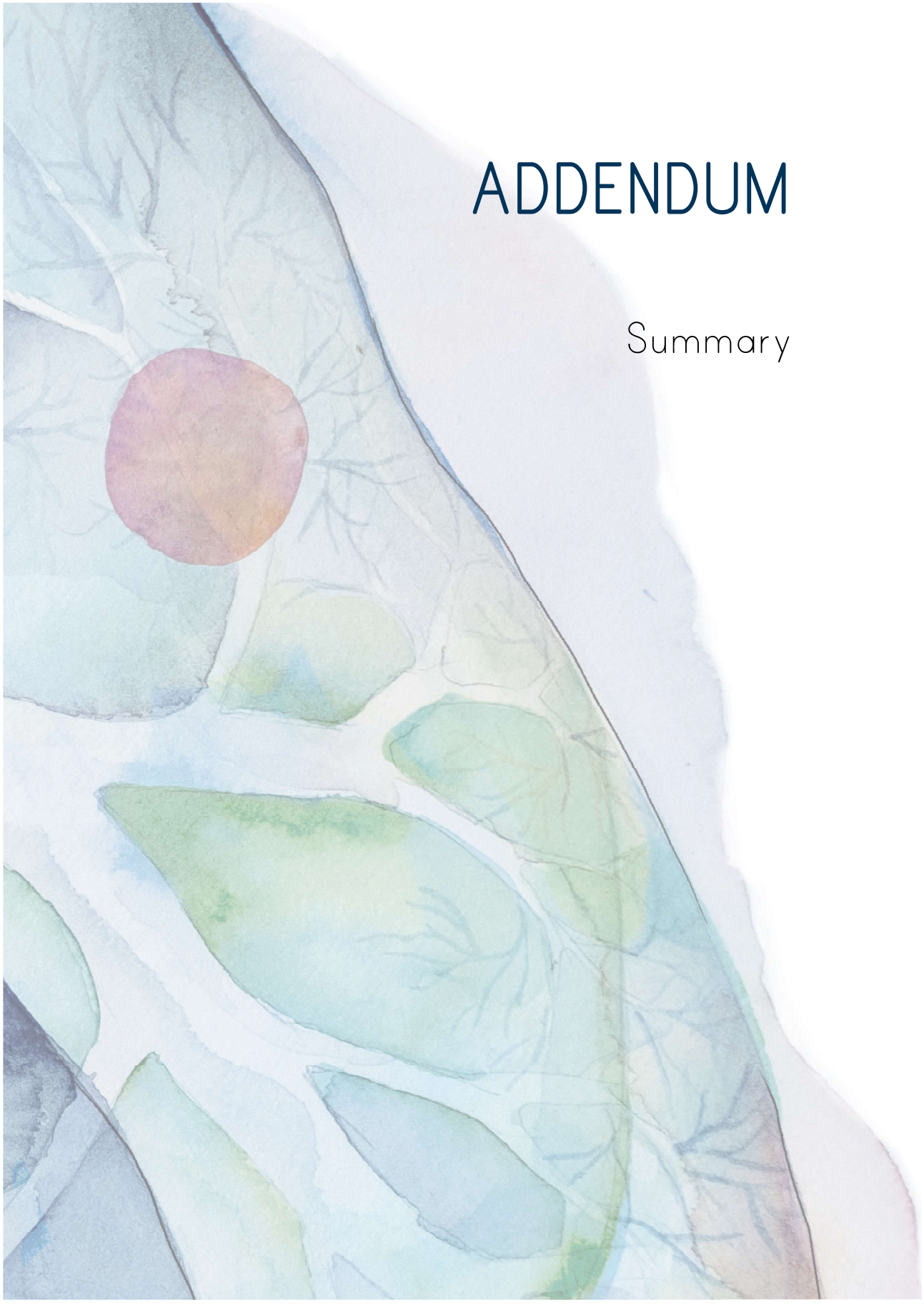
Pulmonary Carcinoids



ADDENDUM

Summary

Summary

Pulmonary neuroendocrine neoplasms (NEN) encompass the well differentiated typical and atypical carcinoids (TC and AC) as well as the poorly differentiated neuroendocrine carcinomas large cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC) (**chapter 1**). Pulmonary carcinoids account for approximately 1-2% of all lung malignancies and their incidence is rising over the last decades. According to the World Health Organization (WHO) 2021 classification criteria, pulmonary carcinoids can be morphologically subdivided into TC and AC based on the mitotic count (TC <2/2mm^2, AC 2-10/2mm^2) and the presence or absence of necrosis. Although pulmonary carcinoids are considered as low- and intermediate grade tumors, distant disease relapse may occur (TC: 1%-6% and AC: 14%-29%) in patients who initially underwent curative surgical resection. Relapse may occur many years after surgical resection, hence the recommendation for long-term surveillance (10-15 years). Known predictors of distant relapse are AC, lymphatic involvement, and incomplete resection status. However, none of them can be reliably used, alone or in combination, to safely exclude patients from long-term follow-up. Moreover, the histopathological classification criteria for pulmonary carcinoids are subject to high-interobserver variation. Together these data indicate the need for new biomarkers to improve both the diagnosis and prediction of prognosis of pulmonary carcinoid patients. Currently, several prognostic markers (OTP, Ki67, CD44) for pulmonary carcinoids have been identified in research settings, but they have not yet been implemented in routine clinical care.

The **aim** of this thesis was 1) to examine the diagnostic workup and prognostication of pulmonary carcinoids in current clinical practice using a unique population-based cohort, 2) to obtain insights into molecular mechanisms underlying carcinoid tumorigenesis, 3) to identify and verify molecular biomarkers to improve the prognostication of pulmonary carcinoid patients, and 4) to develop the first patient-derived tumor organoids for pulmonary NENs to uncover therapeutic vulnerabilities.

Lymph node sampling in relation to disease relapse

The predictive value of extent of per-operative lymph node sampling in relation to disease relapse in pulmonary carcinoid patients is unknown. In addition, post-surgery follow-up recommendations rely on expert opinions and institutional retrospective studies with a rather short follow-up focusing mainly on overall survival instead of relapse free survival. In **chapter 2**, we aimed to address these shortcomings in a population-based cohort with long-term follow-up (median 87.5 months). By combining the Dutch pathology (Palga) and cancer registries (NCR), all patients with surgically resected pulmonary carcinoid disease diagnosed between 2003-2012 were included.

Tumor node metastasis (TNM) staging was updated to TNM8 by screening of complete pathology reports. Both patterns of metastasis and extent of lymph node sampling were evaluated. In total 662 patients were included in the study of which 10% showed disease relapse (26.0% AC vs. 6.2% TC vs. 4.4% carcinoid not otherwise specified (NOS)). Relapses occurred mostly in the liver (50%) and locoregional sites (45%). Median relapse free interval (RFI) was 48.1 months (95% CI 36.8-59.4) thereby underscoring the necessity of long-term follow-up. Poor prognostic factors were AC, pathological nodal stage (pN1/2) and surgical resection margin (R1/R2). Data regarding lymph node dissection were available in 546 patients. A complete mediastinal lymph node sampling according to the European Society of Thoracic Surgeons (ESTS) guidelines was performed in merely 7% of the patients. A slight increase in mediastinal lymph node evaluation was observed over time but this was not associated with the number of relapses. In 477 clinical N0 patients, 5.9% showed pathological N1 disease and 2.5% N2 disease. These data indicate that the extent of lymph node sampling has not a remarkable impact on disease relapse, however, systematic nodal evaluation is advocated as it provides important prognostic information. Furthermore, relapse of disease is not uncommon, and our data show that a long-time follow-up is required whereby surveillance should especially focus on liver and locoregional relapses as these were the most frequent sites of relapse during follow-up.

Preoperative biopsy specimen diagnosis in pulmonary carcinoid

In **chapter 3**, we evaluated the Dutch pathology database to select stadium I-III pulmonary carcinoid patients who underwent a curative resection and of whom both a preoperative biopsy and paired resection specimen were available (n=330). Pathology report conclusions of the biopsy and paired resection were compared. This evaluation showed discordance between the preoperative biopsy and paired resection diagnosis in 57% (n=189/330) of the patients. Moreover, a quarter of preoperatively diagnosed TC and carcinoid NOS patients were reclassified as AC on the resection specimen and these patients exhibited higher relapse rates as compared to non-reclassified TC and carcinoid NOS patients (3% vs. 1% and 16 vs. 6%). Therefore, we advise clinicians to interpret the preoperative biopsy diagnosis with caution in deciding the extent of surgery (e.g., parenchyma-sparing versus non-parenchyma sparing). In **chapter 4**, we emphasize the need for additional preoperative biomarkers that aid in both diagnosis and prognosis of pulmonary carcinoids. We feel that a panel of molecular markers (e.g., OTP, Ki67, CD44) applicable on preoperative biopsies may improve the diagnostic and prognostic accuracy to predict relapse in patients suffering from pulmonary carcinoids disease.

Orthopedia homeobox (OTP) in pulmonary carcinoids

OTP is a member of the homeodomain transcription factor family and has been described as a key player in the development of the neuroendocrine system of the hypothalamus. The current clinical value of OTP expression identified in pulmonary carcinoids, the possible molecular mechanism regulating OTP expression, and the function of OTP are addressed in a literature review in **chapter 5**. This evaluation underscores that OTP is a promising, highly sensitive, and specific marker for pulmonary carcinoid tumors with a favourable prognosis. However, at time of evaluation only a limited number of tumor types had been examined for OTP expression and the regulatory mechanism underlying OTP expression remained undetermined. Hence, in **chapter 7**, we investigated publicly available multi-omics data (whole-exome-, whole-genome-, RNA sequencing and Epic 850K-methylation array) of 58 TC, 27 AC, 69 LCNEC and 51 SCLC patients and TCGA (The Cancer Genome Atlas) data of 33 tumor types 1) to shed light on *OTP* expression patterns in different tumor types and 2) to unravel the mechanisms underlying differential *OTP* expression. Results showed bimodality of *OTP* expression in carcinoids (OTP^{high} vs. OTP^{low} group), with the OTP^{high} group specific to pulmonary carcinoids while absent from all other cohorts analysed. OTP^{low} carcinoids showed a statistically significant worse overall survival. Gene set enrichment analysis (GSEA) for mutated genes related to hallmarks of cancer revealed robust enrichment of three hallmarks in the OTP^{low} group (sustaining proliferative signalling, evading growth suppressor and genome instability and mutation) whereas no robust enrichment was observed within the OTP^{high} group. To date, no gene-inactivating somatic mutations, alterations by chimeric transcripts or genomic rearrangements have been identified in the *OTP* gene. Therefore, we examined epigenetics (e.g., DNA methylation) as a potential regulatory mechanism underlying differential OTP expression by combining transcriptomic and methylomic data of 51 samples (24 OTP^{high}, 10 OTP^{low}, and 17 LCNEC samples). Analyses identified a significantly different methylation level (FDR <0.05 and delta >0.2) between OTP^{high} and OTP^{low} carcinoids for 12/34 OTP 850K Infinium probes. Overall, OTP^{low} carcinoids exhibit higher DNA methylation levels as compared to OTP^{high} carcinoids. Together these data suggest that high OTP expression is a unique feature of pulmonary carcinoids with a favourable prognosis, and that in poor prognostic patients OTP expression is lost, most likely due to changes in DNA methylation levels. Future studies should investigate whether epigenetic therapies might play a role in the treatment of pulmonary carcinoid patients.

Prognostic markers for pulmonary carcinoids

Relapse occurs in 10% of patients with resected pulmonary carcinoid. While TC show relative low relapse rates, safe exclusion from long-term follow-up is impossible as these