Molecular assessment of the cellular origin of Merkel cell carcinoma

Valorization is the estimation in as much the published scientific work can be utilized in the practice and depends on the category of performed research. Whereas data from clinical research, e.g. clinical trials, or translational research might impact clinical practice earlier, it is well know that research data gained from basic research might take longer before they will be transferred or implemented in the clinical practice. Here, I will point out how the main results of this thesis are very likely to improve the diagnosis and clinical management of Merkel cell carcinoma (MCC) in the near future.

Depending on the localization, the rare and aggressive skin cancer MCC is mostly detected at late stage III and IV3. At stage III MCC is metastasized to local lymph nodes. At stage IV it is either metastasized to distant lymph nodes or solid organs e.g. lung3. In the latter it might possibly difficult to distinguish MCCs from small cell lung cancer (SCLC). In chapter 3, we described the complete absence of RE-1 silencing transcription factor REST as well as the achaete-scute family BHLH transcription factor 1 (ASCL1) in MCC. Contrariwise, ASCL1 was shown to be expressed in 70% of SCLCs4. Moreover, the hematopoietic program and the presence of splice variants of the paired box protein PAX5 in MCC which we showed is unique for MCC (chapter 5) might be also used in as marker to distinguish SCLC from MCC.

The main focus of this thesis was to get more insight into the cellular origin of MCC. Irrespective of the cellular origin of MCC, it is expected that the results of this research will contribute to the understanding of its pathogenesis, ultimately possibly leading to novel treatment options. Based on the results presented in this dissertation we now know that the cellular origin should have stem cell activity, is very likely to be of hematopoietic origin and is characterized by a significant, however partially defective hematopoietic PAX5 transcription program. Thus, the pre-pro B-cell is currently the most appropriate candidate for the cellular origin.

The gained knowledge about the cellular origin can now be applied to the treatment of MCC. Because of its rareness clinical trials on MCC treatment of more than 100 people cohorts are rare. Therefore, the most clinical trials for the treatment of MCC are starting from phase 2 or phase 3 where MCC patients are included in a general cohort among others with solid tumors like cutaneous squamous cell carcinoma. A successful example of a nowadays approved

compound for the treatment of MCC is Avelumab which was assessed in clinical trial phase 2 in 200 MCC patients which showed an overall response rate of 50 to 70% 5-7. In chapter 7 we used our gained knowledge about the early B-cell phenotype of MCC and treated the cells with the promising combination of the BCL-2 family member inhibitor Navitoclax and the PI3K p110 Alpelisib. The compounds induced only in combination a stagnation in cell viability. Both compounds are in individual clinical trials phase 2 7-9. Therefore, the costs for the first phase of the clinical trial of about 475,000€ in the Netherlands can be skipped11. The costs of about 11,379€ per patient in clinical trial 2 has still to be considered.12 Clinical trial phase II takes several months with a group of at least 100 people. For MCC to get that many cases are difficult, therefore international cooperation like it was performed with Avelumab are necessary to realize a significant evaluation of the synergism of these compounds in the clinic. Therefore, a clinical trial phase 2 to evaluate the efficacy of Navitoclax/Alpelisib might be conducted within several months and might be approved by the FDA.

After successful treatment, the patients have to be evaluated for recurrence of the cancer by follow-up care. The easiest way is to use biological fluids like blood. The younger DNA methylation age (DNAmAge) of blood cells compared to the chronological age have a significant correlation2. In chapter 2 we characterized that MCCs have a significantly younger DNA methylation age (DNAmAge) compared to the chronological age of the MCC patients. Therefore, it might be possible to establish an assay to determine the DNAmAge of DNA located in the blood. For this screening method only 10 ng of purified DNA is enough for enrichment using the capture enrichment Illumina technology and hybridized with the 353 CpG sites of the Horvath's clock. If the DNAmAge is significant younger, it is most likely that the patient has aberrant cells and the patient has to be screened for recurrence of the cancer.

The knowledge about the synergism of the Navitoclax/Alpelisib combination can be therefore transferred into clinical practice. The combination would not be tested on MCC if we had not observed the hematopoietic pre-pro B-cell origin of MCC. Moreover, the DNAmAge determination blood screening method might be also utilized in the clinic for the follow-up control of MCC. Therefore, this dissertation

has gained important data which potentially will be successfully translated into a pre-clinical setting and transferred further into the clinical practice.

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