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The combination of bortezomib with chemotherapy to treat relapsed/refractory acute lymphoblastic leukaemia of childhood

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Summary

Achieving complete remission (CR) in childhood relapsed/refractory acute lymphoblastic leukaemia (ALL) is a difficult task. Bortezomib, a proteasome inhibitor, has *in vitro* activity against ALL blasts. A phase I-II trial, reported by the Therapeutic Advances in Childhood Leukaemia and Lymphoma (TACL) consortium, demonstrated that bortezomib with chemotherapy has acceptable toxicity and remarkable activity in patients with relapsed ALL failing 2–3 previous regimens. We evaluated bortezomib in combination with chemotherapy in 30 and 7 children with B-cell precursor (BCP) and T-cell ALL, respectively. Bortezomib (1·3 mg/m^2/dose) was administered intravenously on days 1, 4, 8, and 11. Chemotherapy agents were the same as those used in the TACL trial, consisting of dexamethasone, doxorubicin, vincristine and pegylated asparaginase. Three patients (8·1%) died due to infections. Twenty-seven patients (72·9%) achieved CR or CR with incomplete platelet recovery (CRp). Fourteen had minimal residual disease (MRD) lower than 0·1%. Twenty-two of 30 BCP-ALL patients (73·3%) and 5/7 patients (71%) with T-cell ALL achieved CR/CRp. The 2-year overall survival (OS) is 31·3%; CR/CRp patients with an MRD response had a remarkable 2-year OS of 68·4%. These data confirm that the combination of bortezomib with chemotherapy is a suitable/effective option for childhood relapsed/refractory ALL.

Keywords: childhood leukaemia, minimal residual disease, bortezomib, relapsed/refractory disease, acute lymphoblastic leukaemia.

Despite the remarkable improvement in the prognosis of childhood acute lymphoblastic leukaemia (ALL) over the past few decades (Pui & Evans, 2006), achievement of complete remission (CR) in relapsed/refractory (R/R) ALL is hampered by a low response rate and substantial toxicity (Cooper & Brown, 2015). Moreover, as in patients reaching a new CR, the final outcome of paediatric patients with R/R ALL remains unsatisfactory, with the long-term overall survival (OS) ranging between 5% and 50% for patients in first or subsequent relapse (Locatelli *et al*, 2012). In particular, for children with B-cell precursor (BCP)ALL experiencing isolated bone marrow (BM) relapse or first relapse after short remission duration, and for those with relapsed T-cell lineage ALL, the probability of 5-year leukaemia-free (LFS) survival does not exceed 30%, even when they receive allogeneic haematopoietic stem cell transplantation (HSCT) (Parker *et al*, 2010; Tallen *et al*, 2010; Eckert *et al*, 2015).

The most frequent causes of treatment failure for children with relapsed ALL are either resistance to second-line therapy or subsequent BM relapse (Paganin *et al*, 2008; Tallen *et al*, 2010; Eckert *et al*, 2015). Indeed, overall, re-induction chemotherapy after first relapse is successful at inducing CR in only 80–85% of patients (Parker *et al*, 2010; Tallen *et al*, 2010; Locatelli *et al*, 2012; Eckert *et al*, 2015). The Therapeutic Advances in Childhood Leukaemia and Lymphoma (TACL) consortium reported low remission rates of 44%, 27% and 12% for third, fourth and further therapeutic attempts, respectively (Ko *et al*, 2010). Moreover, the outcome of children and adolescents with relapsed ALL and non-response to initial salvage therapy is so far miserable,

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even when therapies with curative intent are employed (von Stackelberg *et al*, 2011).

Standard salvage regimens for relapsed ALL are still largely based on different combinations of the same agents used in frontline therapy in varying doses and schedules. However, also because few randomized trials comparing different re-induction regimens in risk-stratified children with relapsed ALL have been conducted so far (Parker *et al*, 2010; Tallen *et al*, 2010; Locatelli *et al*, 2012), it remains unclear whether any re-induction combination in use today is significantly superior to any other. This suggests that novel approaches are needed for improving CR rates and blast clearance in these patients, and clinical trials are currently ongoing to evaluate the safety and efficacy of adding new agents for re-induction chemotherapy. In particular, recently, some studies have investigated, with promising results, or are investigating alternative treatments for BCP-ALL, including the anti-CD22 antibody-drug conjugate inotuzumab ozogamicin (Kantarjian *et al*, 2016), the bispecific T-cell engager monoclonal antibody blinatumomab (Topp *et al*, 2015) and CD19-specific chimeric antigen receptor T-cell technology (Grupp *et al*, 2013).

The proteasome inhibitors constitute a growing group of compounds currently under clinical use or investigation. Bortezomib (Velcade©, JANSSEN-CILAG INTERNATIONAL NV Beerse, Belgium) was the first proteasome inhibitor approved by the US Food and Drug Administration (FDA) for the treatment of newly diagnosed multiple myeloma (MM), R/R MM and mantle cell lymphoma (Kane *et al*, 2006, 2007). Despite the fact that the anticancer activity of bortezomib is still not completely elucidated, it is clear that multiple mechanisms are involved. Although bortezomib displays only modest single agent activity in children with acute leukemia, *in vitro*, bortezomib is synergic with dexamethasone and additive with asparaginase, vincristine, doxorubicin, and cytarabine (Horton *et al*, 2006). Anecdotally, a child with ALL who previously experienced multiple relapses had a transient clinical response to the administration of bortezomib and dexamethasone (Brown *et al*, 2004). A phase I study by Attar *et al* (2008) suggested that bortezomib is well tolerated in combination with idarubicin and cytarabine in acute myeloid leukaemia (AML) and, when combined with valproic acid (a histone deacetylase inhibitor), the drug was shown to have *in vitro* promising activity against AML blasts (Wang *et al*, 2011; Nie *et al*, 2012). Recently, Buontempo *et al* (2016) demonstrated also that Casein Kinase 2 (CK2) inhibition could be useful in combination with bortezomib as a novel therapeutic strategy in both T-cell and BCP-ALL. Moreover, two TACL phase I and II studies showed that a combination of bortezomib with vincristine, dexamethasone, pegylated L-asparaginase and doxorubicin (VXLD) is active and has acceptable toxicity in children with R/R BCP-ALL (Messinger *et al*, 2010, 2012). Overall, these data indicate that the combination of bortezomib with cytotoxic agents routinely used in the treatment of acute leukaemia has clinical efficacy, which requires confirmation and further investigation.

This report describes the results of a monocentric study carried out in paediatric patients with R/R BCP ALL or T-cell ALL who received the combination of bortezomib with VXLD, according to the scheme published by the TACL group (Messinger *et al*, 2010, 2012), with the aim of re-inducing a new CR.

Patients and methods

Study group

Patients aged 15 years or less at time of diagnosis and 21 years or less at time of treatment were included in the study. Eligibility criteria were a performance status ≤ 2 according to Eastern Cooperative Oncology Group criteria (Oken *et al*, 1982) and absence of any active infection at time of evaluation for enrollment. Other inclusion criteria were: patient serum bilirubin $\leq 2\times$ upper limit of normal (ULN) for age, aspartate transaminase and alanine transaminase $\leq 5\times$ ULN and serum creatinine $<2\times$ ULN.

Disease status of the patients was assessed by history, clinical evaluation, and morphological, cytogenetic and immunophenotyping analysis of both BM and peripheral blood cells. Patients who had previously received allogeneic HSCT were eligible, as long as they were not being treated for active graft-versus-host disease (GvHD). Patients' parents gave written informed consent before treatment. This study was conducted according to guidelines active at the Bambino Gesù Children's Hospital, Rome (Italy), and in accordance with the principles of the Helsinki Declaration.

Treatment plan

The scheme employed was the same as that used in the TACL trial (Messinger *et al*, 2010, 2012). In particular, bortezomib (1·3 mg/m^2/dose) was administered intravenously on days 1, 4, 8 and 11. Dexamethasone 10 mg/m^2/day was given either orally or intravenously for 14 consecutive days. Doxorubicin 60 mg/m^2 was given intravenously on day 1. Vincristine 1·5 mg/m^2/dose (2 mg maximum dose) was administered intravenously on days 1, 8, 15 and 22. Pegylated asparaginase 2500 U/m^2/dose was given intravenously weekly for 4 doses. All patients received intrathecal (IT) methotrexate on day 1. Patients without central nervous system (CNS) ALL involvement were given a second IT injection of methotrexate on day 15. Patients with CNS involvement received additional IT triple-chemotherapy, consisting of methotrexate, methylprednisolone and cytarabine on days 8, 15 and 22. Responding patients were offered allogeneic HSCT if a suitable donor was immediately available or were given consolidation courses of chemotherapy including multiple agents active against ALL, which were chosen according to the treating physician's preference and previous treatment.

Response criteria

Response to treatment was assessed using morphological, cytogenetic and immunophenotyping analysis on day 29. If, at that time, the marrow was hypoplastic, a new BM aspiration and complete blood count (CBC) were performed weekly until recovery or progression. CR was defined as follows: absence of physical signs of leukaemia or detectable leukaemia cells on peripheral blood smear; BM with active haematopoiesis and <5% leukaemia cells; absolute neutrophil count (ANC) $>1 \times 10^9$/l, and platelet count $>100 \times 10^9$/l; normal cerebrospinal fluid findings.

Complete response without platelet recovery (CRp) was defined as M1 BM (<5% bone marrow blasts) with no circulating blasts or extramedullary disease and recovery of ANC ($>1 \times 10^9$/l) but insufficient recovery of platelets ($<100 \times 10^9$/l). Persistent disease (PD) was defined as a BM with more than 5% leukaemia cells, development of new sites of extramedullary disease, or clinical evidence of PD, with or without neutrophil and platelet recovery. The assessment of minimal residual disease (MRD) was performed on BM samples by flow cytometry. BM aplasia was defined as the presence in the peripheral blood of an ANC $<0{\cdot}2 \times 10^9$/l and a platelet count $<20 \times 10^9$/l for at least 3 consecutive days.

The National Cancer Institute (NCI) Common Toxicity Criteria (CTCAE v4.03) were used to evaluate the severity of adverse events recorded on the treating physician's assessement (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf; Accessed June 14, 2010).

Patient monitoring

Pre-treatment evaluations included physical examination, concomitant medication assessment, BM aspirate, lumbar puncture and high-resolution CT scan of the head, chest, abdomen and pelvis. In addition, blood was drawn for serum chemistry, CBC, evaluation of hepatitis viruses and human immunodeficiency virus antibodies. Quantification of adenovirus DNA, Epstein-Barr virus DNA and cytomegalovirus DNA by polymerase chain reaction assay and evaluation of serum galactomannan antigen index were performed at least once a week during the first month. Echocardiogram and electrocardiogram were performed before starting chemotherapy.

Supportive therapy

All patients were initially hospitalized for re-induction treatment, and supportive therapy was administered according to institutional protocols. Given the reported high frequency of infectious complications occurring during treatment (Messinger *et al*, 2010, 2012) and the prevalence of disease-related neutropenia, prophylactic antibiotics (piperacillin-tazobactam) were administered to all children. Broad-spectrum antibiotics were further added if a patient became febrile. All patients were given acyclovir for prevention of herpesvirus infection. Voriconazole or liposomal amphotericin (3 mg/kg twice a week) was administered for preventing fungal infections until neutrophil recovery. Antifungal therapy against both yeasts and moulds was modified if there was clinical evidence of proven/probable fungal infection. Recombinant human granulocyte-colony stimulating factor (G-CSF) at the dosage of 5–10 μg/kg/day was used only in case of occurrence of documented sepsis in a neutropenic patient.

Statistical analysis

Quantitative variables were reported as median value and range, while categorical variables were expressed as absolute value and percentage. Disease-free survival (DFS) was defined as the time from study enrolment to the date of first event or last follow-up. Events were relapse, second neoplasm or death from any cause. Overall survival (OS) was defined as the time from study enrolment to death from any cause or last follow-up. The Kaplan-Meier method was used to estimate DFS and OS probabilities. Differences between groups were compared with the log-rank test. $P < 0{\cdot}05$ were considered to be statistically significant and reported in detail. Patient data were updated in August 2016. Statistical analysis was performed using EZR version 1.32 (Saitama Medical Centre, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (Kanda, 2013).

Results

Patients and treatment

Between February 2012 and June 2016, 37 paediatric patients with R/R ALL were given the treatment detailed above (see *Treatment plan*) at the Haematology/Oncology Department of the IRCCS Bambino Gesù Children's Hospital, Rome, Italy; 30 had BCP and 7 T-cell ALL (2 of them had early T-cell precursor ALL). Patient's characteristics are detailed in Table I. The cohort included 20 males and 17 females, median age at time of latest leukaemia relapse being 10·3 years (2·6–21). Four patients had CNS involvement at diagnosis, while 8/37 experienced combined BM and extramedullary relapse (6 CNS, 1 kidney and 1 gut, respectively). Fifteen children had previously received HSCT, the median time elapsing from HSCT being 218 days (range, 116–1075). Fifteen of the 37 patients (41%) included in the study, had received more than 2 lines of therapy, including clofarabine and blinatumomab, before being enrolled in this study and 7 had refractory disease. The median BM blast percentage in the overall population at time of treatment was 65% (range, 32–95%).

Toxicity

Haematological and non-haematological toxicities are listed in Table II. Infections were the most frequent and severe

Table I. Characteristics of patients enrolled in the study

	N	%	Median	Range
Total	37	100		
Sex				
Male	20	54		
Female	17	46		
Age at diagnosis, years			6·7	0·9–15
Age at study enrolment, years			10·6	2·6–21
Lineage				
B-cell precursor ALL	30	81		
T-cell ALL	7	19		
CNS involvment at diagnosis	4	11		
Genetic abnormalities				
Hypodiploid	2			
STIL	1			
ETV6-RUNX1	2			
t(4;11)	1			
t(9;22)	1			
Previous relapses				
1	15	40		
2	11	30		
≥3	4	11		
Resistant disease	7	19		
Lines of therapy before study enrolment				
1–2 lines	21	57		
>2 lines	16	43		
Previous HSCT				
No	22	60		
Yes	15	40		
Time between last prior treatment attempt and therapy with bortezomib/chemotherapy, months			3	1–25

ALL, acute lymphoblastic leukaemia; CNS, central nervous system; HSCT, haematopoietic stem cell transplantation; *N*, number.

Table II. Toxicity graded according to CTCAE version 4.03 criteria recorded in the study population

	Grade			
Category	3	4	5	Total
Infections	0	3	3	6
Neurological				
Central	1	0	0	1
Peripheral	4	1	0	5
Metabolic				
ALT, AST, bilirubin, GGT	5	0	0	5
Amylase, lipase	5	8	0	13
Hyponatraemia, hypokalaemia, hypocalcaemia	4	3	0	7
Haematological				
Neutrophil	17	13	0	30
Platelet	16	13	0	29
Coagulation				
Fibrinogen decreased	23	0	0	23
Gastrointestinal				
Nausea, vomiting	0	0	0	0

ALT, alanine transaminase; AST, aspartate transaminase; CTCAE, Common Terminology Criteria for Adverse Events; GGT, gamma-glutamyltransferase.

non-haematological toxicities and, remarkably, occurred mainly between day 15 and 22 after the beginning of the treatment.

In our cohort, three septic deaths associated with multi-system organ failure were recorded; all were due to fungal infections (the pathogens demonstrated in blood culture samples were: *Dipodascus capitatus*, *Geotrichum capitatum* and *Sporopachidermia lactativora*). All those patients had received either high-dose liposomal amphotericin (5 mg/kg/day) or voriconazole and G-CSF to accelerate neutrophil recovery. Notably, the severe fungal sepsis developed during the grade 3–4 neutropenic period, and death occurred at a median time of 30 days (range 17–39) from the start of treatment.

During the fourth week of treatment another two patients developed sepsis due to *Candida parapsilosis* and *Stenotrophomonas maltophilia,* respectively, which resolved under appropriate treatment. Another patient experienced *Klebsiella pneumoniae* sepsis on day 13 of the treatment schedule. Intensive combined antibiotic therapy combined with G-CSF was started, and Day 15 chemotherapy was postponed until neutrophil recovery. The patient successfully overcame the sepsis and achieved CR.

The other most common adverse events were neurological complications. One child transiently developed central ataxia, disorientation, headache and dysarthria, which resolved completely within a few days without sequels. Five patients (14·7%) experienced peripheral motor and sensory neuropathy; only one of them had grade 4 sensory neuropathy. All these children had received all 4 doses of bortezomib before the onset of peripheral neuropathy. Neuropathy completely resolved in all these patients using gabapentin, vitamin B1 and B6 supplementation and adequate neuro-physiotherapy.

Metabolic abnormalities, including hypokalaemia, hyponatraemia and hypocalcaemia, were recorded in 15/37 (40·5%) patients.

Nausea, vomiting, or diarrhoea were adequately controlled/prevented with appropriate therapy and never reached grade 3. Low fibrinogen levels and coagulation abnormalities were common, occurring in 83·7% of patients (31/37), but were never higher than grade 2–3. We did not observe pulmonary toxicity, described after bortezomib use in adults (Miyakoshi *et al*, 2006). Thirteen patients (35%) developed BM aplasia during treatment. The median period of hospitalization was 29 days (range 18–51).

Response and outcome

Twenty-three and 4 out of the 37 patients achieved CR (62%) and CRp (10·8%), respectively, leading to an overall response rate of 72·9%. Fourteen of them (52%) had MRD <0·1%, while the remaining 13 patients had MRD levels of

between 0·15% and 2·2%. Seven children had resistant disease, resulting in a failure rate of 18·9%. Three children died during the chemotherapy-induced BM aplasia phase, before adequate evaluation of response/disease status could be determined. Interestingly, in our cohort, not only patients with BCP ALL had a good outcome (22/30, 73·3% CR rate), but also those with T-cell ALL responded well to this combination of drugs (5/7, 71·4% CR rate). The CR/CRp rate of patients who had or had not received allogeneic HSCT before being enrolled in the study was 60% and 81·8%, respectively, while that of patients treated as third-line therapy was 75%. Patients with refractory disease had a CR/CRp rate of 66%, which did not statistically differ from that of patients treated without a documented refractoriness to the last previous treatment. Twenty-three of these patients had long-lasting CR, enabling 18 of them to receive allogeneic HSCT [10 from a human leucocyte antigen (HLA)-haploidentical parent, 5 from a matched unrelated donor and 3 from an HLA-identical sibling]. With a median follow-up of 364 days (range 45–1089), the 2-year OS and DFS were 31·3% (95% confidence interval, CI, 15·3–48·7, Fig 1A) and 27·7% (95% CI 12·4–45·4, Fig 1B), respectively. Children with T-cell lineage ALL had a 2-year OS [53·6% (95% CI 13·2–82·5)] comparable to that of BCP ALL patients [24·4% (95% CI 8·8–44·1)] (P = not significant, Fig 2). Patients who achieved CR after this course of chemotherapy experienced a better OS compared to those who did not respond (see also Fig 3A for details). Among the 27 responding patients, the OS probability of patients who reached a MRD level below 0·1% was significantly better compared with those who did not, being 68·4% (95% CI 35·9–86·8) vs. 0% (P = 0·01, Fig 3B). In patients responding to treatment, the median time to reach a platelet count >20 × 10^9/l and an ANC >0·5 × 10^9/l were 21 (range, 12–43) and 28 days (range, 14–41), respectively.

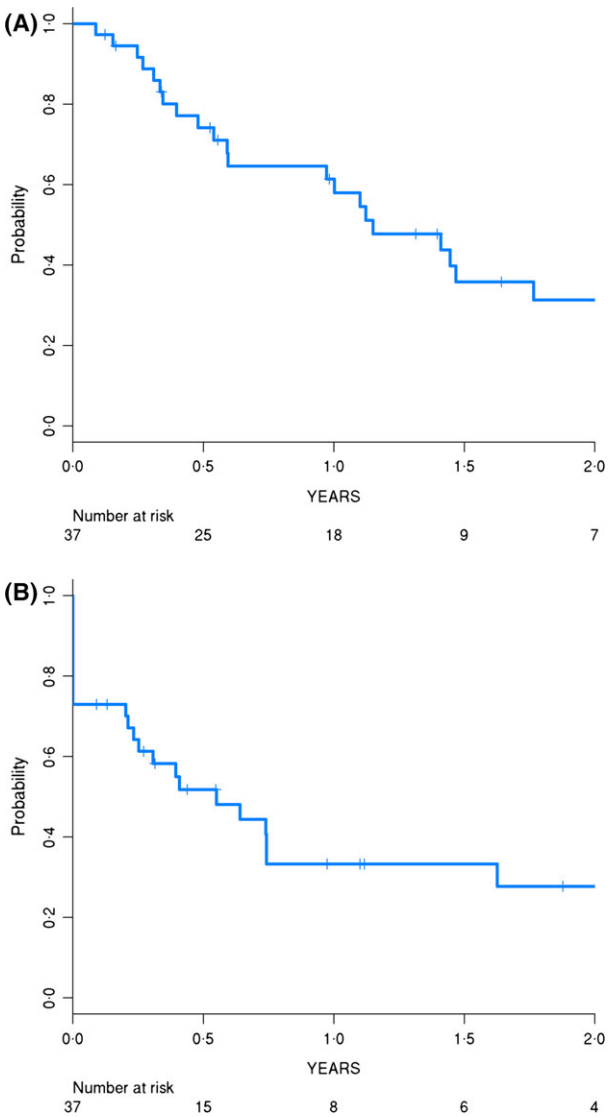

Fig 1. Overall and disease-free survival (DFS) in the whole cohort. (A) 2-year probability of overall survival in the whole cohort of patients. (B) 2-year probability of overall DFS in the whole cohort of patients.

Discussion

This study confirms that, for children with R/R BCP-ALL, the combination of bortezomib with vincristine, dexamethasone, pegylated asparaginase and doxorubicin is associated with acceptable organ toxicity and produces a remarkable overall CR rate, as previously described (Messinger *et al*, 2010, 2012). Interestingly, our data show that this regimen is effective also in T-cell ALL, as 5 out of the 7 patients with T-ALL enrolled in the study reached CR/CRp. Considering the poor prognosis of relapsed T-ALL (Locatelli *et al*, 2012), a response rate of 71·4% for children with T-cell ALL in this group is promising.

The favourable outcome of patients with T-ALL could be explained by the observation that the proteasome inhibitor bortezomib represses the transcription of NOTCH1 and downstream effectors including HES1, GATA3, RUNX3 and nuclear factor-kB (NF-kB) (Espinosa *et al*, 2010; D'Altri *et al*, 2011). Overexpression of the NOTCH1 intracellular domain (NICD) significantly ameliorated bortezomib-induced cytotoxicity against T-ALL cells (Huang *et al*, 2012; Koyama *et al*, 2014). Drug combination studies revealed that bortezomib had a synergistic or additive effect with key drugs for the treatment of T-ALL, such as dexamethasone, doxorubicin and cyclophosphamide, whose cytotoxic effect was readily abolished by NICD overexpression. Notably, the 2-year OS of patients affected by T-ALL was comparable to that of children with BCP-ALL (see also Fig 2B).

In view of the low organ toxicity, the combination of bortezomib and VXLD might also be considered for patients who had previously received allogeneic HSCT and/or monoclonal antibodies, such as blinatumomab (Topp *et al*, 2015). Twelve out of the 16 (75%) BCP-ALL patients enrolled in this study and who had failed blinatumomab responded to

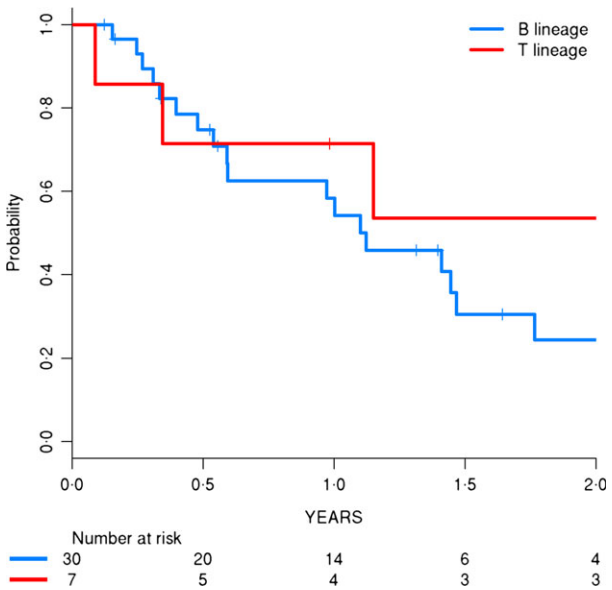

Fig 2. 2-year overall probability of survival in the whole cohort of patients according to the phenotype of acute lymphoblastic leukaemia (ALL) (B-cell precursor –ALL *versus* T-cell ALL).

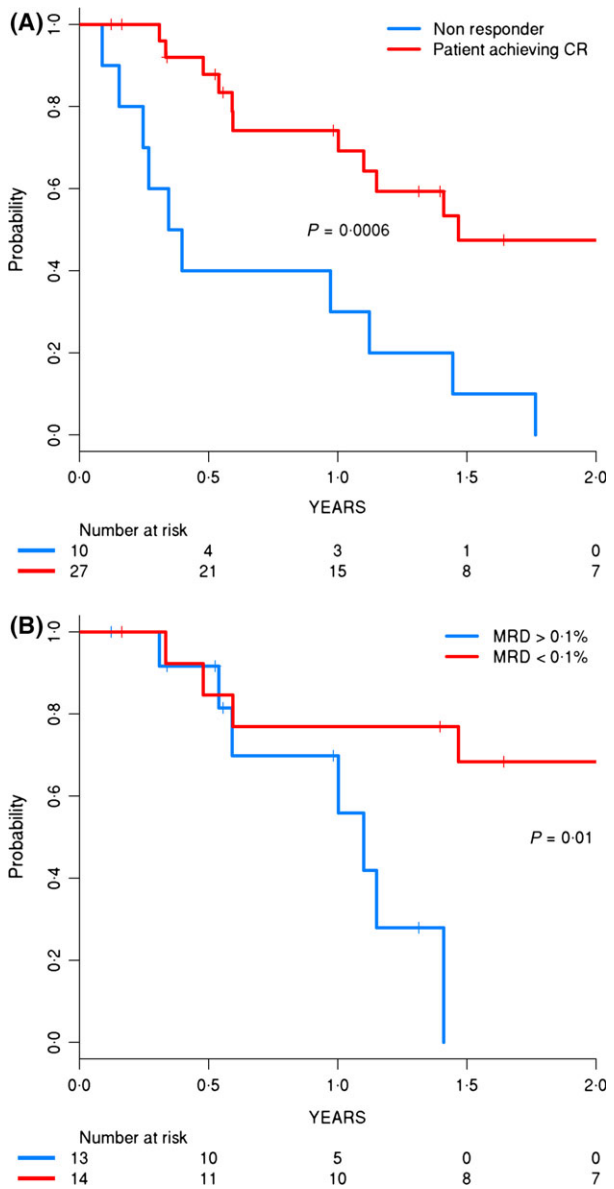

Fig 3. Overall and disease-free survival according to response and minimal residual disease (MRD). (A) 2-year probability of survival according to the achievement of response [i.e. complete response (CR) with or without platelet recovery]. (B) 2-year overall probability of survival in the subgroup of patients who achieved complete response with or without platelet recovery, according to the level of MRD, above or below 0·1%, obtained at the end of treatment.

this combination of drugs, with 5 of them achieving a MRD <0·1%.

Historically, paediatric patients failing 2 or more previous regimens achieved a CR rate of approximately 40% with a variety of multidrug regimens (Gaynon, 2005; Ko *et al*, 2010). In the present study, 43·2% of patients had failed more than 2 previous regimens, including HSCT, as well as novel, effective drugs in ALL, such as clofarabine and blinatumomab; their CR/CRp rate was 75%.

The TACL trials did not measure the MRD of responding patients (Messinger *et al*, 2010, 2012); in our cohort, we found that half of patients who obtained CR/CRp had a MRD level below 0·1%. This finding is noteworthy, considering that a low MRD level is a pre-requisite for benefiting from subsequent allogeneic HSCT (Bader *et al*, 2009). Moreover, in our patients, achievement of MRD response translated into a significantly better probability of 2-year OS (see also Fig 3B).

Infections were a common event, as would be expected in a heavily pre-treated population with profound and long-standing immune- and myelo-suppression. Based on the occurrence of fungal infections observed in the phase I and II studies published by the TACL group (Messinger *et al*, 2010, 2012), we used broad-spectrum antifungal prophylaxis starting from day 1. Despite this prophylaxis and notwithstanding aggressive antifungal treatment started once the infections were diagnosed, 3 patients died of fungal infections. Notably, all fungal sepsis occurred in children who developed chemotherapy-induced BM aplasia. Invasive fungal infections remain an important cause of morbidity and mortality in patients with acute leukaemia (Leventakos *et al*, 2010). The complex scenario in which these infections occur, especially in cases of relapsed or refractory disease, creates diagnostic and treatment challenges (Hale *et al*, 2010). In particular, new strains of yeasts and moulds like those observed in our population are isolated with increased frequency (Brunetti *et al*, 2016; Del Principe *et al*, 2016). We cannot exclude that the prophylaxis with antibiotics that we used may have contributed to these rare fungal infections (Ben-Ami *et al*, 2012). Considering cohort studies published during the past decades, an incidence of fungal infections of about 10% or higher is consistently reported in populations

of patients with AML and R/R ALL (Groll *et al*, 2014). Overall case-fatality rates of invasive fungal disease in the series analysed were between 20% and 70%, with poorest outcomes in patients with disseminated disease, CNS involvement or persistent neutropenia (Dvorak *et al*, 2012; Groll *et al*, 2014).

As previously described (Richardson *et al*, 2009; Messinger *et al*, 2012) and expected, considering that the scheme of treatment includes 2 drugs with known neurotoxicity (vincristine and bortezomib), the other major toxicity was neurotoxicity. Although both sensor and motor neuropathy were transient and resolved under appropriate treatment in our children, we recommend strict monitoring of patients given vincristine with bortezomib, especially when, in addition, they receive voriconazole for mould-active prophylaxis of fungal infections.

Bone marrow aplasia was not seen in both the phase I and II studies conducted by Messinger *et al* (2010, 2012). Here, we report that this regimen leads to a non-negligible rate of BM aplasia. Indeed, in our cohort, 13/37 patients had chemotherapy-induced aplasia. In our study, the median time of platelet and ANC recovery was 21 and 28 days, respectively. Such a delay in haematological recovery is not surprising for heavily pre-treated patients. In light of the number of severe infections occurring during the neutropenic period, the use of G-CSF to hasten neutrophil recovery could be considered, especially in febrile patients.

In conclusion, our study indicates that a standard dose of bortezomib 1·3 mg/m^2 given on days 1, 4, 8 and 11 can be safely combined with an intensive 4-drug re-induction regimen in children with R/R ALL, of both BCP and T-cell lineage. This study also supports further evaluation of bortezomib in children with R/R-ALL, including those experiencing first relapse, or even in newly diagnosed patients at high risk of treatment failure due to either high level of MRD or poor-risk cytogenetics. Considering the high risk of life-threatening/fatal infections in such a vulnerable population, accurate microbiological monitoring and aggressive antibacterial and antifungal prophylaxis/treatment are recommended.

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Authorship

F.L. designed the study; A.B. and F.L. wrote the paper, analysed and interpreted data; P.M. provided statistical analysis; V.B. performed immunophenotyping analysis. A.B., L.V., S.G., L.S., R.C., V.C., K.G., M.T.R. and M.G.C. treated the patients. A.B., S.G., C.G., M.A., V.T. collected the data, and helped with data analysis.

Conflict-of-interest disclosure

The authors do not have any potential conflict of interest to disclose.

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