Impaired Overall Survival in Young Patients With Acute Myeloid Leukemia and Variants in Genes Predisposing for Myeloid Malignancies

Martin Kirschner1,2, Benjamin Rolles1,2, Martina Crysandt1,2, Christoph Röllig3, Friedrich Stölzel3, Michael Kramer3, Martin Bornhäuser3, Hubert Serve4, Uwe Platzbecker6, Carsten Müller-Tidow6, Kim Kricheldorf1,2, Tim H. Brümmendorf1,2, Edgar Jost1,2, Fabian Beier1,2

Correspondence: Martin Kirschner (mkirschner@ukaachen.de).

Acute myeloid leukemia (AML) is an aggressive myeloid neoplasm with a relevant subgroup evolving from inherited disorders. According to the WHO 2016 classification, the latter group is categorized as “Myeloid neoplasms with germ line predisposition (MNGLP),” comprising various syndromes based on germline mutations in genes such as \( \text{CEBPA} \), \( \text{GATA2} \), \( \text{RUNX1} \), or \( \text{SAMD9} \) as well as bone marrow failure syndromes such as telomere biology disorders (TBD).\(^1\) The increased risk of AML development at younger age is a common criteria of this category.\(^2\)

Our study focused on the incidence of TBD as a subcohort of MNGLP in younger AML patients with aberrant karyotype. TBD patients are at particularly high risk of malignant transformation both toward solid tumors and hematologic neoplasms, with the risk of MDS and AML development increased up to 2700- and 200-fold, respectively.\(^3\) The identification of classical TBD such as dyskeratosis congenita (DKC) is based on family history and the typical clinical triad (leukoplakia, nail dystrophy, abnormal skin coloring) mostly predominant in younger patients. Due to a less specific and more heterogeneous spectrum of phenotypes in adult-onset TBD, classical DKC signs are often missing and consequently, accurate diagnosis can be challenging. This together with an overall limited awareness of late-onset genetic disorders with first manifestation in adult age results in significant underdiagnosis.\(^4\) As a result, adult AML may often be the first manifestation of TBD in selected cases.\(^5\)\(^6\)

TBDs are characterized by impaired telomere maintenance eventually leading to accelerated and functionally critical telomere shortening which in return is associated with chromosomal instability.\(^1\) Therefore, AML development in TBD is supposed to be driven mostly by chromosomal aberrations resulting from telomere-mediated chromosomal fusion events or aneuploidy.\(^6\) In line with this model, AML arising from TBD is supposed to go along with an increased frequency of aberrant karyotypes probably predominantly involving chromosome arms with short telomeres.\(^7\) While the risk of AML development in TBD is known, the reverse incidence of an underlying TBD in adult patients with AML is unclear to date.\(^1\)

Based on these considerations, the present study aimed to determine the incidence of underlying TBD cases in young newly diagnosed AML patients with aberrant karyotype. Telomere length (TL) screening via PCR in nonclonal cells was performed in remission samples following induction therapy to investigate the relationship between the degree of preexistent telomere shortening and onset of AML. In order not to miss other additional MNGLP, we performed a comprehensive genetic screening for non-TBD-MNGLP in this preselected cohort.

The database of the German Study Alliance Leukemia (SAL) registry including 5207 patients with AML was screened for patients below 35 years (n = 577) fulfilling the following criteria: (1) blast-free state/remission after chemotherapy, (2) aberrant karyotype (≥3 aberrations) detected in diagnostic karyotype or FISH analysis, and (3) available samples of peripheral blood or bone marrow (Figure 1A). Detailed methods are described in the Suppl. section.

Using the screening approach mentioned above, we were able to identify 29 patients with DNA for next generation sequencing (NGS) analysis and available data for overall survival (OS) in 23 patients and. All patients have been classified as de novo AML by the treating physicians (for detailed characteristics, see Table 1).
First, we analyzed whether TL prescreening in remission samples can be used to “enrich” for patients with underlying TBD (\(n = 21\), see Supplement for details). Mean TL of all patients was not significantly shortened compared to age-adjusted controls in line with previous data.\(^8\) However, using the 1% percentile as cutoff, we found 6 patients with significantly shortened TL. NGS analysis of this subcohort revealed two variants of unknown significance (VUS) in the TBD-associated genes \(TINF2\) and \(RPA1\) according to the American College of medical genetics (Table 1). In order not to miss a TBD or other MNGLP case, we expanded...
| Patient No, n = 29 | Sex | Age | Cytogenetics | Allogenic SCT | CR After Induction | AML-Relapse | Death | Affected Genes Hereditary (ACMG class) | ACGM Criteria (Detail) | Affected Genes Somatic; Pathogenic Variants | TL Below 1st Percentile | OS (mo) |
|-------------------|-----|-----|--------------|---------------|-------------------|-------------|-------|-------------------------------------|-----------------------|------------------------------------------|----------------------|--------|
| 1                 | M   | 33  | 46.XY[7]; 46.Y(X;19)p11.2;p24,t(11;12)(p34;p13), del(12)(p11-12) | Yes           | Yes              | No           | No    | /                                   | TINF2 c.62A>G;        | PM1, PP4                                   | 92.04                | 104.73 |
| 2                 | M   | 24  | 46.XY[3]; 46.XY(del[7]q22), inc[1]; 46.XY, del[7](q22), inv(16)(p33;22), +mar, inc[1] | No            | Yes              | Yes          | Yes   | /                                   | SBDS c.258+2T>G/C    | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 4.80                 | 9.96   |
| 3                 | F   | 22  | 50.X,add(2p?), del(3)(p14), der(3)(p711), +6,mar, inc[p10] | Yes           | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 103.12               | 12.10  |
| 4                 | F   | 26  | 45.XX(6;15)(q17;1q11), der(10)(13q?)q31?q25, q11(1q11-1q25) | Yes           | Yes              | Yes          | Yes   | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 104.73               | 105.33 |
| 5                 | F   | 30  | 46.XX[6]; 47.XX, +22, +mar(16)p13q23, +mar(18)p22q24 | No            | Yes              | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 131.56               | 4.67   |
| 6                 | F   | 19  | 49.XX[2]; 49.XX, +4, +mar(22p34,q21) | Yes           | Yes              | Yes          | Yes   | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 129.06               | 131.56 |
| 7                 | M   | 33  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | Yes           | Yes              | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 18.44                | 4.53   |
| 8                 | M   | 22  | 48,XY[7]; 48,XY, +8, +9, +16, +mar(22p34,q21), +mar(22p34,q21) | Yes           | Yes              | Yes          | Yes   | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 22.12                | 22.12  |
| 9                 | F   | 27  | 48,XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 27.37                | 27.37  |
| 10                | F   | 30  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | Yes           | Yes              | Yes          | Yes   | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 22.12                | 22.12  |
| 11                | F   | 22  | 48,XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | Yes              | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 27.37                | 27.37  |
| 12                | M   | 26  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | Yes           | Yes              | Yes          | Yes   | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 22.12                | 22.12  |
| 13                | M   | 33  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 27.37                | 27.37  |
| 14                | M   | 20  | 43-47.XX, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | Yes           | Yes              | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 22.12                | 22.12  |
| 15                | M   | 24  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 27.37                | 27.37  |
| 16                | M   | 24  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | Yes           | Yes              | Yes          | Yes   | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 22.12                | 22.12  |
| 17                | M   | 32  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 27.37                | 27.37  |
| 18                | M   | 22  | 48,XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 22.12                | 22.12  |
| 19                | M   | 32  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 27.37                | 27.37  |
| 20                | M   | 32  | 46.XY, t(11;17)(q23;q25), 46.Y,idem,+i(8)(q10)x2, 47.Y,idem,+8[4] | No            | No               | No           | No    | /                                   |                       | PM1, PP2; TP5, two variants (NAP 6% and 78%) | 27.37                | 27.37  |
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Affected Genes

Table 1. (Continued)

| Patient | Sex | Age | TL | Cytogenetics | AML-Relapse | Death | CR after Induction | SCT | Allogenic | CR After | Acute Myeloid Leukemia | Percentile OS (mo) | ACMG Criteria (Detail) |
|---------|-----|-----|----|-------------|-------------|-------|-------------------|-----|-----------|-----------|------------------------|------------------|------------------------|
| 21*     | F   | 29  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 32.67            | P.M1, P.M2, P.M3, P.M5 |
| 22      | F   | 25  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 85               | P.M1, P.M2, P.M3, P.M5 |
| 23      | F   | 28  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 1.0              | P.M1, P.M2, P.M3, P.M5 |
| 24      | F   | 29  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 5.98             | P.M1, P.M2, P.M3, P.M5 |
| 25*     | M   | 28  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 23.47            | P.M1, P.M2, P.M3, P.M5 |
| 26      | F   | 28  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 22.54            | P.M1, P.M2, P.M3, P.M5 |
| 27      | M   | 29  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 31.27            | P.M1, P.M2, P.M3, P.M5 |
| 28      | F   | 29  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 23.47            | P.M1, P.M2, P.M3, P.M5 |
| 29      | F   | 29  | No | 46,XX       | Yes         | Yes    | No                | No  | No        | No        | No                     | 23.47            | P.M1, P.M2, P.M3, P.M5 |

**Table 1.** Patients with class 5 variants showed a significantly impaired OS compared to non-MNLGP patients (HR: 0.0005; 95% CI: <0.0001 to 0.029, P ≤ 0.001; Figure 1C). Relapse as a possible cause for impaired survival was reported in 50% (4/10) of our patients with detected variants. Due to the retrospective nature of the cohort, data on the causes of death were unfortunately not available.

In summary, we analyzed the proportion of patients with underlying MNLGP with a particular focus on TBD in young AML patients with aberrant karyotype. Indeed, TL prescreening was able to narrow down the cohort to 6 patients with shortened TL of whom 2 patients were eventually found to have variants in TBD-associated genes. However, it was not possible to identify the additional 3 cases identified with variants in TBD-associated genes. One reason for this limitation possibly resides in the impaired diagnostic value of telomere PCR compared to the gold standard diagnostic method flow-FISH (requiring living cells). Obviously, due to the relatively small sample size of this clinically preselected cohort, larger studies including functional testing are needed to substantiate the false positive/negative test ratio and the final diagnostic value of TL prescreening in this setting.

Few data are available about the incidence of germine predisposition in AML in general. Two recent studies in predominantly older AML patients showed a different distribution pattern of the detected variants compared to non-MNLGP patients (HR: 0.30; 95% CI 0.09-0.96, P = 0.043; Figure 1B). Patients with pathogenic variants only (n = 2) showed an even more pronounced difference in OS compared to non-MNLGP patients (HR: 0.0005; 95% CI: <0.0001 to 0.029, P ≤ 0.001; Figure 1C). Relapse as a possible cause for impaired survival was reported in 50% (4/10) of our patients with detected variants. Due to the retrospective nature of the cohort, data on the causes of death were unfortunately not available.

Interestingly, we observed a significantly impaired OS in the patients with variants in MNLGP genes compared to the rest of the cohort. This is somewhat in contrast to previous data showing a more favorable outcome at least for patients with DDX41 mutations.11

We were not able to provide detailed analysis for the causes of death in our MNLGP cohort. Patients with bone marrow failure associated MNLGP have in general an increased risk for treatment related toxicity and mortality.12 Based on the even further impaired OS of MNLGP patients with confirmed pathogenic variants, it is possible, that at least some of our identified VUS might be pathogenic thus explaining their obvious impact on OS. However, additional functional analyses are needed to further characterize these variants.

Our data support the need to develop specific screening but also treatment protocols for AML patients with underlying MNLGP to both reduce toxicity and improve response to therapy. In addition, genetic counseling of affected families is crucial and specific screening for MNLGP-related genes in HLA-identical or haploidentical family donors for allogenic HSCT is mandatory.13

Current guidelines recommend genetic screening for all MNLGP in adults only in the presence of a positive family
history or characteristic physical abnormalities. Late-onset adult MNGLP patients often present clinically with few characteristic symptoms leading to substantial underdiagnosis. In line with this finding, we were able to retrospectively confirm with the treating hematologist that the two cases with now confirmed pathogenic variants had initially been classified as de novo AML by the treating physician all located at experienced centers. Similar observations were found in a study of young adult patients with MDS where in a relevant subset of patients identified to have underlying SBDS presented with no relevant phenotype beside short body size.

Our study was limited by the retrospective nature of this analysis. Gold standard germline samples could not be obtained and had to be substituted by remission samples following induction therapy. Nevertheless, the data showed that samples in remission provide a suitable source to identify variants in MNGLP genes by NGS using the expected VAF cutoff for germline variants (see Supplement).

Given the significant difference of survival in AML patients with and without variants in MNGLP, our study clearly indicates the need of prospective screening for MNGLP in young patients with AML. Age and aberrant karyotype (potentially complemented by TL screening for TBD) might provide simple parameters to trigger genetic screening for inherited MNGLP in addition to the actual recommendations based on mere family history and clinical findings. Along this line, larger trials are needed to weigh the individual parameters and clarify the added value of TL measurement in this setting.

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AUTHOR CONTRIBUTIONS

MK performed the experiments, analyzed and interpreted the data and wrote the manuscript. MC, CR, FS, MK, HB, HS, UP, CM, CDB provided patient samples, clinical data and revised the manuscript. BR, AM, KK, MB, MV performed the experiments and analyzed the data. MWW, SSS performed the experiments and analyzed the data. THB, EJ, FB conceived and planned the study design, interpreted the data, and wrote the manuscript.

DISCLOSURES

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