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LETTER TO THE EDITORS

Impact of 9q deletions on the classification of patients with acute myeloid leukemia

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Deletions in the long arm of chromosome 9, del(9q), are recurrent but rare cytogenetic aberrations in myeloid neoplasms including acute myeloid leukemia (AML), where they were observed with a frequency of ~ 2% (Langabeer et al. 1998; Grimwade et al. 2010; Naarmann-de Vries IS et al. 2018). Del(9q) is considered as marker of intermediate risk according to the MRC classification (Grimwade et al. 2010; Döhner et al. 2010, Grimwade et al. 2016). Cytogenetically, del(9q) can be observed as a sole abnormality or in association with other cytogenetic aberrations. In more detail, a significant association with t(8;21)(q22;q22) (*RUNX1-RUNX1T1* rearrangement) and t(15;17)(q24;q21) (*PML-RARA* rearrangement) was described (Langabeer et al. 1998; Döhner et al. 2010). Moreover, AML with del(9q) was characterized by frequent mutations of *NPM1*, *DNMT3A*, *CEBPA* and *WT1*, and mutations affecting *NPM1* and *DNMT3A* were exclusively identified in patients with del(9q) as the sole abnormality (Fröhling et al. 2005; Herold et al. 2017). A minimally deleted region of del(9q) was detected in patients with AML that comprises seven genes potentially involved in leukemogenesis (*GKAP1*, *KIF27*, *C9ORF64*, *HNRNPK*, *RMI1*, *SLC28A3* and *NTRK2*). Expression of these genes was found to be significantly reduced in patients with del(9q) compared to AML patients with normal karyotype (Naarmann-de Vries IS et al. 2018). Moreover, two genes closely related to the commonly deleted region of del(9q) (*TLE1* and *TLE4*) were identified to contribute to leukemogenesis due to haploinsufficiency in patients with t(8;21)(q22;q22) (Dayyani et al. 2008). In the 2016 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia, del(9q) was excluded as a defining cytogenetic abnormality for AML with myelodysplasia-related changes due to its frequent association with *NPM1* and biallelic *CEBPA* mutations, which themselves define AML subgroups (Arber et al. 2016). Thus, the aim of the present project was the investigation of the frequency of del(9q) in AML and its accompanying molecular and cytogenetic abnormalities and assessment whether or not del(9q) is associated with a myelodysplasia-related mutation profile.

We evaluated 9762 AML patients for which bone marrow and/or peripheral blood samples had been sent for diagnosis to the MLL Munich Leukemia Laboratory between 2005 and 2017. Detection of del(9q) was performed using chromosome banding analysis (CBA) as previously described according to standard methods (Schoch et al. 2002). Patients agreed with the use of laboratory data for research studies. The study followed the rules of the Helsinki Declaration. Patients with del(9q) were further analyzed for mutations in *NPM1*, *CEBPA* and *RUNX1* with amplicon next-sequencing (NGS) to categorize them according to the WHO classification. Patients without a class defining aberration or a complex aberrant karyotype were screened for AML- or MDS-related mutations *(ASXL1*, *BCOR*, *DNMT3A*, *EZH2*, *FLT3*-ITD, *FLT3*-TKD, *IDH1*, *IDH2*, *KMT2A*-PTD, *KRAS*, *NRAS*, *SF3B1*, *SRSF2*, *STAG2*, *TET2*, *TP53*, *U2AF1*, *WT1* and *ZRSR2)*. The template library was generated with the TruSeq Custom Amplicon Low Input Kit and sequenced with the NextSeq (Illumina, San Diego, CA; sensitivity: 3%). NGS data were analyzed using the Sequence Pilot (version 4.1.1 Build 510 for the Illumina platform, JSI Medical systems, Kippenheim, Germany). SPSS (version 19.0.0) software (IBM Corporation, Armonk, NY) was used for

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statistical analysis. All reported p values are two-sided and were considered significant at $p \leq 0.05$. Variants of unknown significance were excluded from statistical analysis. The size of the deleted region on chromosome 9 was evaluated by genomic arrays (SurePrint G3 ISCA CGH + SNP Microarray, Agilent, Waldbronn, Germany). Images were analyzed using the DEVA Software v1.2.1 (Roche Nimblegen) and Nexus Copy Number 6.1 (Biodiscovery, Inc., El Segundo, CA). Aberrations were evaluated in each sample using BioDiscovery's Fast Adaptive States Segmentation Technique (FASST2) algorithm. Furthermore, *NUP98*-rearrangements were investigated by fluorescence in situ hybridization (FISH) analysis using probes for *NUP98* (MetaSystems, Altlussheim, Germany).

In 114/9762 patients (1.2%) a del(9q) was detected by CBA, which is in line with previous results describing a frequency of ~ 2% in AML patients (Naarmann-de Vries IS, 2018). Our cohort with del(9q) comprised 60 male and 54 female patients, median age was 63 years (range 16–90 years). Del(9q) was accompanied by the following rearrangements: *RUNX1-RUNX1T1* rearrangement in 20/114 patients (18%), *PML-RARA* in 5/114 patients (4%), *KMT2A* in 2/114 patients (2%) and *MECOM* in 1/114 patients (1%). In 20/114 patients (18%) a *NPM1* mutation and in 7/114 patients (6%) a biallelic *CEBPA* mutation was observed. Furthermore, *RUNX1* mutations were found in 3/114 patients (3%). The remaining 56 patients (49%) were divided into the following three groups: patients with del(9q) as a sole cytogenetic abnormality (del(9q) sole, 8/114 patients, 7%), patients with one or two additional cytogenetic aberrations (del(9q) other, 19/114 patients, 17%) and patients with complex aberrant karyotype (complex, 29/114 patients, 20%) harboring 4–29 cytogenetic aberrations (median: 8). Moreover, two patients with del(9q) showed AML specific rearrangements (t(8;16)(p11;p13) and t(3;5)(q25;q35)). Cases with del(9q) sole and other ($n = 27$) were further characterized by FISH analysis using probes for *NUP98*, which revealed a *NUP98*-rearrangement in 5/27 patients (19%) in the del(9q) sole and other group. *NSD1* located on the long arm of chromosome 5 was identified as the partner gene in 3 of these patients, leading to the translocation t(5;11)(q35;p15). In the remaining two cases, the translocations t(9;11)(q34;p15) and t(11;20)(p15;q11) were observed. Mutation frequencies revealed as most frequently mutated genes in del(9q) sole and del(9q) other: *FLT3*-ITD (8/25 patients, 32%), *TET2* (7/27, 26%), *NRAS* (5/25, 20%), *KRAS* (5/27, 19%), *FLT3*-TKD (4/27, 15%) and *WT1* (3/26, 11%) (Fig. 1). To identify whether patients with del(9q) sole and other resemble a molecular mutation profile typical for secondary AML arising from MDS (s-AML), the presence of gene mutations related to s-AML was evaluated, as mutations in *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, or *STAG2* were found to be > 95% specific for the diagnosis of s-AML (Lindsley, et al. 2015). 9/25 patients (36%) had a mutation in at least one of these genes, whereas the majority of 16/25 patients (64%) showed no such mutation. Determination of the size and localization of del(9q) by genomic arrays showed that in patients with del(9q) sole the deletion size ranged from 14 to 42 Mb (median: 28 Mb), in patients with del(9q) other from 19 to 70 Mb (median: 32 Mb). A minimal deleted region for patients with del(9q) sole and other could not be determined for all patients due to the strong variation between the respective deleted regions. For investigation of the prognostic impact of del(9q) sole and del(9q) other, follow-up data were available for 75 patients with a median follow-up of 27 months (Fig. 2). The median overall survival (OS) for patients with *RUNX1-RUNX1T1*, *NPM1* and biallelic *CEBPA* mutation was not reached. It was 73 months for patients with del(9q) sole and del(9q) other and 16 months for patients with

Fig. 1 Summary of mutations detected in patients with 9q deletion (sole and other). The mutation status of each gene (lines) is depicted for the cohort of 27 cases with del(9q) sole and del(9q) other, each column represents one patient. The genes marked with an asterisk are s-AML-related (according to Lindsley et al. 2015). Red: mutated, grey: wild-type, orange: variant of unknown significance, white: not analyzed

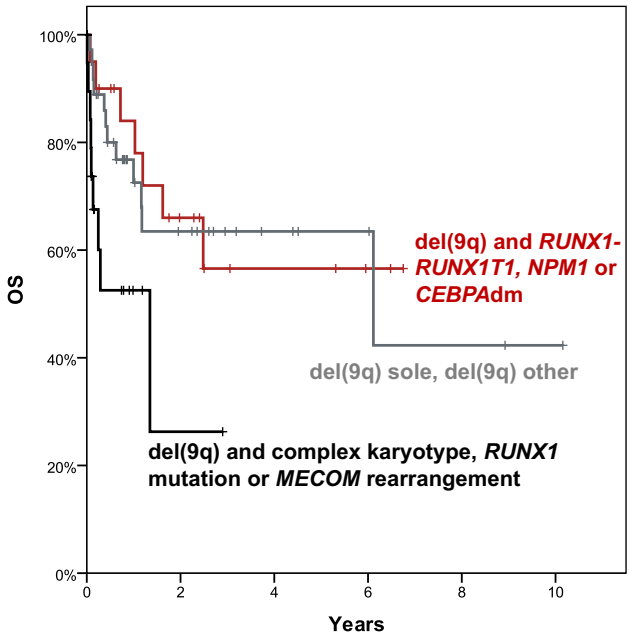

Fig. 2 Overall survival (OS) in patients with 9q deletion according to the absence or presence of additional abnormalities. Grey line: cases with del(9q) sole and del(9q) other ($n=20$); red line: cases with del(9q) and *RUNX1-RUNX1T1*, *NPM1* or *CEBPA*dm (double mutation) ($n=36$); black line: cases with del(9q) and complex karyotype, *RUNX1* mutations or *MECOM* rearrangement ($n=19$)

complex karyotype, *RUNX1* mutations or *MECOM* rearrangements. The OS of first two groups did not differ significantly, but was significantly distinct from the third group ($p=0.03$ and 0.03, respectively).

In summary, in 1.2% of AML patients a del(9q) was present and del(9) frequently co-occurred with *RUNX1-RUNX1T1*, biallelic *CEBPA* and *NPM1* mutations, *NUP98*-rearrangements and other AML-typical translocations. Moreover, a mutation signature typical for s-AML was rather infrequent. Thus, it seems reasonable that the del(9q) is no longer regarded as a defining cytogenetic abnormality for AML with myelodysplasia-related changes, in particular, as prognosis in del(9q) cases with non-complex karyotype is favorable.

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Compliance with ethical standards

Conflict of interest CH, WK and TH declare part ownership of Munich Leukemia Laboratory (MLL). BB, AS and MM are employed by the MLL Munich Leukemia Laboratory.

Informed consent Informed consent was obtained from all individual participants included in the study.

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