Evaluation report of Ph.D. thesis *Next Generation Sequencing-based Analysis of Immunoglobulin and T-cell Receptor Genes Rearrangements Repertoire in Haematological Malignancies* by Michaela Kotrová

The proposed dissertation thesis addresses the hot topic and dynamic field of minimal residual disease (MRD) in acute lymphoblastic leukaemia (ALL). As the importance of MRD for clinical risk assessment is well established in current treatment protocols, Michaela Kotrová focuses on further refinement of MRD measurements using novel technologies and their interpretation.

The aims are:

1. To design, develop and validate a novel next generation sequencing (NGS)-based assay for detecting Ig/TCR rearrangements.
2. To generate and interpret pilot data in screening for MRD targets, MRD measurements and sequencing of the entire immune repertoire using NGS-based assay.
3. To asses the prognostic value of NGS-MRD and the repertoire diversity in paediatric B-ALL.

The hypotheses are clearly stated. In the methods section, Kotrová demonstrates a consummate mastery of the NGS method including primer design, library preparation, template sequencing, and complex data analysis with open-source bioinformatics tools. It is to be emphasized, that the candidate does not employ commercially available sets of primers which would compromise data interpretation, but has developed her own in-house multiplex fusion primer sets for the Ion Torrent platform. At the same time, she takes an active part in the EuroClonality-NGS Consortium which aims to standardize and validate NGS assays for Ig/TCR MRD detection in haematological malignancies. The differences between in-house technology and the EuroClonality approach are discussed.

Results that should be stressed include:

1. High correlation of NGS-MRD measurements with standard qPCR proven on 210 samples from 76 children treated with the AIEOP BFM-ALL 2000 protocol with the majority of discrepancies in samples below the sensitivity of both methods.
2. NGS-MRD at day 33 is more specific for predicting relapse in the above mentioned protocol.
3. NGS helps to overcome difficulties with interpretation of low positive MRD results on qPCR, particularly in samples from regenerating bone marrow and early after allogeneic stem cell transplantation.
4. Identification of the immune repertoire diversity (measured as a ratio of the number of clones to the number of total reads) on day 78 as a novel prognostic marker independent of MRD.

The thesis refers to 78 relevant and highly topical articles. It also leaves no doubt that the candidate has a broad theoretical background, and has mastered a number of relevant methods. She has authored four papers in prestigious journals (total IF = 22,29), and personally presented the results of her research at several prestigious meetings, including oral presentation at the annual conference of the European Haematology association. Her paper on NGS-MRD in ALL published in Blood is of seminal importance and is repeatedly quoted at haematological conferences. These facts reflect the dynamic and creative atmosphere of the CLIP laboratory as well as the inspirational and committed training of Dr. Eva Froňková.

As a member of the EuroClonality-NGS Consortium, Michaela Kotrová directly works on standardization of this novel method for MRD measurements which will affect clinical standards in a near future. She has demonstrated a creative approach to problem solving, and has an excellent independent research perspective.

On the basis of what is stated above, I recommend that the permission for a public defence of the dissertation can be granted. In addition, I suggest that after successful defence of her thesis, Michaela Kotrová should be awarded the Ph.D. degree.

Questions for Ph.D. defence:

1. Do all regions of Ig gene amplify with the same intensity? If not, how do you approach this issue when interpreting quantitative results?
2. How does the methodology for NGS-MRD assessment differ when it is measured in aplastic vs normocellular sample? Is the information about cellularity of the sample needed ahead or can it be assessed from the sequencing results themselves?
3. What was the reason for not including IgK targets into your NGS-based assay?
4. Have you performed any functional assay to evaluate potential differences in immunologic response between patients with high and low repertoire diversity?

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