# The oncogenetic landscape and clinical impact of *BCL11B* alterations in adult and pediatric T-cell acute lymphoblastic leukemia

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive and heterogenous hematological cancer representing 15% of pediatric and 25% of adult ALL.[1] It arises from the transformation of T-cell precursors arrested at specific stages of maturation. T-ALL are associated with a multitude of acquired genetic abnormalities that contribute to this developmental arrest and exacerbated proliferation, including the loss of major tumor suppressive pathways such as inactivating alterations of *PTEN* and *CDKN2A/B* and activation of oncogenic pathways (e.g., activating mutations in *NOTCH1, IL7R/JAK/STAT*).[2]

Most patients respond well to polychemotherapy, but at the cost of acute adverse effect and sequelae notably in the case of allogeneic stem cell transplantation (allo HSCT). Despite this initial favorable response, about 20-30% of pediatric[3] and 40% of adult T-ALL relapse, with 5-year overall survival (OS) rates below 20%.[4] Hence, identifying prognostic markers at diagnosis is a critical medical need to refine the treatment protocols and improve the patients' outcomes.

*BCL11B* belongs to the Kruppel-like C2H2 type zinc finger transcription factor family that contains six C2H2 zinc fingers and proline-rich and acidic regions with 95% identity in their zinc finger domains. It encodes two different protein isoforms consisting of 823 and 894 aa in humans. These structures include DNA-binding and protein-interacting regions. BCL11B is critical in the development and maintenance of T-cell identity. It was initially discovered as a potential suppressor of radiation-induced T-cell lymphoma.[5] The role of BCL11B in leukemogenesis was first pointed out through its cryptic translocation with *TLX3*, positioning this oncogene under the control of *BCL11B* regulatory elements.[6] However, monoallelic *BCL11B* deletions or missense mutations were reported in 9% of T-ALL cases, showing that *BCL11B* is a haplo-insufficient tumor suppressor that could collaborate with additional oncogenic lesions during thymocyte development.[7] Nevertheless, the incidence and clinical data on *BCL11B* alterations in large cohorts of patients are still lacking. Hence, we described the mutational landscape and clinical outcome related to BCL11B alterations in two cohorts of adult and pediatric T-ALL patients treated in GRAALL03/05 protocols or according to FRALLE2000T recommendations, respectively.

We took advantage of an extensively annotated cohort of 476 patients included in the GRAALL03/05 French protocol for adults aged up to 60 years (n=215) or treated according to FRALLE2000T recommendations for children (n=261). *BCL11B* alterations were identified in cases of 476 T-ALL cases by gene mutation screening and next-generation sequencing (NGS)-based analysis of copy number variation (CNV) as previously described. Array-comparative genomic hybridization (array-CGH) was also performed for 310 patients. All deletions identified in array-CGH were confirmed by NGS-based analysis of CNV. These alterations were more frequent in adults: 48 cases (22%) in GRAALL03/05 than in children: 38 cases (15%) in FRALLE2000T-treated patients ($P$=0.03) (Figure 1A). *BCL11B* mutations were the most frequent alterations: 73 patients (for a total of 99 mutations) (Figure 1C). *BCL11B* is a zinc finger transcription factor that binds DNA via its Cys2His2 zinc (C2H2 Zn) finger domains. Most mutations were missense (66%) within a mutational hotspot in exon 4 (83 mutations) affecting predominantly amino acids 452, 465, and 472 located in the C2H2 Zn finger domain (Figure 1C). Frameshift or non-sense mutations (34%) in exons 1-4 were also detected and predicted to produce truncated forms of the protein. *BCL11B* deletions were detected in 13 cases (3%) (Figure 1B). Only seven of 13 (54 %) presented focal intragenic deletions. Other cases harbored pan-genic deletions leading to haplo-insufficiency. The distribution of the alterations types was similar between adults and children, with a preponderance of mutations in both cohorts: 38 mutations (18%) and ten deletions (5%) in GRAALL03/05 and 35 mutations (13%) and three deletions (1%) in FRALLE2000T.

The clinical and biological characteristics of the patients according to *BCL11B* status are described in Table 1. Patients carrying BCL11B alterations (*BCL11B*$^{alt}$) are slightly older with a median age at diagnosis of 19.3 years (range, 1.8-57.0) compared to 14.8 years (range, 1.1-59.1) ($P$=0.045), with no difference in the sex ratio (Table 1; *Online Supplementary Tables S1* and *S2*). *BCL11B*$^{alt}$ patients presented with reduced leukocytosis at diagnosis compared to the wild-type (wt) group (median white blood cell [WBC] count: 36.4x10$^9$/L *vs*. 67.6x10$^9$/L; $P$=0.004). No difference was observed in the central nervous system (CNS) involvement rate.

*BCL11B*$^{alt}$ patients had a better good prednisone response (69% *vs*. 53%; $P$=0.008), a higher rate of early chemotherapy response (85% *vs*. 69%; $P$=0.005), and presented less detectable minimal residual disease (MRD) at the end of induction (10% *vs*. 42%; $P$<0.001) as compared to BCL11B$^{wt}$ patients. Similar allo HSCT in the first complete remission were performed in both groups (17% *vs*. 23%).

Phenotypically, *BCL11B*$^{alt}$ T-ALL were less often described as ETP (8% *vs*. 21%; P=0.017) and mostly exhibited a cortical IMβ/Pre-αβ stage (76%) (Table 1; *Online Supplementary Tables S1* and *S2*). Consequently, more *TLX1/TLX3* deregulations (57%) were identified in this group. Conversely, *TAL1* (5%) or *HOXA9* (15%) overexpression were rare events in this subgroup, with no *PICALM::MLLT10* rearrangement detected. While lesions of the NOTCH1 pathway or genetic deletions of *CDKN2A/B* are frequent oncogenetic traits of T-ALL (70% in our cohort), almost all *BCL11B*$^{alt}$ patients harbored these lesions (94% of NOTCH1 signaling alterations, 87% of *CDKN2A/B* deletions) (*Online Supplementary Figure S1*).

Regarding JAK/STAT signaling, the gene mutation profile is more heterogeneous with more *PTPN2* (16% *vs*. 7%) and *DNM2* mutations (29% *vs*. 14%,) but less *JAK3* mutations (8% *vs*. 21%) in *BCL11B*$^{alt}$, as expected due to more TLX1/3 deregulations. Epigenetic regulators have a specific profile with more *KMT2D* (9%) and *PHF6* mutations (46%) and fewer *SUZ12* alterations (5%). No significant difference was observed concerning PI3K signaling between *BCL11B*$^{wt}$ and *BCL11B*$^{alt}$.

In consistency with these results, patients with *BCL11B*$^{alt}$ were fewer and scored a higher risk profile defined by the *NOTCH1/FBXW7/RAS/PTEN* (N/F/R/P) oncogenetic classifier: 28% *versus* 47% (*P*=0.001) (Table 1).

In order to investigate the prognostic value of *BCL11B* alterations, survival analyses were performed in the entire cohort of 476 patients treated according to the GRAALL03/05 protocol and FRALLE2000T recommendations.

Patients with *BCL11B*$^{alt}$ T-ALL have a favorable outcome in

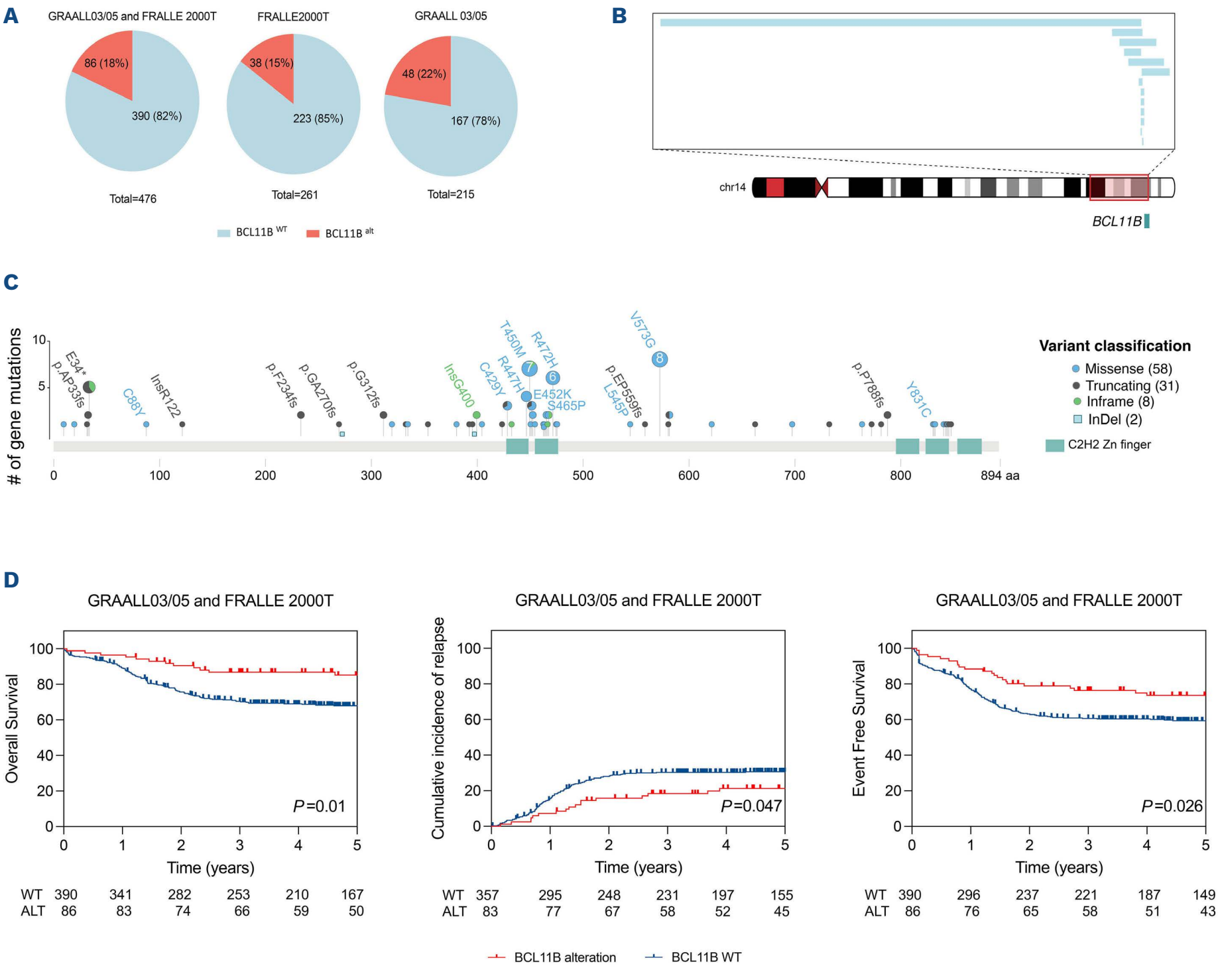


**Figure 1. *BCL11B* alterations and incidence in T-cell acute lymphoblastic leukemia.** (A) Incidence of *BCL11B* alterations in GRAALL03/05 and FRALLE2000T treated patients. (B) *BCL11B* deletion mapping representation in chromosome 14. (C) Lollipop plot representing intragenic mutational patterns according to patients occurrence. (D) Kaplan-Meier curves according to *BCL11B* status in GRAALL03/05 and FRALLE2000T treated patients: overall survival (left), cumulative incidence of relapse (middle), event-free survival (right).

terms of OS, cumulative incidence of relapse (CIR) and event-free survival (EFS) to *BCL11B*$^{WT}$ patients (Figure 1D). For *BCL11B*$^{alt}$ patients, the 5-year OS was 85.2% (95% confidence interval [CI]: 75.3-91.4) *versus* 67.9% (95% CI: 62.9-72.4) for *BCL11B*$^{WT}$ (hazard ratio [HR]=0.53; 95% CI: 0.35-0.80; *P*=0.01). The 5-year CIR was 21.4% (95% CI: 8.3-38.5) *versus* 30.7% (95% CI: 23.9-37.7) (HR=0.60; 95% CI: 0.39-0.92; *P*=0.047). The 5-year EFS was 73.5% (95% CI: 62.6-81.7) *versus* 59.4% (95% CI: 54.2-64.1) (HR=0.60; 95% CI: 0.44-0.90; *P*=0.025). A similar trend was observed in adults and children separately (Figure 2). However, in multivariate analysis, considering variables associated with OS in the univariate analysis as covariates (including oncogenetic classifier), the *BCL11B* status does not remain significantly associated with a better OS.

In this large cohort of adult and pediatric homogeneously treated T-ALL, *BCL11B* alterations were associated with an overall good response to treatment and a favorable long-term prognosis.[8] These results have to be interpreted in the context of a favorable mutational landscape associated with this alteration.[9] Indeed, *BCL11B* alterations were identified in a subgroup of T-ALL presenting a low-risk oncogenetic classifier with frequent *NOTCH1* and rare *NRAS* or *PTEN* alterations.

Recurrent cryptic t(5;14)(q35;q32) translocations juxtaposing *BCL11B* and *TLX3* result in *BCL11B* gene regulatory elements driving the aberrant overexpression of *TLX3*. This translocation is responsible for the majority of *TLX3* overexpression in T-ALL, thereby, inactivating one functional allele of *BCL11B* and leading to a haplo-insufficient phenotype in some cases. *TLX3* alterations are observed in 20% of T-ALL in children and 13% in adults. The prognosis value

**Table 1.** Biological and clinical characteristics of patients according to *BCL11B* status.

| GRALL03/05 and FRALLE2000T | Overall N=476 | *BCL11B*$^{wt}$ N=390 (82%) | *BCL11B*$^{alt}$ N=86 (18%) | *P*[1] |
|---|---|---|---|---|
| **Biological characteristics** | | | | |
| ETP status[2], N (%) | 56 (18) | 51 (21) | 5 (8) | 0.017 |
| Phenotypic classification,[3] N (%) | | | | <0.001 |
| IM0/δ/γ | 89 (21) | 86 (26) | 3 (4) | |
| IMβ/Pre-αβ | 211 (50) | 149 (44) | 62 (76) | |
| TCR γδ | 53 (13) | 45 (13) | 8 (10) | |
| TCR αβ | 66 (16) | 57 (17) | 9 (11) | |
| Molecular classification,[4] N (%) | | | | <0.001 |
| *PICALM::MLLT10* | 13 (3) | 13 (4) | 0 (0) | |
| *TLX1* | 54 (13) | 22 (7) | 32 (39) | |
| *TLX3* | 72 (17) | 57 (17) | 15 (18) | |
| *SIL-TAL1* | 57 (14) | 53 (16) | 4 (5) | |
| *HOXA9* overexpression | 79/336 (24) | 69/270 (26) | 10/66 (15) | |
| Negative | 219 (53) | 188 (56) | 31 (38) | |
| Risk classifier, N (%) | | | | 0.001 |
| High | 209 (44) | 185 (47) | 24 (28) | |
| Low | 267 (56) | 205 (53) | 62 (72) | |
| **Clinical characteristics** | | | | |
| Age at Diagnosis in years, median (range) | 15.33 (1.08-59.15) | 14.8 (1.1-59.1) | 19.3 (1.8-57.0) | 0.045 |
| Sex, N (%) | | | | 0.49 |
| Male | 357 (75) | 295 (76) | 62 (72) | |
| Female | 119 (25) | 95 (24) | 24 (28) | |
| WBC x10$^9$/L, median (range) | 63.80 (0.30-980.00) | 67.60 (0.30-965.00) | 36.40 (4.25-980.00) | 0.004 |
| CNS Involvement, N/N (%) | 51/474 (11) | 38/388 (10) | 13/86 (15) | 0.18 |
| Response to therapy, N/N (%) | | | | |
| Good prednisone response | 259/467 (55) | 202/384 (53) | 57/83 (69) | 0.008 |
| Chemosensitivity | 337/467 (72) | 266/383 (69) | 71/84 (85) | 0.005 |
| MRD1 >10$^{-4}$ | 123/340 (36) | 117/280 (42) | 6/60 (10) | <0.001 |
| Complete remission | 440/476 (92) | 357/390 (92) | 83/86 (97) | 0.17 |
| Allogeneic HSCT | 101/456 (22) | 87/372 (23) | 14/84 (17) | 0.19 |

[1]Welch two-sample *t*-test; Fisher's exact test for count data; Fisher's exact test for count data with simulated *P* value. [2]ETP status was available for 307 samples; [3]phenotypic classification was available for 419 samples; [4]molecular classification was available for 415 samples. CNS: central nervous system; ETP: early T-cell precursor; IM: immature; MRD1: minimal residual disease; SCT: stem cell transplantation; TCR: T-cell receptor; WBC: white blood cell count; wt: wild-type; alt: alterations.

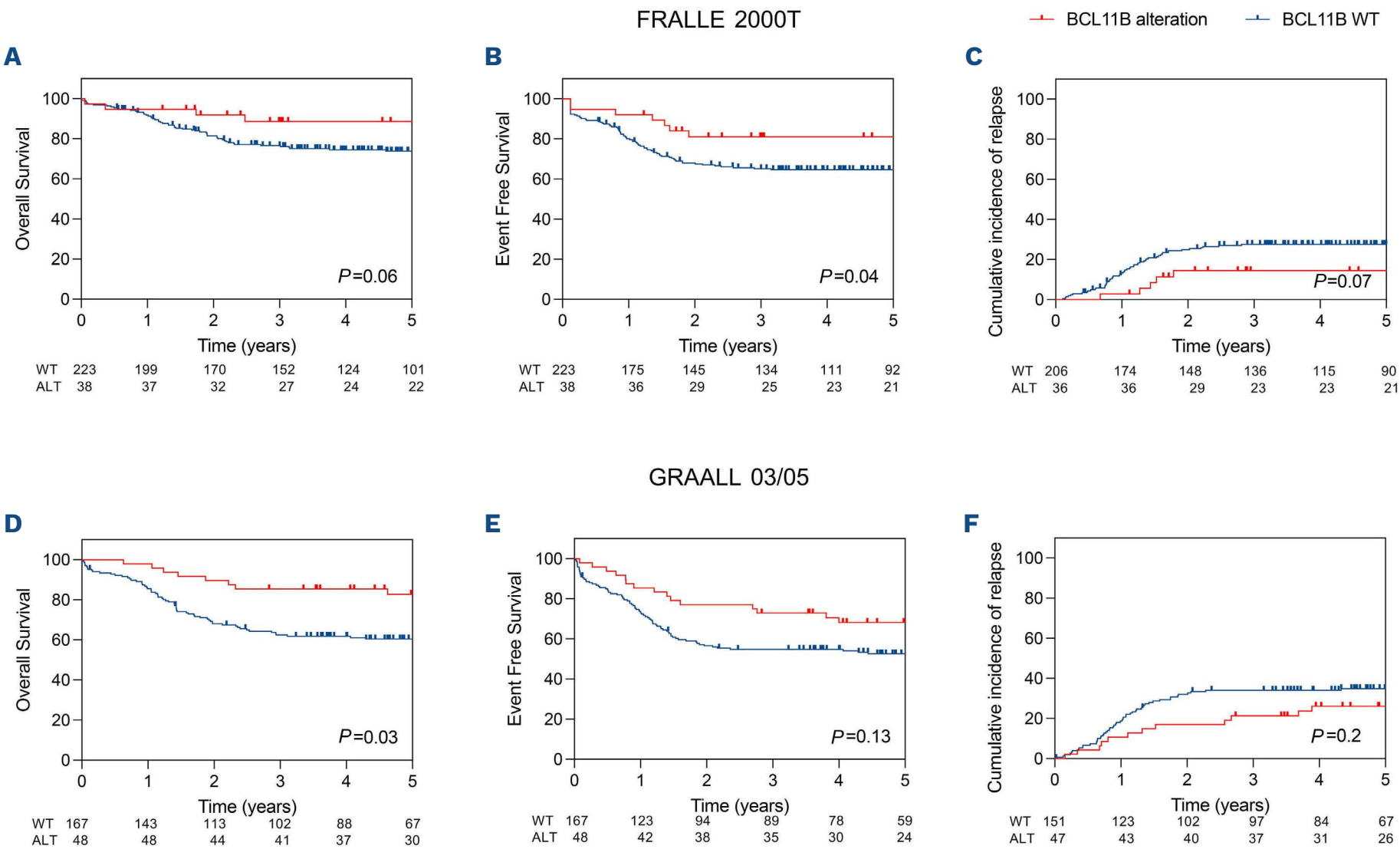


**Figure 2. Kaplan-Meier curves according to *BCL11B* status in FRALLE2000T or GRAALL03/05 treated patients.** (A, D) Kaplan Meier curves representing overall survival (OS) according to *BCL11B* status. (B, E) Kaplan Meier curves representing event-free survival (EFS) according to *BCL11B* status. (C, F) Kaplan Meier curves representing cumulative incidence of relapse (CIR) according to *BCL11B* status.

of *TLX3* overexpression is either neutral or dismal in several adults and pediatric series.[1,10–12] Previous work considered that *BCL11B* inactivation could be secondary to this translocation and lead to pathogenic consequences.[7] However, our results showed that *BCL11B* alterations are associated with a favorable outcome, contrary to *TLX3* overexpression, suggesting that *TLX3* overexpression could mitigate the favorable profile of *BCL11B* alterations. In line with this, heterozygous loss of *Bcl11b* was reported to reduce lethal thymic lymphoma in ATM$^{-/-}$ mice by suppressing lymphoma progression, but not the initiation of the disease.[13]

During physiological hematopoiesis, BCL11B expression in T-cell precursors is maximal during the onset of the DN2 phase, then maintained throughout the subsequent maturation stages. In human T-ALL cell lines, BCL11B loss of function led to apoptosis.[14] On the contrary, BCL11B overexpression has been reported to convey a chemo-protective effect. Interestingly, recent studies reported on the role of BCL11B in lineage ambiguous stem cell leukemia, T/M MPAL and ETP-ALL. Several mechanisms were described. The translocation t(2;14)(q22;q32) yields an in-frame ZEB2-BCL11B fusion product that leads to the misexpression of BCL11B in early progenitor cells where the *BCL11B* enhancer is not normally active. Another rearrangement identified a transcriptional regulatory sequence hijacked by the *BCL11B* gene itself. All these rearrangements result in high expression of BCL11B.[15–17] Altogether, these data supported multiple roles for BCL11B in the pathogenesis of acute leukemia according to maturation arrest: as a tumor suppressor in “typical” T-lineage ALL with loss of function mutation or deletion, or as a stage-specific oncogene in hematopoietic stem or early progenitors by existing or *de novo* super-enhancers maximally active in hematopoietic stem cell progenitors.

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**Disclosures**

No conflicts of interest to disclose.

**Contributions**

MED, GA and VA conceived the study and oversaw the project. MED, MS, AP, NB, AB provided study materials or patients. MED and GA performed molecular analyses. MED, GA, AP, JG, EB and LC collected and assembled data. MED and GA performed statistical analysis. MED, GA, AP, JG, EB and VA analyzed and interpreted data. MED, GA and VA wrote the manuscript. All authors approved the manuscript.

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**Data-sharing statement**

No data will be shared.

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