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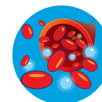

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RESEARCH

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Low expression of T-cell transcription factor *BCL11b* predicts inferior survival in adult standard risk T-cell acute lymphoblastic leukemia patients

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Abstract

Background: Risk stratification, detection of minimal residual disease (MRD), and implementation of novel therapeutic agents have improved outcome in acute lymphoblastic leukemia (ALL), but survival of adult patients with T-cell acute lymphoblastic leukemia (T-ALL) remains unsatisfactory. Thus, novel molecular insights and therapeutic approaches are urgently needed.

Methods: We studied the impact of *B-cell CLL/lymphoma 11b (BCL11b)*, a key regulator in normal T-cell development, in T-ALL patients enrolled into the German Multicenter Acute Lymphoblastic Leukemia Study Group trials (GMALL; n = 169). The mutational status (exon 4) of *BCL11b* was analyzed by Sanger sequencing and mRNA expression levels were determined by quantitative real-time PCR. In addition gene expression profiles generated on the Human Genome U133 Plus 2.0 Array (affymetrix) were used to investigate *BCL11b* low and high expressing T-ALL patients.

Results: We demonstrate that *BCL11b* is aberrantly expressed in T-ALL and gene expression profiles reveal an association of low *BCL11b* expression with up-regulation of immature markers. T-ALL patients characterized by low *BCL11b* expression exhibit an adverse prognosis [5-year overall survival (OS): low 35% (n = 40) vs. high 53% (n = 129), P = 0.02]. Within the standard risk group of thymic T-ALL (n = 102), low *BCL11b* expression identified patients with an unexpected poor outcome compared to those with high expression (5-year OS: 20%, n = 18 versus 62%, n = 84, P < 0.01). In addition, sequencing of exon 4 revealed a high mutation rate (14%) of *BCL11b*.

Conclusions: In summary, our data of a large adult T-ALL patient cohort show that low *BCL11b* expression was associated with poor prognosis; particularly in the standard risk group of thymic T-ALL. These findings can be utilized for improved risk prediction in a significant proportion of adult T-ALL patients, which carry a high risk of standard therapy failure despite a favorable immunophenotype.

Keywords: Adult T-cell acute lymphoblastic leukemia, BCL11b, Outcome, Standard risk, Expression, Mutation

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Background

Improved treatment strategies, integrating risk stratification and minimal residual disease (MRD) monitoring, have improved survival of adult patients with acute lymphoblastic leukemia (ALL) over the last decades [1,2]. Nevertheless, overall survival (OS) remains unsatisfactory with about 40-70%, depending on protocol and age group. Thus far, novel therapy approaches have mainly been developed in B-lineage ALL, where new targeted therapies with monoclonal antibodies like Rituximab or small molecule tyrosine kinase inhibitors (TKI) such as imatinib for Philadelphia chromosome/BCR-ABL-positive patients have been established [3-6]. In T-cell acute lymphoblastic leukemia (T-ALL), less progress has been made despite the introduction of nelarabine in relapsed and refractory disease [7,8]. Other molecular driven approaches, including γ-secretase inhibitors, have until now been less successful [9].

In the German Multicenter Study Group for Adult ALL (GMALL), immunologic subtypes are routinely used as prognostic factors for the risk stratification of T-ALL. Within the high risk group of early T-ALL patients the recently identified subgroup of Early T-cell precursor (ETP-) ALL has been characterized by an immature immunophenotype with a high rate of *FLT3*-mutations, suggesting a potential role for TKI in the treatment for these high risk patients [10-12]. In contrast, T-ALL patients with a thymic phenotype have a more favorable prognosis [1] and are therefore classified as standard risk. Nevertheless, this large group (56% of adult T-ALL) [3] of standard risk T-ALL contains a molecularly and clinically heterogeneous group of patients. Thus, novel insights into the molecular stratification will further aid in refining treatment options.

The *B-cell CLL/lymphoma 11b* (*BCL11b*) gene, a Krüppel-like C_2H_2 zinkfinger transcription factor located on chromosome 14q32.2, is a key player in physiologic T-cell development with potential impact on T-ALL leukemogenesis. In normal hematopoiesis, the onset of *BCL11b* expression in T-cell progenitors occurs during the onset of DN2 phase and is maintained throughout subsequent differential stages (Figure 1) [13,14]. For several target genes BCL11b acts as repressor (p21, p57), for others as activator of transcription (IL-2) [15-17]. *In vitro* studies demonstrated that knockdown inhibited proliferation and led to apoptosis in human T-ALL cell lines [18,19] and a chemo-protective effect of *BCL11b* overexpression was also observed [20].

In addition, *BCL11b* is proposed to act as haploinsufficient tumor-suppressor, as its disruption was found to be associated with lymphomagenesis in mice [21,22]. In humans, chromosomal translocations involving the *BCL11b* gene locus were identified in patients with acute myeloid leukemia (AML), T-ALL and T/myeloid acute bilineage leukemia [23-28]. Likewise, deletions and missense mutations, disrupting gene function, were reported in 9 to 16% of pediatric T-ALL patients [29,30]. One study found *BCL11b* more frequently mutated in adult patients compared to children (20% vs. 5.3%) [31], with a lower mutation rate in early immature (3.6%) and a higher rate (12%) in cortical/mature adult T-ALL [32]. Studies on the prognostic impact of *BCL11b* mutations gave diverging results: a small study reported a favorable outcome for adult T-ALL patients with *BCL11b* mutations (n = 4) [32], however, studies in pediatric patients reported no prognostic effect of mutations [29,30].

We hypothesized that deregulated expression of *BCL11b*, which is tightly regulated in normal T-cell differentiation, and *BCL11b* mutations play an important role in T-ALL. Therefore, we analyzed *BCL11b* mRNA expression levels in a large cohort of adult T-ALL patients and screened for mutations in the zinc finger region.

Results

BCL11b is heterogeneously expressed in adult T-ALL

BCL11b mRNA expression levels were not detectable in CD34 positive hematopoietic progenitor cells or unselected bone marrow (BM) samples from healthy donors, whereas high expression levels were found in CD3 positive mature T-cells (median: 2.5, Figure 2). In contrast, diagnostic BM samples of adult T-ALL patients (n = 195) showed an aberrant and highly heterogeneously *BCL11b* expression pattern (median: 0.5 range = 0-12.3; Figure 2).

As explorative approach, we divided the samples into *BCL11b*-low and *BCL11b*-high expression groups by quartiles. Expression levels in the lowest quartile Q1 (≤0.2) were regarded as *BCL11b*-low, and samples with levels in quartiles Q2-4 (>0.2) as *BCL11b*-high.

BCL11b associated global gene expression profile

To explore the underlying transcriptional profile associated with aberrant *BCL11b* expression in T-ALL, we analyzed microarray expression data of an independent cohort of 86 adult T-ALL patients [33]. Samples were classified into a low and a high *BCL11b* expression group as described in the material and methods section. We identified 229 up-regulated probe sets (corresponding to 183 unique genes, hypothetical genes/proteins and open reading frames) and 200 down-regulated probe sets (corresponding to 166 genes, hypothetical genes/proteins and open reading frames) in the *BCL11b*-low group compared to the *BCL11b*-high group (Figure 3A, Additional file 1: Table S2 and Additional file 1: Table S3). In the *BCL11b*-low group, genes reported to be suppressed by *BCL11b* were highly expressed: cyclin-dependent kinase inhibitor 1A (p21) and cyclin-dependent kinase inhibitor 1C (p57) [15,17]. Interestingly, genes associated with an immature stem cell-like phenotype were up-regulated in the *BCL11b*-low cohort including *IGFBP7*,

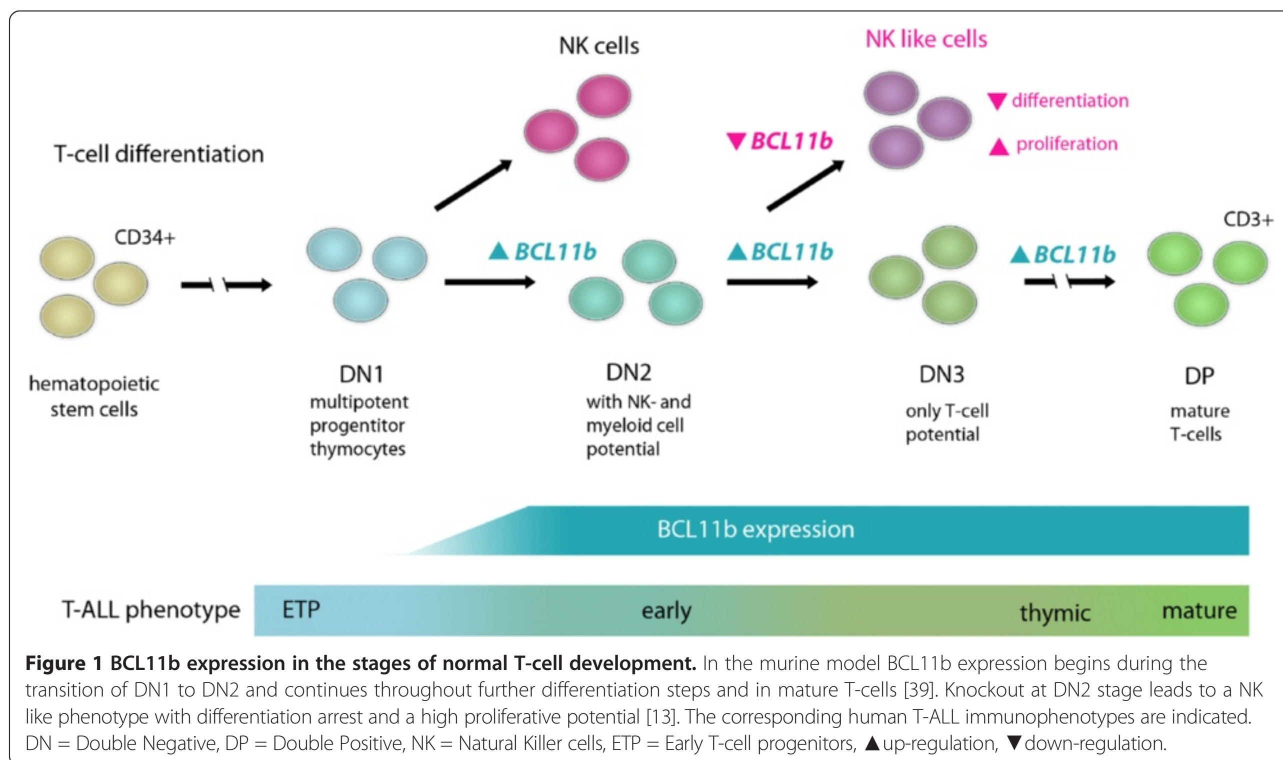

Figure 1 BCL11b expression in the stages of normal T-cell development. In the murine model BCL11b expression begins during the transition of DN1 to DN2 and continues throughout further differentiation steps and in mature T-cells [39]. Knockout at DN2 stage leads to a NK like phenotype with differentiation arrest and a high proliferative potential [13]. The corresponding human T-ALL immunophenotypes are indicated. DN = Double Negative, DP = Double Positive, NK = Natural Killer cells, ETP = Early T-cell progenitors, ▲up-regulation, ▼down-regulation.

BAALC, CD34, and *FLT3* [34-36]. In contrast, the *BCL11b*-high group showed co-expression of markers of a mature T-cell phenotype including several T-cell receptor genes as well as CD8 and CD6. This was further underscored in gene set enrichment analysis (GSEA), in which gene sets associated with physiological T-cell development were enriched in *BCL11b*-high (P < 0.01) and genes down regulated in normal T-cells were enriched in *BCL11b*-low group (P = 0.01; Figure 3B) [37].

BCL11b expression with respect to molecular and clinical characteristics

We further explored molecular characteristics associated with *BCL11b* expression in the T-ALL GMALL patient cohort by quantitative RT-PCR. Samples in the lowest expression quartile (Q1; n = 49; *BCL11b* expression range = 0-0.2) were compared to samples with aberrantly high *BCL11b* expression levels defined as Q2-4 (n = 146; *BCL11b* expression range >0.2-12.3, Additional file 1: Table S4). In concordance with the gene expression profiles (GEP) data, these molecular studies by RT-PCR confirmed *IGFBP7* to be overexpressed in the *BCL11b*-low compared to the *BCL11b*-high group. No significant difference between *BCL11b*-low and *BCL11b*-high patients was observed for the previously described negative prognostic factors including *BAALC, ERG*, and *WT1* [34,38]. *GATA3*, a transcription factor up-regulated in DN1 phase of normal T-cell development [39] was significantly lower expressed in *BCL11b*-low (median: 2.1 vs. 5.7, P < 0.01) compared to *BCL11b*-high patients. The frequency of TCR rearrangements was significantly lower (50% vs. 80%, P = 0.01) in *BCL11b*-low compared to the *BCL11b*-high group. The *BCL11b* low and the high expressing groups did not differ regarding *NOTCH1* or *WT1* mutation frequencies (Additional file 1: Table S4).

There was no difference with respect to age or sex of T-ALL patients enrolled on the GMALL 06/99 and 07/03 trials, but within the *BCL11b*-low group significantly more patients had a low white blood cell count (WBC; 62% vs. 18% <30.000 × 10^9/l WBC; P = 0.01; Table 1). Patients with early T-ALL showed a significantly lower *BCL11b* expression (median: 0.3) compared to patients with mature (median: 0.6, P = 0.03) or thymic (median: 0.6, P = 0.01) T-ALL (Additional file 1: Figure S1).

BCL11b expression is associated with outcome in adult T-ALL

While there was no difference between *BCL11b*-low and *BCL11b*-high within the overall GMALL cohort of T-ALL patients with respect to the response to induction therapy (Table 1), the overall survival probability of *BCL11b*-low patients was significantly lower compared to *BCL11b*-high patients (Q1: n = 40, 35% at 5 yrs; Q2-4: n = 129, 53% at 5 yrs; P = 0.02; Figure 4A). When outcomes were analyzed separately for each quartile, T-ALL patients with *BCL11b*-low (Q1) showed an inferior outcome (Q1: n = 40; 35% OS at 5 yrs) compared to patients in the remaining quartile groups (Q2: n = 42; 52% OS at 5 yrs; Q3: n = 42;

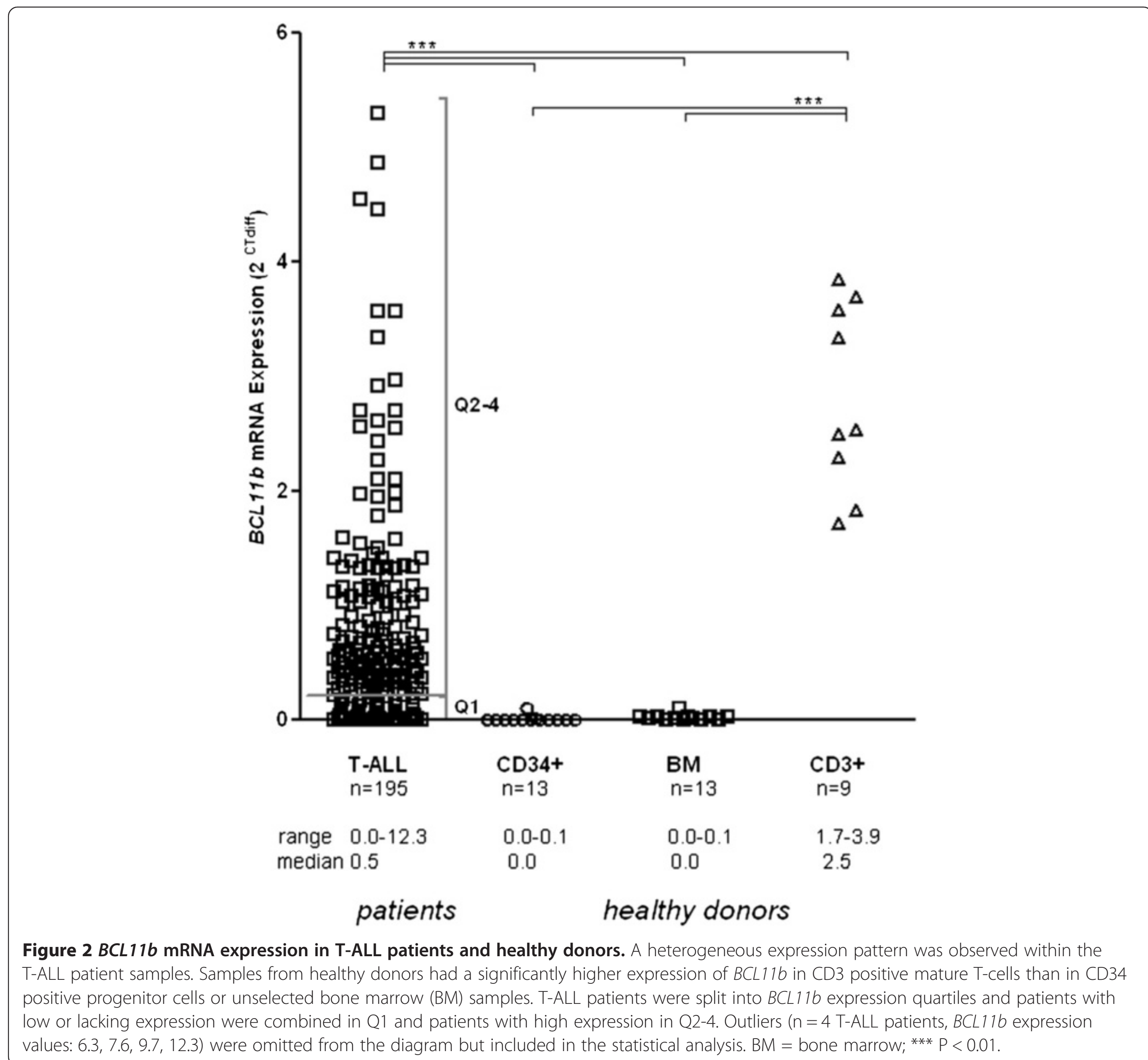

Figure 2 ***BCL11b*** **mRNA expression in T-ALL patients and healthy donors.** A heterogeneous expression pattern was observed within the T-ALL patient samples. Samples from healthy donors had a significantly higher expression of *BCL11b* in CD3 positive mature T-cells than in CD34 positive progenitor cells or unselected bone marrow (BM) samples. T-ALL patients were split into *BCL11b* expression quartiles and patients with low or lacking expression were combined in Q1 and patients with high expression in Q2-4. Outliers (n = 4 T-ALL patients, *BCL11b* expression values: 6.3, 7.6, 9.7, 12.3) were omitted from the diagram but included in the statistical analysis. BM = bone marrow; *** P < 0.01.

52% OS at 5 yrs; Q4: n = 45; 55% OS at 5 yrs; Additional file 1: Figure S2A).

Low *BCL11b* defines high risk patients within the standard risk group of thymic T-ALL

The identification of novel prognostic markers is in particular important for the largest subgroup of standard risk thymic T-ALL. As these patients are regarded as standard risk, allogeneic stem cell transplantation is not recommended in first complete remission within GMALL trials. In thymic T-ALL, *BCL11b* expression groups did not differ in the expression levels of molecular markers including *BAALC*, *ERG*, *IGFBP7* and *WT1*. The *BCL11b*-low group showed significantly lower expression of T-cell regulator *GATA3* compared to *BCL11b*-high (median Q1: 1.6, median Q2-4: 5.1, P < 0.01). Significantly fewer thymic *BCL11b*-low patients remained in continuous complete remission at five years compared to *BCL11b*-high thymic T-ALL patients (at 5 yrs: Q1: n = 15, 38%; Q2-4: n = 78, 72%; P = 0.02; Additional file 1: Figure S2B). Moreover, *BCL11b*-low thymic T-ALL had a significantly inferior OS compared to *BCL11b*-high thymic T-ALL patients: while only 20% (n = 18) of *BCL11b*-low patients were alive at 5 years, the 5 year OS of the *BCL11b*-high group was 62% (n = 84; P < 0.01, Figure 4B). Similar to the entire cohort, patients with thymic T-ALL *BCL11b*-low (Q1) showed a significantly inferior outcome compared to higher expression quartiles (Q1: n = 18, 20% at 5 yrs; Q2 n = 26, 67% at 5 yrs¸ Q3 n = 18, 50% at 5 yrs; Q4 n = 30, 70% at 5 yrs; P = 0.01; Additional file 1: Figure S2C).

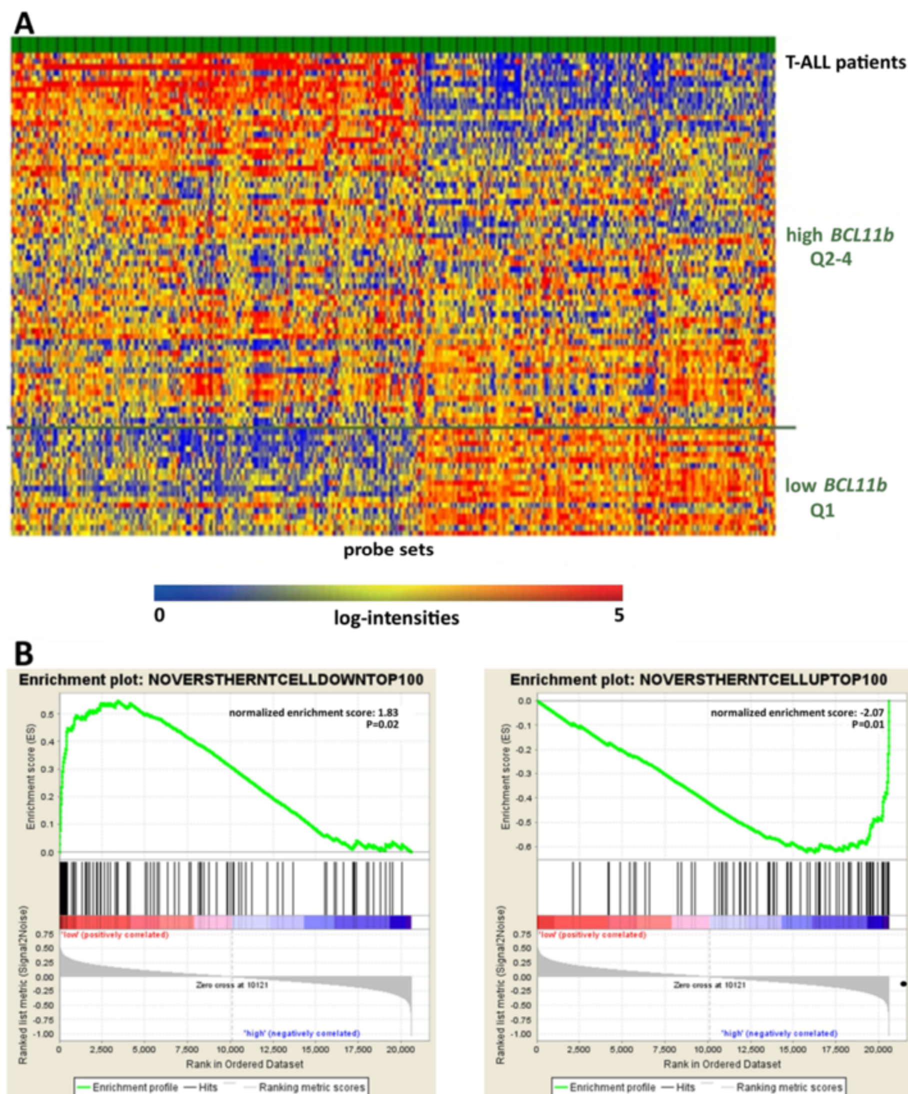

Figure 3 Gene expression profiles reveal an immature phenotype in *BCL11b-low* group. A: Heat map of two-fold or greater differentially expressed genes between *BCL11b* quartile 1 (Q1) and quartiles 2-4 (Q2-4). **B**: Gene set enrichment analysis for differentially regulated genes in physiological T-cell development according to their *BCL11b* expression. On the left side the top hundred down-regulated genes are shown, on the right side the top hundred up-regulated genes. The lists were taken from Noversthern et al. [37].

High frequency of *BCL11b* mutations in adult T-ALL

We sequenced *BCL11b* exon 4 and identified in 14% (24/178) of the T-ALL patients protein modifying alterations (Figure 5, Additional file 1: Table S6). A single T-ALL patient carried two mutations. Sixteen of the mutations were point mutations with single base pair exchanges leading to amino acid exchanges in 13 and a translation stop in three cases. Seven patients carried deletions and two insertions causing frame shifts. 23 of 24 mutations were either located within zinc finger domains or had an impact on these domains through frame shift or stop of translation, and thus have a likely impact on protein function. Nearly all patients with *BCL11b* mutations were in the *BCL11b*-high group (n = 22/23, P = 0.01). The presence of *BCL11b* mutations was associated with the maturation stage of the T-ALL. Of the 21 patients with *BCL11b* mutations, 18 were classified as thymic T-ALL (85.5%; P = 0.03; Additional file 1: Table S7). *WT1*, a negative prognostic factor, was significantly lower expressed in the *BCL11b* mutated group and other known oncogenes or molecular factors were not associated with *BCL11b* mutation status (not shown). However, outcome analyses showed no significant impact of *BCL11b* mutations and deletions on overall survival (Additional file 1: Figure S3).

Discussion

During normal T-cell development, T-cell progenitors pass through separate differentiation stages and this process is

Table 1 Clinical characteristics of GMALL T-ALL patients with respect to *BCL11b* expression

Characteristics	*BCL11b*-low	*BCL11b*-high	
	Q1	Q2-4	P-value
n	40	129	
BCL11b expression			
Median	0.1	0.8	
Range	0-0.2	0.2-12.3	
Age			n.s.
15-35 yrs	27	70	
36-55 yrs	13	48	
56-65 yrs	0	11	
Sex			n.s.
Female %	20%	26%	
WBC, $\times 10^9$/l			0.01
<30,000	24	42	
30-100,000	10	53	
>100,000	5	30	
T-ALL subtype			0.01
Early n (%)	17 (42%)	23 (18%)	
Mature n (%)	5 (13%)	22 (17%)	
Thymic n (%)	18 (45%)	84 (65%)	
Response to induction therapy			n.s.
CR n (%)	37 (95%)	115 (94%)	
ED n (%)	1 (3%)	3 (2%)	
Failure n (%)	1 (3%)	5 (4%)	

Abbreviations: *CR* = complete remission, *ED* = early death, *WBC* = white blood cell count.

tightly regulated by lineage specific transcription factors. While onset of *GATA3* and *TCF-1* expression characterize the early DN1 (ETP) phase in T-cell differentiation, *BCL11b* is expressed at the later DN2 stage [39]. As a gatekeeper of T-cell development, it is essential for the correct αβ T-cell development [3,40]. Loss of *BCL11b* at various stages was shown to induce a natural killer cell-like phenotype with a differentiation arrest and was associated with a high proliferative potential [14,40]. In addition, *BCL11b* is necessary for T-cell identity maintenance. Alteration of different stage-specific transcription factors and key regulators of T-cell differentiation by silencing, ectopic expression or mutations have shown to contribute in oncogenic transformation in T-ALL. For example, aberrant *NOTCH1* signaling through activating mutations found in 50-60% of T-ALL cases [41,42], is a prominent example of a potent driver event in T-cell leukemogenesis. For *TAL1*, which plays a key role in hematopoietic stem cell development, rearrangements and aberrant expression were found in T-ALL patients [43]. In this case, *TAL1* expression was positively correlated with survival in pediatric patients [44]. Likewise for the homeobox transcription factor *TXL-1* deregulation and gene locus abnormalities had been associated with improved outcome for *TXL*-1-high T-ALL patients [45]. Understanding of the molecular processes involved in T-ALL pathology offers the possibility to refine prognosis and stratify therapeutic algorithms.

For *BCL11b* aberrant expression levels, deletions and mutations have been reported in T-ALL [29-32,46,47]. Here we comprehensively investigated the implications of altered *BCL11b* expression and loss of function mutations in a large cohort of adult T-ALL patients (n = 169). While $CD34^+$ hematopoietic progenitor cells and unselected BM samples of healthy donors lack *BCL11b* expression, T-ALL patients showed an aberrant and highly heterogonous *BCL11b* expression pattern. Similar to normal T-cell differentiation, the expression of *BCL11b* reflected the maturation stage in T-ALL and thus was significantly lower in the early compared to thymic and mature T-ALL subgroups (Figure 1, Additional file 1: Figure S1). Analysis of microarray expression data confirmed this observation revealing up-regulation of genes associated with an immature phenotype in *BCL11b*-low and an enrichment of markers of a mature T-cell phenotype in *BCL11b*-high T-ALL.

While the combined patient data suggested that *BCL11b* expression reflects the differentiation arrest of leukemic cells, expression was heterogeneously distributed and patients that lacked or had very low *BCL11b* expression were found in all immunophenotypic subgroups. This suggests that *BCL11b* is not just a mere marker of genetically more differentiated blasts, but may act as a maturation dependent tumor suppressor, which is supported in other studies [29,30,47]. If deregulated during differentiation, disruption of normal *BCL11b* function may contribute to malignant transformation.

In this study the cohort of uniformly treated adult T-ALL patients, the *BCL11b*-low subgroup had a significantly inferior OS compared to the *BCL11b*-high patients. In particular, in the standard risk group of thymic T-ALL, *BCL11b* was of relevant prognostic impact: 62% of *BCL11b*-high patients were alive after 5 years, whereas survival was only 20% at 5 years in the *BCL11b*-low subgroup. Remission duration was also significantly shorter for patients within the *BCL11b*-low subgroup. This contrasts a study in pediatric T-ALL patients, which showed that *BCL11b* expression had no impact on OS [30], although the difference may be due to the smaller sample size of the study and that patients were not classified into immunophenotypic subgroups. Nevertheless, low *BCL11b* expression was associated with chemotherapy induction failure in the same study.

While immunophenotypic classification of T-ALL has improved outcome prediction, a relevant percentage of patients classified as standard risk based on their thymic

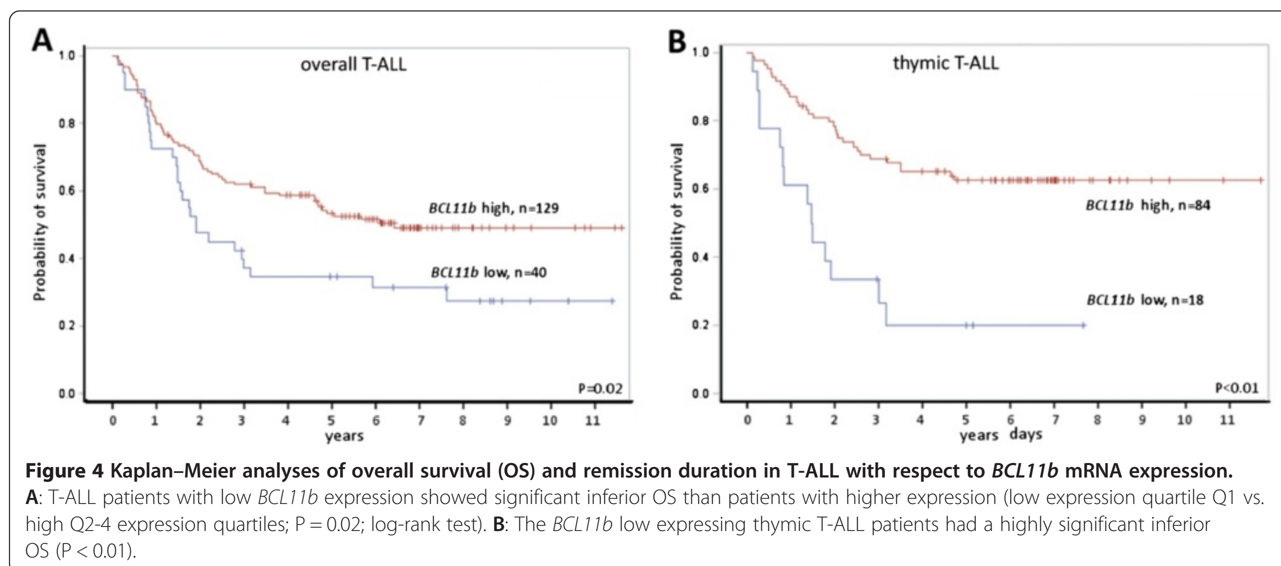

Figure 4 Kaplan–Meier analyses of overall survival (OS) and remission duration in T-ALL with respect to *BCL11b* mRNA expression. **A**: T-ALL patients with low *BCL11b* expression showed significant inferior OS than patients with higher expression (low expression quartile Q1 vs. high Q2-4 expression quartiles; P = 0.02; log-rank test). **B**: The *BCL11b* low expressing thymic T-ALL patients had a highly significant inferior OS (P < 0.01).

immunophenotype, fail conventional chemotherapy. As thymic T-ALL on the molecular and clinical levels compromises a highly heterogeneous cohort, it remains essential to identify patients with a high risk of relapse to adjust treatment stratification. Our results indicate that thymic *BCL11b*-low T-ALL patients represent a high risk subgroup which would benefit from intensified MRD monitoring and treatment stratification including allogeneic stem cell transplantation. Since lack of *BCL11b* expression proved to indicate inferior survival, we investigated disruption of the gene's function on the mutational level. *BCL11b* mutations in pediatric and adult patients had been reported in T-ALL in the zinc finger structures of exon 4 [29,30,32,46]. In agreement with these studies, we discovered a high rate (14%) of *BCL11b* mutations in this large cohort of T-ALL patients. We found an association of immunophenotype and mutation frequency: only 2% mutational rate in early T-ALL patients and 19% mutational rate in thymic T-ALL patients. Our results support a recent study in which adult T-ALL patients characterized as "early/immature" had fewer *BCL11b* mutations [32]. The number of *BCL11b*

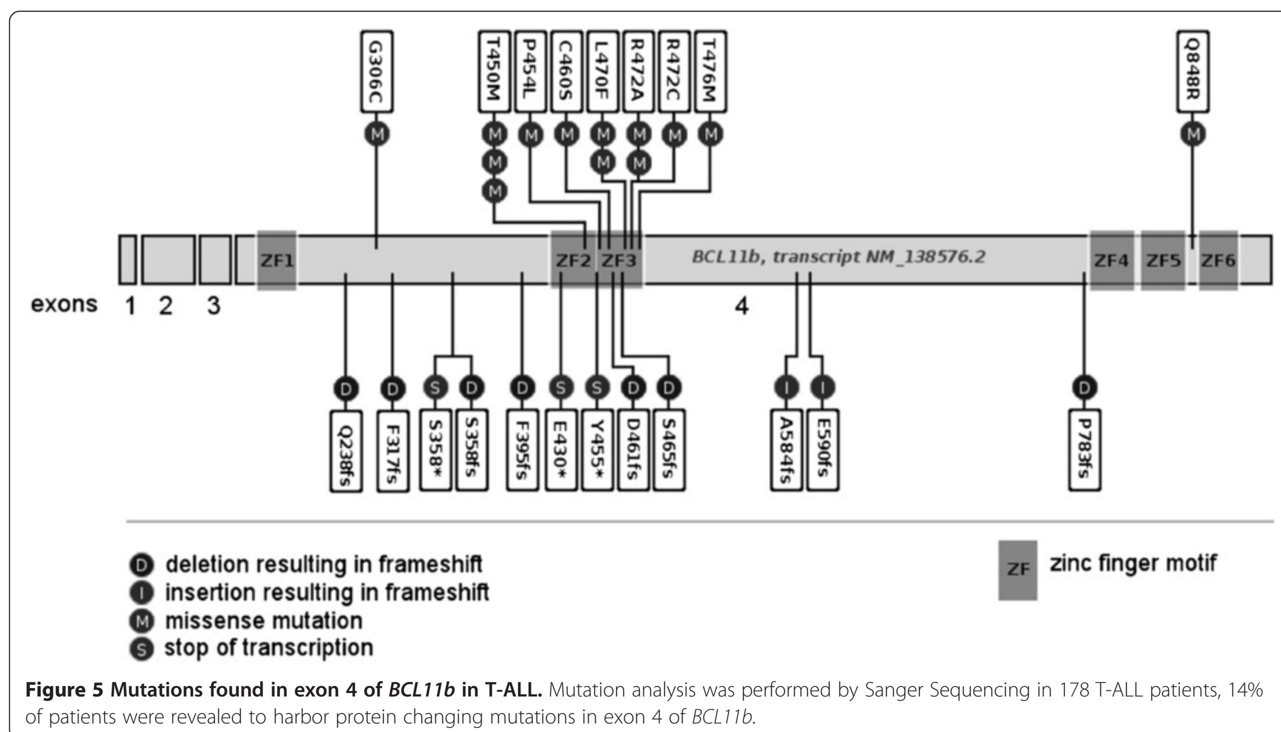

Figure 5 Mutations found in exon 4 of *BCL11b* in T-ALL. Mutation analysis was performed by Sanger Sequencing in 178 T-ALL patients, 14% of patients were revealed to harbor protein changing mutations in exon 4 of *BCL11b*.

mutations in thymic T-ALL samples in this report was low, limiting the statistical significance regarding outcome. Also, gene expression studies may be more sensitive to identify patients with the specific outcome-associated phenotype caused by *BCL11b* down-regulation.

Further studies are needed to fully understand the biological relevance of *BCL11b* mutations, and to explore potential directed therapies.

In summary, we identified *BCL11b* expression as a potent prognostic factor in the overall cohort and in particular in the standard risk subgroup of thymic T-ALL. These findings will help to identify patients with an enhanced risk of failure to standard therapy, however, standardized detection analyses of aberrant gene expression levels in a routine diagnostic setting remains challenging. More importantly, alterations in critical transcription factors contribute to leukemogenesis and may be regarded as ideal candidates for differentiation directed therapies in the future.

Methods

Patients

We analyzed diagnostic BM material of 195 adult T-ALL patients sent to the GMALL reference laboratory [48]. Immunophenotyping of the samples was centrally performed in the GMALL reference laboratory at the Charité, University Hospital Berlin, Germany, as previously described [49,50], and classified into the T-ALL subgroups: early (n = 50), mature (n = 33) and thymic (n = 112). Of these, 169 T-ALL patients were consecutively enrolled in the GMALL trials 06/99 and 07/2003 [51] with available clinical follow-up data. Additionally, samples of healthy adult donors were used, 13 BM samples, 13 CD34+ progenitors and nine CD3+ selected T-cell samples. Written informed consent according to the declaration of Helsinki had been given. Studies were approved by the ethics board and registered in a public registry (clincaltrials.gov NCT00199056, NCT0098991).

Sample preparation and qRT-PCR

Total RNA and DNA extractions were performed using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. To analyze *BCL11b* expression, complementary DNA was synthesized and quantitative real-time polymerase chain reaction (qRT-PCR) was performed, using *Glucose-6-Phosphate Isomerase* (*GPI*) as internal control, as previously described [52]. For *BCL11b* amplification forward primer AACCCGCAGCACTTGTCC, reverse-primer ATTTGACACTGGCCACAGGT and probe FAM-CTCATCACCCCAGAGGCTGACCAT-BHQ1 spanning exons 1 and 2 were used. Expression levels of *BCL11b* were calculated using the mean of ΔCT from two replicates and expressed as $2^{\mu(\Delta CT)}$. The mRNA expression levels for *IGFBP7*, *WT1*, *BAALC*, *ERG*, and *GATA3* by qRT-PCR, as well as mutation status of *WT1* and *NOTCH1* and TCR-rearrangements had been determined in previous studies [12,34,35,52-54].

Gene expression profiles

BCL11b-associated GEP of an independent set of 86 adult T-ALL samples were generated from raw data obtained from the Microarrays Innovations in Leukemia (MILE) multicenter study (HG-U133 Plus 2.0; Affymetrix, Santa Clara, CA, USA) [33]. For the GEP-analysis, samples were divided into quartiles (Q) according to *BCL11b* expression [median of the two probe sets (219528_s_at, 222895_s_at)]. To identify *BCL11b*-associated GEP signatures, the lowest expression quartile (Q1) was compared to quartiles 2 to 4 united (Q2-4). Lists of genes with a 2-fold under- or over-expression were generated. Statistical significance was calculated by the non-parametric *t*-test with a *P*-value ≤0.05. The data analyses were carried out with GeneSpring software version 4.2 (Silicon Genetics, Redwood City, CA, USA).

Mutational analysis

Quantity and quality of genomic DNA was sufficient for the mutational analysis of *BCL11b* in 178 T-ALL samples. As previous studies had detected only very few mutations outside exon 4, which harbors all six of the gene's zinc finger domains, we focused on this region [29,30]. Primer pairs were newly designed or used as previously published for bidirectional Sanger sequencing of exon 4 (complete list see Additional file 1: Table S1) [29]. Geneious version 5.4.3 software (Biomatters Ltd., Auckland, NZ) was used for analysis.

Additional file

Additional file 1: Statistical analysis. Figure S1. BCL11b mRNA expression in T-ALL immunophenotypic subtypes. **Figure S2.** Kaplan–Meier analysis of overall survival (OS) in T-ALL with respect to BCL11b mRNA expression. **Figure S3.** Kaplan–Meier analysis of overall survival (OS) in T-ALL with respect to BCL11b mutation status. **Table S1.** Primer sets designed for human BCL11b exon 4 (NP_612808.1). **Table S2.** Probe sets up-regulated in the BCL11b-low group of T-ALL patients. **Table S3.** Probe sets up-regulated in the BCL11b-high group of T-ALL patients. **Table S4.** Molecular characteristics of T-ALL patients with respect to BCL11b expression. **Table S5.** Molecular characteristics of thymic GMALL T-ALL patients in respect to BCL11b expression. **Table S6.** BCL11b exon 4 mutations in T-ALL. **Table S7.** Clinical characteristics of GMALL T-ALL patients in respect to BCL11b mutations. **Table S8.** Members of the German Multicenter Study Group for Adult ALL Supplemental Methods: Statistics. References.

Abbreviations

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BM: Bone marrow; DN: Double negative; ETP-ALL: Early t-cell precursor ALL; GEP: Gene expression profile; GSEA: Gene set enrichment analysis; GMALL: German multicenter acute lymphoblastic leukemia study group; MILE: Microarrays innovations in leukemia; MRD: Minimal residual disease; OS: Overall survival; qRT-PCR: Quantitative real-time polymerase chain reaction; T-ALL: T-cell acute lymphoblastic leukemia; TKI: Tyrosine kinase inhibitors.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
IB performed laboratory work, data analysis and wrote the manuscript. NG supervised the GMALL study center, performed statistical analysis and reviewed the manuscript. CS performed laboratory work for this study. SH helped to design the study. LF provided expression data. SS, RS, KSE, MS, AR and DH recruited the study patients and performed the study procedures. CDB coordinate the research and reviewed the manuscript. MN performed statistical analysis and reviewed the manuscript. All authors read and approved the final manuscript.

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