Molecular genetic characterization of myeloid/lymphoid neoplasms associated with eosinophilia and rearrangement of *PDGFRA, PDGFRB, FGFR1* or *PCM1-JAK2*

Myeloid or lymphoid neoplasms associated with eosinophilia (MLN-Eo) are a clinically heterogeneous class of hematologic diseases but present with a limited number of characteristic fusion genes. While the underlying chromosomal aberrations are well characterized, we extended the genetic analysis of MLN-Eo to mutation screening and show that 23% (14/61) of patients carry at least one mutation in addition to the World Health Organization (WHO) defining genetic changes and that *RUNX1* mutations were recurrently found.

The WHO classification defines MLN-Eo if a genetic rearrangement affects *PDGFRA-, PDGFRB- or FGFR1*.[1,2] Cases with *PCM1-JAK2*[3] are included as provisional entities.[1] The presence of the rearrangements are essential to the current classification, whereas eosinophilia is frequently observed, but not a prerequisite for diagnosis.[1] That emphasizes the role of *PDGFRA-, PDGFRB-, FGFR1* and *PCM1-JAK2* rearrangements as drivers of the malignant development and attractive drug targets. The latter has been impressively demonstrated for tyrosine kinase inhibitors (TKI) in patients with rearrangements involving *PDGFRA* and *PDGFRB*. For *PCM1-JAK2* patients, ruxolitinib has shown efficacy. Besides the detection of the rearrangements, risk-adapted therapy requires morphological, histopathological and clinical evaluation. Patients with *FGFR1* rearrangements have the most aggressive course and can present with acute myeloid leukemia and T-cell or B-cell lymphoblastic leukemia/lymphoma. Treatment could therefore require intensive chemotherapy or allogeneic transplantation.[2,4]

Many hematologic malignancies have been extensively characterized on a molecular genetic basis over the last years, which has unraveled a large number of typically mutated genes and a deeper understanding of disease development or targeted treatment options. A study by Pardanani *et al.* on ten patients with *FIP1L1-PDGFRA* indicated that somatic mutations also exist in MLN-Eo.[5] The goal of our study was to extend the molecular characterization to a larger cohort and to include patients with all four types of genetic rearrangements. Furthermore, we aimed to use follow-up samples in order to get a more detailed view of the clonal relationship between genetic rearrangements and somatic mutations and their sequential development.

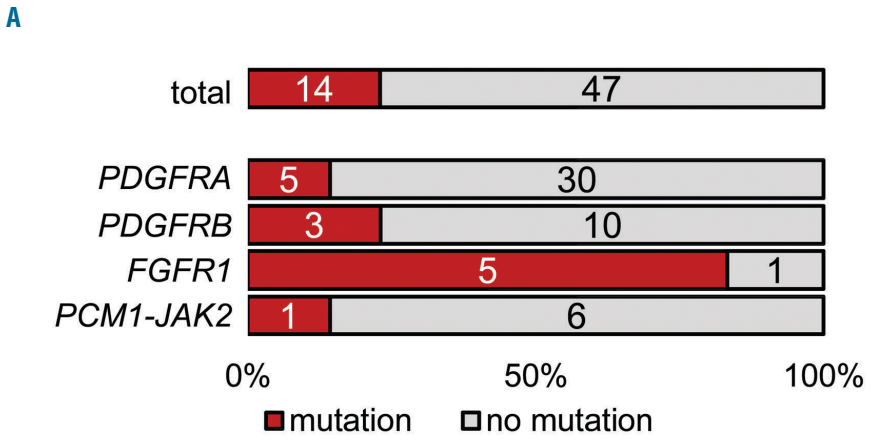

Figure 1. MLN-Eo patients with mutations. (A) Given is the percentage of patients with one or more pathogenic variant in each rearrangement group. Variants of uncertain significance (VUS) are excluded. (B) Genes with a mutation are shown in red, VUS in orange (gray: unmutated; white: not analyzed). Vertical columns are individual patients with *PDGFRA*- (green), *PDGFRB*- (blue), *FGFR1*- (brown) and *PCM1-JAK2* (purple) rearrangement.

We received material from 61 patients, either at initial diagnosis or at a treatment naïve time point between March 2006 and December 2016, for routine diagnostic assessment including detection of the typical genetic rearrangements by fluorescent *in situ* hybridization (FISH), chromosome banding and/or reverse transcriptase polymerase chain reaction (RT-PCR).[3,6-8] The different techniques used for genetic analysis are a consequence of the heterogeneous genetic aberration types (translocations and deletions).[4] FISH analysis can prove the involvement of *PDGFRA, PDGFRB* and *FGFR1*, however, the identification of the exact fusion partner could require e.g., individual RT-PCRs or novel sequencing approaches.[9] All four types of rearrangements were included: *PDGFRA* (n=35), *PDGFRB* (n=13), *FGFR1* (n=6) and *PCM1-JAK2* (n=7). Our cohort had a male predominance (56 males, 5 females), a broad age range (median: 52; range: 23-78 years), and a median of 39% (range: 1-87%) eosinophils in the peripheral blood (data on eosinophils available for n=45). Eosinophilia was not a prerequisite for inclusion. Follow-up data was available for 55 cases. In accordance with the Declaration of Helsinki, patients gave written informed consent for genetic analysis and research studies. The study herein was approved by the Munich Leukemia Laboratory (MLL) institutional review board. Further patient information, including the final diagnosis given at the time of diagnosis, can be found in *Online Supplementary Table S1* and *Online Supplementary Table S2*.

DNA and ribonucleic acid (RNA) was isolated from peripheral blood or bone marrow samples according to standard protocols.[10] We analyzed 48 genes using TruSeq Custom Amplicon library preparation (Illumina, San Diego, CA, USA). The panel included genes which are frequently mutated in myeloid malignancies, and genes which are described in lymphatic malignancies (*Online Supplementary Table S3*). Panels were sequenced on the NextSeq or MiSeq platform (Illumina). Alignment and variant calling was performed by SeqNext 4.3 (JSI Medical Systems, Kippenheim, Germany) with a 3% detection limit.

In addition to *PDGFRA-, PDGFRB-, FGFR1-* and *PCM1-JAK2* rearrangements, 24 non-synonymous genetic variants were detected (*Online Supplementary Table S4*). Of these, protein truncating variants (PTV) were defined as somatic/pathogenic. Non-PTVs were only counted as pathogenic if they occurred at functionally characterized positions or were well annotated in the COSMIC or ClinVAR database (several submissions) and no opposing classifications could be made by dbSNP or ExAC databases. Other non-PTVs were classified as variants of uncertain significance (VUS). VUS (n=5) were excluded from further analysis.

Pathogenic mutations were detected in 14 of 61 patients (23%), and multiple mutations were found in three cases. Patients with mutations were significantly older (mean: 60 *vs.* 51 years; P=0.03). Within the largest subgroups (*PDGFRA, PDGFRB*) no significant effect on event-free survival (EFS) was observed for mutations (*Online Supplementary Figure S1A*). Six patients had shown the morphology of an acute leukemia (*Online Supplementary Table S2*). There was no significant differences in mutation frequencies compared to samples, which were initially classified as hypereosinophilic syndrome (HES) or myeloproliferative neoplasms (MPN) (*Online Supplementary Figure S2*). We observed no increased mutation frequency with a higher degree of eosinophilia, and no association of mutations with white blood cell counts, thrombocyte counts or hemoglobin levels (*data not shown*). However patients with *FGFR1* rearrangements had a significantly higher frequency of mutations in comparison to the three other groups (P=0.001, χ^2 test). Mutation rates were 14% for the *PDGFRA-* (5/35), 23% for the *PDGFRB-* (3/13), 83% for the *FGFR1-* (5/6) and 14% for the *PCM1-JAK2* (1/7) subgroup (Figure 1A). For *PDGFRA* rearrangements, somatic KIT and CSF3R mutations are known from previous work on ten samples,[5] which we did not find herein. While a previous study of three *PDGFRB* rearranged cases[11] did not show any mutation, we identified mutated cases. Both discrepancies could be the consequence of small cohort sizes and a heterogeneous disease group.

The only gene which was recurrently mutated was *RUNX1* (6/19 mutations, 32%). All mutations in the *FGFR1* rearranged group were *RUNX1* mutations (Figure 1B). Among the other patients, only one *RUNX1* mutation was found in a *PDGFRA* patient (P<0.001, χ^2 test). Until now, one case was reported in 2013 with *ZMYM2-FGFR1* and also a *RUNX1* mutation detected by Sanger sequencing.[12] In addition, work from 2015 identified a patient with *FGFR1* rearrangement and a second translocation, which involved the *RUNX1* gene.[13]

The other mutations did not show a similar recurrence: *ETV6, NRAS, STAT5B* and *ZRSR2* were mutated only

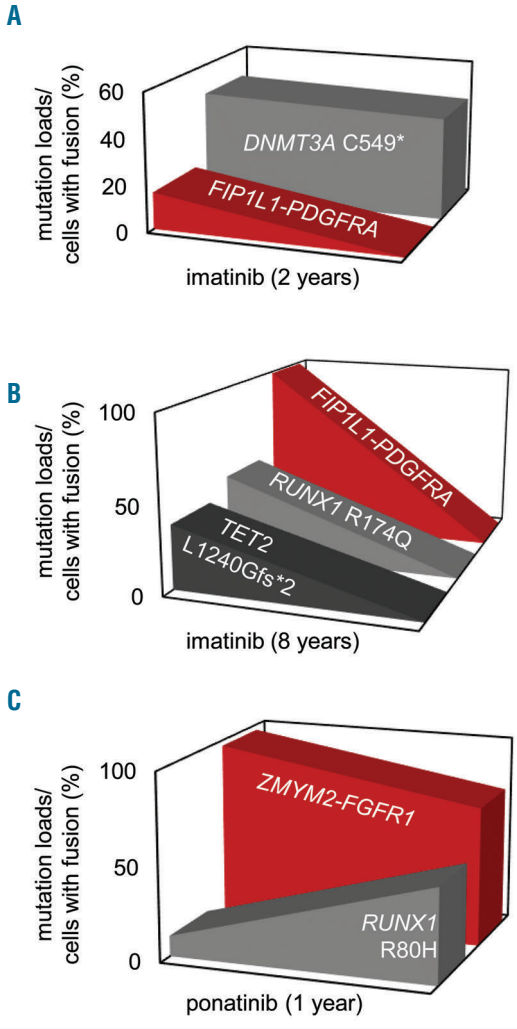

Figure 2. Follow-up of mutation loads and cells with the fusion gene. Shown are examples of different clonal developments. Mutation loads (mutated/all reads in %) are indicated as gray areas. Cell number of cells with the fusion gene (by FISH or qPCR for negative results) are displayed in red.

once. However, nine of 19 mutations (47%) were found in the functional group of epigenetic regulators (*ASXL1*, *BCOR, DNMT3A* and *TET2*, Figure 1B). Epigenetic regulators are frequently mutated in a broad set of other hematological diseases, but especially in myeloid malignancies. Of note, *ASXL1, DNMT3A* and *TET2* are also mutated in older individuals without a hematologic malignancy, which was recently described as clonal hematopoiesis of indeterminate potential (CHIP).

Our next goal was the identification of clonal development. Therefore, the mutated positions were sequenced in available treatment samples (n=8). Follow-up monitoring for MLN-Eo was performed by FISH, chromosome banding analysis or *PDGFRB* expression testing by quantitative PCR (primers: CGTCAAGATGCTTAAATCCACAGC, TGATGATATAGATGGGTCCTCCTTTG, probes: 5'-GCTGAAGATCATGAGTCACCTTGGGC-fluorescein 3', 5' LCRed 640-CCACCTGAACGTGGTCAA-CCTGTTG-Pho 3'). Complete molecular remission (CR) was assessed by negative RT-PCR. We observed different types of clonal development. Type A (*Online Supplementary Table S5*) in cases with CR of *FIP1L1-PDGFRA* (n=3), the mutations (*DNMT3A*, n=2; *TET2*, n=1) were detectable at levels comparable to the initial diagnosis. Type A represents mutations that developed prior to or independently of the *FIP1L1-PDGFRA* rearrangement (Figure 2A). Type B (n=3, *Online Supplementary Table S5*) are mutation loads which follow the quantitative course of the rearrangement; mutations were not detectable upon reduction or eradication of cells with the *PDGFRA* or *PDGFRB* rearrangement and are thus expected to be part of the MLN-Eo clone (Figure 2B). Finally, type C was observed for *RUNX1* mutations in patients with *ZMYM2-FGFR1* or *BCR-FGFR1*. At initial diagnosis or before treatment the *RUNX1* mutations were subclonal. While the proportion of *FGFR1* rearranged cells was almost stable in the two cases with available follow-up, the *RUNX1* mutated fractions strongly expanded (Figure 2C, *Online Supplementary Table S5*). This suggests that the clone with both the mutation and the rearrangement had a proliferative advantage over cells with only a *FGFR1* rearrangement. More work will be necessary to understand the role of the myeloid transcription factor *RUNX1* in the functional and biological context with *FGFR1* rearrangements. The high frequency of mutations in patients with *FGFR1* rearrangements and the critical role that *RUNX1* plays in hematopoiesis could contribute to the inferior outcome or acute myeloid leukemia (AML)-like presentation of patients with *FGFR1* rearrangements. Our *FGFR1* subgroup showed the poorest EFS (*Online Supplementary Figure S1B*). The current WHO classification acknowledges the importance of *RUNX1* mutations, especially for AML, by including a provisional entity of AML with mutated *RUNX1*.[14] In general, *RUNX1* mutations are also known to be associated with inferior outcome in AML or myelodysplastic syndromes (MDS).[15]

In conclusion, detecting *PDGFRA, PDGFRB, FGFR1* and *PCM1-JAK2* rearrangements is a prerequisite for up-to-date WHO classification, and an essential step in the differential diagnosis of neoplasms with eosinophilia.[4] However, the identification of additional somatic mutations might allow a clinically relevant risk stratification in the future. Thus, an integrative genetic approach could provide a better understanding of disease biology, with insights including clonal evolution, and should therefore guide personalized and risk-adapted therapy decisions.

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