Improving Diagnostic Accuracy and Follow-up of Neuroendocrine Neoplasms using a novel technology

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Neuroendocrine neoplasms (NEN) are a heterogenous group of relatively rare tumors that pose significant diagnostic challenges due to their clinical, molecular and (cyto)morphological diversity, as well as their variable anatomical locations. Moreover, NEN often evolve slowly, therefore demanding long-term follow-up to aid treatment strategy decisions. Current diagnostic and follow-up methods rely on imaging, (repeated) tissue biopsies and circulating protein biomarkers, which are suboptimal in terms of sensitivity and specificity. Therefore, there is an urgent need for novel, minimally invasive biomarkers that can offer more accurate and comprehensive information about the tumor.

A promising source of biomarkers that has garnered growing attention is the methylome. While advances have been made in DNA methylation detection, traditional approaches still depend on bisulfite conversion, a harsh chemical process that damages DNA, and are limited to analyzing only a few targets simultaneously. To overcome these limitations, our research group developed IMPRESS (Improved Methylation Profiling using Restriction Enzymes and smMIP Sequencing). Unlike conventional methods, IMPRESS employs methylation-sensitive restriction enzymes (MSREs) rather than bisulfite and uses single molecule Molecular Inversion Probes (smMIPs), enabling much higher multiplexing capacity.

We aim to develop a more accurate diagnostic and follow-up test by applying the IMPRESS approach to NEN. First, through *in silico* analyses we demonstrated that the DNA methylation changes in NEN can differentiate NEN samples from controls (healthy tissue and other cancer types) and can trace the tissue of origin. We identified general NEN-specific and tissue-specific differentially methylated CpGs (DMCs) and selected those suited for IMPRESS. Next, smMIPs were designed for all selected DMCs (n = 2304). At the moment, we evaluating the efficiency and discriminatory power of these smMIPs in tissue samples and will soon start the analysis in liquid biopsies. The best performing smMIPs will be incorporated into a model, which will then be validated in liquid biopsies from an independent patient cohort for which two clinical trials are currently ongoing.