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Characteristics and outcome of patients with acute myeloid leukaemia and t(8;16)(p11;p13): results from an International Collaborative Study*

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Summary

In acute myeloid leukaemia (AML) t(8;16)(p11;p13)/*MYST3–CREBBP* is a very rare abnormality. Previous small series suggested poor outcome. We report on 59 patients with t(8;16) within an international, collaborative study. Median age was 52 (range: 16–75) years. AML was *de novo* in 58%, therapy-related (t-AML) in 37% and secondary after myelodysplastic syndrome (s-AML) in 5%. Cytogenetics revealed a complex karyotype in 43%. Besides *MYST3–CREBBP*, whole-genome sequencing on a subset of 10 patients revealed recurrent mutations in *ASXL1*, *BRD3*, *FLT3*, *MLH1*, *POLG*, *TP53*, *SAMD4B* ($n = 3$, each), *EYS*, *KRTAP9-1 SPTBN5* ($n = 4$, each), *RUNX1* and *TET2* ($n = 2$, each). Complete remission after intensive chemotherapy was achieved in 84%. Median follow-up was 5·48 years; five-year survival rate was 17%. Patients with s-/t-AML ($P = 0{\cdot}01$) and those with complex karyotype ($P = 0{\cdot}04$) had an inferior prognosis. Allogeneic haematopoietic cell transplantation (allo-HCT) was performed in 21 (36%) patients, including 15 in first complete remission (CR1). Allo-HCT in CR1 significantly improved survival ($P = 0{\cdot}04$); multivariable analysis revealed that allo-HCT in CR1 was effective in *de novo* AML but not in patients with s-AML/t-AML and less in patients exhibiting a complex karyotype. In summary, outcomes of patients with t(8;16) are dismal with chemotherapy, and may be substantially improved with allo-HCT performed in CR1.

Keywords: acute myeloid leukaemia, t(8;16)(p11;p13)/*MYST3–CREBBP*, whole-genome sequencing, allogeneic haematopoietic cell transplantation, outcome.

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Introduction

In adult patients with acute myeloid leukaemia (AML) the balanced translocation t(8;16)(p11;p13) is a rare abnormality (incidence <1%) resulting in the fusion of the MYST histone acetyltransferase 3 (*MYST3*, formerly known as *MOZ* or *KAT6A*) gene on the short arm of chromosome 8 to the *CREBBP* gene on the short arm of chromosome 16.[1] Both genes encode proteins with histone acetyltransferase activity[2–4] and are involved in transcriptional regulation and cell cycle control.[4–7] Several fusion transcripts have been described that fuse exon 15 or 16 of *MYST3* to exon 3–8 to *CREBBP*. MYST3 acts as co-activator of several transcription factors related to haematopoiesis, such as RUNX1, PU.1 and NF-KB.[8,9] CREBBP is a transcriptional co-activator of some haematopoietic transcription factors,[10,11] interacts with NF-kB and p53, and has an essential role in regulating haematopoietic stem cell stemness.[6] Although the precise pathogenic pathways of t(8;16) AML are mostly unknown, one of the essential mechanisms underlying the disease may be the disruption of several haematopoietic pathways owing to the interaction of the MYST3–CREBBP chimeric protein with several key transcription factors.[13] Of note, it is currently unknown if further genetic abnormalities are complementing the molecular make-up.

Previous case reports have suggested that t(8;16) AML occurs as therapy-related AML, often with extramedullary disease and with a high rate of disseminated intravascular co-agulation, mimicking acute promyelocytic leukaemia (APL).[7] AML with t(8;16) occurs in children and adults. Surprisingly, in paediatric patients with congenital t(8;16)(p11;p13) spontaneous remissions have been observed, though the relapse rate seems to be high, suggesting a need for long-term monitoring.[14] In adults, previous small series suggested a poor outcome.[1] However, neither prospective trials nor larger retrospective cohort studies are available to support these results from small series. Particularly, it is unclear if allogeneic haematopoietic cell transplantation (allo-HCT) improves survival if applied during first complete remission (CR). The aims of our study were to characterize AML with t(8;16) and to assess outcomes according to different treatment strategies.

Methods

Patients and treatment

Information on 59 patients with AML and t(8;16) diagnosed between 1992 and 2016 was collected from eight study groups/institutions in the US and Europe. Participating centres were chosen based on network relationships of the first and last author. Detailed case report forms (including information on baseline characteristics, chemotherapy, allo-HCT, response and survival) were collected from all participating centres. Inclusion criteria were adolescent or adult patients with t(8;16) and all patients who fulfilled these criteria were included by the participating groups/institutions. Diagnosis of AML was based on French–American–British Cooperative Group criteria,[15] and, after 2003, on revised International Working Group criteria.[16] Chromosome banding was performed using standard techniques, and karyotypes were described according to the International System for Human Cytogenetic Nomenclature.[17] *FLT3* mutation screening for internal tandem duplications (ITD) and point mutations within the tyrosine kinase domain (TKD) was carried out at each institution per local practice.[18,19] Data collection and analysis were approved by the Institutional Review Boards of the participating centres.

In a cohort of ten patients with available genomic DNA from bone marrow at baseline, we performed whole-genome sequencing (WGS). Whole-genome libraries were prepared using the TruSeq Nano kit (100 ng input; Illumina, San Diego, CA, USA) following the manufacturer's recommendations. Sequencing was performed on Illumina HiSeq X machines in 150 paired-end mode. Each library was sequenced on two lanes resulting in a 60-fold minimum coverage.

Processing of whole-genome sequencing data

Whole-genome sequencing data were processed as described.[20] In brief, paired sequencing reads were mapped to the reference sequence hs37d5 from the 1000 Genomes Project (Phase II) by using bwa-mem 0.7.15.[21] Duplicate reads were marked with sambamba (v.0.5.9).[22] Single nucleotide variants (SNVs) and insertions/deletions (indels) were called using in-house pipelines based on samtools/bcftools version 0.1.19 and platypus version 0.8.1.[23] Common SNVs/indels that could be found in polymorphism databases were filtered out. This includes mutations found in dbSNP 147 with the 'COMMON=1' tag, which were rescued, however, if they had a corresponding Online Mendelian Inheritance in Man (OMIM) record. We additionally removed mutations found in the Exome Aggregation Consortium database 0.3.1 (>0·1%),[24] in the Exome Variant Server (EVS) ESP6500SI-V2 (>1%), or in our in-house control dataset (>2%, among 280 controls). Based on the Annovar annotations, only the SNVs and indels found in coding regions were selected for further analysis.

Treatment

Fifty-one of the 59 patients (86%) received intensive induction treatment either within clinical trials (n = 27) or according to local institutional standards (n = 24). Treatment protocols included the Study Alliance Leukemia (SAL) AML2003 (n = 2)[25] trial, the United Kingdom Medical Research Council (MRC) trials AML10 (n = 1),[26] AML11 (n = 2),[27] AML12 (n = 4),[26] AML15 (n = 8),[26] AML16 (n = 1)[28] and AML17 (n = 9)[29] protocols. Induction therapy consisted of the 7 + 3 regimen (n = 38) or comparable intensive treatment (n = 13). Eight patients were not eligible for

intensive induction therapy at diagnosis and received best supportive care (BSC) including one patient who was treated with the multikinase inhibitor pazopanib within a clinical trial.

Response was assessed according to International Working Group recommendations.[16] All studies were approved by the Institutional Review Boards of the participating centres. All patients provided written informed consent for participation in one of the treatment trials or for therapy according to local standards.

Statistical analyses

Survival end-points including overall survival (OS), relapse-free survival (RFS), cumulative incidence of relapse (CIR) and cumulative incidence of death in CR (CID) were defined according to the revised recommendations of the International Working Group.[16] Comparisons of patient characteristics were performed with the Wilcoxon-test for continuous variables and Fisher's exact test for categorical variables. The median follow-up time was computed using the reverse Kaplan–Meier estimate.[30] The Kaplan–Meier method was used to estimate the distribution of RFS and OS.[31] Confidence interval (CI) estimation for survival curves was based on the cumulative hazard function using Greenwood's formula for variance estimation. Log-rank tests were employed to compare survival curves between groups. The effect of allo-HCT on OS as a time-dependent intervening event was assessed by using the Mantel–Byar method[32] for univariable and the Andersen–Gill model for multivariable analyses stratified per decade (1992–1999, 2000–2009, 2010–2016).[33] The method of Simon and Makuch was used to estimate survival distributions with respect to time-dependent interventions.[34] The individuals at risk were initially all represented in the chemotherapy group. If patients received an allo-HCT, they were censored at this timepoint in the chemotherapy group and further followed up within the allo-HCT group.

CIR and CID and their standard errors were computed according to the method described by Gray[35] and included only patients attaining CR. All statistical analyses were performed within the statistical software environment R, version 3.5.1, using the R packages *rms*, version 5.1-2 and *survival*, version 2.42-3.[36]

Results

Study cohort

Overall demographic and clinical data were collected from 59 patients (MRC, $n = 25$; SAL, $n = 12$; CETLAM, $n = 8$; Czech Leukemia Centres, $n = 6$; Johns Hopkins University, Baltimore, $n = 4$; University of Maryland Greenebaum Comprehensive Cancer Center, $n = 2$; Fred Hutchinson Cancer Research Center, Seattle, $n = 1$; and Massachusetts General Hospital, Boston, $n = 1$) diagnosed with t(8;16) AML between 1992 and 2016. Baseline characteristics are summarized in Table I; median age was 52 years (range, 16–75 years) and 40 patients (68%) were female. AML was *de novo* in 34 (58%), therapy-related in 22 (t-AML; 37%) and secondary after previous myelodysplastic syndrome in three (s-AML; 5%) patients. Data on extramedullary disease were available in 31/59 (53%) patients. Of those, six had extramedullary disease (19%).

Primary diseases, previous therapy and latency period to the occurrence of t-AML

Information on the onset of the prior malignancy was available in 11 of the 22 patients (50%) with t-AML. The median latency period between diagnosis of the primary malignancy and the occurrence of t-AML was 1·6 years (range, 1–11 years). Thirteen (59%) of the 22 patients had a previous solid cancer, including two patients with two prior malignancies [breast cancer and meningioma; small-cell lung cancer and diffuse large B-cell lymphoma (DLBCL)]. Breast cancer was the most common neoplasm ($n = 7$; 54%), followed by small-cell lung cancer ($n = 2$; 15%) and Ewing sarcoma ($n = 1$; 8%) (missing data in $n = 3$; 23%). Of note, with 86% ($n = 19/22$) the association of t-AML and female gender was very high. Most of the solid tumours had been treated with chemotherapy with ($n = 3$) or without ($n = 4$) radiation (missing data in $n = 3$); radiotherapy only was applied in $n = 3$.

Eight (36%) of the 22 patients had a previous lymphoma: seven (88%) with non-Hodgkin lymphoma (DLBCL, $n = 4$; Burkitt lymphoma, $n = 1$; not classified, $n = 2$) and one (14%) with Hodgkin lymphoma. Of those, one patient received radiotherapy only and all others were treated with chemotherapy. In addition, one (5%) patient had received cytotoxic therapy for the treatment of ulcerative colitis.

Cytogenetic and molecular analyses

Cytogenetics were available in 58 (98%) patients and t(8;16)(p11;p13) was detected by fluorescence *in situ* hybridization in one (2%) patient without successful cytogenetics. The balanced translocation t(8;16) was the sole abnormality in 21 (36%) patients, while additional cytogenetic abnormalities were present in 37 (64%), most frequently within a complex karyotype ($n = 25$). In 54 (93%) of 58 patients, t(8;16) was the founding/parenteral clone and in only four (7%) patients a secondary event.

Mutational status on *NPM1* and *FLT3*-ITD were available in 59% ($n = 35/59$). None of the patients was *NPM1*-positive and only one (3%) patient had a *FLT3*-ITD. *FLT3*-TKD mutational status was available in 17 (29%) patients and seven (41%) harboured a *FLT3*-TKD mutation (Table I).

In ten AML patients with available DNA, WGS was performed. These cases included seven patients with typical *MYST3–CREBBP* fusions as well as three cases with atypical

Table I. Baseline characteristics of patients with acute myeloid leukaemia and t(8;16).

	All patients (n = 59)	Subset of patients with s-AML/t-AML (n = 25)
Median age (years) (range)	52 (16–75)	53 (20–75)
Male gender, n (%)	19 (32)	6 (24)
Median WBC, 10^9/l (range)	9·7 (1·8–235·9)	9·6 (1·8–76)
Missing	3	2
Median haemoglobin, g/dl (range)	10·0 (2·8–15·5)	9·7 (6·2–14·0)
Missing	3	2
Median platelets, 10^9/l (range)	56 (10–388)	54 (10–346)
Missing	3	2
Median BM blasts, % (range)	80 (12–100)	73·5 (24–98)
Missing	4	3
Cytogenetics, n (%)		
t(8;16) as sole abn	21 (36)	10 (42)
+ additional abn	37 (64)	14 (58)
Complex	25 (43)	9 (37·5)
Missing	1	1
Disease type, n (%)		
De novo AML	34 (58)	
s-AML	3 (5)	3 (12)
t-AML	22 (37)	22 (88)
FLT3-TKD		
n (%)	7 (41)	5 (83)
Missing	42	19

abn, aberration; allo, allogeneic; AML, acute myeloid leukaemia; BM, bone marrow; FLT3, fms-related tyrosine kinase 3; s-AML, AML after previous myelodysplastic syndrome; t-AML, therapy-related AML; TKD, tyrosine kinase domain; WBC, white blood cell count.
Results may not add-up to 100 due to rounding.

breakpoints. Altogether, additional mutations were detected in *ASXL1* (n = 3), *BCORL1* (n = 1), *BRD3* (n = 3), *CBL* (n = 1), *EYS* (n = 4), *FLT3* (n = 3), *IDH1* (n = 1), *KRTAP9-1* (n = 4), *MLH1* (n = 3), *NUP98* (n = 1), *PSIP1* (n = 1), *POLG* (n = 3), *RUNX1* (n = 2), *SAMD4B* (n = 3), *SETD2* (n = 1), *SPTBN5* (n = 4), *TET2* (n = 2), *TP53* (n = 3) and *WT1* (n = 1), suggesting a high frequency of complementing mutations. Interestingly, two of the three cases with atypical breakpoints harboured *TP53* mutations (Fig 1).

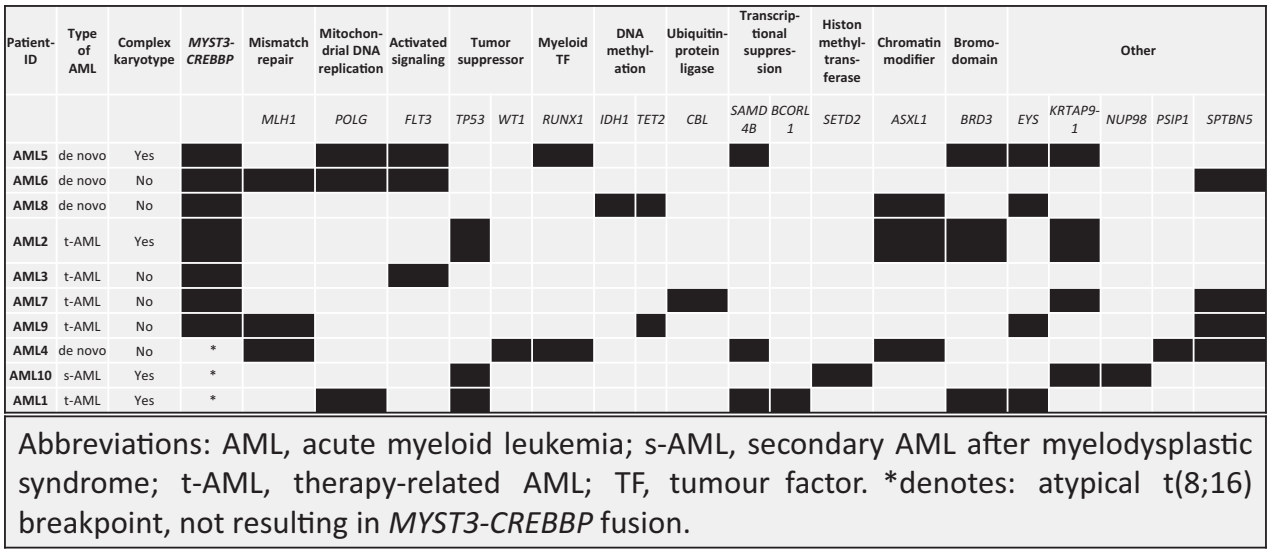

Fig 1. Oncoprint depicting the functional categorization of mutated genes as measured by whole-genome sequencing as well as type of acute myeloid leukaemia per patient.

Response to induction therapy

All patients who could not be treated intensively ($n = 8$) died early (median six days, range 1–23 days). The main reason for receiving no treatment was very early death (median one day, range, 1–6 days) due to haemorrhage/bleeding ($n = 5$; 63%). All patients, who died early were younger than 70 years (median age, 62 years; range, 51–68 years). The median age of patients with haemorrhagic *versus* non-haemorrhagic death was 62 years vs. 72 years, respectively. Disseminated intravascular co-agulation at diagnosis was present in 53% ($n = 10$/20) and contributed to early death.

Data on response to intensive induction therapy were available in $n = 51$ patients. Three patients (8%) died early after induction therapy, again one patient due to cerebral haemorrhage. CR was achieved in 43 of the 51 patients (84%), including four patients who received high-dose cytarabine as salvage therapy upon failure of first induction therapy. Five patients (10%) were refractory. In trend, CR rates were higher in younger patients (≤60 years; $n = 35$) with 90% as compared to those in older patients ($n = 8$) with 67% ($P = 0{\cdot}07$).

Further therapy including intensive consolidation and allo-HCT

Twenty-two (51%) of 43 patients in CR received intensive consolidation chemotherapy without transplantation, whereas 21 (49%) proceeded to allo-HCT. Of those, allo-HCT was performed in 15 patients in CR1 as well as in three patients in CR2. Additionally three patients were transplanted after relapse. Patients with chemo-consolidation were significantly older as compared to transplanted patients ($P = 0{\cdot}02$), whereas other variables, such as gender ($P = 0{\cdot}75$), median white blood cell (WBC) count ($P = 0{\cdot}14$), median bone marrow blasts ($P = 0{\cdot}93$) and type of disease ($P = 0{\cdot}87$) were comparable. Conditioning was myeloablative including total body irradiation in nine (43%) and dose-reduced in 12 (57%) patients. Source of donor was matched related in seven (33%), matched unrelated in nine (43%), haplo-identical in four (19%) and unknown in one (5%) of the 21 patients.

Table II. Relapse-free and overall survival according to treatment strategy in first complete remission.

	Five-year RFS (%)	95% CI	Five-year OS (%)	95% CI
Allo-HCT ($n = 15$)	26	9–74	38	17–81
Consolidation chemotherapy ($n = 24$)	7	2–28	11	4–32

allo-HCT, allogeneic haematopoietic cell transplantation; CI, confidence interval; OS, overall survival; RFS, relapse-free survival.

Survival

Of 51 patients receiving intensive therapy, $n = 41$ died. Forty-three patients achieved a first CR, of whom 28 relapsed and six experienced treatment-related mortality (four after allo-HCT and two after intensive consolidation therapy). The median follow-up of the entire cohort was 5·48 years. Median and five-year OS of the entire cohort were 6·7 months (95% CI, 5·7–13·0 months) and 17% (95% CI, 9–33%). Five-year RFS and OS were 26% (95% CI, 9–74%) and 38% (95% CI, 17–81%) in patients proceeding to allo-HCT in CR1 ($n = 15$), as compared to 7% (95% CI, 2–28%); and 11% (95 CI, 4–32%), respectively, in those who received consolidation chemotherapy alone ($n = 24$; Table II).

Survival of patients with s-/t-AML

Patients with s-AML or t-AML had a significant inferior RFS ($P = 0{\cdot}02$) and OS ($P = 0{\cdot}01$); five-year RFS and OS rates were 24% (95% CI, 12–48%) and 28% (95% CI, 16–50%) in patients with *de novo* AML as compared to 0% each in s-AML/t-AML, respectively.

Impact of complex karyotype on survival

The only additional variable with significant prognostic impact was a complex karyotype for OS ($P = 0{\cdot}04$) but not RFS ($P = 0{\cdot}50$). No prognostic impact was identified for older age (>60 years; OS, $P = 0{\cdot}23$; RFS, $P = 0{\cdot}99$), type of translocation (OS, $P = 0{\cdot}57$; RFS, $P = 0{\cdot}80$), WBC count (OS, $P = 0{\cdot}77$; RFS, $P = 0{\cdot}84$) and sex (OS, $P = 0{\cdot}16$; RFS, $P = 0{\cdot}40$).

Influence of allo-HCT on survival

The influence of allo-HCT assessed as a time-dependent covariable as post-remission therapy on OS is illustrated by a Simon–Makuch plot (Fig 2). To assess the impact of allo-HCT performed in CR1 as a time-dependent event, we performed a Mantel–Byar analysis. This analysis revealed a significant improvement of OS in patients proceeding to allo-HCT in CR1 ($P = 0{\cdot}04$).

In a multivariable Anderson–Gill model on OS including allo-HCT performed in first CR1 as a time-dependent covariable, we identified s-AML/t-AML ($P = 0{\cdot}01$), transplant status ($P = 0{\cdot}02$) as well as complex karyotype ($P = 0{\cdot}09$) as significant prognostic covariables (Table III). In subgroup analysis ($n = 16$) presence of *FLT3*-TKD had no prognostic impact on OS. In addition, Figure 3 shows a Kaplan–Meier plot on OS according to type of AML with t(8;16).

CIR and CID

In patients achieving CR1, CIR was significantly lower after allo-HCT ($n = 15$) as compared to those who were treated

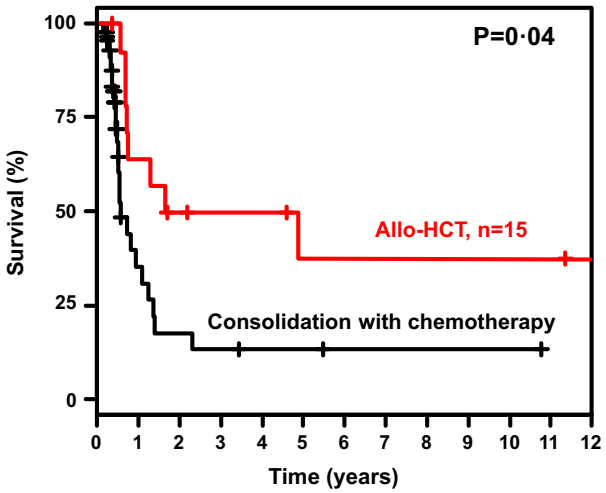

Fig 2. Simon–Makuch plot illustrating the influence of allogeneic haematopoietic cell transplantation (allo-HCT) assessed as a time-dependent covariable in first complete remission on overall survival. [Colour figure can be viewed at wileyonlinelibrary.com]

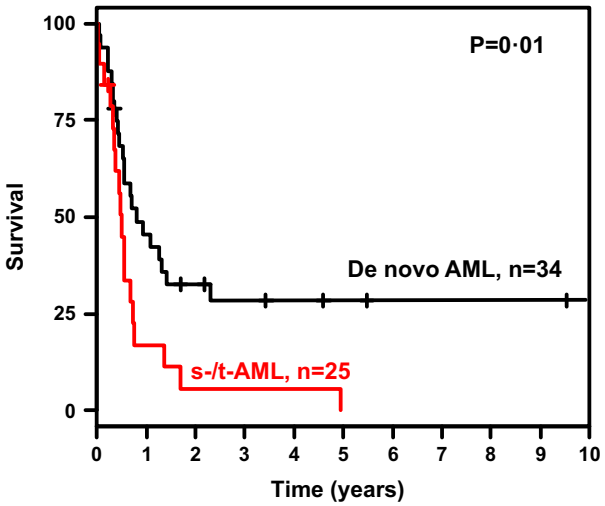

Fig 3. Overall survival according to type of acute myeloid leukaemia (AML) with t(8;16). [Colour figure can be viewed at wileyonlinelibrary.com]

Table III. Multivariable Andersen–Gill model on overall survival.

	HR (95% CI)	*P*-value
s-AML/t-AML	2·56 (1·24–5·25)	0·01
Complex karyotype	1·77 (0·91–3·47)	0·09
Transplant status	0·34 (0·13–0·87)	0·02

Analysis was stratified for decade, 1992–1999, 2000–2009, 2010–2016. AML, acute myeloid leukaemia; CI, confidence interval; HR, hazard ratio; s-AML, secondary AML after myelodysplastic syndrome; t-AML, therapy-related AML.

with consolidation chemotherapy (n = 24; P = 0·002; Fig 4, Panel A). As expected, CID was in trend higher in patients proceeding to allo-HCT as compared to those receiving consolidation chemotherapy (P = 0·06; Fig 4, Panel B).

Neither type of conditioning (P = 0·60) nor donor type (matched related donor *versus* matched unrelated/haploidentical; P = 0·40) had an impact on outcome. Patients proceeding to allo-HCT in CR1 with a typical breakpoint (n = 11) had a favourable OS at four years, whereas none of the patients (n = 4) with an atypical breakpoint survived beyond two years (Fig 5).

Discussion

The aim of our study was to better characterize adult AML patients with the rare translocation t(8;16) in the largest reported cohort study so far (n = 59) and compare outcomes according to treatment strategies.

We found a high proportion of t-AML (37%) as well as of female patients (68%). Of note, with 86% the incidence of t-AML in female patients was very high, which may be related to the widespread use of anthracycline-based chemotherapy for breast cancers. In addition, we found a high incidence of t-AML after non-Hodgkin-lymphoma,[37] particularly after treatment for DLBCL,[38] suggesting that long-time monitoring should be considered for non-Hodgkin lymphoma survivors.[38] Like other t-AMLs arising after treatment with topoisomerase-II inhibitors, t-AMLs with t(8;16) were characterized by a short latency period from the onset of the prior malignancy.[39,40] These findings suggest that there might be a higher susceptibility towards leukaemogenesis in patients with t(8;16) and that this abnormality frequently occurs therapy-related.[41–43] Indeed, we found a high frequency of mutations in clonal haematopoiesis of indeterminate potential-associated genes or MDS-related genetics, which encompass a large number of diverse mutations that cluster upon epigenetic regulation of transcription either from DNA methylation, post-translational chromatin modifications, or altered RNA splicing. This suggests a high frequency of genomic instability, leading to a high frequency of complementing mutations in patients with t(8;16).

Previous studies in AML patients with t(8;16) reported an incidence of additional abnormalities ranging from 39% to 54%.[1,14,43,44] The incidence of 64%, most frequently complex karyotypes, observed in our cohort even exceeds previously published data.[1,14,43,44] A complex karyotype, however, can frequently be found in patients with t-AML[39] and may also point to a higher degree of chromosomal instability and thus susceptibility towards leukaemogenesis. Strikingly, concurrent *FLT3*-TKD mutations were present in 41%. The high incidence of TKD mutations are a potential target for treatment with tyrosine kinase inhibitors, such as midostaurin[45] or gilteritinib[46] although we acknowledge that this finding should be interpreted with caution due to the low number of patients with available data. Additional chromosomal abnormalities had no impact on OS, whereas the presence of a

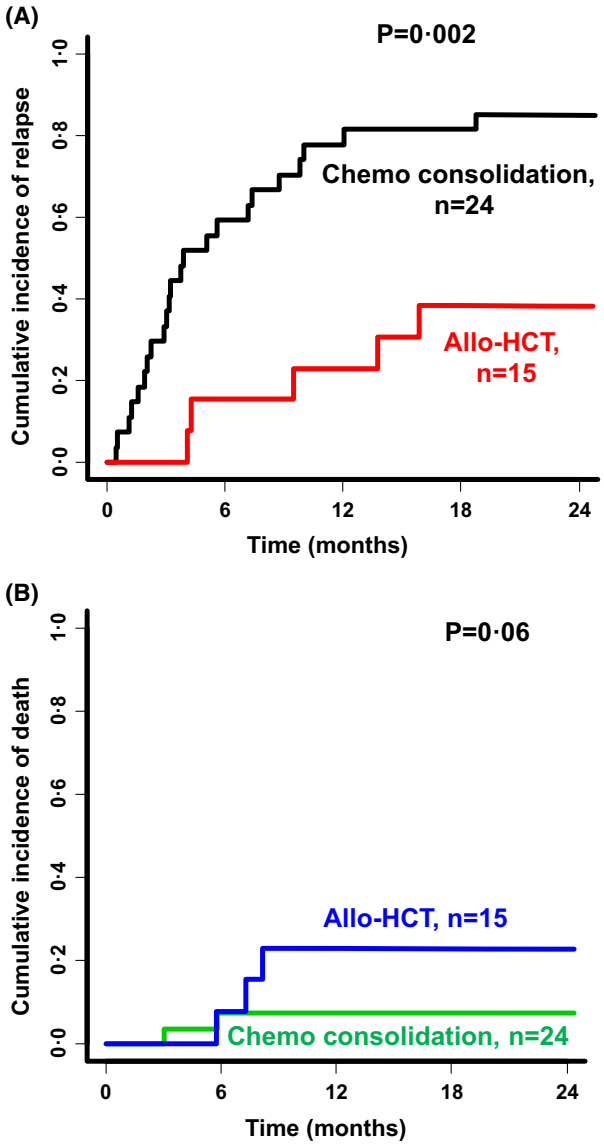

Fig 4. Cumulative incidence of relapse (CIR, Panel A) and cumulative incidence of death (CID, Panel B) according to treatment strategy. CIR and CID included only patients attaining complete remission. allo-HCT, allogeneic haematopoietic cell transplantation. [Colour figure can be viewed at wileyonlinelibrary.com]

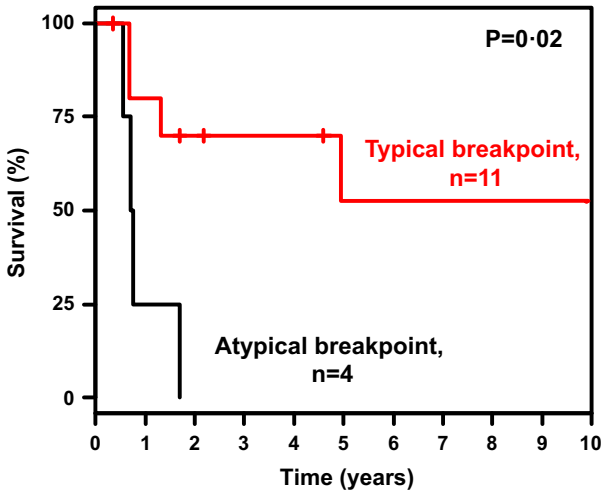

Fig 5. Impact of t(8;16) breakpoint in patients proceeding to allogeneic haematopoietic cell transplantation in first complete remission. [Colour figure can be viewed at wileyonlinelibrary.com]

complex karyotype (≥3 abnormalities) was associated with a negative impact on OS (P = 0·04).

We further addressed the question if other mutations are present that may contribute to the negative outcome of patients with a *MYST3–CREBBP* translocation. Thus, we performed WGS in a cohort of ten patients with available DNA at diagnosis. We found a large number of secondary mutations, including mutations in tumour suppressor and myeloid tumour factor genes as well as transcriptional suppressor and chromatin modifier genes. Besides well-described mutations in AML, we found mutations in *POLG*, which is responsible for mitochondrial DNA replication[47] as well as mutations in *MLH1*, which belongs to the DNA mismatch repair system and plays an important role in maintaining genomic stability.[48–52] Whereas both mutations were described to increase the risk of hereditary and sporadic cancers,[48,50,52,53] their incidence and clinical impact in AML are less well defined. Both mutations were not described by Ley *et al.*, who performed WGS or whole-exome sequencing on 200 adult, *de novo* AML patients, along with RNA and microRNA sequencing and DNA-methylation analysis.[54] In addition neither mutations in *EYS*, *KRTAP9-1*, *PSIP1* nor *SPTBN5* were described before in AML.[54] Recent publications on *MLH1* mutations, however, suggest that they may play an important role in AML,[55] particularly in the evolution of the disease.[56] Thus, it is tempting to speculate that the leukaemogenic effect of the *MYST3–CREBBP* fusion gene may rely on a deregulated modulation of downstream targets, probably due to impaired histone acetyltransferase activity of the proteins involved in this translocation[57] and that further gene mutations may contribute to the aggressive nature of the disease.

Relatively high rates of disseminated intravascular co-agulation ranging from 39% to 64% were reported previously.[14,44] Indeed, severe bleeding complications were the major cause of early death in our cohort, necessitating urgent treatment, as in APL.[58]

In contrast to previous reported data,[44] our cohort of AML patients with t(8;16) showed a high CR rate after intensive induction therapy. However, most patients relapsed rapidly and succumbed to their disease. In contrast to previously published data in neonates,[14] a spontaneous remission was not observed in any adolescent or adult patient with t(8;16)(p11;p13) in prior small series[1,44,59] or in our cohort study.

Despite allo-SCT in six patients, all patients with t-AML who achieved CR (n = 13) died, mostly due to AML relapse (n = 10) or transplant-associated mortality (n = 3),

necessitating alternative treatment strategies within clinical trials in these patients.

In contrast to previously published data on small series of AML patients with t(8;16) reporting a median OS of 4·7[1]–8·5[44] months, our data are in line with a recent publication by Xie *et al*. reporting a median OS of 18·2 months in a series of 15 patients.[59] Notably, in their analysis, OS was even higher in patients with *de novo* AML and/or non-complex karyotype.[59] In our analysis, only patients with *de novo* AML who proceeded to allo-SCT in first CR were able to achieve meaningful OS rates, suggesting that early transplant in first CR should be considered as standard of care if possible. As expected, CIR was significantly lower in our cohort after allo-HCT performed in first CR as compared to intensive consolidation chemotherapy. Since supportive care might have impacted outcome, we have included the decade of treatment in the multivariable analysis. However, this had no impact on OS. In addition, neither type of conditioning nor donor type had an impact on outcome. Nevertheless, we would like to emphasize that retrospectively collected data have several limitations since the factors for allocating patients to allo-HCT, such as comorbidities, individual assessment of the treating physician, choice of conditioning and availability of a donor remain unknown.

Conclusions

As in APL, bleeding issues are the main reason for early death. Thus, treatment and supportive measures should be started as soon as possible. Our cohort of AML patients with t(8;16) showed a high CR rate after intensive induction therapy, suggesting that these patients should be candidates for intensive induction therapy whenever possible. Despite the initial high chemo-sensitivity of the disease, treatment with consolidation chemotherapy alone resulted in dismal survival outcomes. Those patients with *de novo* AML and t(8;16) who proceeded to allo-SCT in first CR achieved encouraging OS rates, suggesting that an early transplant in first CR should be standard of care when possible for these patients. New targeted therapies, such as venetoclax in combination with hypomethyling agents,[60] may be an appealing avenue.

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Conflicts of interest

All authors declare no competing conflict of interest.

Author contributions

SK and RFS were responsible for the concept of this paper, contributed to the literature search data collection, analysed and interpreted data, and wrote the manuscript. MJL was responsible for the concept of this paper, contributed to the literature search data collection, contributed patients, analysed and interpreted data, and critically revised the manuscript. DG performed research. GW analysed and interpreted data. AS, BB, CTh and RL performed research and critically revised the manuscript. RKH, MK, FG, ZS, EHE, CS, ZR, JM, PZ, MRB, AMB, TS, PC, RBW, AKB, ADH, GE, CMT, UP, CR, JE and NHR contributed patients and critically revised the manuscript. All authors reviewed and approved the final manuscript.

References

1. Haferlach T, Kohlmann A, Klein HU, Ruckert C, Dugas M, Williams PM, et al. AML with translocation t(8;16)(p11;p13) demonstrates unique cytomorphological, cytogenetic, molecular and prognostic features. *Leukemia*. 2009;**23**:934–43.
2. Borrow J, Stanton VP, Andresen JM, Becher R, Behm FG, Chaganti RS, et al. The translocation t(8;16)(p11;p13) of acute myeloid leukemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat Genet*. 1996;**14**:33–41.
3. Champagne N, Pelletier N, Yang X-J. The monocytic leukemia zinc finger protein MOZ is a histone acetyltransferase. *Oncogene*. 2001;**20**:404–9.
4. Jacobson S, Pillus L. Modifying chromatin and concepts of cancer. *Curr Opin Genet Dev*. 1999;**9**:175–84.
5. Allard S, Masson J-Y, Coté J. Chromatin remodeling and the maintenance of genome integrity. *Biochim Biophys Acta*. 2004;**1677**:158–64.
6. Camos M, Esteve J, Jares P, Colomer D, Rozman M, Villamor N, et al. Gene expression profiling of acute myeloid leukemia with translocation t (8;16)(p11;p13) and MYST3-CREBBP rearrangement reveals a distinctive signature with a specific pattern of HOX gene expression. *Cancer Res*. 2006;**66**:6947–54.
7. Gervais C, Murati A, Helias C, Struski S, Eischen A, Lippert E, et al. Acute myeloid leukaemia with 8p11 (MYST3) rearrangement: an integrated cytologic, cytogenetic and molecular study by the groupe francophone de cytogénétique hématologique. *Leukemia*. 2008;**22**:1567–75.
8. Yang XJ, Ullah M. MOZ and MORF, two large MYSTic HATs in normal and cancer stem cells. *Oncogene*. 2007;**26**:5408–19.
9. Chan EM, Chan RJ, Comer EM, Goulet RJ 3rd, Crean CD, Brown ZD, et al. MOZ and MOZ-CBP cooperate with NF-kappaB to activate transcription from NF-kappaBdependent promoters. *Exp Hematol*. 2007;**35**:1782–92.
10. Vo N, Goodman RH. CREB-binding protein and p300 in transcriptional regulation. *J Biol Chem*. 2001;**276**:13505–8.
11. Blobel GA. CREB-binding protein and p300: molecular integrators of hematopoietic transcription. *Blood*. 2000;**95**:745–55.
12. Wadgaonkar R, Phelps KM, Haque Z, Williams AJ, Silverman ES, Collins T. CREB-binding protein is a nuclear integrator of nuclear factor-kappaB and p53 signaling. *J Biol Chem*. 1999;**274**:1879–82.
13. Chen J, Odenike O, Rowley JD. Leukaemogenesis: more than mutant genes. *Nat Rev Cancer*. 2010;**10**:23–36.
14. Coenen EA, Zwaan CM, Reinhardt D, Harrison CJ, Haas OA, de Haas V, et al. Pediatric acute myeloid leukemia with t(8;16)(p11;p13), a distinct

clinical and biological entity: a collaborative study by the International-Berlin-Frankfurt-Münster AML-study group. *Blood*. 2013;**122**:2704–13.
15. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. a report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;**103**:620–5.
16. Cheson BD, Bennett JM, Kopecky KJ, Büchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, And Reporting Standards For Therapeutic Trials In Acute Myeloid Leukemia. *J Clin Oncol*. 2003;**21**:4642–9.
17. Mitelman F. ISCN: An international system for human cytogenetic nomenclature. Basel, Switzerland: S. Karger, 1995.
18. Yokota S, Kiyoi H, Nakao M, Iwai T, Misawa S, Okuda T, et al. Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome among various hematological malignancies. A study on a large series of patients and cell lines. *Leukemia*. 1997;**11**:1605–9.
19. Thiede C, Steudel C, Mohr B, Schaich M, Schäkel U, Platzbecker U, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002;**99**:4326–35.
20. Jabs J, Zickgraf FM, Park J, Wagner S, Jiang X, Jechow K, et al. Screening drug effects in patient-derived cancer cells links organoid responses to genome alterations. *Mol Syst Biol*. 2017;**13**(11):955.
21. Abecasis GRA, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;**491**(7422):56–65.
22. Tarasov A, Vilella AJ, Cuppen E, Nijman IJ, Prins P. Sambamba: fast processing of NGS alignment formats. *Bioinformatics*. 2015;**31**(12):2032–4.
23. Jones DTW, Jäger N, Kool M, Zichner T, Hutter B, Sultan M, et al. Dissecting the genomic complexity underlying medulloblastoma. *Nature*. 2012;**488**(7409):100–5.
24. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;**536**(7616):285–91.
25. Schaich M, Parmentier S, Kramer M, Illmer T, Stölzel F, Röllig C, et al. High-dose cytarabine consolidation with or without additional amsacrine and mitoxantrone in acute myeloid leukemia: results of the prospective randomized AML2003 trial. *J Clin Oncol*. 2013;**31**:2094–102.
26. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;**116**:354–65.
27. Wheatley K, Brookes CL, Howman AJ, Goldstone AH, Milligan DW, Prentice AG, et al. Prognostic factor analysis of the survival of elderly patients with AML in the MRC AML11 and LRF AML14 trials. *Br J Haematol*. 2009;**145**:598–605.
28. Burnett AK, Russell NH, Culligan D, Cavanagh J, Kell J, Wheatley K, et al. The addition of the farnesyl transferase inhibitor, tipifarnib, to low dose cytarabine does not improve outcome for older patients with AML. *Br J Haematol*. 2012;**158**:519–22.
29. Burnett AK, Russell NH, Hills RK, Kell J, Cavenagh J, Kjeldsen L, et al. A randomized comparison of daunorubicin 90 mg/m^2 vs 60 mg/m^2 in AML induction: results from the UK NCRI AML17 trial in 1206 patients. *Blood*. 2015;**125**:3878–85.
30. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials*. 1996;**17**:343–6.
31. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;**53**:457–81.
32. Mantel N, Byar D. Evaluation of response-time data involving transient states: an illustration using heart transplant data. *J Am Stat Assoc*. 1974;**69**:81–6.
33. Andersen P, Gill RD. Cox's regression model for counting processes: a large sample study. *Ann Stat*. 1982;**10**:1100–20.
34. Simon R, Makuch RW. A non-parametric graphical representation of the relationship between survival and the occurrence of an event: application to responder versus non-responder bias. *Stat Med*. 1984;**3**:35–44.
35. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;**16**:1141–54.
36. R Development Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2014.
37. Gibson CJ, Lindsley RC, Tchekmedyian V, Mar BG, Shi J, Jaiswal S, et al. Hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. *J Clin Oncol*. 2017;**35**:1598–605.
38. Bari A, Marcheselli L, Marcheselli R, Liardo EV, Pozzi S, Sacchi S, et al. Therapy-related myeloid neoplasm in non-Hodgkin lymphoma survivors. *Mediterr J Hematol Infect Dis*. 2011;**3**:e2011065.
39. Kayser S, Döhner K, Krauter J, Köhne C-H, Horst HA, Held G, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. *Blood*. 2011;**117**:2137–45.
40. Kayser S, Krzykalla J, Elliott MA, Norsworthy K, Gonzales P, Hills RK, et al. Characteristics and outcome of patients with therapy-related acute promyelocytic leukemia front-line treated with or without arsenic trioxide. *Leukemia*. 2017;**31**:2347–54.
41. Takahashi K, Wang F, Kantarjian H, Doss D, Khanna K, Thompson E, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *Lancet Oncol*. 2017;**18**:100–11.
42. Gillis NK, Ball M, Zhang Q, Ma Z, Zhao YL, Yoder SJ, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. *Lancet Oncol*. 2017;**18**:112–21.
43. Quesnel B, Kantarjian H, Bjergaard JP, Brault P, Estey E, Lai JL, et al. Therapy-related acute myeloid leukemia with t(8;21), inv(16), and t(8;16): a report on 25 cases and review of the literature. *J Clin Oncol*. 1993;**11**:2370–9.
44. Diab A, Zickl L, Abdel-Wahab O, Jhanwar S, Gulam MA, Panageas KS, et al. Acute myeloid leukemia with translocation t(8;16) presents with features which mimic acute promyelocytic leukemia and is associated with poor prognosis. *Leuk Res*. 2013;**37**:32–6.
45. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. *N Engl J Med*. 2017;**377**:454–64.
46. Perl AE, Altman JK, Cortes J, Smith C, Litzow M, Baer MR, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1–2 study. *Lancet Oncol*. 2017;**18**:1061–75.
47. Yehudai D, Liyanage SU, Hurren R, Rizoska B, Albertella M, Gronda M, et al. The thymidine dideoxynucleoside analog, alovudine, inhibits the mitochondrial DNA polymerase γ, impairs oxidative phosphorylation and promotes monocytic differentiation in acute myeloid leukemia. *Haematologica*. 2019;**104**:963–72.
48. Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. *Curr Opin Genet Dev*. 1999;**9**:89–96.
49. Kunkel TA, Erie DA. DNA mismatch repair. *Annu Rev Biochem*. 2005;**74**:681–710.
50. Modrich P, Lahue R. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu Rev Biochem*. 1996;**65**:101–33.
51. Li GM. The role of mismatch repair in DNA damage-induced apoptosis. *Oncol Res*. 1999;**11**:393–400.
52. Li GM. Mechanisms and functions of DNA mismatch repair. *Cell Res*. 2008;**18**:85–98.
53. Tervasmäki A, Mantere T, Hartikainen JM, Kauppila S, Lee HM, Koivuluoma S, et al. Rare missense mutations in RECQL and POLG associate with inherited predisposition to breast cancer. *Int J Cancer*. 2018;**1**(142):2286–92.
54. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;**368**:2059–74.
55. Yu G, Yin C, Wu F, Jiang L, Zheng Z, Xu D, et al. Gene mutation profile and risk stratification in AML1-ETO-positive acute myeloid leukemia based on next-generation sequencing. *Oncol Rep*. 2019;**42**:2333–44.

56. Mao G, Yuan F, Absher K, Jennings CD, Howard DS, Jordan CT, et al. Preferential loss of mismatch repair function in refractory and relapsed acute myeloid leukemia: potential contribution to AML progression. *Cell Res*. 2008;**18**:281–9.
57. Kitabayashi I, Aikawa Y, Nguyen LA, Yokoyama A, Ohki M. Activation of AML1-mediated transcription by MOZ and inhibition by the MOZ-CBP fusion protein. *EMBO J*. 2001;**20**:7184–96.
58. Kayser S, Schlenk RF, Platzbecker U. Management of patients with acute promyelocytic leukemia. *Leukemia*. 2018;**32**:1277–94.
59. Xie W, Hu S, Xu J, Chen Z, Medeiros LJ, Tang G. Acute myeloid leukemia with t(8;16)(p11.2;p13.3)/KAT6A-CREBBP in adults. *Ann Hematol*. 2019;**98**:1149–57.
60. DiNardo CD, Pratz K, Pullarkat V, Jonas BA, Arellano M, Becker PS, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. *Blood*. 2019;**133**:7–17.

