Letter to *Blood*

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TO THE EDITOR:

Somatic *TP53* mutations are preleukemic events in acute lymphoblastic leukemia

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Acute lymphoblastic leukemia (ALL) is an aggressive hematological malignancy with especially dismal outcomes in adults and at the recurrence of the disease. Somatic mutations in the tumor suppressor gene *TP53* occur in 6% to 19% of ALL cases at diagnosis and are enriched in low hypodiploid-near triploid ALL and at reoccurrence of the disease. *TP53* mutations in ALL play an important role in the evolution of treatment-resistant clones, associated with early relapses and poor overall survival.[1-6] Furthermore, *TP53* mutations have been reported as an early leukemogenic event in patients with acute myeloid leukemia, representing acute myeloid leukemia-initiating mutations and mediators of resistant disease, even at subclonal levels.[7,8] However, for ALL, very limited data are available. Here, we retrospectively analyzed the kinetics of *TP53* mutations in the bone marrow and peripheral blood during follow-up in a group of 43 adult patients with *TP53*-mutated ALL and compared the *TP53* mutation burden with minimal residual disease (MRD) levels assessed by EuroMRD-standardized real-time quantitative polymerase chain reaction (PCR) of clonal immune gene rearrangements (IG/TR MRD).[9,10]

Analyses were performed in a cohort of 43 adult patients with ALL with known *TP53* mutations at diagnosis or at relapse (supplemental Data, available on the *Blood* website),[6] with available longitudinal MRD measurements[9,10] and leftover DNAs of the respective bone marrow and/or blood samples in the Hematology Lab Kiel. All patients received treatment according to GMALL protocols and provided informed consent for the use of left-over samples for scientific purposes (supplemental Data). For each patient, the leukemic *TP53* mutation profile was determined in at least 1 tumor-positive diagnostic and/or tumor-positive follow-up (54 samples) sample using a UMI-based amplicon next-generation sequencing approach (supplemental Table 1). The same method was applied to the 40 follow-up samples obtained from 30 patients who achieved molecular remission. All the samples were obtained before allogeneic hematopoietic stem cell transplantation. In patients with *TP53* mutation positivity in an MRD-negative follow-up sample and availability of archived left-over viable cells in the respective samples, distinct precursor and mature hematopoietic populations were isolated using the FACS Aria sorter and analyzed for the presence of *TP53* mutations using mutation-specific digital droplet PCR.

We analyzed the diagnostic and follow-up samples of 43 patients with ALL (36 B-cell-precursor ALL, 3 Burkitt leukemia, and 4 T-ALL) with known *TP53* mutations at diagnosis or relapse. As expected, most of these patients (65%, 28/43) had ALL cases with a low hypodiploid-near triploid karyotype,[5,6] all with the loss of 1 *TP53* copy in a diploid or 2 copies in a tetraploid chromosome set. In total, 48 *TP53* mutations (71% missense, 6% nonsense, 8% splice site, and 15% frameshift mutations) were identified as subclonal or clonal alterations, primarily in the DNA-binding domain (Figure 1A; supplemental Table 1). A total of 94 samples from these patients assessed for IG/TR MRD were retrospectively analyzed for *TP53* mutation load at the time of diagnosis and during follow-up at the time of MRD-positivity (n = 54 samples) or in molecular remission (n = 40 samples), if applicable (Figure 1B). The IG/TR MRD persisted in 13 patients (30%) at quantifiable (MRD-positive, 7 patients, 16%) or nonquantifiable levels (MRD <Q, 6 patients, 14%). Thirty patients achieved molecular remission at some point (MRD-negative, 70%). Discrepancies in MRD kinetics between IG/TR MRD and *TP53* mutant-allele burden were detected in 9 of the 30 MRD-negative patients (30%, Figure 1B). In 14 samples of these 9 patients, *TP53* mutations were detected at levels between 2.0% and 42.9% (median 14.1%, upper left Figure 1C) despite IG/TR MRD-negativity. These data suggest that *TP53* mutations occur as an early event in the preleukemic compartment, which can generate differentiated progeny and persist in nonleukemic cells during remission. Similarly, Salmoiraghi et al[11] recently reported that *TP53* alterations were correlated with very early rearrangements of the immunoglobulin receptor in ALL, also pointing to the occurrence of *TP53* mutations as an early leukemogenic event and potentially their preleukemic origin. We selected 5 out of these 9 IG/TR MRD-negative and *TP53*-mutated patients (all B-ALL, Figure 2A) and analyzed the *TP53* mutation load using

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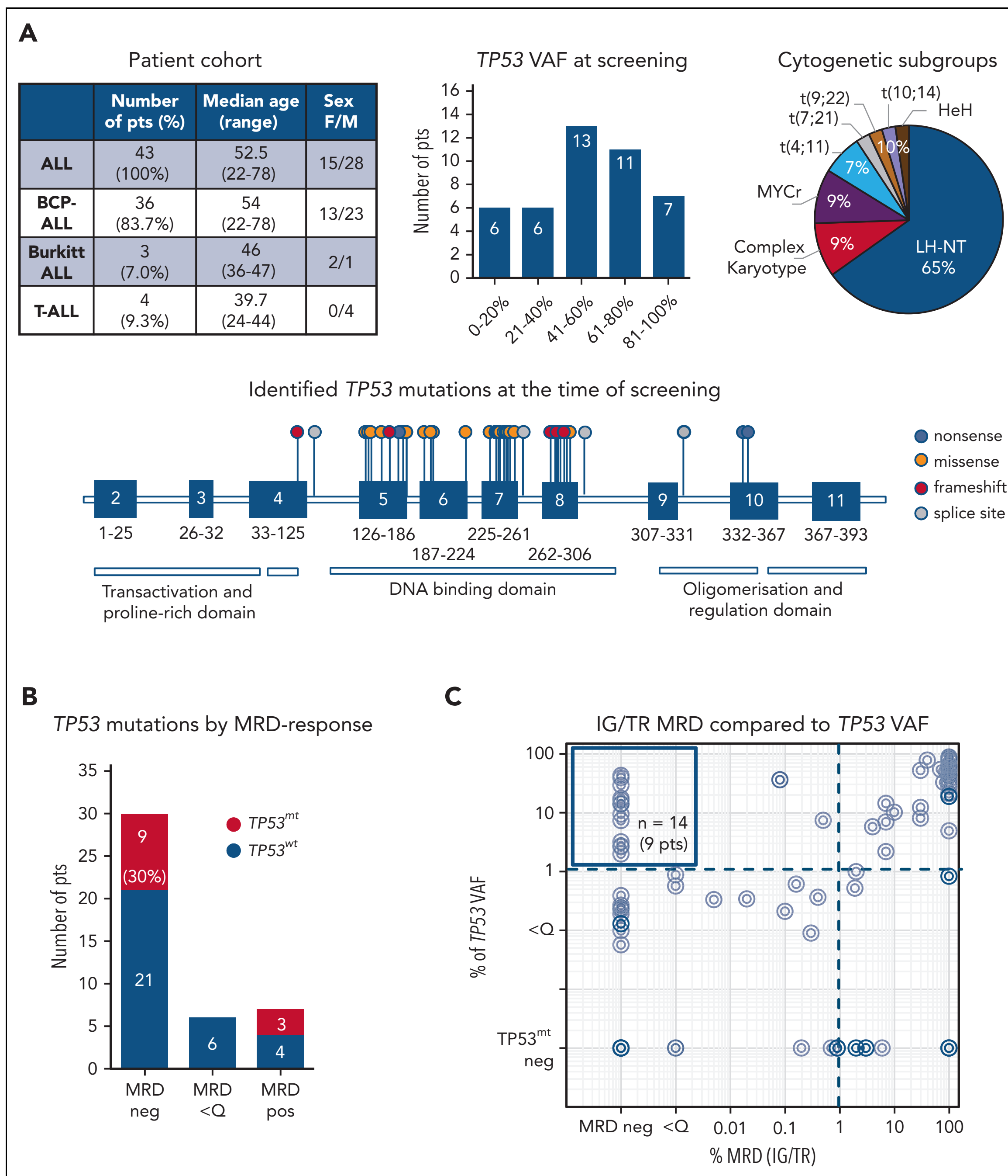

Figure 1. *TP53* mutation profiles in a cohort of patients with ALL. (A) Description of a cohort of 43 patients with ALL with known somatic *TP53* mutations at the time of diagnosis or relapse (top left), regarded as the screening timepoint. Distribution of *TP53* mutant-allele burden (top middle) and cytogenetic subgroups (top right). Forty-two distinct *TP53* mutations are shown on lollipop chart and color-coded as nonsense, missense, frameshift and splice site mutations (bottom). Some *TP53* mutations occurred more than once, in total 48 *TP53* mutations were detected. *TP53* mutation load compared with IG/TR MRD in diagnostic and follow-up samples. (B) Patients are stratified based on the best molecular response (MRD-negative, MRD <Q, or MRD-positive) achieved before allogeneic stem cell transplantation. The proportion of patients with persisting *TP53* mutations (red bars) in the respective follow-up samples is shown (left). IG/TR MRD (x-axis) is compared with the mutated *TP53* variant allele frequency (VAF, y-axis) in all diagnostic and follow-up samples (94 samples). The dotted lines represent the sensitivity threshold for *TP53* mutations (right). $TP53^{mt}$, mutated *TP53*; $TP53^{wt}$, wild-type *TP53*.

mutation-specific digital droplet PCR (supplemental Table 2) in all available follow-up samples. The results were compared with the MRD kinetics over time (Figure 2A, top). In all 5 cases, *TP53* mutations persisted in the follow-up samples despite MRD-negativity. To specify the origin of *TP53* mutations, we sorted distinct mature and progenitor hematopoietic populations based on their characteristic immunophenotypes (supplemental Figure 1) in molecular remission at the indicated time point. Interestingly, early hematopoietic cells, including multipotent progenitors, common myeloid/lymphoid progenitors, myeloid progenitors, and B-cell precursors, as well as differentiated cells such as granulocytes and monocytes, contained *TP53* mutations at levels ranging between 1.1% and 54.3% (Figure 2A, bottom), indicating the preleukemic origin of *TP53* mutations

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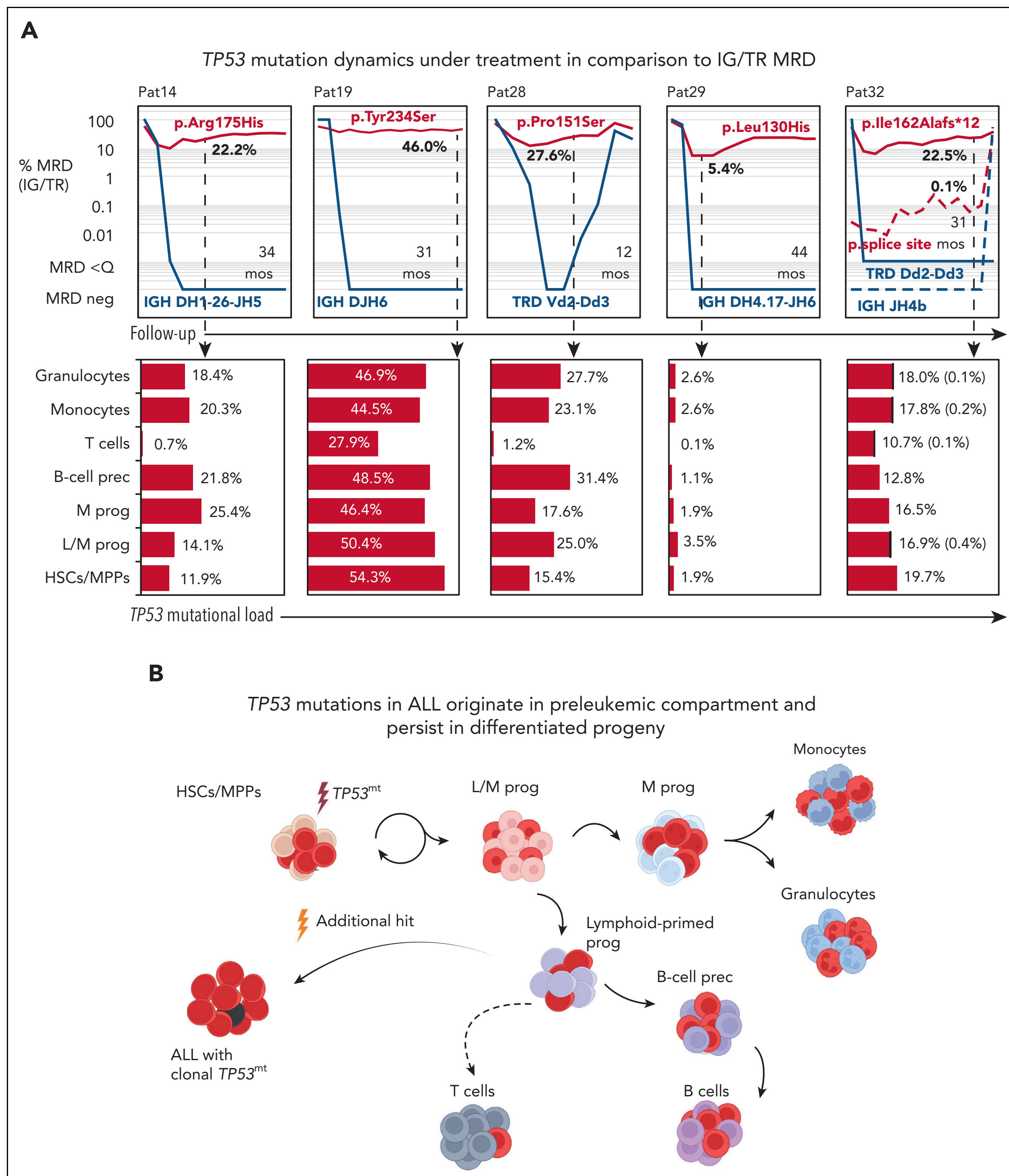

Figure 2. Discrepant dynamics of *TP53* mutations during follow-up compared with IG/TR MRD. (A) Longitudinal DNA samples of 5 patients with ALL selected based on *TP53* mutation positivity in an MRD-negative follow-up sample and availability of archived left-over viable cells were analyzed for the presence of specific *TP53* mutations using mutation-specific digital droplet PCR. In all 5 cases, the respective *TP53* mutations (red lines) were sustained despite MRD-negativity (blue lines) during remission (top panel). Pat32 relapsed with a clone harboring an entirely unrelated IG/TR (dotted blue line) rearrangement and gained a second *TP53* mutation (dotted red line) while sustaining the diagnostic *TP53* mutation. In patients 14, 19, and 29, false MRD negativity owing to potential clonal evolution of the MRD marker was excluded by IGH amplicon next-generation sequencing analysis that neither showed the diagnostic IGH clonotype nor a related IGH or any other unrelated highly abundant IGH clonotype in the remission sample (data not shown). Distinct hematopoietic cell populations (early hematopoietic cells; hematopoietic stem cells/multipotent progenitors [HSCs/MPPs]), common myeloid/lymphoid progenitors (L/M prog), myeloid progenitors (M prog), and B-cell precursors (B-cell prec), as well as the mature immune cell populations such as T cells, monocytes, and granulocytes of an MRD-negative follow-up sample, were isolated using the FACS Aria sorter. Sorted subpopulations were analyzed for the presence of specific *TP53* mutations by mutation-specific digital droplet PCR. Values in percentages indicate the *TP53* mutant-allele burden in the isolated populations. (B) *TP53* mutations in ALL originate in a preleukemic compartment that can self-renew and cause clonal expansions in hematopoietic compartments; thus, they persist in tumor-free cells during remission and increase at the (re)occurrence of the disease. IGH, immunoglobulin heavy locus; *TP53*^{mt}, mutated *TP53*; *TP53*^{wt}, wild-type *TP53*. Panel B was created with BioRender.

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(graphically shown in Figure 2B). Along this line, patients with preleukemic mutation patterns generally harbored a high *TP53* mutation load (variant allele frequency >30%, supplemental Table 1) at the time of diagnosis, suggesting the presence of *TP53* mutations in leukemia-originating cells and subsequently in all leukemic cells. *TP53* mutations were generally very low/absent in mature T cells, except for patients 19 and 32 with late follow-up samples used in sorting experiments at 31 and 24 months after diagnosis, respectively, reflecting distinct developmental route and delayed recovery kinetics of T cells under/following ALL treatment.[12,13]

Half of the relapses in ALL evolve from an ancestral subclone present at diagnosis.[14,15] *TP53* mutations are often enriched in relapse and contribute to resistance development in ALL.[15,16] To that end, we observed that 1 patient with ALL (Figure 2A, Pat32), who relapsed after 2 years with a leukemic clone showing a completely different karyotype and IG/TR rearrangement fully unrelated to the initial diagnostic clone, gained a second *TP53* mutation while sustaining the diagnostic one. This suggests underlying clonal heterogeneity and involvement of preleukemic *TP53* mutations in ALL progression. To address the role of preleukemic *TP53* and tumor-associated mutations in ALL progression, we compared the mutation profiles of 141 genes involved in leukemia pathogenesis in available paired samples of 9 patients obtained at diagnosis and/or disease progression (supplemental Figure 2; supplemental Data). Three patients had characteristic preleukemic *TP53* mutation patterns and available remission samples. The initial *TP53* mutations persisted in all patients with disease recurrence. Two patients gained (additional) *TP53* mutations (Pat15; Pat32) at the time of relapse. The mutational profiles differed between the initial and paired relapse samples; however, no specific, reccurrent mutation patterns were identified in this relatively small patient group. In addition to *TP53*, candidate preleukemic genes (supplemental Figure 2) were identified as they persisted during remission in patients with ALL, requiring further studies to confirm their preleukemic origin.

In conclusion, we showed that a considerable number of adult patients with ALL harbor somatic *TP53* mutations in a preleukemic compartment, are able to self-renew and clonally expand in different hematopoietic cells, and persist during remission. Therefore, the consideration of *TP53* mutations as MRD markers requires careful interpretation. The primary selection criteria in this retrospective cohort were the presence of *TP53* mutations in ALL and the availability of longitudinal MRD kinetics. Thus, valid statements regarding the frequency or clinical relevance of the preleukemic *TP53* mutation pattern in ALL are not possible. Understanding the role of preleukemic *TP53* mutations in ALL evolution and disease progression has broad clinical implications for leukemia treatment, MRD monitoring, treatment selection, and the prediction of treatment outcomes. Further studies in more homogeneous and prospective settings are needed to elucidate the clinical implications of preleukemic *TP53* mutations in ALL.

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Authorship

Contribution: G.C., C.J-K., J.B., K.P., and W.W. performed the experiments; G.C., J.B., W.W., H.T., and L.B. analyzed the results; B.K., H-H.O., M. Kotrova, F.D., M. Kelm, A-S.S., and N.D. helped with the experiments and data analysis; A.S., C.B., M.M., and C.H. provided the relevant patient information for this study; G.C., M.B., and C.H. designed the research; G.C. drafted the first version of the manuscript; and all authors discussed the results and contributed to the final manuscript.

Conflict-of-interest disclosure: M.B. is contracted to carry out research for Affimed, Amgen, Regeneron, and is a member of the advisory boards of Amgen and Incyte and the speaker bureaus of Amgen, Janssen, Pfizer, and Roche. C.D.B. is contracted to carry out research for Novartis and is a member of the advisory board of Amgen. C.H. declares partial ownership of the Munich Leukemia Laboratory. A.S., C.B., and M.M. are employed by Munich Leukemia Laboratory. P.M.A. is a consultant for CRISPR Therapeutics. The remaining authors declare no competing financial interests.

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Footnotes

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Mutation profiles of each patient may be found in a data supplement available with the online version of this article.

Data are available on reasonable request from the corresponding author, Monika Brüggemann (m.brueggemann@med2.uni-kiel.de)

The online version of this article contains a data supplement.

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