Introduction
Minimal residual disease (MRD - also now called measurable residual disease in some publications) is of increasing importance in hematologic malignancies. It has been demonstrated to be an independent, post-diagnosis, prognostic indicator for several leukemias including AML and ALL and is of increasing and new importance for CLL and multiple myeloma (Table 1).\(^1\)\(^-\)\(^5\) It is the backbone for treatment strategies in CML.\(^6\) Its results provide important information for risk stratification and treatment planning.
MRD can be evaluated by multi-parameter flow-cytometry (MFC) and by molecular techniques. However, standardization, qualitatively as well as quantitatively, still needs a lot of efforts for broad application and routine clinical use in several diseases. Although many publications have proven the power of MRD measurement by either MFC or molecular techniques, before or even after allogeneic stem cell transplantation, additional studies implementing this tools for adapted treatment strategies are warranted. No new study should start without at least the option to study MRD.

Current state-of-the-art
Minimal residual disease, measured by MFC or molecular techniques demonstrate a sensitivity of 1:10^4 to 1:10^6 and is thus much more sensitive than cytomorphology, cytogenetics or FISH. Several approaches are used and have been published to definitely detect the malignant cells. Harmonisation of protocols as well as of antibody combinations for MFC can be implemented. Individual, laboratory based strategies, however, still are much more often applied.

One of the two approaches of MRD analysis by MFC relies on the detection and knowledge of the respective character of the leukemic cells at diagnosis, leading to the definition of the so-called “leukemia associated immunophenotype (LAIP)”, while the “different-from-normal-approach” relies on the knowledge of normal bone marrow and differences from this detected at the time of MRD assessment. The same diversity is true for molecular techniques, such as real time quantitative polymerase chain reaction (RQ-PCR). Newer techniques are still being tested for broad clinical applications such as digital PCR (d-PCR) or next generation sequencing (NGS).\(^1\)

Multi-parameter flow-cytometry
Different panels at diagnosis have to be used for the respective disease entities and normally include several additional, specific CD markers in addition to the mandatory markers for diagnosis. This leads to a much higher sensitivity and specificity for follow up investigations. In acute leukemias also several different combinations of CD markers can lead to more than one LAIP, even increasing sensitivity. Based on the definition of the specific characteristics of these specific leukemia cells of the individual patient, MRD by MFC, at least for acute leukemias should be based on a minimum of 8 colours, and can widely be used in dedicated laboratories. Several guidelines have been published in the last years to support antibody selection, read out and reporting.\(^7\)\(^-\)\(^8\) If possible, peripheral blood and bone marrow should be investigated at diagnosis, both materials could also be used at follow up. Samples can be analyzed up to 72 hours after drawing, however, shorter travel times should be preferred. Standardization of gating as well as analysis and report should be improved. If possible, MRD studies by MFC should be implemented in all future clinical studies, especially to foster post-remission treatment strategies, proactively including or excluding transplantation for the respective patient based on his MRD level.\(^2\)\(^-\)\(^9\)\(^-\)\(^15\)
Molecular techniques

Increasing efforts have been undertaken to standardize molecular techniques for MRD, especially in CML by introducing the international scale (IS) and standardized ring trials. Several ELN guidelines have been demonstrated to serve as best treatment stratification information.\(^1\)\(^6\)\(^,\)\(^16\) Also in ALL post remission MRD has proven to be of important clinical relevance. Due to the increasing knowledge about molecular markers in hematology new targets for MRD have been detected and need further clinical evaluation. Sensitivity in comparison to MFC is in most cases comparable, in some diseases or in comparison to some antibody-combinations vs. molecular markers the one or the other technique is superior and has to be defined for the individual patient and its leukemic cells at diagnosis in comparison.

As many new molecular markers have been described in the last years, several prospective studies should be initiated to define and prove the best marker and its clinical relevance in MRD post remission, for guiding indication to transplant as well as for post-transplant follow-up. In addition to RT-PCR new techniques such as digital PCR and NGS based assays will show their clinical relevance in the next years to define not only the best MRD marker by molecular approaches but also the respective most sensitive and reliable technique. Further guidelines in addition to those already published especially for CML and ALL but also for some markers in AML such as NPM1, are needed as well as bioinformatic support and definitions how to report results and how often and when MRD should be investigated. This is true for molecular techniques as well as for MFC. A recent review for AML summarizes state-of-the-art.

Future perspective

MRD by immunophenotyping or by molecular techniques will gain much more influence in post remission strategies for hematological malignancies in the next years. Both techniques have advantages as well as disadvantages, however as they in many cases can be used complementarily, patients at diagnosis can be defined and stratified to the best MRD technique to be followed during treatment and in remission. MRD will also gain more importance, as the clinical decision to transplant or not to transplant in first CR or in later CR can be guided. Further, therapeutic approaches after allogeneic transplantation such as the use of DLI, can be guided by MRD. Further, as demonstrated by CML already, MRD levels can lead to stop treatment or avoid further chemotherapy what automatically reduces side effects for the patient.

It is foreseeable that the use and the clinical importance of MRD studies, in clinical trials but also in daily practice will not only lead to more specific, individualized treatment and patient follow up but also reduce toxicity and costs. Therefore, efforts should be taken much more to define the respective and best technique to use for MRD to improve patients care and also to reduce costs in hematology in the next years.

### Table 1. Methods to detect MRD according to disease entities.

| Method (MRD) | Sensitivity | AML | ALL | CML | CLL | Myeloma |
|--------------|-------------|-----|-----|-----|-----|---------|
| Multi-parameter flow cytometry | \(1 \times 10^{-6} - 1 \times 10^{-9}\) | + | + | - | + | + |
| Molecular approaches | \(1 \times 10^{-6} - 1 \times 10^{-9}\) | + | + | - | + | - |

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