Guidelines for EuroMRD participantship (ALL section)

RQ-PCR analysis of Ig/TCR gene rearrangements for quantitative MRD diagnostics in lymphoid malignancies such as ALL is a highly complex technology, which needs extensive knowledge and experience. The complexity and required knowledge cannot be compared with RQ-PCR analysis of fusion gene transcripts.

EuroMRD has defined the following guidelines for participantship of the ALL section:

A. Extensive knowledge on Ig/TCR gene rearrangements

1. *Extensive knowledge on structure of Ig/TCR genes and immunobiology of Ig/TCR gene rearrangement processes*
   e.g. structure of each human Ig/TCR locus: *IGH, IGK, IGL, TCRD/A, TCRB, TCRG;* stepwise rearrangement process; hierarchical order of rearrangements; incomplete and complete rearrangements; gene segment usage.

2. *Insight into Ig/TCR gene rearrangement patterns in lymphoid malignancies*
   e.g. Ig/TCR rearrangement patterns per disease category; specific characteristics, such as ongoing rearrangements, oligoclonality, somatic hypermutations (SHM), etc. per disease category.

B. Extensive experience in Ig/TCR gene analysis and RQ-PCR for MRD detection

1. *Extensive knowledge on methods for detection and identification of Ig/TCR gene rearrangements*
   e.g. Southern blotting; PCR analysis of the various types of Ig/TCR gene rearrangements; heteroduplex analysis (mixed heteroduplex analysis); Sanger and next generation sequencing and interpretation of sequences (including definition of the junctional regions).

2. *Knowledge and experience on RQ-PCR analysis of Ig/TCR junctional regions*
   e.g. RQ-PCR methods: possibilities and limitations; sample handling; control samples; sensitivity testing; interpretation of RQ-PCR results (according to EuroMRD guidelines).

3. *Evidence of experience to provide reliable RQ-PCR-based MRD results*
   - at least 25 ALL patients completely analyzed
   - at least 5 patients analyzed in full parallel to an EuroMRD laboratory (with comparable results)

4. *Experience in MRD diagnostics for NHL patients is not necessarily sufficient for MRD diagnostics in ALL patients*
   A EuroMRD laboratory competent in analysis of a range of Ig and TCR targets is competent to analyze IGH within NHL protocols, but an NHL laboratory with experience limited to analysis of mature B lymphoid disorders will require additional training (see point 3) in order to undertake MRD analysis of ALL.

C. Size of MRD laboratory and minimum number of ALL patients per year

1. *Statement by chairman of national treatment protocol concerning central MRD laboratory*.
   - MRD diagnostics according to guidelines of EuroMRD
   - sufficient experience level of MRD laboratory (at least 25 patients completely analyzed, including 5 patients in full parallel to a EuroMRD laboratory)

2. *A MRD laboratory should consist of at least one scientist and preferably at least two technicians* in order to guarantee sufficient supervision, continuity, sample throughput, and quality control. One technician can handle 30-40 ALL patients per year depending on the number of targets and follow-up samples investigated.

3. *Preferably an MRD laboratory should cover a population of at least 10 to 12 x 10^6 inhabitants* (or a country, in case of lower number of inhabitants).

4. *Preferably at least 50 to 60 ALL patients should be evaluated for protocol-based MRD per year* to maintain a *sufficiently high experience level*. For reasons of efficiency and costs, preferably more patients should be evaluated per laboratory.

D. Continuation of EuroMRD participantship

1. *Continuous participation in EuroMRD meetings and QA rounds* Each EuroMRD participant is obliged to participate in the 6-monthly QA rounds and in the corresponding EuroMRD meetings. Failure to participate in two consecutive QA rounds/meetings will terminate the EuroMRD participantship. Only by exception and with explanation, the EuroMRD participantship can be continued.

2. *Continuous supervision and education*. Each EuroMRD laboratory should have sufficient supervision and continuity in order to guarantee sufficiently high quality and experience level.

3. *Annual number of new patients*. At least 50 to 60 new patients should be evaluated each year to maintain a sufficiently high experience level. Preferably more patients should be evaluated per laboratory.

4. *Two-year evaluation of EuroMRD participantship.* Every two years the status of the EuroMRD participantship will be evaluated for all members via the form "*Continuation of EuroMRD Participantship*".

Questions about the EuroMRD Participantship can be sent to info@euromrd.org