bjh short report

# An effective modestly intensive re-induction regimen with bortezomib in relapsed or refractory paediatric acute lymphoblastic leukaemia

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## Summary

This trial explored the efficacy of re-induction chemotherapy including bortezomib in paediatric relapsed/refractory acute lymphoblastic leukaemia. Patients were randomized 1:1 to bortezomib (1.3 mg/m$^2$/dose) administered early or late to a dexamethasone and vincristine backbone. Both groups did not differ regarding peripheral blast count on day 8, the primary endpoint. After cycle 1, 8 of 25 (32%) patients achieved complete remission with incomplete blood count recovery, 7 (28%) a partial remission and 10 had treatment failure. Most common grade 3–4 toxicities were febrile neutropenia (31%) and pain (17%). Bortezomib was safely combined with vincristine. Bortezomib rarely penetrated the cerebrospinal fluid.

**Keywords:** bortezomib, childhood leukaemia, acute leukaemia, pharmacokinetics, proteasome inhibitor.



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Paediatric relapsed/refractory acute lymphoblastic leukaemia (rrALL) is the fourth most common paediatric malignancy and outcome after relapse remains poor with remission rates that range from 40% to as low as 10% for bad-prognostic subtypes such as T cell ALL and patients with an early relapse (Ko *et al*, 2010). Improvement of survival requires the introduction of novel agents with a new mechanism of action.

Bortezomib (BTZ) is a proteasome inhibitor that has been reported to sensitize malignant cells to other agents, including glucocorticoids (GC), both *in vitro* for leukaemias (Horton *et al*, 2006) and *in vivo* for multiple myeloma (MM) patients (Richardson *et al*, 2010).

We here report the results of this multicentre, multinational, open label, comparative and randomized phase II study on the efficacy of BTZ with conventional chemotherapy in rrALL (www.trialregister.nl: *NTR1881/ITCC021*. When this study started it was essentially unknown if BTZ could be combined safely with vincristine (VCR) in children. In adults, peripheral neuropathy had emerged as a significant side-effect of BTZ. This study addressed the combination of BTZ and VCR plus dexamethasone (DXM), looking at the day 8 GC-response. The sensitivity for GC is a well-known prognostic factor in ALL patients and is used in many protocols to define risk-group stratification (Riehm *et al*, 1987). Furthermore, response on day 8 has been shown to be predictive for outcome in relapsed ALL patients (Oudot *et al*, 2008).

The methods are detailed in the Data S1, Table S1, Table S2 and Fig 1).

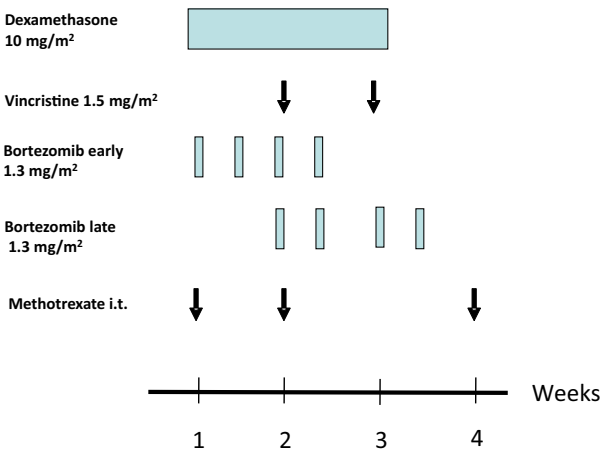


**Fig 1.** Treatment scheme. Standard re-induction chemotherapy consisted of 2 weeks dexamthasone at 10 mg/m$^2$/day in 3 doses plus vincristine given twice, on days 8 and 15, at 1·5 mg/m$^2$/dose. In addition, patients were randomized in a 1:1 ratio for receiving 'early' bortezomib: on days 1, 4, 8 and 11, or 'late' bortezomib: on days 8, 11, 15 and 18 at 1·3 mg/m$^2$. Methotrexate was given intrathecally (i.t.) as routine central nervous system prophylaxis. [Colour figure can be viewed at wileyonlinelibrary.com]

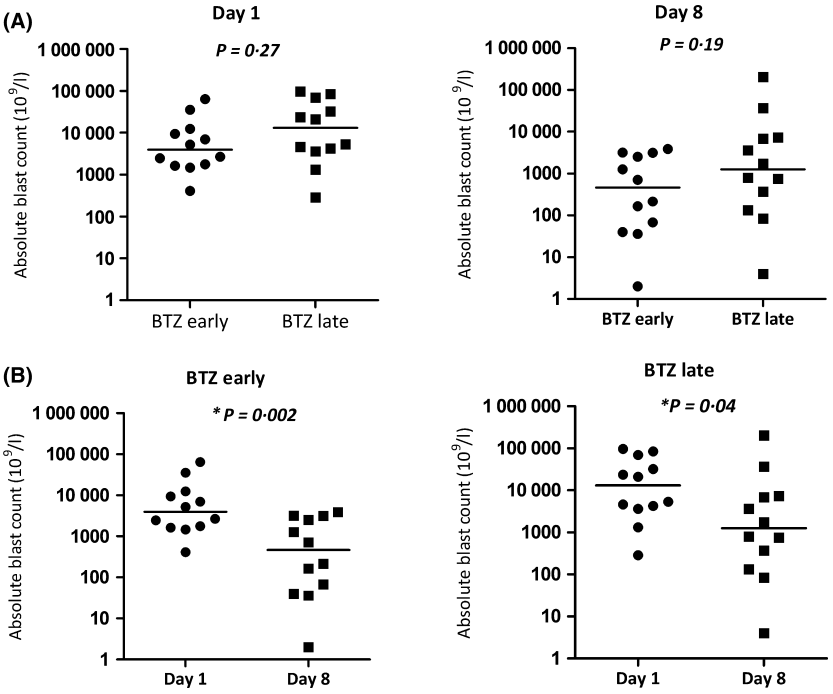


**Fig 2.** Absolute peripheral blood blast count after 1 week of treatment for BTZ early and BTZ late. (A) Comparison of absolute peripheral blood blast count between patients allocated to bortezomib (BTZ) early ($n$ = 12) and BTZ late ($n$ = 12) at day 1 and day 8 of treatment. (B) Comparison of the decrease in absolute blast count between patients allocated to BTZ early ($n$ = 12) and BTZ late ($n$ = 12) at day 8. The horizontal line represents the median.

## Results and discussion

Baseline patient characteristics are shown in Table S3. Fourteen patients were allocated to early BTZ, 15 patients to the BTZ late group. All patients received at least 2 injections of BTZ; 13 patients received a second cycle of BTZ; 4 patients received 3 cycles, and 2 patients received 4 cycles of BTZ. The patients received a median of 4 injections of BTZ (range 2–16). Five patients were excluded from the primary endpoint analysis, two for not having efficacy data on day 8, one patient for meeting an exclusion criterion and two patients for having an absolute peripheral blood blast count on day 1 lower than $0{\cdot}1 \times 10^9$/l.

The primary endpoint, day 8 blast count, was thus evaluable in 24 patients.

Day 8 blast count was not different between the 'BTZ early' (median $0{\cdot}714 \times 10^9$/l, range $0{\cdot}002–3{\cdot}9 \times 10^9$/l, $n$ = 12) and 'BTZ late' groups (median $0{\cdot}774 \times 10^9$/l, range $0{\cdot}004–203{\cdot}8 \times 10^9$/l, $n$ = 12) (Fig 2A; $P$ = 0·19). Thus, no benefit was seen with BTZ and DXM, compared to DXM alone after these first 8 days. Absolute peripheral blast count decreased significantly in both 'BTZ early' ($P$ = 0·002) and 'BTZ late' ($P$ = 0·04) after the first 8 days of treatment from median $4{\cdot}9 \times 10^9$/l (range, 0·288–96·7) to $0{\cdot}732 \times 10^9$/l (0·002–203·8), median 85% decrease, (range 0-100%), and was similar for both groups (Fig 2B). In the BTZ early group, 7 patients were DXM good responders, versus 5 patients in the BTZ late group.

No differences in bone marrow response were found between the arms for days 1, 8 and 22 and after 43 days (Fisher's exact $P > 0{\cdot}60$ for time points considered)(Table S4 and Figure S1) and no differences were found in absolute peripheral blast counts between both arms on day 22 (Mann–Whitney-$U$ $P$ = 0·23).

Twenty-five patients were evaluable for response rate after cycle 1 (2 patients discontinued treatment due to toxicity, 2 patients did not have efficacy data on day 22). Eight patients (32%) achieved a complete remission with incomplete blood count recovery (CRi), 7 (28%) a partial remission (PR), and 10 had treatment failure. There was no association between remission status and the early or late administration of BTZ (Fisher's exact $P$ = 0·53), nor was there an association between response and disease status, being either refractory or relapsed, although numbers were small (Fisher's exact $P$ = 0·96). Although this study design did not show that early BTZ leads to greater efficacy compared to late BTZ, the overall response rate (ORR) was 60% (CRi+PR) with a low intensity schedule, in this cohort of heavily pretreated patients.

Of patients who received a second treatment cycle ($n$ = 13), 4 patients recovered from CRi to CR, two patients remained in CRi, one patient improved from PR to CRi, one patient had treatment failure after cycle 2; no data on bone marrow status was available after the 2nd cycle for 5 patients.

The TACL (Therapeutic Advances in Childhood Leukaemia) study, evaluating BTZ with VCR, DXM, plus pegylated asparaginase and doxorubicin in paediatric rALL patients who failed at least 2 prior regimens, achieved a CR rate of 64% (CR+partial CR [CRp]) (Messinger *et al*, 2012), while in our study only 32% of patients achieved CRi after cycle 1 and half of those improved to CR after a second cycle. In the TACL study, the 2 T-ALL patients did not respond to therapy, while in another study that used the same TACL

regimen, 5 out of 7 patients with T-ALL achieved CR or CRp (Bertaina *et al*, 2017). In our study, with 3 out of 4 T-ALL patients in the BTZ late group, one of these four patients achieved a PR after cycle 1 and a CRi after cycle 2 (Table S5).

The median follow-up time was 20·6 months. No differences were found regarding overall survival (log-rank $P = 0{\cdot}68$). As for event-free survival (EFS), most events took place before 5 months from randomization (29% EFS survival rate for early BTZ vs. 13% for late BTZ at 5 months). The disease-free survival analysis is based on only 11 patients who achieved CR after start of study treatment and tends to be more favourable in the BTZ early group ($P = 0{\cdot}08$; see Figures S2A–C for the Kaplan–Meier analysis results). Due to the small sample size in this study, these results must be interpreted with caution.

The incidence of toxicity of any kind was not different between both randomized arms. Table S6 summarizes the occurrence of adverse events grade ≥3 in cycle 1. Febrile neutropenia was the principal toxicity ($n = 9$), pain the most common neurotoxicity and one patient went off-study following these events. Only two patients experienced grade 3-4 peripheral neuropathy in cycle 1. Peripheral neuropathy appears to be less common in children than in adults (Blaney *et al*, 2004); it was not observed in a paediatric trial with single-agent BTZ (Horton *et al*, 2007) and was not frequently seen in paediatric rrALL patients treated with BTZ and combination chemotherapy including VCR and BTZ (Messinger *et al*, 2012; Bertaina *et al*, 2017).

The BTZ dose was reduced or withdrawn in cycle 1 in 4 patients, which was due to toxicity in three of them (10·3%).

In total, BTZ dose was reduced or withdrawn because of toxicity at any cycle in 5 (17·2%) patients. All adverse events are listed in Table S7, and serious adverse events (SAE) in Table S8. Adverse events reported that were definitely related to BTZ were vomiting (one patient, cycle 1), and fatigue and pain concomitantly reported for one patient in cycle 2. Toxicity in cycle 1 was not different between the arms. No SAEs were reported to be definitely related to BTZ. Grade 3/4 adverse events in cycle 2 are displayed in Table S9. Similar to cycle 1, no differences were noted in toxicities between the arms.

Pharmacokinetic parameters demonstrated substantial intra- and inter-individual variability (Table S10). Median peak plasma concentrations and area under the plasma concentration curve (AUC) were higher (19·1 vs. 45·8 ng/ml, $P = 0{\cdot}004$ and 109·3 vs. 275·1 μg/h/l, $P < 0{\cdot}001$, respectively), and clearance was lower after the last dose of BTZ compared to the first dose (7·5 vs. 2·6 l/h, $P < 0{\cdot}001$), which is consistent with data in adults receiving BTZ only (Reece *et al*, 2011) or BTZ and DXM (Osawa *et al*, 2014). A recent study in paediatric ALL and acute myeloid leukaemia patients showed that clearance of BTZ in children is similar to that of adults with multiple myeloma and requires careful monitoring of toxicity over time (Hanley *et al*, 2017).

We are the first to measure BTZ levels in the cerebrospinal fluid (CSF). BTZ penetrated the CSF in only 9 out of 25 patients, and only to a very limited extent (0·07–5·2 ng/ml). It is therefore unlikely that BTZ significantly adds to the treatment or prophylaxis of leptomeningeal involvement in leukaemia.

No correlation between systemic AUC and concentration in CSF was found ($r^2 = 0{\cdot}11$).

Pharmacodynamic analysis showed a maximum inhibitory effect at the time maximum peak plasma concentration was reached at the first time point after BTZ administration (median 68% inhibition, range 37–80%), with a recovery of 20S proteasome activity over time (median 86% recovery after 24 h, range 50–127%), consistent with previous observations in children and adults treated with single-agent BTZ (Papandreou *et al*, 2004; Hamilton *et al*, 2005; Reece *et al*, 2011). None of the pharmacodynamic and pharmacodynamic parameters correlated with response and were not different between both arms.

Given that early or late administration of BTZ showed similar treatment responses, and the ORR to this regimen was encouraging, randomized studies comparing conventional chemotherapy with or without BTZ are necessary to determine the additive value of BTZ.

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## Author contribution

GJLK, DN, and MZ wrote the paper. GJLK, JB, JC, RAM, MZ designed the clinical trial. AJW, PSvH, MLY, VdH, analysed the data. GJLK, AA, AB, EdB, FF, CR, TK, BdM, BN, GP, DR, PR, PS, AvS, and MZ included patients and contributed to the data.

## Conflict of Interest

The authors declare no conflicts of interest.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig S1.** The absolute BM blast percentages on day 1, 8, 22, and 43 of treatment.

**Fig S2.** Overall survival, defined as the time of first dose of study treatment to death of any cause compared between treatment arms and the total group.

**Table S1.** In- and exclusion criteria

**Table S2.** Response definitions

**Table S3.** Patient and disease characteristics according to randomization arm.

**Table S4.** Absolute bone marrow blasts (M1, M2 or M3) at days 1, 8, 22 and 43

**Table S5.** Response data after cycle 1 by immunophenotype, randomization arm and disease status

**Table S6.** Hematological toxicities grade ≥3 and non-hematological toxicities (cycle 1)

**Table S7.** All Adverse events

**Table S8.** Listing of Deaths, Other Serious and Significant Adverse Events

**Table S9.** Hematological toxicities grade ≥3 and non-hematological toxicities (cycle 2)

**Table S10.** Pharmacokinetic and pharmacodynamic parameters

**Table S11.** List of hospitals and investigators involved in the study

**Data S1.** Supplemental Methods

## References


Bertaina, A., Vinti, L., Strocchio, L., Gaspari, S., Caruso, R., Algeri, M., Coletti, V., Gurnari, C., Romano, M., Cefalo, M.G., Girardi, K., Trevisan, V., Bertaina, V., Merli, P. & Locatelli, F. (2017) The combination of bortezomib with chemotherapy to treat relapsed/refractory acute lymphoblastic leukaemia of childhood. *British Journal of Haematology*, **176**, 629–636.

Blaney, S.M., Bernstein, M., Neville, K., Ginsberg, J., Kitchen, B., Horton, T., Berg, S.L., Krailo, M. & Adamson, P.C. (2004) Phase I study of the proteasome inhibitor bortezomib in pediatric patients with refractory solid tumors: a Children's Oncology Group study (ADVL0015). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, **22**, 4804–4809.

Hamilton, A.L., Eder, J.P., Pavlick, A.C., Clark, J.W., Liebes, L., Garcia-Carbonero, R., Chachoua, A., Ryan, D.P., Soma, V., Farrell, K., Kinchla, N., Boyden, J., Yee, H., Zeleniuch-Jacquotte, A., Wright, J., Elliott, P., Adams, J. & Muggia, F.M. (2005) Proteasome inhibition with bortezomib (PS-341): a phase I study with pharmacodynamic end points using a day 1 and day 4 schedule in a 14-day cycle. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, **23**, 6107–6116.

Hanley, M.J., Mould, D.R., Taylor, T.J., Gupta, N., Suryanarayan, K., Neuwirth, R., Esseltine, D., Horton, T.M., Aplenc, R., Alonzo, T.A., Lu, X., Milton, A. & Venkatakrishnan, K. (2017) Population pharmacokinetic analysis of bortezomib in pediatric leukemia patients : model-based support for body surface area-based dosing over the 2- to 16-year age range. *The Journal of Clinical Pharmacology*, **57**, 1183–1193.

Horton, T.M., Gannavarapu, A., Blaney, S.M., D'Argenio, D.Z., Plon, S.E. & Berg, S.L. (2006) Bortezomib interactions with chemotherapy agents in acute leukemia *in vitro*. *Cancer Chemotherapy and Pharmacology*, **58**, 13–23.

Horton, T.M., Pati, D., Plon, S.E., Thompson, P.A., Bomgaars, L.R., Adamson, P.C., Ingle, A.M., Wright, J., Brockman, A.H., Paton, M. & Blaney, S.M. (2007) A phase 1 study of the proteasome inhibitor bortezomib in pediatric patients with refractory leukemia: a Children's Oncology Group study. *Clinical Cancer Research*, **13**, 1516–1522.

Ko, R.H., Ji, L., Barnette, P., Bostrom, B., Hutchinson, R., Raetz, E., Seibel, N.L., Twist, C.J., Eckroth, E., Sposto, R., Gaynon, P.S. & Loh, M.L. (2010) Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a Therapeutic Advances in Childhood Leukemia Consortium study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, **28**, 648–654.

Messinger, Y.H., Gaynon, P.S., Sposto, R., van der, G.J., Eckroth, E., Malvar, J. & Bostrom, B.C. (2012) Bortezomib with chemotherapy is highly active in advanced B-precursor acute lymphoblastic leukemia: Therapeutic Advances in Childhood Leukemia & Lymphoma (TACL) Study. *Blood*, **120**, 285–290.

Osawa, T., Naito, T., Kaneko, T., Mino, Y., Ohnishi, K., Yamada, H. & Kawakami, J. (2014) Blood distribution of bortezomib and its kinetics in multiple myeloma patients. *Clinical biochemistry*, **47**, 54–59.

Oudot, C., Auclerc, M.-F., Levy, V., Porcher, R., Piguet, C., Perel, Y., Gandemer, V., Debre, M., Vermylen, C., Pautard, B., Berger, C., Schmitt, C., Leblanc, T., Cayuela, J.-M., Socie, G., Michel, G., Leverger, G. & Baruchel, A. (2008) Prognostic factors for leukemic induction failure in children with acute lymphoblastic leukemia and outcome after salvage therapy: the FRALLE 93 study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, **26**, 1496–1503.

Papandreou, C.N., Daliani, D.D., Nix, D., Yang, H., Madden, T., Wang, X., Pien, C.S., Millikan, R.E., Tu, S.-M., Pagliaro, L., Kim, J., Adams, J., Elliott, P., Esseltine, D., Petrusich, A., Dieringer, P., Perez, C. & Logothetis, C.J. (2004) Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, **22**, 2108–2121.

Reece, D.E., Sullivan, D., Lonial, S., Mohrbacher, A.F., Chatta, G., Shustik, C., Burris, H., Venkatakrishnan, K., Neuwirth, R., Riordan, W.J., Karol, M., von Moltke, L.L., Acharya, M., Zannikos, P. & Keith Stewart, A. (2011) Pharmacokinetic and pharmacodynamic study of two doses of bortezomib in patients with relapsed multiple myeloma. *Cancer chemotherapy and pharmacology*, **67**, 57–67.

Richardson, P.G., Weller, E., Lonial, S., Jakubowiak, A.J., Jagannath, S., Raje, N.S., Avigan, D.E., Xie, W., Ghobrial, I.M., Schlossman, R.L., Mazumder, A., Munshi, N.C., Vesole, D.H., Joyce, R., Kaufman, J.L., Doss, D., Warren, D.L., Lunde, L.E., Kaster, S., Delaney, C., Hideshima, T., Mitsiades, C.S., Knight, R., Esseltine, D.L. & Anderson, K.C. (2010) Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. *Blood*, **116**, 679–686.

Riehm, H., Reiter, A., Schrappe, M., Berthold, F., Dopfer, R., Gerein, V., Ludwig, R., Ritter, J., Stollmann, B. & Henze, G. (1987) Corticosteroid-dependent reduction of leukocyte count in blood as a prognostic factor in acute lymphoblastic leukemia in childhood (therapy study ALL-BFM 83). *Klinische Pädiatrie*, **199**, 151–160.