written informed consent, and the study was approved by the institutional review board according to the Declaration of Helsinki.

Procedures

We used a 3 + 3 dose escalation strategy. We planned to enrol cohorts of three patients at each dose level. The initial dose of BP1001 was 5 mg/m^2 (cohort 1). If one patient experienced toxic effects of grade 3 or worse, three more patients were accrued at that dose level. If two or more patients experienced toxic effects of grade 3 or worse, that dose level was deemed toxic. If dose-limiting toxicity was not observed, patients would be sequentially enrolled into cohort 2 (10 mg/m^2), cohort 3 (20 mg/m^2), cohort 4 (40 mg/m^2), cohort 5 (60 mg/m^2), or cohort 6 (90 mg/m^2). Eligible patients with refractory or relapsed acute myeloid leukaemia were enrolled into cohort 7 or cohort 8. In phase 1b, the doses for cohorts 7 and 8 were not predefined. We planned to use the maximum tolerated dose, as determined by the previous BP1001 cohorts, to determine the doses for cohorts 7 and 8. Low-dose cytarabine was administered as twice-daily subcutaneous injections, beginning on day 10 and lasting until day 19 of each cycle.

Grb2 antisense oligodeoxynucleotide (5′-ATATTT-GGCGATGGCTTC-′3) was manufactured by Nitto Denko Avecia Inc (Cincinnati, OH, USA) and incorporated into DOPC lipid (Coldstream Laboratories Inc, Lexington, KY, USA) with a lyophilisation protocol. On the day of drug infusion, 0·9% normal saline was added to achieve a final BP1001 concentration of 2·5 mg/mL. In each cycle, participants were given intravenous infusions of BP1001 over 2–3 h twice weekly (every 3 or 4 days) for 28 days. BP1001 was given with a target of at least one cycle. Participants had the option of being given up to six cycles of treatment if they experienced clinical benefit as assessed by clinical investigator. Because this was a phase 1 study without experience of any similar agent, the regulatory authorities at the time of protocol approval recommended that patients receive only up to six cycles.

We assessed bone marrow at baseline (within 14 days of study start), at completion of cycle one, cycle two, and as clinically indicated. Cytogenetic analysis was done before the start of treatment at the MD Anderson Cancer Center clinical cytogenetics laboratory. Molecular analysis was also done at the MD Anderson Cancer Center clinical molecular diagnostics laboratory to detect somatic mutations in bone marrow at baseline.

We collected peripheral blood samples from patients in cohorts 3 to 6 who received BP1001 monotherapy, before they were dosed on cycle one, days 1, 8, 15, 22, or end of treatment, or as clinically indicated. This was an optional procedure that was offered to all participants from these cohorts (ie, those receiving doses expected to have levels with possible biological activity). Only patients who consented to this optional study had samples collected and processed, and only samples from patients who were assessable for dose-limiting toxicity were analysed for. White blood cells were isolated from peripheral blood using BD Vacutainer CPT cell preparation tubes with sodium heparin (BD Biosciences, San Jose, CA, USA), fixed and stored in a –80°C freezer. Samples were then shipped to Flow Contract Site Laboratory LLC (Bothell, WA, USA). Flow cytometry was used to determine Grb2 and phosphorylated ERK1 and ERK2 (pERK) concentrations in CD33-expressing cells (clone WM53; Biolegend, San Diego, CA, USA). Grb2 concentrations were investigated with anti-Grb2 primary antibody (clone 81; BD Transduction Laboratories, San Jose, CA, USA) and visualised by secondary antibody conjugated with fluorescein thiocyanate (Millipore Sigma, Burlington, MA, USA). pERK concentrations were assessed using anti-pERK antibody conjugated with phycoerythrin (clone 20A; BD Transduction Laboratories).

We collected blood samples for measurement of Grb2 antisense oligodeoxynucleotide concentrations from patients in all cohorts, pre-dose, and 1, 2, 4, 6, 8, 24, and 72 h after the start of infusion. We planned to process the samples to plasma and analyse them with a sandwich ELISA to quantify Grb2 antisense oligodeoxynucleotide concentration (Charles River Laboratories, Montreal, QC, Canada). We estimated pharmacokinetic parameters using Phoenix pharma-cokinetic software (Certara, Princeton, NJ, USA). We used a non-compartmental approach with an estimated 2·5 h infusion time for parameter estimation. All parameters were generated from Grb2 antisense oligodeoxynucleotide individual concentrations in plasma. We estimated parameters using nominal sampling times relative to the start of infusion. We calculated the area under the concentration versus time curve (AUC) using the linear trapezoidal method with linear interpolation. We determined the slope of the terminal elimination phase using log linear regression on the unweighted concentration data. Parameters relying on the determination of the terminal elimination phase were not reported if the coefficient of determination (R^2) was less than 0·800, or if the extrapolation of the AUC to infinity represented more than 20% of the total area. Parameters estimated from plasma were: T_{max}, the time after dosing at which the maximum observed concentration was observed; C_{max}, the maximum observed concentration measured after dosing; $t_{1/2\ apparent}$, terminal elimination half-life; $AUC_{(0–24)}$, AUC from the dosing to 24 h after administration; $AUC_{(0–t)}$, AUC from dosing to the time after dosing at which the last quantifiable concentration was observed; $AUC_{(0–inf)}$, estimated AUC from dosing to infinity; CL, apparent clearance rate of Grb2 antisense oligodeoxynucleotide; and Vz, apparent volume of distribution of Grb2 antisense oligodeoxynucleotide.

We collected urine samples for measurement of Grb2 antisense oligodeoxynucleotide concentrations from all patients, at start of dose to 4 h, 4–8 h after dose, and 8–24 h after dose. Urine samples were also analysed using sandwich ELISA methodology (Charles River Laboratories). We estimated urine parameters using Microsoft Excel (2007). Reported parameters were: total U, cumulative amount of Grb2 antisense oligodeoxynucleotide excreted in urine over the entire sampling period; CLr, renal clearance relative to plasma; and percentage recovered, relative amount of Grb2 antisense oligodeoxynucleotide excreted in the urine compared with the total drug administered.

Outcomes

The objectives of the phase 1 BP1001 monotherapy portion of the trial were to assess the toxicity and tolerance of escalating doses of BP1001 in patients with leukaemia, and to determine the maximum tolerated dose (defined as the dose immediately below the one producing dose-limiting toxicity in two or more patients) and the optimal biologically active dose of BP1001 (which was defined as a 50% reduction in Grb2 expression in circulating leukaemia cells). We graded toxicity according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 3.0). Other objectives were to assess the in-vivo pharmacokinetics of BP1001 and tumour response. For the phase 1b study of BP1001 and low-dose cytarabine, we aimed to assess safety and toxicity of the combination of BP1001 and low-dose cytarabine in patients with refractory or relapsed acute myeloid leukaemia, or those who were refractory to at least one previous therapy regimen and no more than one previous salvage regimen.

For all diseases, we classified stable disease as an absence of clinically significant progression of the disease status in the patient. We defined progressive disease as a clinically significant deterioration (eg, increase disease burden or increased disease-related complications) in the disease status of the patient.

For acute myeloid leukaemia, we defined complete remission as a morphological leukaemia-free state (absolute neutrophil count $>1 \times 10^9$ cells per L, platelet count $>100 \times 10^9$ cells per L, normal marrow differential with <5% blasts with no Auer rods, and a normocellular or hypercellular marrow). Complete recovery with incomplete platelet recovery required all defining criteria as per complete recovery, but with a platelet count less than 100×10^9 cells per L. We defined complete recovery with incomplete blood count recovery as fulfilling all the same criteria for complete recovery, except for residual neutropenia (<1000 cells per μL) or thrombocytopenia (<100 000 cells per μL). A partial remission required all of the same haematological values as a complete recovery, but with a decrease of at least 50% in the percentage of blasts in the bone marrow aspirate, to 5–25%. Definitions of haematological and cytogenetic responses for chronic myeloid leukaemia have been described elsewhere.[17] We defined major molecular responses as a BCR-ABL to ABL ratio less than 0·02, and we defined complete molecular responses as having undetectable BCR-ABL. For myelodysplastic syndrome, response criteria were as per the International Working Group response criteria for myelodysplasia.[18]

Overall survival was an exploratory endpoint and was calculated from the time of treatment initiation to the date of death from any cause. Survival was censored at the time that

treatment was discontinued, for all patients who received a bone marrow transplant or at the last recorded contact or assessment when patients were alive at the time of analysis.

Statistical analysis

This phase 1 study used a 3 + 3 design to determine the maximum tolerated dose and optimally biological active dose of BP10001. Grb2 expression was measured in patients before and after therapy, by measuring reduction in median fluorescence intensity with low cytometry. In the first part of the study only, if at three consecutive dose levels the optimal biologically active dose response was reached in at least two-thirds of patients, dose escalation was intended to stop and the middle dose that provided optimal biologically active dose would be selected.

We generated the pharmacokinetic analysis, tables, and graphs using Phoenix (version 1.4). We generated descriptive statistics (n, arithmetic mean, SD) for appropriate grouping and sorting variables using Phoenix (version 1.4), and Microsoft Excel (2007). Overall survival was a prespecified exploratory endpoint and was calculated, by the Kaplan-Meier method, from the time of treatment initiation to the date of death from any cause.

Role of the funding source

The funder of the study had a role in data analysis, data interpretation, and writing of the report. The funder of the study had no role in study design or data collection. The corresponding author had full access to all of the data and the final responsibility for the decision to submit for publication.

Results

Between July 23, 2010, and Feb 23, 2016, we enrolled 39 patients. 32 patients were treated with escalating doses of BP1001 (cohorts 1–6) and seven were treated with BP1001 plus low-dose cytarabine in the combination cohorts (cohorts 7 and 8; figure). The 32 patients treated with single-agent BP1001 included 13 patients in cohort 1, six in cohort 2, three in cohort 3, three in cohort 4, three in cohort 5, and four in cohort 6. The seven patients treated in the combination cohorts included four patients in cohort 7 and three in cohort 8. Five patients were diagnosed with chronic myeloid leukaemia in blast phase, 30 with acute myeloid leukaemia, and four with myelodysplastic syndrome (table 1). The median age was 65 years (IQR 57–75). Before enrolment, patients had received a median of four treatment regimens. Patients had diverse cytogenetic findings, including six with t(9;22) mutations, 13 with diploid mutations, 11 with complex mutations, and six with miscellaneous mutations. Patients receiving monotherapy had a median number of one treatment cycle, and those receiving combination therapy had a median of three cycles. Of the 39 patients, 27 (69%) were assessable for dose-limiting toxicity. 12 patients were removed from study before the end of cycle one because of disease progression or death, without dose-limiting toxicity, and were replaced per-protocol guidelines. Two patients did not receive any infusions of BP1001 and were not included in the study. Among the 27 assessable patients, 21 received BP1001 monotherapy and six received BP1001 plus low-dose cytarabine combination therapy. None of the patients receiving BP1001 plus low-dose cytarabine had previously

received low-dose cytarabine before initiating therapy during the study. Mutations for all patients included *ASXL1* (n=1), *BCR-ABL* (n=6, including T315I in two patients), *CEBPA* (n=4), *DNMT3A* (n=1), *FLT3-ITD* (n=2), *FLT3* (n=1), *GATA2* (n=1), *IDH1* or *IDH2* (n=4), *JAK2* (n=2), *NOTCH1* (n=1), *NPM1* (n=2), *KRAS* (n=1), *NRAS* (n=3), *TET2* (n=2), and *TP53* (n=3; appendix p 1). Four patients receiving BP1001 monotherapy had received a previous allogeneic stem-cell transplant, including one haploidentical stem-cell transplant. The median number of previously received tyrosine kinase inhibitors for patients in chronic myeloid leukaemia in blast phase was 3 (IQR 2–3). Previously received tyrosine kinase inhibitors included imatinib, dasatinib, nilotinib, bosutinib, and ponatinib. Two patients had T315I mutations, one of whom also had a concurrent F359V mutation. Another patient had both Y253 and F317V mutations.

Table 2 shows the adverse events for patients receiving BP1001 monotherapy and those receiving BP1001 plus low-dose cytarabine combination therapy. One patient treated with 5 mg/m^2 BP1001 (patient 1) experienced dose-limiting toxicity consisting of grade 4 mucositis and grade 4 hand–foot syndrome while receiving concurrent hydroxyurea 3 g per day for rapidly proliferative chronic myeloid leukaemia in blast phase (table 2). The patient had a previous history of hydroxyurea-induced mucositis. Being the first patient to receive BP1001, these toxic effects were considered possibly related to BP1001, and the event was reported as dose-limiting toxicity. After cohort expansion to six patients, no other patient developed dose-limiting toxic effects. 13 patients were enrolled before six patients completed one cycle of BP1001, so that a sufficient number of patients were assessable for dose-limiting toxicity. The remaining seven patients dropped out because of disease progression or death. Hyperleukocytosis was managed during the study with hydroxyurea in 17 other patients during the first cycle of treatment, none of whom had any dose-limiting toxic effects. Two other patients in cohort 1 and cohort 5 developed mucositis of grade 1–2. No dose-limiting toxicity was noted in any other patient receiving monotherapy or combination therapy. No patient required dose reduction, and there were no treatment-related deaths. Only one patient (number 1) discontinued for possible drug-related toxic effects. Therefore, a maximum tolerated dose was not identified. The highest tested dose for BP1001 was 90 mg/m^2. Otherwise, toxic effects were overall manageable.

Four patients died as a result of adverse events (table 2). Patient nine died with sepsis and multiple organ failure (table 3). However, there was only one death that we considered to be related to study treatment (patient 1; multiple organ failure in the setting of progressive chronic myeloid leukaemia in blast phase). All other deaths (n=7) occurred in the context of progressive or refractory leukaemia, including respiratory distress (n=1), pneumonia (n=1), sepsis (n=1), and cardiac arrest (n=1; table 3). Other toxicities possibly related to BP1001 were mostly grade 1 or 2 and manageable (table 2).

After one cycle, BP1001 monotherapy decreased the percentage of peripheral blood blasts by at least 50% in nine (33%) of 27 patients who had peripheral blood blasts at the start of therapy, within a median of 9 days (IQR 7–18), and decreased bone marrow blasts by at least 50% in three (10%) of 29 patients who had bone marrow blasts at the start of therapy, within a median of 30 days (IQR 30–35; table 3). Per protocol, patients could receive BP1001 treatment for up to 6 months if they exhibited stable disease (ie, <50% increase

in their white blood cell count during the first 4 weeks of therapy), or had improvement of their disease, defined as haematological improvement, or achievement of either partial or complete haematological remission. Four patients receiving monotherapy completed two cycles of treatment and three patients completed five cycles of treatment (table 3). The cytogenetic and molecular profile of these patients is shown in the appendix (p 1).

Preclinical data suggest that BP1001 increased the sensitivity of acute myeloid leukaemia cell lines to cytarabine but not to hypomethylating agents. In phase 1b, we studied the effects of a BP1001 plus low-dose cytarabine combination regimen in patients with refractory or relapsed acute myeloid leukaemia. For all patients but one, the dose-limiting toxicity was not reached in the phase 1 monotherapy cohorts, up to the maximum dose of 90 mg/m^2 BP1001. However, before the initiation of phase 1b, we did not know whether the BP1001 plus low-dose cytarabine combination could induce unexpected toxicity. Since the C_{max} and AUC of BP1001 were very similar between the 60 mg/m^2 and the 90 mg/m^2 doses (appendix pp 2, 3), we decided to compare the safety and toxicity of the two doses of BP1001 (60 *vs* 90 mg/m^2) combined with low-dose cytarabine in phase 1b. Of the seven patients who received combination therapy, three received three cycles of treatment and one received five cycles of treatment (table 3). The BP1001 plus low-dose cytarabine combination was well tolerated, with a toxicity profile similar to that of BP1001 monotherapy, including the absence of identifiable dose-limiting toxicity (table 2). Five (83%) of six assessable patients receiving BP1001 plus low-dose cytarabine experienced at least a 50% reduction in bone marrow blasts, which occurred in a median of 29 days (IQR 28–55) and over a median of one cycle (1–2). Two patients achieved a complete remission, one achieved complete remission with incomplete haematological recovery, and two had stable disease. Of the three patients who achieved complete remission or incomplete haematological recovery, this occurred after a median of 2 months (range 1–5·5; median of two cycles [range 1–3]).

Because of technical issues with storage of plasma and urine samples from patients in cohorts 1–6, we used samples from patients in cohorts 7 and 8 for the pharmacokinetic analysis. The lower limit of quantitation of Grb2 antisense oligodeoxynucleotide concentration in plasma is 0·50 ng/mL. There was no quantifiable Grb2 antisense oligodeoxynucleotide at the pre-study timepoint. The concentrations of Grb2 antisense oligodeoxynucleotide in plasma declined bi-exponentially, and the T_{max} was observed 1 h after infusion (appendix p 2). At 72 h after infusion, the plasma concentrations of Grb2 antisense oligodeoxynucleotide were below the lower limit of quantitation or slightly above it (appendix p 2). Plasma $t_{1/2}$ ranged from 22·2 h to 37·7 h for the 60 mg/m^2 dose, and from 4·6 h to 14·2 h for the 90 mg/m^2 dose (appendix p 3). The C_{max}, $AUC_{(0–24)}$, $AUC_{(0–t)}$, and $AUC_{(0–inf)}$ were similar for both doses (appendix p 3). However, Vz decreased by approximately 1·5 times and the CL increased by approximately 1·5 times when the dose increased from 60 mg/m^2 to 90 mg/m^2 (appendix p 3). The C_{max} and AUC of BP1001 were very similar between the two doses, suggesting that the uptake of BP1001 might have reached its highest level at the 60 mg/m^2 dose, thus resulting in higher CL and lower $t_{1/2}$, even when the higher drug dose was administered. The percentage of Grb2 antisense oligodeoxynucleotide recovered in the urine was lower for the 60 mg/m^2 dose (range 0·04–3·23%) than for the 90 mg/m^2 dose (0·36–4·77%; appendix p 3).

Flow cytometry was used to determine the protein concentrations of Grb2 and pERK in CD33-positive peripheral blood cells at baseline, on days 15 and end of treatment (appendix p 4). On day 15, Grb2 concentrations decreased by a mean of 61% (range 47–85%) in seven of 11 samples tested, and pERK concentrations by 52% (28–82%) in six of 11 samples. Thus, relative to baseline, six samples had concomitant decrease in Grb2 and pERK concentrations, whereas one sample had a decrease in Grb2 but no change in pERK. On day 15, the median decrease in Grb2 concentrations in all the samples was 54% (IQR 0–63) and the median decrease in pERK concentrations in all samples was 28% (0–54) relative to baseline. At the end of treatment, BP1001 decreased Grb2 concentrations relative to baseline by at least 25% (mean decrease 49% [range 28–91%]) in ten (83%) of 12 samples, and pERK concentrations by at least 25% (mean decrease 52% [27–91%]) in seven (58%) of 12 samples. Seven samples therefore had concomitant decrease in Grb2 and pERK concentrations, whereas three samples had Grb2 decrease but not pERK decrease. At end of treatment, Grb2 concentrations in all samples decreased by a median of 42% (IQR 28–54), and pERK concentrations decreased by a median of 31% (0–47), relative to baseline.

We cannot formally state that we identified the optimal biologically active dose for BP1001 because in at least two-thirds of patients, there were no three consecutive levels by which a decrease of 50% or more was reached. On day 15, a Grb2 decrease of at least 50% was observed in two-thirds of patients receiving BP1001 doses of 60 and 90 mg/m^2 (appendix p 4). At end of treatment, a Grb2 decrease of at least 50%, relative to baseline, was observed in a third of patients across the BP1001 20–90 mg/m^2 doses (cohorts 3–6; appendix p 4).

The median overall survival for all assessable patients (n=27) was 2·89 months (IQR 1·7–4·9). Overall survival was 2·5 months (1·3–3·1) for patients receiving monotherapy, and 6·21 months (3·3–14·0) for combination therapy. The median follow-up of the two surviving patients in the BP1001 plus low-dose cytarabine combination treatment group was 15·6 months (range 14·0–17·2).

Grade 5 adverse events were cardiopulmonary disorders (two [5%] of 39 patients), fever (including neutropenic fever) and infections (two [5%] of 39 patients), and multiple organ failure (one [3%] of 39 patients). We considered no serious adverse event to be probably or definitely related to BP1001.

Discussion

In this phase 1/1b study of BP1001, an inhibitor targeted against GRB2, the drug was well tolerated both as monotherapy and in combination with low-dose cytarabine. We did not identify a maximum tolerated dose. The most common grade 3–4 adverse events were cardiopulmonary disorders, and fever (including neutropenic fever) and infections. BP1001 showed potential anti-leukaemic effects: nine (33%) of 27 patients in monotherapy cohorts had at least a 50% reduction in peripheral blood blasts, three (10%) of 29 patients in monotherapy cohorts had at least a 50% reduction in bone marrow blasts, and seven (22%) patients benefited from mono therapy (as per investigator’s assessment) and had extended cycles of treatment. Furthermore, three (50%) of six assessable patients receiving BP1001 plus low-dose cytarabine combination therapy achieved complete recovery or

incomplete haematological recovery. These data suggest that BP1001 has early evidence of anti-leukaemic activity in combination with low-dose cytarabine.

We postulate that inhibition of Grb2 could curtail the progression of haematological malignancies because Grb2 is essential to the signalling of BCR-ABL, KIT, FLT3, and JAK2 tyrosine kinases, which are overexpressed or mutated in several leukaemias.[5–7] We selected an antisense strategy to inhibit Grb2 because Grb2 is an intracellular protein with no enzymatic activity. To target the *GRB2* transcript, we used P-ethoxy antisense oligodeoxynucleotides, which do not contain locked nucleic acids or sulphur groups. Phosphorothioate antisense oligodeoxynucleotide,[19,20] gapmer antisense oligonucleotide containing locked nucleic acids,[21] 2′-O-methoxy ethyl,[22] constrained ethyl residues,[23] and 2′-O-methyl RNA-containing mixed backbone antisense oligoucleotide[24,25] have been associated with complement activation,[19] coagulation,[19,20] and increased concentrations of serum transaminases[19,21–25] in oncology clinical trials. The Grb2 P-ethoxy antisense oligodeoxynucleotide was incorporated in the neutral DOPC lipid to enhance its biodistribution and intracellular uptake.[15] When complexed with negatively charged antisense oligodeoxynucleotides, cationic lipids could deposit aggregates in lung capillaries and cause an embolism.[26]

Results from preclinical studies[15,16] showed that BP1001, administered at 15 mg/kg dose (equivalent to 45 mg/m^2 in human beings), extended the survival of mice bearing leukaemia xenografts without inducing excessive toxicity. The US Food and Drug Administration recommended the starting dose of BP1001 as a tenth of the efficacy dose used in this mouse survival study.[15,16] Thus, we used 5 mg/m^2 as the starting dose of BP1001 in phase 1. BP1001 was well tolerated. A maximum tolerated dose was not reached, even when BP1001 was administered at doses of 90 mg/m^2. Contrary to many antisense oligodeoxynucleotides,[19,21–25] BP1001 did not induce an increase in serum transaminase concentrations. This finding might be due do the fact that BP1001 is packaged in a neutral liposome, made of naturally occurring DOPC lipids, and the P-ethoxy structure of the drug does not induce ribonuclease H activity, which has been linked with the hepatotoxicity of some antisense oligodeoxynucleotides.[27] However, this possibility remains to be tested. Thrombocytopenia[19,20,23,25] and prolongation of activated partial thrombin time (aPTT)[19,22,24] have also been reported for some antisense analogues, sometimes as dose-limiting toxicity.[21,23,24] Three patients in our study had grade 3–4 thrombocytopenia, but this was probably disease related since thrombocytopenia is common in this setting and was present at baseline. BP1001 did not seem to affect thrombocytopenia or aPTT. The two patients who had prolonged aPTT were given the lowest BP1001 dose, and aPTT was not observed in patients given higher BP1001 doses. These findings show that BP1001, which is composed of P-ethoxy oligodeoxynucleotide, has a very different toxicity profile to other antisense oligodeoxynucleotide analogues, which have been associated with thrombocytopenia,[19,20,23,25] aPTT prolongation,[22,24] and neurotoxicity.[28,29]

Grb2 is upstream of pERK. A concomitant decrease in Grb2 and pERK concentrations was observed in 58% of assessed samples. However, there were a few instances in which a Grb2 decrease was not associated with pERK decrease, suggesting that in some instances, Grb2 might not be operating through pERK, or that other pathways in addition to Grb2

were activating pERK, but this warrants further investigation. Additionally, BP1001 did not reduce Grb2 concentrations in two patients. We speculate that this finding could have been possibly due to insufficient plasma concentrations of BP1001.

Seven patients benefited from BP1001 monotherapy and had extended cycles of treatment while receiving monotherapy. These patients, who were refractory to at least one previous therapy regimen and no more than one previous salvage regimen, had diverse cytogenetic backgrounds: BCR–ABL, diploid, or complex mutations. Two patients had the *JAK2*-V617F mutation, and two patients had the *NRAS* mutation. Increased Grb2 binding to the *JAK2*-V617F mutant is expected because of the enhanced tyrosine kinase activity and phosphorylation of mutant *JAK2*.[6] Since Grb2 is upstream of RAS, BP1001 benefiting patients with an *NRAS* mutation would not be expected. The three RAS family members have different post-translational modifications and subcellular localisations, resulting in consequent differential signaling.[30] NRAS signals through Raf and RhoA to regulate cell adhesion, whereas KRAS signals through Akt and Cell division control protein 42 (Cdc42) to regulate motility.[31] The function of NRAS is not clear, but paradoxically, patients with acute myeloid leukaemia with the *NRAS* mutation have been reported to have increased cytarabine sensitivity.[32] Patients with *NRAS* mutation might experience clinical benefit from BP1001 plus low-dose cytarabine. Further preclinical studies are evidently required in these subsets of patients to understand any possible role and the mechanism of action BP1001 might have in such instances.

In this study, the highest tested dose of BP1001 was 90 mg/m^2. BP1001 seemed to be cleared more rapidly at the 90 mg/m^2 dose than at the 60 mg/m^2 dose, decreasing its Vz and $t_{1/2}$. It seemed that the uptake of BP1001 might have plateaued at 60 mg/m^2, limiting BP1001 absorption even when a higher drug dose was administered. Preclinical and clinical reports show that liposomes are primarily taken up by mononuclear phagocytic cells of the liver, followed by those of the spleen, lungs, and bone marrow.[33] When liver uptake of liposomes is saturated, the excess liposomes are taken up by the spleen, leading to enhancement of liposome uptake.[26,33] This activity could result in a higher CL and shorter $t_{1/2}$ at higher BP1001 doses, although this has not been directly tested. A similar phenomenon has been observed with liposomal amphotericin B administered at 2·5 mg/kg compared with 1·0 mg/kg.[34] Our data indicate that Grb2 downregulation and potential anti-leukaemic effect in combination with low-dose cytarabine could be attained in participants at both doses, and safety and tolerability were not substantially different. Therefore, BP1001 is being administered at 60 mg/m^2 in combination with low-dose cytarabine in our ongoing phase 2 study.

One of the limitations of this study was that the pharmacokinetic analysis was not reported for patients receiving BP1001 monotherapy. This was because of technical issues relating to the development of the sandwich ELISA to quantify Grb2 antisense oligodeoxynucleotide concentration in plasma and urine took some time to resolve, and prevented us from measuring these concentrations from the start of the study. When we tried to analyse the plasma and urine concentrations of BP1001 from patients in cohorts 1–6, the samples had been stored too long and were beyond their storage stability. Although we only report BP1001 samples from patients in the BP1001 plus low-dose cytarabine cohorts,

samples were taken from patients on cycle one, day 1, before low-dose cytarabine was administered. We felt that the pharmacokinetic values of these samples reflected those of BP1001 monotherapy. We have recently acquired preliminary data suggesting that low-dose cytarabine might not interfere with our BP1001 ELISA, but this remains to be validated. Another limitation of the study is that we cannot formally state that we have identified the optimal biologically active dose. On day 15, the Grb2 and pERK downregulation effects appeared grossly similar between the 60 and 90 mg/m^2 BP1001 doses. At the end of treatment, a similar effect appeared to be reached with the 20, 60, and 90 mg/m^2 BP1001 dose range, because a 44–46% decrease in Grb2 concentrations was observed between these doses. We interpreted these results to suggest that further dose escalation was unwarranted as it appeared that we had reached a plateau in biological activity. Also, it would have been ideal to determine whether BP1001 could have a potential effect on leukaemia stem cells by determining whether BP1001 decreased Grb2 and pERK concentrations in the CD34-positive–CD38-negative cell population.

To our knowledge, this is the first phase 1 study of a liposome-incorporated P-ethoxy antisense oligodeoxynucleotide targeted against Grb2. Since Grb2 is crucial to BCR-ABL signalling, the safety and efficacy of BP1001 in combination with tyrosine kinase inhibitors will also be studied in patients with advanced chronic myeloid leukaemia, including chronic myeloid leukaemia in blast phase. Furthermore, the favourable safety profile and encouraging pharmacodynamics of BP1001 suggest that the liposome-incorporated P-ethoxy antisense oligodeoxynucleotide approach could serve as a template to potentially target other so-called non-druggable proteins, including anti-apoptotic proteins and transcription factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of interests

ATA reports personal fees and other from Bio-Path Holdings Inc during the conduct of the study. ATA also has patent 7,309,692 (liposomal Grb2 antisense) licensed to Bio-Path Holdings Inc. JC reports grants and personal fees from Bio-Path Holdings Inc during the conduct of the study. All other authors declare no competing interests.

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Research in context

Evidence before this study

Results from preclinical studies indicate that the growth factor receptor-bound protein 2 (Grb2) is crucial for the signalling of oncogenic tyrosine kinases. Inhibition of the function or expression of Grb2 results in inhibition of cancer cell growth and enhanced survival of mice bearing leukaemia xenografts. It has been proposed that Grb2 is a potential therapeutic target for haematological malignancies, and that inhibition of Grb2 is an appropriate treatment strategy to pursue in the context of a haematological clinical trial. We did a systematic review of the scientific literature, using PubMed to find studies published in English up to Sept 30, 2016, using the term “growth factor receptor bound protein-2 (Grb2) inhibitor” in the subject search. We also searched ClinicalTrials.gov up to Sept 30, 2016, for clinical studies, using “Grb2” as the search term.

Added value of this study

To our knowledge, this is the first phase 1/1b study of a Grb2 inhibitor, BP1001. BP1001 is comprised of a liposome-incorporated antisense oligodeoxynucleotide targeted against the *GRB2* transcript. We found that BP1001, when administered intravenously twice weekly for 28 days at doses up to 90 mg/m^2, was well tolerated in patients with refractory or relapsed haematological malignancies. Furthermore, we provide preliminary evidence that BP1001 in combination with low-dose cytarabine induced complete remission in patients with refractory or relapsed acute myeloid leukaemia who were ineligible for induction therapy.

Implications of all the available evidence

Results of this study support the preclinical evidence that Grb2 is a potential therapeutic target for haematological malignancies. The favourable safety profile of BP1001 strongly suggests that BP1001, which is composed of P-ethoxy oligodeoxynucleotide, has a very different toxicity profile than other antisense oligonucleotide analogues, which have been associated with serum transaminase activation, thrombocytopenia, and activated partial thrombin time prolongation. The tolerability of BP1001 could aid use of BP1001 in clinical combination settings.

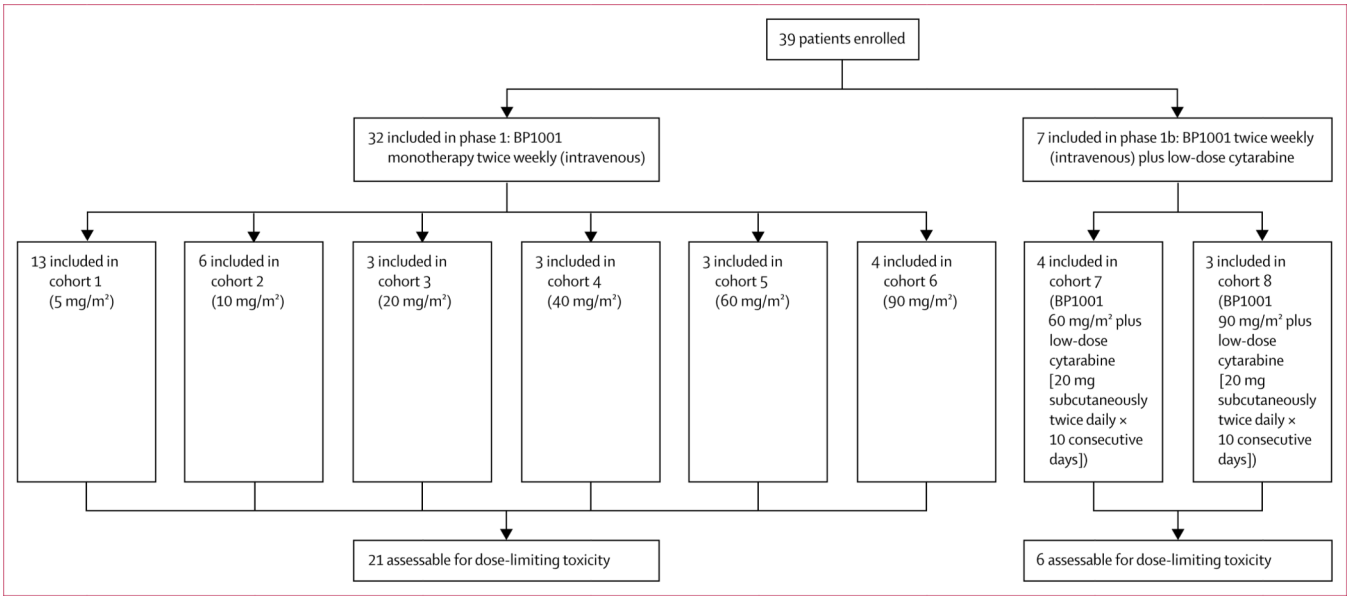

Figure 1: Trial profile

Patients who were not in the study at day 28 were not included in assessment for dose-limiting toxicity.

Table 1:

Baseline characteristics of the total patient cohort

	Phase 1 (n=32)	Phase 1b (n=7)
Median age (years)	63 (56–73)	72 (70–76)
Sex		
Male	22 (69%)	5 (71%)
Female	10 (31%)	2 (29%)
Number of previous regimens	4 (5–6)	1 (1–1)
Diagnosis at study entry		
Chronic myeloid leukaemia in blast phase	5 (16%)	0
Acute myeloid leukaemia	23 (72%)	7 (100%)
Myelodysplastic syndrome	4 (13%)	0
Performance status		
Median peripheral blasts	32% (5–71)	4% (0–10)
Median bone-marrow blasts *	43% (23–69)	25% (18–31)
Median white blood cell count ($\times 10^6$ per mL) †	3·7 (1·5–9·2)	2·2 (0·6–2·3)
Median haemoglobin (g/mL)	9·4 (8·8–9·9)	9·1 (8·9–9·2)
Median platelets ($\times 10^6$ per mL)	16 (12–28)	14 (2–80)
Cytogenetics		
Total t(9;22) ‡ t(9;22) plus complex	6 (19%) 4 (13%)	0 0
Diploid	8 (25%)	5 (71%)
Complex (total)	10 (31%)	1 (14%)
Complex plus trisomy 8	1 (3%)	0
Complex plus del(20q)	1 (3%)	0
Complex plus del(5) or del(7)	2 (6%)	0
Complex plus del(5q)	4 (13%)	1 (14%)
Complex plus miscellaneous	2 (6%)	0
Abnormal (non-complex)	8 (25%)	1 (14%)
del(7q)	1 (3%)	0
del(7)	1 (3%)	0
Trisomy 8	1 (3%)	0
Miscellaneous	5 (16%)	1 (14%)

Data are median (IQR), n (%), n (IQR), or % (IQR).

* Calculated with data from 36 participants because three patients in phase 1 were not assessable.

† For the patients with chronic myeloid leukaemia in blast phase (n=5) and Philadelphia chromosome-positive acute myeloid leukemia (n=1), the median white blood cell count was $8{\cdot}96 \times 10^6$ per mL (IQR 3·8–9·2).

‡ Four patients had t(9;22)-positive chronic myeloid leukaemia and complex cytogenetics, one patient had t(9;22)-positive acute myeloid leukaemia, and one patient had t(9;22) mutation but no complex cytogenetics.

Table 2:

Numbers of adverse events

	Patients receiving BP1001 monotherapy				Patients receiving BP1001 plus low-dose cytarabine combination therapy			
	All grades	Grade 1–2	Grade 3–4	Grade 5	All grades	Grade 1–2	Grade 3–4	Grade 5
Cardiopulmonary	61	42	17	2	11	4	8	0
Fevers (including neutropenic fever) and infections	34	21	11	2	12	6	6	0
Electrolyte and blood chemistry abnormalities	49	36	13	0	1	1	0	0
Gastrointestinal	35	34	1	0	5	4	1	0
Pain (including generalised, musculoskeletal, or flank pain)	21	17	4	0	4	1	2	0
Oedema	18	18	0	0	2	1	1	0
Bleeding or coagulation test abnormality	9	7	2	0	5	3	2	0
Renal	9	4	5	0	1	1	0	0
Leukocytosis	8	0	8	0	1	0	1	0
Asthenia or fatigue	5	3	2	0	4	2	2	0
Cognitive disturbance	4	4	0	0	0	0	0	0
Myelosuppression	4	0	4	0	0	0	0	0
Mucositis	3	2	1*	0	0	0	0	0
Hepatic	2	0	2	0	0	0	0	0
Tumor lysis syndrome	2	0	2	0	0	0	0	0
Hypovolaemia	1	0	1	0	1	0	1	0
Leukaemia cutis	1	0	1	0	1	1	0	0
Hand–foot syndrome	1	0	1*	0	0	0	0	0
Agitation	1	0	1	0	0	0	0	0
Hypervolaemia	0	0	0	0	1	0	1	0
Multiple organ failure	1	0	0	1	0	0	0	0
Syncope	1	0	1	0	0	0	0	0
Decubitus ulcer	0	0	0	0	1	0	1	0
Hallucination	0	0	0	0	1	0	1	0
Platelet count decreased	0	0	0	0	1	0	1	0

We included grade 1–2 events affecting more than 10% of participants, and all events of grade 3 and worse.

*Because these events were assessed as possibly related to study drug (BP1001) and were grade 3, they were assessed as dose-related toxic effects. Both events occurred in patient 1, at the 5 mg/m^2 dose level (cohort 1). Per protocol, we expanded the cohort to six assessable patients. All six patients have been reviewed, with no recurrences of grade 3 or worse of either of these events in any treated patient.

Table 3:

Peripheral blood or bone-marrow blast percentages of patients receiving BP1001 monotherapy or BP1001 plus low-dose cytarabine combination therapy, by patient number

	Cohort	BP1001 (mg/m^2)	Receiving low-dose cytarabine	Diagnosis	Number of previous therapies	Peripheral blood blasts (%)			Bone marrow blasts (%)			Treatment cycles completed	Reason for treatment discontinuation
						Baseline	Nadir	Off-Rx	Baseline	Nadir	Off-Rx		
1	1	5	No	CML-BP	4	51	NR	97	78	ND	ND	<1	Dose-limiting toxicity (mucositis and hand–foot syndrome); death (disease progression)
2	1	5	No	CML-BP	4	65	12	9	85	ND	ND	<1	Death (respiratory distress)
4	1	5	No	AML	5	67	43	76	67	ND	ND	<1	Withdrawn (disease progression)
5	1	5	No	CML-BP	8	46	43	93	49	ND	ND	<1	Withdrawn (disease progression)
6*	1	5	No	AML	1	15	2	5	NE	NE	23	5	Withdrawn (disease progression)
7	1	5	No	MDS	6	0	0	0	8	4	6	5	Withdrawn (disease progression)
8	1	5	No	AML	7	51	NR	37	70	ND	ND	<1	Withdrawn (disease progression)
9	1	5	No	AML	5	NE	NR	100	98	ND	ND	<1	Death (sepsis)
10	1	5	No	AML	6	1	0	1	23	10	10	1	Withdrawn (disease progression)
11	1	5	No	CML-BP	6	7	NR	50	11	ND	ND	1	Withdrawn (disease progression)
12	1	5	No	AML	5	93	NR	100	98	ND	ND	<1	Withdrawn (disease progression)
13	1	5	No	CML-BP	4	7	NR	65	37	ND	ND	<1	Withdrawn (disease progression)
14	1	5	No	AML	3	48	5	21	33	ND	ND	1	Withdrawn (disease progression
15	2	10	No	AML	2	54	31	72	85	76	76	1	Withdrawn (disease progression)
17	2	10	No	MDS	4	7	NR	24	4	NR	41	<1	Withdrawn (disease progression)
18	2	10	No	AML	5	31	NR	88	35	ND	ND	<1	Withdrawn (disease progression)

	Cohort	BP1001 (mg/m^2)	Receiving low-dose cytarabine	Diagnosis	Number of previous therapies	Peripheral blood blasts (%)			Bone marrow blasts (%)			Treatment cycles completed	Reason for treatment discontinuation
						Baseline	Nadir	Off-Rx	Baseline	Nadir	Off-Rx		
19	2	10	No	AML	2	1	NR	ND	46	ND	ND	<1	Withdrawn (disease progression)
20	2	10	No	AML	3	76	5	63	50	NR	80	1	Withdrawn (disease progression)
21	2	10	No	AML	4	71	43	74	40	38	38	2	Withdrawn (disease progression)
22	3	20	No	AML	2	1	0	1	8	3	ND	2	Withdrawn (disease progression)
23[†]	3	20	No	MDS	6	NE	NE	NE	NE	NE	NE	1	Death (disease progression)
24	3	20	No	MDS	1	0	0	0	NE	NE	NE	5	Withdrawn (disease progression)
25	4	40	No	AML	3	10	3	19	25	NR	36	2	Withdrawn (disease progression)
26[‡]	4	40	No	AML	6	11	NR	80	25	NR	80	1	Withdrawn (disease progression)
27	4	40	No	AML	5	93	NR	97	87	ND	ND	1	Withdrawn (disease progression)
28	5	60	No	AML	5	96	93	98	89	88	88	1	Withdrawn (disease progression)
29	5	60	No	AML	3	35	7	24	28	ND	ND	1	Withdrawn (disease progression)
30	5	60	No	AML	4	51	17	82	72	NR	92	1	Death (disease progression)
31	6	90	No	AML	4	0	0	0	17	NR	17	1	Withdrawn (disease progression)
32	6	90	No	AML	3	2	NR	42	24	22	22	2	Death (pneumonia)
33	6	90	No	AML	6	88	58	36	69	80	80	<1	Withdrawn (disease progression)
34	6	90	No	AML	6	5	NR	92	66	ND	ND	1	Death (cardiac arrest)
35	7	60	Yes	AML	1	0	0	0	17	2	2	1	Complete remission with incomplete haematological recovery
36	7	60	Yes	AML	4[§]	68	50	ND	72	ND	ND	<1	Withdrawn (disease progression)
37	7	60	Yes	AML	1	10	NR	31	25	33	ND	1	Death (disease progression)
38	7	60	Yes	AML	1	4	0	ND	23	2	3	5	Complete remission

	Cohort	BP1001 (mg/m^2)	Receiving low-dose cytarabine	Diagnosis	Number of previous therapies	Peripheral blood blasts (%)			Bone marrow blasts (%)			Treatment cycles completed	Reason for treatment discontinuation
						Baseline	Nadir	Off-Rx	Baseline	Nadir	Off-Rx		
39	8	90	Yes	AML	3[§]	70	0	52	36	16	58	3	Stable disease
40	8	90	Yes	AML	1	0	0	0	31	2	2	3	Complete remission
41	8	90	Yes	AML	1	0	0	0	18	9	14	3	Stable disease

Two patients did not receive any infusions of BP1001 and were not included in the study: patient 3 withdrew consent because of screening failure, and patient 16 withdrew because of clinical deterioration, per clinical investigator's assessment. Off-Rx=off treatment. CML-BP=chronic myeloid leukaemia in blast phase. NR=no reduction in blasts. ND=not done. AML=acute myeloid leukaemia. NE=not evaluable. MDS=myelodysplastic syndrome.

[*] Patient was diagnosed with myelofibrosis with acute myeloid leukaemia.

[†] Patient did not enter cycle two because of drug supply issues.

[‡] Patient was diagnosed with Philadelphia-chromosome-positive AML.

[§] Includes previous therapy for antecedent haematological disorder (eg, MDS and chronic myelomonocytic leukemia).