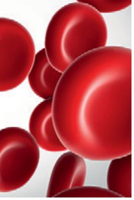

Letters to *Blood*

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TO THE EDITOR:

Topoisomerase 2β mutation impairs early B-cell development

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Mutations impairing early B-cell development cause monogenic primary immunodeficiencies that manifest with markedly reduced or absent B cells, hypogammaglobulinemia, and recurrent bacterial infections from childhood. Approximately 85% of such patients have mutations in *BTK*, the gene responsible for X-linked agammaglobulinemia.[1] Current research focuses on patients with unknown genetic defects, because the identification of the causative genes not only will facilitate diagnosis of primary immunodeficiencies but also can reveal new biological roles of the affected proteins in human B-cell development and point at novel drug targets.

The autosomal dominant syndrome of unknown etiology called BILU (B-cell Immunodeficiency, Limb anomalies and Urogenital malformations) was previously described in 2 unrelated families originating from Cyprus and France[2,3] (families A and B; Figure 1A; supplemental Tables 1 and 2, available on the *Blood* Web site). The BILU patients have absent or reduced B cells, but normal T and myeloid cells[2,3] (supplemental Figure 1). Here we studied these 2 families. Given that in family A, both parents of patient II:4 are healthy, we hypothesized that the BILU syndrome was caused by a de novo mutation. We sequenced exomes of 4 subjects in family A (Figure 1A) and identified the only mutation in the coding part of the genome that was absent in healthy parents I:3 and I:4, appeared de novo in their affected daughter II:4, and then was transmitted to her affected daughter III:1. This heterozygous G>C mutation at the cDNA nucleotide 1453 of the *TOP2B* gene (ENST00000435706) resulted in the alanine-to-proline substitution at position 485 (A485P) of topoisomerase 2β (TOP2B). Next, we used Sanger sequencing and found that exactly the same mutation was present in patients from family B (Figure 1B). Given that this mutation was never found in any healthy subject (eg, absent from more than 90 000 subjects in the gnomAD database[4]), but is present in the BILU patients from 2 unrelated families, this novel mutation is the cause of the BILU syndrome.

TOP2B is a type II topoisomerase, an enzyme that generates transient DNA double-strand breaks and solves topological problems during replication and transcription (eg, removes DNA supercoils, knots, and catenanes).[5] TOP2B and the other human type II topoisomerase 2α (TOP2A) can make active homodimers and heterodimers.[6,7] First, we studied how the newly discovered A485P mutation interfered with the structure of the TOP2B protein. The mutation affects alanine that is conserved in eukaryotic and even in prokaryotic type II topoisomerases (Figure 1C). Its substitution with proline is predicted to destabilize an α helix within the TOPRIM domain (Figure 1D-E) that is essential for the catalytic activity of the TOP2B protein.[8,9]

Because BILU patients have no or few B cells in the blood, we studied T cells, skin fibroblasts, and induced pluripotent stem cells and found reduced amounts of the TOP2B protein in patients' cells in comparison with cells of healthy control individuals (Figure 2A). We then used CRISPR-Cas9 to knock-out TOP2B in HEK-293 cells and expressed in these cells the wild-type and mutant proteins, $TOP2B^{WT}$ and $TOP2B^{A485P}$. We found a low-molecular-weight product of $TOP2B^{A485P}$ degradation, suggesting that the mutant protein is less stable than wild-type TOP2B (supplemental Figure 2). Co-immunoprecipitation showed that both $TOP2B^{WT}$ and $TOP2B^{A485P}$ interact with endogenous TOP2A (supplemental Figure 2).

To find out whether the mutation affects enzymatic activity, we then studied purified recombinant full-length $TOP2B^{WT}$ and $TOP2B^{A485P}$ proteins using DNA relaxation and decatenation in vitro assays.[10] We found that enzymatic activities of $TOP2B^{A485P}$ were reduced more than 10-fold (Figure 2B-C; supplemental Figure 3A-B). In human cells, DNA relaxation can be performed by both type I and type II topoisomerases, whereas DNA decatenation is performed only by type II topoisomerases TOP2B and TOP2A. Therefore, we studied cell lysates using only the decatenation assay. The decatenation activity of lysed patient's T-cell blasts was reduced (Figure 2D; supplemental Figure 4). Likewise, the decatenation activity of lysed wild-type HEK-293 cells transiently expressing $TOP2B^{A485P}$ was lower than the activity of cells expressing $TOP2B^{WT}$ (Figure 2E; supplemental Figure 5). Interestingly, it was also lower than the decatenation activity of untransfected wild-type HEK-293 cells that expressed

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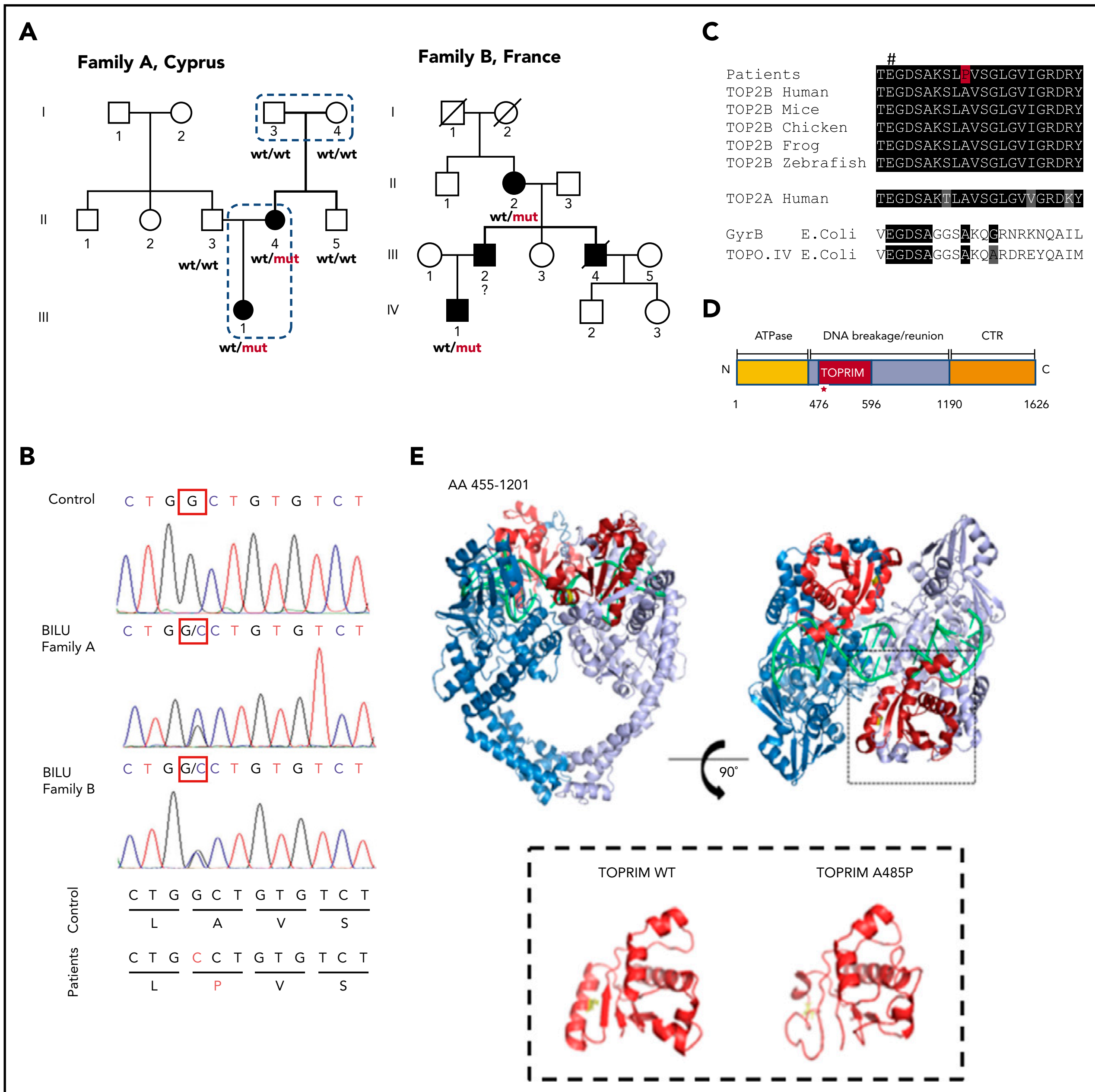

Figure 1. Novel dominant mutation A485P affects TOP2B catalytic site and causes the BILU syndrome. (A) Two families with the BILU syndrome: ○ and □, unaffected; ● and ■, affected; wt, wild-type allele; mut, A485P mutation in TOP2B. Exome sequencing was performed in 4 subjects, shown by dotted lines. (B) Electrophoregrams of the TOP2B genomic DNA sequence showing the same mutation in patients from the 2 BILU families. (C) Multispecies protein sequence alignment of type II topoisomerases. #Conserved glutamate essential for TOP2B activity. (D) Schematic representation of domains of the TOP2B protein. The A485P mutation in the TOPRIM domain is shown by a red star. The TOPRIM domain is part of the DNA gate that catalyzes DNA cleavage and religation. (E) Structure of the TOP2B homodimer (amino acid residues 455 to 1201) in complex with DNA (green), the TOPRIM domain (red), and the alanine at codon 485 (yellow; upper). I-TASSER-modeled structures[25] of the TOP2B TOPRIM domain (lower).

only endogenous TOP2B, as well as endogenous TOP2A (Figure 2E; supplemental Figure 5). Similarly, in TOP2B-knockout HEK-293 cells, co-expression of TOP2B^{WT} and TOP2B^{A485P} proteins resulted in lower decatenation activity than the expression of TOP2B^{WT} alone (Figure 2F; supplemental Figure 6). These results indicate that mutant TOP2B^{A485P} protein itself not only has reduced intrinsic enzymatic activity but also exerts a dominant negative effect on the activities of wild-type TOP2B and TOP2A. Thus, our experimental data are consistent with the dominant negative effect of the TOP2B mutation that causes BILU syndrome, rather than with haploinsufficiency. Moreover, haploinsufficiency of TOP2B is an unlikely causative mechanism of BILU because multiple subjects with various heterozygous loss-of-function *TOP2B* mutations have been detected in population cohorts (eg, the gnomAD database[4] has 42 such unaffected subjects; https://gnomad.broadinstitute.org/).

Recently, other dominant mutations affecting the TOPRIM domain of TOP2B have been shown to cause Hoffman syndrome, characterized by B-cell deficiency, limb abnormalities, and facial dysmorphism[11] (supplemental Tables 1 and 2). Our results indicate that BILU and Hoffman syndrome are manifestations of the same

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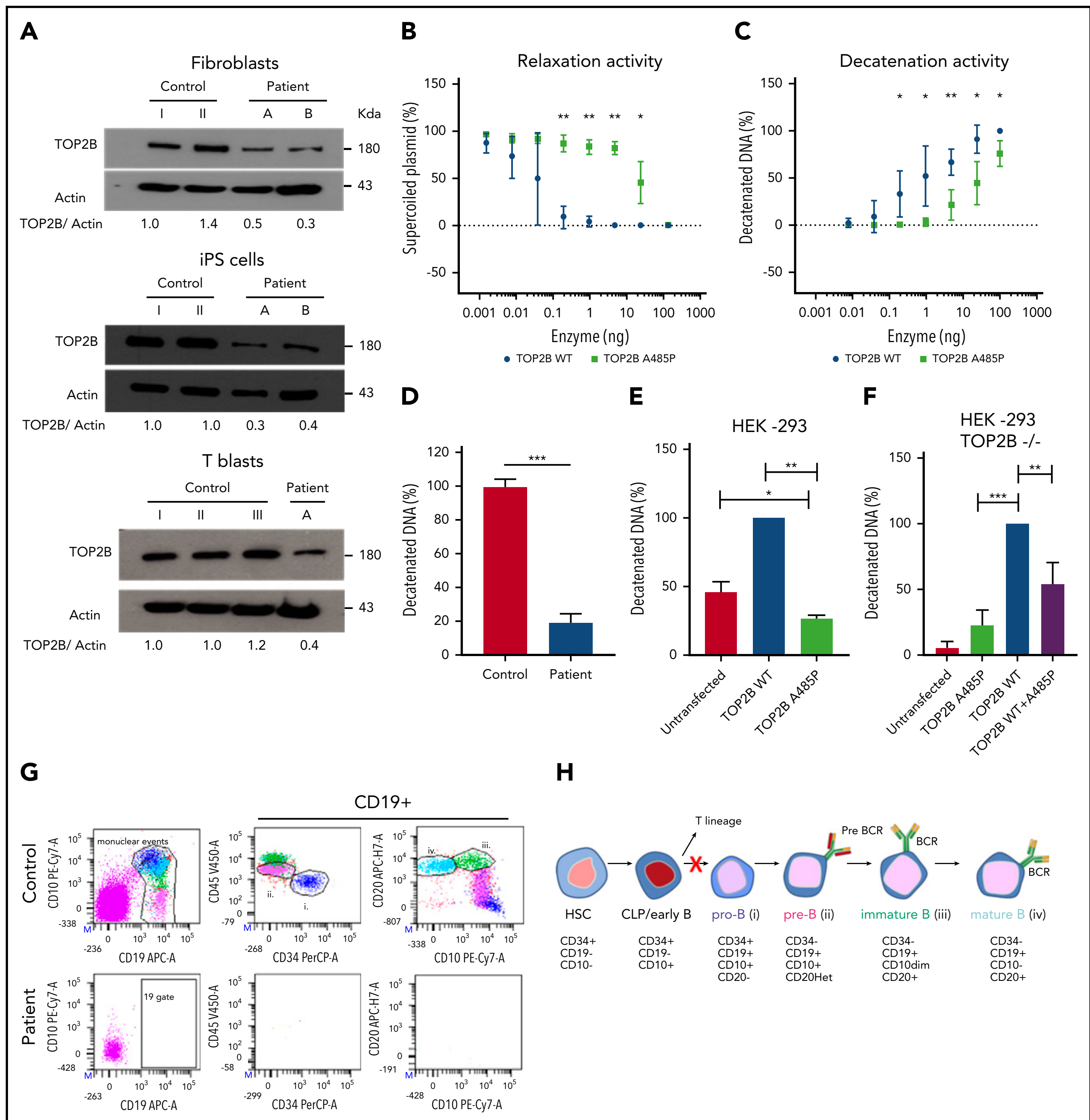

Figure 2. Mutation A485P reduces expression and activity of the TOP2B protein and impairs early B-cell development. (A) Western blots showing expression of the TOP2B protein in primary dermal fibroblasts, induced pluripotent stem cells (iPS cells) dedifferentiated from dermal fibroblasts, and T-cell blasts derived from peripheral blood mononuclear cells. Patients: A is III.1 in family A; B is IV.1 in family B. Fold change of band densitometry is shown. (B) Relaxation of negatively supercoiled DNA by the purified recombinant TOP2B^{WT} and TOP2B^{A485P} proteins. (C) Decatenation of kinetoplast DNA by the purified recombinant TOP2B^{WT} and TOP2B^{A485P} proteins. (D) Decatenation of kinetoplast DNA by nuclear extracts from T-cell blasts. (E-F) Decatenation of kinetoplast DNA by nuclear extracts from wild-type HEK-293 cells (E) or TOP2B-knockout HEK-293 cell (F), untransfected or transfected with plasmids encoding TOP2B^{WT} and TOP2B^{A485P}. *P* values were calculated using 2-tailed (E) and 1-tailed (F) paired *t* tests. Graphs show averages ± SD. **P* < .05; ***P* < .01; ****P* < .001. (G) Bone marrow immunophenotyping of the BILU patient and a healthy unrelated control individual showing pro-B (i), pre-B (ii), immature B (iii), and mature B (iv) cells. (H) B-cell development stages; red X shows defect in the BILU patients. CLP, common lymphoid progenitor; HSC, hematopoietic stem cell.

disease, TOP2B deficiency. Importantly, these findings demonstrate a previously unknown critical role of TOP2B in B-cell development.

The developmental defect leading to B-cell deficiency in patients with BILU and Hoffman syndromes has not been investigated previously. To reveal the affected stage of B-cell development, we studied a bone marrow aspirate of the BILU patient II:4 from family A, using multicolor flow cytometry, and found a complete absence of any CD19^{+} cells, including pro-B, pre-B, and immature and mature B cells, but normal T, NK, and myeloid cell lineages (Figure 2G; supplemental Figure 7). This finding for the first time shows that TOP2B is critical during the earliest stages of B-cell lineage after the common lymphoid progenitor stage (Figure 2H). The early block in B-cell development distinguishes patients with

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TOP2B deficiency from most other B-cell immunodeficiencies that either impair later stages of B-cell differentiation, resulting in the accumulation of $CD19^{+}$ pro-B cells (eg, BTK deficiency), or affect multiple hematopoietic cell lineages (eg, ADA or GATA2 deficiencies).[12] Rather, it is reminiscent of the early block of B-cell differentiation seen in patients with dominant *TCF3* mutations and recessive *PIK3R1* mutations.[13,14] Nevertheless, in TOP2B deficiency, this block is leaky, because immunoglobulins and small numbers of B cells have been found in peripheral blood of several patients (supplemental Table 2).

TOP2B had been shown to produce signaling-induced double-strand breaks at gene promoters[15-18] and was involved in activation of transcription[16-18] and transcription of long genes,[19] as well as formation and maintenance of topologically associated domains and chromatin loops.[20,21] Although these TOP2B functions may contribute to the B-cell developmental defect, the exact molecular mechanism affecting specifically B cells, but not other immune cell lineages, remains unclear.

TOP2B and TOP2A are the targets of the anticancer drug etoposide that traps these enzymes in a complex with cleaved DNA, which eventually leads cells to apoptosis. Etoposide, in combination with clofarabine and cyclophosphamide, had been used for chemotherapy of acute lymphoblastic leukemia (ALL).[22-24] Interestingly, in pediatric patients with refractory/multiple relapse ALL, this chemotherapy regimen was found to be more effective against B-cell precursor ALL than T-cell ALL.[23] This clinical observation is consistent with the particularly important role of TOP2B in B-cell precursors, rather than in T-cell lineage. Thus, etoposide, as well as other inhibitors of type II topoisomerases, may be especially effective for the treatment of B-cell malignancies.

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Authorship

Contribution: O.P. planned and performed the experiments and analyzed the data with help from A.C., D.E., M.M., A.A., J.C., E.B., and D.C.-L.; S.I. analyzed bone marrow aspirate of the BILU patient; A.S.A. analyzed lymphocytes in peripheral blood of the BILU patients; V.P. performed bioinformatics analysis of the exome data; O.H., C.P., A.F., A.D., S.K., and S.O.B. looked after the BILU patients and collected clinical data; K.O. participated in the planning of experiments and analyzed the data; and S.N. conceived the study, identified the TOP2B mutation, planned the experiments, and analyzed the data.

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Footnotes

For original data, please e-mail the corresponding author.

The online version of this article contains a data supplement.

REFERENCES

1. Conley ME, Dobbs AK, Farmer DM, et al. Primary B cell immunodeficiencies: comparisons and contrasts. *Annu Rev Immunol*. 2009;27(1):199-227.
2. Edery P, Le Deist F, Briard ML, et al. B cell immunodeficiency, distal limb abnormalities, and urogenital malformations in a three generation family: a novel autosomal dominant syndrome? *J Med Genet*. 2001;38(7):488-493.
3. Tischkowitz M, Goodman F, Koliou M, et al. Autosomal dominant B-cell immunodeficiency, distal limb anomalies and urogenital malformations (BILU syndrome) - report of a second family. *Clin Genet*. 2004;66(6):550-555.
4. Lek M, Karczewski KJ, Minikel EV, et al; Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536(7616):285-291.
5. Pommier Y, Sun Y, Huang SN, Nitiss JL. Roles of eukaryotic topoisomerases in transcription, replication and genomic stability. *Nat Rev Mol Cell Biol*. 2016;17(11):703-721.
6. Biersack H, Jensen S, Gromova I, Nielsen IS, Westergaard O, Andersen AH. Active heterodimers are formed from human DNA topoisomerase II alpha and II beta isoforms. *Proc Natl Acad Sci USA*. 1996;93(16):8288-8293.
7. Gromova I, Biersack H, Jensen S, Nielsen OF, Westergaard O, Andersen AH. Characterization of DNA topoisomerase II alpha/beta heterodimers in HeLa cells. *Biochemistry*. 1998;37(47):16645-16652.
8. Aravind L, Leipe DD, Koonin EV. Toprim--a conserved catalytic domain in type IA and II topoisomerases, DnaG-type primases, OLD family nucleases and RecR proteins. *Nucleic Acids Res*. 1998;26(18):4205-4213.
9. Dong KC, Berger JM. Structural basis for gate-DNA recognition and bending by type IIA topoisomerases. *Nature*. 2007;450(7173):1201-1205.
10. Nitiss JL, Soans E, Rogojina A, Seth A, Mishina M. Topoisomerase assays. *Curr Protoc Pharmacol*. 2012;Chapter 3:Unit 3.3.
11. Broderick L, Yost S, Li D, et al. Mutations in topoisomerase IIβ result in a B cell immunodeficiency. *Nat Commun*. 2019;10(1):3644.
12. Durandy A, Kracker S, Fischer A. Primary antibody deficiencies. *Nat Rev Immunol*. 2013;13(7):519-533.
13. Boisson B, Wang YD, Bosompem A, et al. A recurrent dominant negative E47 mutation causes agammaglobulinemia and BCR(-) B cells. *J Clin Invest*. 2013;123(11):4781-4785.
14. Conley ME, Dobbs AK, Quintana AM, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85α subunit of PI3K. *J Exp Med*. 2012;209(3):463-470.
15. Haffner MC, Aryee MJ, Toubaji A, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat Genet*. 2010;42(8):668-675.
16. Ju BG, Lunyak VV, Perissi V, et al. A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. *Science*. 2006;312(5781):1798-1802.

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17. Madabhushi R, Gao F, Pfenning AR, et al. Activity-induced DNA breaks govern the expression of neuronal early-response genes. *Cell*. 2015; 161(7):1592-1605.

18. Trotter KW, King HA, Archer TK. Glucocorticoid receptor transcriptional activation via the BRG1-dependent recruitment of TOP2β and Ku70/86source [published correction appears in *Mol Cell Biol*. 2017; 37(18):e00348-17]. *Mol Cell Biol*. 2015;35(16):2799-2817.

19. King IF, Yandava CN, Mabb AM, et al. Topoisomerases facilitate transcription of long genes linked to autism. *Nature*. 2013;501(7465):58-62.

20. Uusküla-Reimand L, Hou H, Samavarchi-Tehrani P, et al. Topoisomerase II beta interacts with cohesin and CTCF at topological domain borders. *Genome Biol*. 2016;17(1):182.

21. Canela A, Maman Y, Jung S, et al. Genome organization drives chromosome fragility. *Cell*. 2017;170(3):507-521.e18.

22. Hijiya N, Gaynon P, Barry E, et al. A multi-center phase I study of clofarabine, etoposide and cyclophosphamide in combination in pediatric patients with refractory or relapsed acute leukemia. *Leukemia*. 2009; 23(12):2259-2264.

23. Locatelli F, Testi AM, Bernardo ME, et al. Clofarabine, cyclophosphamide and etoposide as single-course re-induction therapy for children with refractory/multiple relapsed acute lymphoblastic leukaemia. *Br J Haematol*. 2009;147(3):371-378.

24. Hijiya N, Thomson B, Isakoff MS, et al. Phase 2 trial of clofarabine in combination with etoposide and cyclophosphamide in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. *Blood*. 2011; 118(23):6043-6049.

25. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. The I-TASSER Suite: protein structure and function prediction. *Nat Methods*. 2015;12(1): 7-8.

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TO THE EDITOR:

Blinatumomab for infant acute lymphoblastic leukemia

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Unlike older children with acute lymphoblastic leukemia (ALL), there has been almost no improvement in outcome for infants in the last 2 decades. Six-year event-free survival (EFS) and overall survival (OS) in successive international infant trials, Interfant99[1] and Interfant06,[2] were 46.4% and 53.8%, and 46.1% and 58.2%, respectively. High risk patients in Interfant06 had a 6-year EFS and OS of 20.9% and 29.9%, respectively, despite hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1). Outcome after relapse is dismal, with a 3-year OS of 20.9%.[3]

Among novel approaches, immune therapies, such as chimeric antigen receptor (CAR) T cells and blinatumomab, offer the greatest potential for improving cure rates. The bispecific CD3/CD19-engaging antibody, blinatumomab, was found to result in complete and often minimal residual disease (MRD)-negative remission in children with relapsed/refractory B-cell ALL (B-ALL). Better responses were observed in patients with <50% bone marrow blasts (55.6% vs 32.7%; 95% confidence interval, 30.8-78.5 and 20.3-47.1, respectively),[4] and an adult study showed a complete MRD response rate of 78% when blinatumomab was used to treat MRD-positive ALL in hematological remission.[5] Because the risk of relapse after HSCT is predicted by MRD status prior to transplant, deeper molecular remissions achieved by using blinatumomab might improve posttransplant outcomes.

Here, we report the outcome of 11 infants who received blinatumomab for persistent MRD prior to HSCT. To our knowledge, this is the largest experience reported in this rare subgroup of patients.

This retrospective analysis included patients from the United Kingdom and the Republic of Ireland with B-ALL, whose initial diagnosis was before the first birthday. Patients were identified from the minutes of a national tumor board, supplemented by a survey of pediatric hematologists in the 2 countries. All children were initially treated according to the Interfant 06 protocol.[2] Between 2016 and 2019, patients in first remission or after relapse received blinatumomab for MRD reduction prior to HSCT. None of the patients had received a prior HSCT. Individual patient MRD at all time points was measured in the same laboratory using a Euro-MRD Consortium–accredited and standardized technique for real-time quantitative polymerase chain reaction of immunoglobulin gene rearrangements. EFS was defined as time from diagnosis to relapse, secondary tumor, or death, and OS was defined as time to death. OS and EFS were reported using the Kaplan-Meier function. Analysis was performed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA).

Eleven patients were identified who met the eligibility criteria for analysis, which was treatment of *KMT2A*-rearranged infant ALL with blinatumomab in first remission or relapse, regardless of the age at which it was administered. The median age at the time of blinatumomab administration was 0.5 years (range, 0.2-2.9). One patient had a late relapse of *KMT2A*- rearranged infant ALL (2.9 years) and was included in the analysis as per the intended aim. All patients had *KMT2A* rearrangement. Seven patients received blinatumomab after relapse, and 4 patients received it as first-line therapy for resistant or refractory disease. Of the 8 patients who were in first or second MRD-positive CR, median MRD was 0.2% (range, 0.06-1) (Table 1).

Nine patients received a single 28-day cycle of blinatumomab, and the other 2 patients received a second cycle, pending