Identification of PML-RARA, CBFB-MYH11 and RUNX1-RUNX1T1 genomic fusion sequences is feasible and enables unambiguously interpretable and sensitive monitoring of minimal residual disease

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Introduction

Minimal residual disease (MRD) monitoring enables the evaluation of therapy response and guides clinical decisions in acute myeloid leukemia (AML). In AML with PML-RARA/CBFB-MYH11/RUNX1-RUNX1T1 fusions, mainly fusion transcripts (FT) have been used for MRD monitoring so far. Despite inconstant target-to-cell ratio and variable diagnostic expression (both hampering MRD interpretation), simple FT identification and the availability of standardized quantification assays have favored FT as MRD targets over genomic (DNA) breakpoint sequences of the respective fusion genes (FG).

Aims

1) to assess the feasibility of identification of genomic fusion sequences using targeted sequencing
2) to assess the feasibility of subsequent FG-based MRD monitoring using patient-specific qPCR systems
3) to assess the concordance of MRD data obtained by FG- versus FT-based approaches

Approach

Identification of genomic fusion sequences by targeted massive parallel sequencing:

We applied next generation sequencing (NGS), to identify FG genomic breakpoints in 23 PML-RARA-/CBFB-MYH11-/RUNX1-RUNX1T1-positive patients. Targeted sequencing utilizing hybridization to a custom designed probe set for target enrichment was performed (Fig.1). The median sequencing output was 504 k reads mapped to target region.

Quantification of fusion genes breakpoints and fusion gene transcripts by qPCR:

The genomic fusion sequences were used to design patient-specific qPCR assays. Experimental set-up, assessment of QR and sensitivity as well as result interpretation followed the standards of EuroMRD international network. The ALB gene was used to normalize FG level to DNA input.

Quantification of the PML-RARA, RUNX1-RUNX1T1 and CBFB-MYH11 fusion transcripts was performed using primers, probes, PCR conditions and experimental set-up according to the standards of the Europe Against Cancer consortium. The GUS transcript was used to normalize FT level to cDNA input.

Quantifiable MRD levels were expressed relative to diagnosis, as a ratio of normalized FG level/FT level detected in follow-up samples to that detected in diagnostic sample.

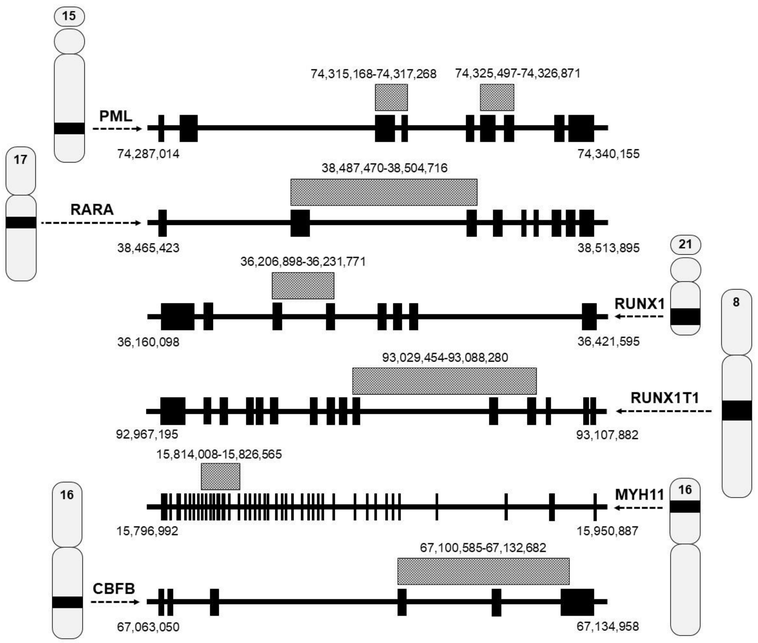

Figure 1. Custom-designed probe sets utilized for targeted sequencing. Custom-designed probe sets are depicted as hatched grey boxes above individual genes (PML, RARA, RUNX1, RUNX1T1, MYH11, CBFB). Arrows below the gene symbols correspond to orientation of the gene coding strands. Exons are shown as black boxes. Genomic coordinates of the genes and targeted regions are shown using hg19 reference.

Results

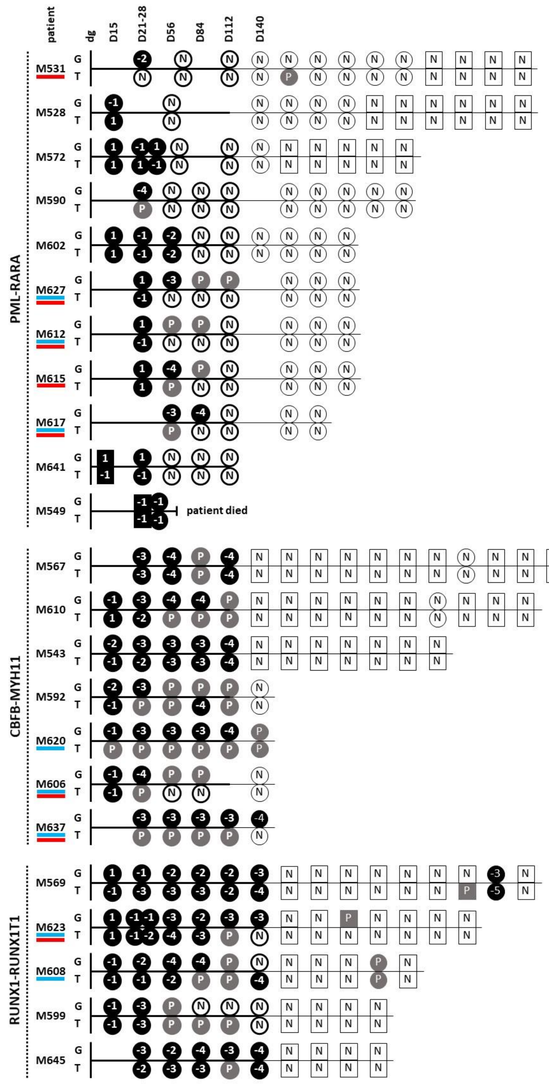

Figure 3. Schematic representation of MRD levels in individual patients harboring the PML-RARA, CBFB-MYH11 and RUNX1-RUNX1T1 fusions during their treatment courses as assessed by the FG-based approach (G) versus by the FT-based approach (T). Bone marrow (BM) samples are shown as circles, peripheral blood (PB) samples as squares. If paired BM and PB samples were analyzed at the particular time-point, only BM is shown. MRD levels ≥0.5 are coded as "1", <0.5 - ≥0.05 as "-1", <0.05 - ≥0.005 as "-2", <0.005 - ≥0.0005 as "-3", <0.0005 - ≥0.00005 as "-4", <0.00005 as "-5". All samples with quantifiably positive MRD levels are shown as black symbols, samples with non-quantifiably positive and negative MRD levels are shown as grey symbols with "P" code and white symbols with "N" code, respectively. Bold versus thin symbol borders and time-lines represent intensive versus maintenance treatment phases. Time course in days (D) is shown at the top of scheme. dg – diagnosis.

We identified FG breakpoints in all 23 patients (11 PML-RARA-positive, 7 CBFB-MYH11-positive, 5 RUNX1-RUNX1T1-positive). In 20 patients we found two (n=14) or three (n=6) fusion sequences. Both reciprocal fusions were found in 9/11 PML-RARA-positive and 4/5 RUNX1-RUNX1T1-positive and 2/7 CBFB-MYH11-positive patients.

FG breakpoints were utilized as targets with constant target-to-cell ratio for MRD monitoring in 265 follow-up samples. Patient-specific quantification assays were successfully optimized for all 23 patients. MRD data were compared with results of standardized assays for FT-quantification (Fig.2)

Data were discrepant in 27% samples; MRD was underestimated in 56 and overestimated in 12 samples by FT-quantification. Importantly, FT-quantification skewed the evaluation of **early treatment response** or of **molecular remission achievement** in **8/22 (36%)** and **8/18 (44%)** patients, respectively (Fig.3).

The FG-based assays were more sensitive for MRD detection compared to the FT-based approach. FT levels in diagnostic samples varied significantly, over > 3 logs, among the patients (Fig.4). Importantly, low diagnostic FT expression dramatically limits the sensitivity of FT detection in follow-up samples. Surprisingly, the discrepancies in MRD levels could not be attributed only to the differences in sensitivities.

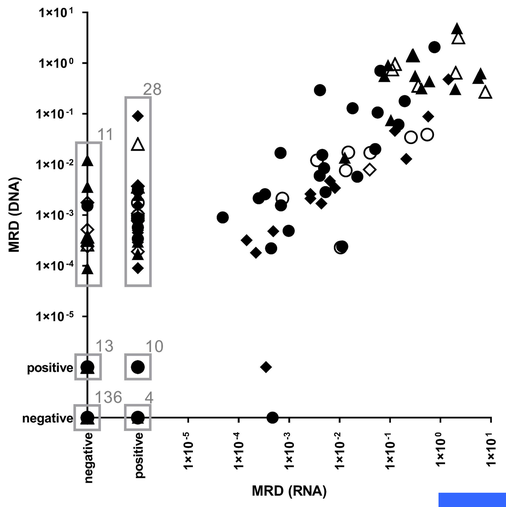

Figure 2. Comparison of MRD levels in 265 follow-up samples of patients harboring the PML-RARA, CBFB-MYH11 and RUNX1-RUNX1T1 fusions measured by the FG-based approach (DNA) versus by the FT-approach (RNA). Grey boxes surround specific clusters of samples whose counts are indicated by the numbers at top right corners.

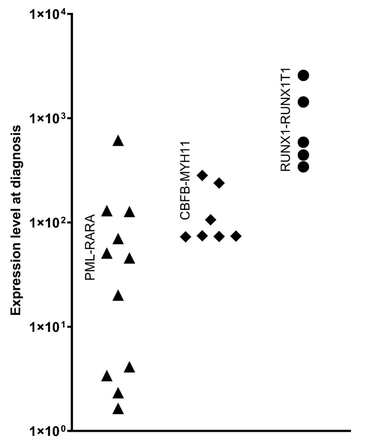

Figure 4. Fusion transcript expression levels at diagnosis in patients with PML-RARA (n=11), CBFB-MYH11 (n=7) and RUNX1-RUNX1T1 (n=5).

Conclusions

In summary, our study shows that both the identification of genomic fusion sequences and the FG-based MRD monitoring are highly feasible in PML-RARA-/CBFB-MYH11-/RUNX1-RUNX1T1-positive AML and represent a superior tool for the evaluation of therapy response than the so far widely used FT-based monitoring. We believe that our data provides rationale for (and could stimulate) additional studies addressing the question whether such an improvement of evaluation of response to therapy could translate into an improvement of risk prediction and therapy tailoring – and, finally, of patients' outcome.

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There are no relationships to disclose.