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Investigation of Small-Cell Lung Cancer Epidemiology in Sweden and Analysis of Clinical and Tumor Specific Prognostic Biomarkers

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To my parents and grandparents.

ABSTRACT

Small-cell lung cancer (SCLC) accounts for approximately 13% of all newly diagnosed lung cancer (LC) cases. This disease is correlated with heavy smoking. It is characterized by a rapid doubling time combined with a propensity to metastasize quickly and by neuroendocrine differentiation. In patients with locally advanced disease, the standard of care is concurrent radiation and platinum-doublet chemotherapy (PDCT). Patients with stage IV disease have also until recently been treated with PDCT. Even though a majority of SCLC cases initially responds to PDCT, almost all patients inevitably relapse. This thesis aims to deepen the knowledge of SCLC.

Paper I was a population-based study, where we aimed to investigate the possible association between educational levels and overall death of Swedish SCLC patients. The patient population consisted of 4256 subjects. The key findings showed that educational level is an independent prognostic factor in Swedish men diagnosed with SCLC and among patients with Limited disease.

In **paper II**, we performed a validation of the 8th TNM staging system on 706 SCLC cases and compared the system´s prognostic performance to the 6th and 7th TNM editions as well as to the older two-stage system that segmented patients as either having Limited Disease (LD) or Extensive Disease (ED). The study provided additional information supporting the robustness of the 8th TNM edition in prognostically categorizing SCLC and confirms its usefulness in clinical practice.

In **paper III**, we conducted a real-world study on 545 consecutive cases during an eight-year period. The aim was to understand in depth the treatment patterns of SCLC patients from Karolinska University Hospital. Another goal was to examine the outcome of SCLC patients upon re-challenge with PDCT. The survival outcomes for LD and ED SCLC patients were poor, correlating with previous studies. The results also showed that SCLC patients with sensitive relapse after first line PDCT may benefit from re-challenge.

In **paper IV**, the expression of multiple biomarkers, including Notch1, Hes1, Ascl1, and DLL3, were analysed in a selected cohort of 46 SCLC patients. The study, in part, focused on how the expression patterns differed based on patients' resistance or sensitivity to PDCT. We evaluated the prevalence of expression of these four biomarkers in human samples using biopsies and studied the potential association with survival and benefit from 1st line PDCT. The study showed that Notch1 seems to be an independent prognostic factor in SCLC. Furthermore, a negative association between Notch1 and Ascl1 expression was observed.

In summary, this thesis expands on the understanding of SCLC by analysing epidemiological trends, the impact of socioeconomic status, changes to classifying patients, and possible prognostic biomarkers.

LIST OF SCIENTIFIC PAPERS

I. **Tendler S**, Holmqvist M, Wagenius G, Lewensohn R, Lambe M, De Petris L. Educational level, management and outcomes in small-cell lung cancer (SCLC): A population-based cohort study. *Lung Cancer 139: 111-117, 2020*

II. **Tendler S**, Grozman V, Lewensohn R, Tsakonas G, Viktorsson K, De Petris L. Validation of the 8th TNM classification for small-cell lung cancer in a retrospective material from Sweden. *Lung Cancer 120:75–81, 2018, Corrigendum 123; 178-179, 2018.*

III. **Tendler S**, Zhan Y, Pettersson A, Lewensohn R, Viktorsson K, Fang F, De Petris L. Treatment patterns and survival outcomes for small-cell lung cancer patients- a Swedish single center cohort study. *Acta Oncologica 1-7, 2020*

IV. **Tendler S**, Kanter L, Lewensohn R, Ortiz-Villalón C, Viktorsson K, De Petris L. The prognostic implications of Notch1, Hes1, Ascl1, and DLL3 protein expression in SCLC patients receiving platinum-based chemotherapy. *Manuscript.*

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Tsakonas G, Hellman F, Gubanski M, Friesland S, **Tendler S**, Lewensohn R, Ekman S, De Petris L. Prognostic factors affecting survival after whole brain radiotherapy in patients with brain metastasized lung cancer. *Acta Oncologica Feb; 57(2):231-238, 2018.*

CONTENTS

LIST OF ABBREVIATIONS

1st line	First-line
2nd line	Second-line
3rd line	Third-line
Bid	Twice per day
BSC	Best Supportive Care
CDR	Cause of Death Register
CRT	Chemo- and Radio-Therapy
CT	Chemotherapy
CI	Confidence Interval
DLL3	Delta-like ligand 3
ED	Extensive Disease
^{18}F-FDG	^{18}F-fluorodeoxyglucose (C6H1118FO5)
FDA	Food and Drug Administration
FFPE	Formalin-Fixed, Paraffin-Embedded
Gy	Gray
HR	Hazard Ratio
Hes1	Hairy/Enhancer of Split 1
LISA	Longitudinal Integration Database for Health Insurance, Labor Market Studies
LD	Limited Disease
IASLC	International Association for the Study of Lung Cancer
IHC	Immunohistochemistry
LCBaSe	Lung Cancer Data Base Sweden
LC	Lung Cancer
MRI	Magnetic Resonance Imaging
Notch1	Notch Homolog 1 Translocation-Associated
NLCR	Swedish National Lung Cancer Register
NSCLC	Non-Small Cell Lung Cancer
ORR	Objective Response Rate
OS	Overall Survival

PDX	Patient-Derived Xenograft
PCI	Prophylactic Cranial Irradiation
PDCT	Platinum-Doublet Chemotherapy
PE	Platinum and Etoposide
PET	Positron Emission Tomography
PFS	Progression-Free Survival
PS	Performance Status
RCT	Randomized Clinical Trial
Rova-T	Rovalpituzumab Tesirine
RT	Radiotherapy
SCLC	Small-Cell Lung Cancer
SCR	Swedish Cancer Register
SEI	Socioeconomic Index
TTF	Thyroid Transcriptor Factor
TNM	Tumor, Node, and Metastasis
VALSG	Veterans' Administration Lung Study Group

1 Background

1.1 Introduction

Lung cancer (LC) is the deadliest form of cancer worldwide in both sexes. (1) The World Health Organization (WHO) divides LC into two main categories: non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). (2, 3) The majority of LC patients are diagnosed with NSCLC, with adenocarcinoma being the largest subtype (44%) followed by squamous cell carcinoma (26%) and large cell carcinoma (15%). (4) The proportion of LC that is classified as SCLC has decreased over the past decades from 20%-25% in the 1980s to around 13% today. (5, 6) SCLC is a rare disease in non-smokers, representing only 3% of cases. (7) The proportion of women diagnosed with SCLC has increased to 50% over the past few decades due to changes in tobacco consumption habits. (8) The risk of developing SCLC increases with age, with approximately 45% of cases are above 70 years. (5)

1.2 Origins of SCLC

SCLC cells originate from a sensory cell called neuroendocrine cell. (9) In 1926, Dr. WG Barnard identified certain mediastinal cells in the lungs. (10) At the time, such cells were thought to be “oat cell sarcomas”. However, in 1959, researchers correctly recognized that these neuroendocrine cells were in fact a separate and distinct form of LC, which today is known as SCLC. (11)

SCLC is diagnosed by immunohistochemical staining, a diagnostic tool used to distinguish SCLC from other malignancies. The disease is characterized by small homogenous malignant cells, which are larger in size compared to lymphocytes. (12) The diagnosis SCLC can usually be determined by testing for thyroid-transcriptor factor-1 (TTF-1), and established neuroendocrine markers such as, synaptophysin, chromogranin, and CD56. (12-15)

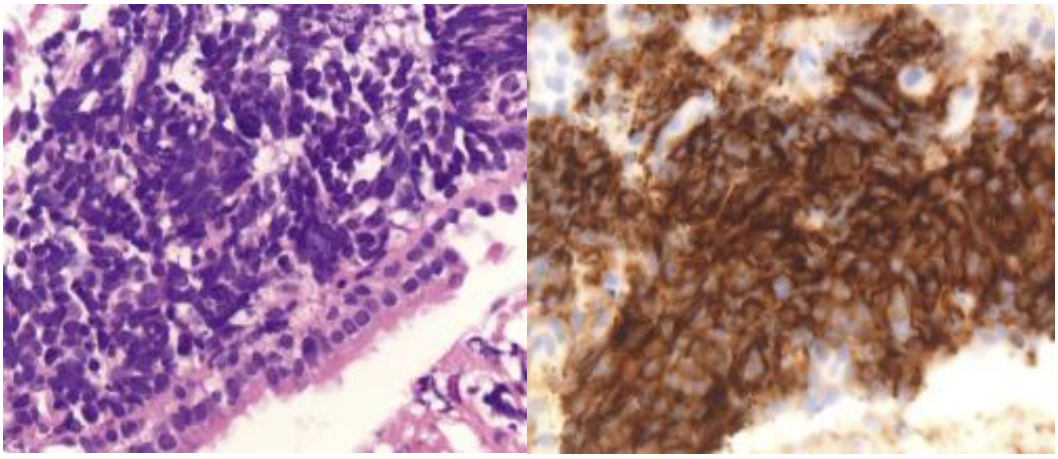

Figure 1 Morphology of SCLC

Hematoxylin and eosin stained SCLC section (left) and positively stained CD56 in brown color of a SCLC section (right). Magnification 40x. *Reprinted with permission from Elsevier*

1.3 LC registries

The Swedish Cancer Register (SCR) is maintained by the Swedish National Board of Health and Welfare. The SCR's purpose is to monitor cancer incidence and survival in the Swedish population for medical research. (16) Since 1958, the SCR has been prospectively collecting data on new cancer cases, including linked information on medical data (anatomical site, method and date of diagnosis, and morphology), patient data (age, sex, and place of residence), and follow-up time (cause and date of death). (16) A notification of clinical, morphological, and autopsy-based cancer diagnoses is mandated by Swedish law, which ensures a high national coverage of around 95% when validated against the National Patient Register (NPR). (16) The SCR is considered extensive, with similar registries from other countries having significantly lower coverage percentages. For example, the Surveillance, Epidemiology, and End Results (SEER) program accounts for around 28% of the United States population. (17)

The Swedish National Lung Cancer Register (NLCR) was founded in 2002 and aims to include all cases classified as LC according to the International Classification of Diseases for Oncology (code C34). (18) The NLCR collects a variety of patient data, including age, sex, smoking status, performance status (PS), diagnostic procedures, histopathology, and stage of disease at diagnosis. (19) The registry also includes information regarding planned treatment, lead times of referrals, and primary treatment decisions. The NLCR covers around 96% of Swedish lung cancer cases. (16, 19)

The Longitudinal Integration Database for Health Insurance and Labor Market studies (LISA) was established in 1990 and contains annual registers on highest achieved education for all residents aged 16 and older in Sweden. In addition to information on educational level, the LISA also has data on country of birth, date of emigration, employment, cohabitation status and number of persons per household. (20, 21)

Since 1961, the Swedish Cause of Death Register (CDR) has collected information on the date and underlying causes of death of every person in Sweden. According to recent statistics, the percentage of missing death certificates is less than 1%. (21, 22)

The Lung Cancer Data Base Sweden (LCBaSe) was started for research purposes and is based on all LC patients registered in the NLCR from 2002-2016. The LCBaSe is composed of linkages between the NLCR and the SCR, CDR, LISA, NPR, Prescribed Drug Registry and Total Population Registry. (20)

1.4 Epidemiology and socioeconomic factors

Socioeconomic factors, including income, educational level, and accessibility to healthcare providers, contribute to health disparities. The literature has historically used income as the main measure of socioeconomic index (SEI). (23) However, using educational level as the SEI instead can avoid the risk of reverse causation bias. Thus, while a cancer diagnosis may result in reduced income, it should not affect educational level, which the subject in almost all

cases has already achieved. (23, 24) In addition, education may to a certain extent facilitate a lifelong choice of health attitude and behavior. (23)

Other studies have investigated the potential association between SEI and cancer-specific mortality. (23, 25) In Sweden, everyone should have equal access to healthcare through a tax-funded National healthcare system. However, studies on Swedish prostate cancer, breast cancer, and NSCLC patients have shown differences in survival outcomes among the various SEI subgroups. (20, 24, 26) Furthermore, a recent report on Swedish NSCLC patients showed a difference in diagnostic intensity and mortality among different SEI groups. (27)

Less educated patients have been shown to be diagnosed with a more advanced stage of their cancer diagnosis. (28) This could possibly be explained by poorer compliance to certain screening programs (breast-, cervical-, colorectal-, and prostate- cancer). (29, 30) Another study has shown that patients with high education have a greater probability of receiving curative treatment compared to low educated cancer patients. (31) These factors may be less relevant to SCLC cases, since the majority of SCLC patients are diagnosed with metastatic disease. (32, 33)

The aim of paper I was to investigate the potential influence of educational level (as the measure of SEI) on treatment patterns, lead times, and outcomes in Swedish SCLC patients.

1.5 Clinical symptoms and prognosis

The most frequent symptoms of SCLC include a cough, dyspnea, weight loss, and fatigue. Most patients are diagnosed within three months of symptom onset, reflecting a short tumor doubling time. (34) For stage I-III disease, the median survival ranges from 15 to 20 months and the five-year survival rate is approximately 10%. (35) In contrast, for patients with stage IV disease, the median survival is 8 to 13 months and the five-year overall survival (OS) is only 1%. (36) In **Figure 2**, the five-year OS for SCLC and NSCLC patients diagnosed in Sweden between 2012 and 2018 are presented. (19)

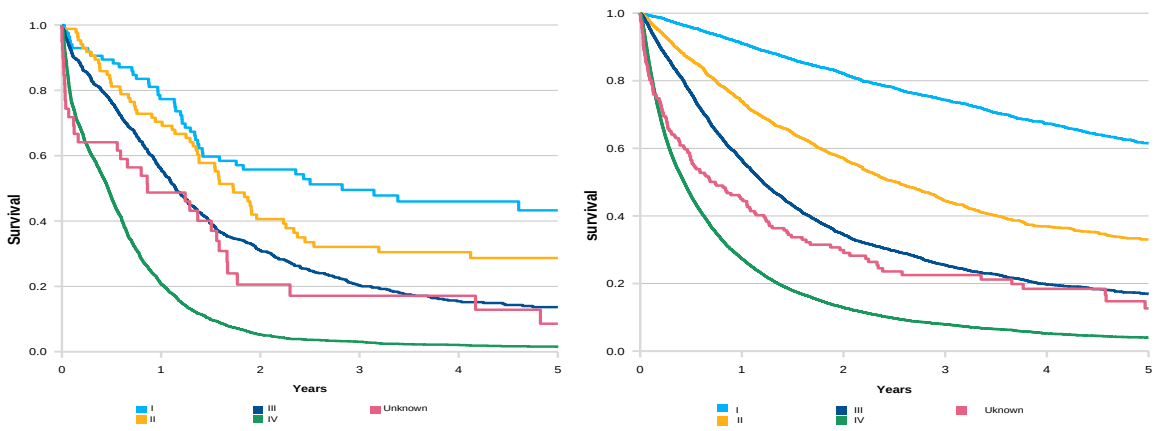

Figure 2 Prognosis for Lung Cancer patients according to stage of the disease

The overall survival for patients diagnosed with SCLC (n=3362) (left) and NSCLC (n=22132) (right) by stage of the disease (7th TNM) in Sweden between 2012-2018. *Picture from the Swedish National Lung Cancer Register.*

1.6 Classification of SCLC according to the VALSG classification and TNM

The classification systems used for SCLC patients are constructed according to the assessment of tumor burden at diagnosis and are key to determining prognosis. (37, 38) Early attempts to classify patients into subgroups have been performed to separate potentially curative patients from palliative ones. (5) In the late 1950s, Veterans' Administration Lung Study Group (VALSG) developed the first staging system for SCLC, which divided cases into Limited Disease (LD) or Extensive Disease (ED). (38) LD encompasses tumors confined to one hemithorax with or without regional lymph node involvement and where the tumor burden can be effectively included in the same radiation portal. ED comprises everything beyond LD. (38, 39) The VALSG classification system was later elaborated by the International Association for the Study of Lung Cancer (IASLC) to encompass the contralateral mediastinal, hilar, and supraclavicular nodes into the definition of LD. (37)

The tumor, node, and metastasis (TNM) classification system was developed in the 1980s to better stratify LC patients. (40) Since then, it has been periodically revised with updated editions. A major revision resulted in the 7th TNM edition in 2007, which included 81,495 LC patients out of which 12,000 were SCLC cases. (37, 41) The 7th TNM edition added new subcategories to the tumor size (T)- and metastasis pattern (M)-descriptors. (42) No new stage categories were introduced. (37) In the 7th TNM edition, pleural or pericardial dissemination (effusions or nodules) were no longer classified as having localized disease (T4). (42) This subgroup was instead included into the metastatic descriptor (M1a) in order to distinguish patients treated with chemotherapy (CT) alone. (43) Despite certain improvements, the 7th TNM edition had several weaknesses, including the fact that the population used in this edition was largely limited to patients of European or North American descent and radiological tools, such as ^{18}F-FDG-PET/CT scan, were not yet implemented for clinical staging. (44)

To further improve the TNM classification system, IASLC developed the 8th TNM edition, analyzing data of approximately 70,000 LC cases (among them 6,000 SCLC) diagnosed between 1999 and 2010 in 16 countries. (45) In the 8th TNM edition several new subgroups were added according to the size of the primary tumor (T-descriptor). (46) Two new subcategories were also added to the M-descriptor, with patients designated with a single metastasis into M1b, while subjects with more than one extra-thoracic metastasis were classified as M1c. (47) There were no changes to the lymph node (N-descriptor) between the TNM editions because of adequate prediction of prognosis. (48)

The changes introduced between the 7th and 8th TNM editions are presented in **Table 1**, and the new stages introduced in the 8th TNM edition are outlined in **Table 2**. (45, 49)

Table 1 The changes between the 7th and 8th TNM editions.

Descriptor	7th TNM	8th TNM
T-descriptor		
</=1cm	T1a	T1a
>1-2 cm	T1a	T1b
>2-3 cm	T1b	T1c
>3-4 cm	T2a	T2a
>4-5 cm	T2a	T2b
>5-7cm	T2b	T3
>7 cm	T3	T4
Bronchus <2 cm from carina	T3	T2
Total atelectasis	T3	T2
Invasion of diaphragm	T3	T4
Invasion of mediastinal pleura	T3	-
M-descriptor		
Metastasis within the thoracic cavity	M1a	M1a
Single extrathoracic metastasis	M1b	M1b
Multiple extrathoracic metastasis	M1b	M1c

Table 2 New stages introduced in the 8th TNM edition.

Stage	T-descriptor	N-descriptor	M-descriptor
IA1	T1a	N0	M0
IA2	T1b	N0	M0
IA3	T1c	N0	M0
IB	T2a	N0	M0
IIIC	T3/T4	N3	M0
IVA	Any T	Any N	M1a/M1b
IVB	Any T	Any N	M1c

Besides the VALSG classification, which was specifically developed for SCLC, the 8th TNM classification system was validated on predominantly NSCLC patients. (45) The VALSG classification remains in use for guiding the treatment of SCLC patients. Despite this, for diagnosing SCLC patients, the TNM system is generally encouraged. (45) Therefore, the goal of paper II was to validate the 8th TNM edition, specifically as it relates to SCLC patients.

1.7 Radiological tools used in SCLC diagnosis

The diagnosis of SCLC is highly dependent on the different radiological modalities. (50) All radiological tools described below are important for accurately staging SCLC patients and are used either separately or in combination to determine the appropriate treatment option for each patient.

1.7.1 The role of CT scan

The first step of identifying and staging SCLC patients is to perform a computed tomography scan (CT scan) of the thorax and upper abdomen. This radiological modality is performed to evaluate the tumor burden and the effect of oncological treatments. (50)

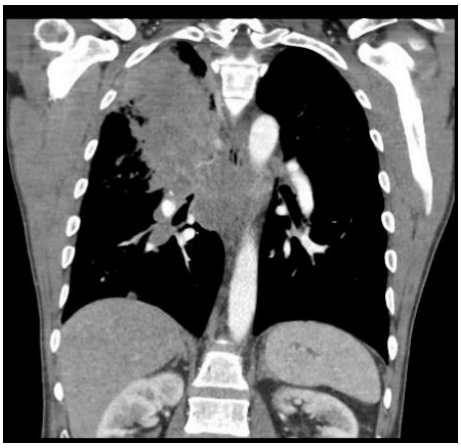

Figure 3a CT scan thorax/abdomen of a SCLC patient. A CT thorax/abdomen scan performed on a SCLC patient showing a centrally localized tumor prior to initiating palliative chemotherapy.

1.7.2 The role of ^{18}F-FDG-PET/CT scan

Fluorodeoxyglucose (FDG)-positron emission tomography scan (^{18}F-FDG-PET/CT scan) is a radiological method using the fluorodeoxyglucose radioisotope that is administered into the patients. It is then taken up by high-glucose- using cells, including tumor cells. (51) Therefore, this radiological modality combines functional (by ^{18}F-FDG-PET) and anatomical information (by CT scan). (52) ^{18}F-FDG-PET/CT scan is used in SCLC patients to more precisely assess mediastinal lymph node status and exclude distant metastasis prior to initializing therapy with curative intent. (52, 53) Previous reports have shown that adding ^{18}F-FDG-PET to CT scan improves accuracy of staging and can result in a change of treatment. (44, 54, 55) The usage of ^{18}F-FDG-PET/CT scan became part of clinical practice in Sweden in 2008.

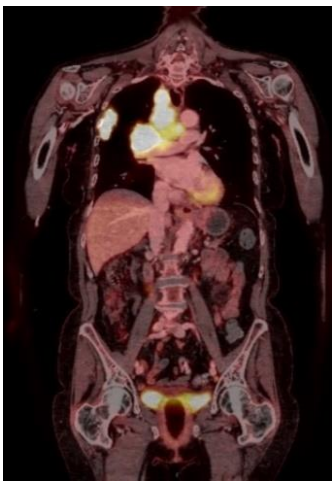

Figure 3b ^{18}F-FDG-PET/CT scan of a SCLC patient ^{18}F-FDG-PET/CT scan performed on a SCLC patient with a lymph node conglomerate located in the mediastinum with a high FDG-uptake and a primary tumor in the upper right lobe.

1.7.3 The role of CT scan and MRI of the brain

At the time of diagnosis, 15%-20% of SCLC patients have brain metastases, and the risk of development increases during the course of the disease. (56) The absence or presence of brain metastasis guides the treatment of SCLC patients. (57, 58) Therefore, it is recommended that SCLC patients receive a CT scan and MRI of the brain prior to initializing therapy to identify potential intracranial metastases. (56, 59) An MRI is better at finding small brain lesions compared to a CT scan of the brain. (56)

1.8 Tumor biology and signaling aberrations in SCLC

SCLC is characterized by a rapid tumor growth and a high degree of genomic alterations. (60) It has been suggested that most of the mutations observed in SCLC are passengers; they do not contribute to growth. This makes identifying the relevant driver mutations more difficult. (60) Whole genome sequencing performed on human SCLC samples has not found evidence of mutually exclusive targetable driver oncogenes. (61) The tumor suppressor genes *TP53* and *RB1* are inactivated in 90% and 65% of SCLC cases, respectively. (62) Mutations in targetable oncogenes, such as epidermal growth factor receptor (EGFR), are rarely found in SCLC. (63) However, recent publications suggest that SCLC may be subcategorized into four distinct types; SCLC- Ascl1(A) - NeuroD1(N), - YAP1(Y), and - POU2F3(P), with each letter representing the main transcription factor involved in each subtype. (63) The neuroendocrine-derived subgroup, SCLC- A, is the most common (70%). (63) The SCLC-Y and SCLC-P subtypes lack neuroendocrine features and have a different origin and growth pattern when compared to the SCLC-A subtype. (64, 65) The SCLC-Y and SCLC-P subtypes include a small proportion of SCLC patients. (63)

1.9 Notch signaling pathway activation (Notch1 and Hes1)

The Notch signaling pathway regulates several cellular processes in healthy individuals, including cell differentiation during both embryonic and adult development. (66, 67) The Notch pathway is also relevant in solid tumors, being either pro-tumorigenic or tumor-

suppressive, depending on the cellular context. (68) In certain cancers, including malignant melanoma and mesothelioma, the Notch signaling pathway acts as an oncogenic driver. (69, 70)

The Notch pathway is regulated by a short-range cell to cell signaling. (71) It consists of several receptors (Notch-1 to 4), and is inactivated in a majority of SCLC cases. (67, 72) The Notch1 receptor has been described as the main Notch receptor involved in regulating the proliferation of neuroendocrine cells. (66, 73) Notch1 has a higher affinity at the ligand-binding site compared to other Notch receptors. (74, 75) Some of the proteins regulated by Notch1 have been identified. One such protein is the hairy/enhancer of split 1 (Hes1), a transcription factor that controls neuroendocrine cell differentiation. (76) The consequence of Hes1 is among other things inhibition of transcription of the neuroendocrine transcription factor, achaete-scute complex homologue 1 (Ascl1). (67, 76) This subsequently leads to a switch towards proliferation of non-neuroendocrine cells, which are slow growing and have a seemingly chemo-resistant feature to platinum. (71, 77)

Notch signaling activation occurs between different cells when a ligand binds to its receptor, *in trans*. (78) The Golgi apparatus is the cellular localization for generation of Notch receptor proteins in which both protein synthesis and peptidase processing takes place generating an intermediate form of the Notch receptor. When a ligand binds onto the Notch receptor at the cell surface, proteolytic cleavage (S3 cleavage) of the Notch receptor by γ-secretase is triggered, resulting in release of the Notch intracellular domain (NICD) into the nucleus, where it induces transcription of the target genes, including Hes1. (78) This results in activation of the Notch signaling pathway. The activation is transient as the endogenous NICD degrades rapidly and is present at Notch target promoters only in response to the ligand. (67, 72) A simplified illustration of this Notch signaling activation is illustrated in **Figure 4a**, below.

1.10 Notch1 signaling pathway inactivation (Ascl1 and DLL3)

The Notch signaling pathway is inactivated by Notch ligands, which includes the transmembrane proteins; Delta-like ligand-1, 3, and 4 (DLL1, DLL3, DLL4) and Jagged 1 and 2 (JAG1 and 2). (72) DLL3 differs from the other Notch ligands by having a smaller amount of epidermal growth factor (EGF)-like repeats. (79) DLL3 expression is regulated by Ascl1, a transcription factor that induces neuroendocrine cell proliferation and regulates the proliferation of pro-oncogenes such as *Sox2, Myc-L and BCL-2*. (80-82) When expression of Ascl1 is silent, the DLL3 expression also seems to be suppressed. (83) Furthermore, studies have shown that ASCL1 and Notch1 expression have a mutually exclusive expression pattern. (84, 85)

Notch signaling inactivation occurs when DLL3 binds to its Notch receptor within the same cell (cis-interaction), in an autonomous pattern. (86, 87) The DLL3 expression is found in the cytoplasm of normal tissues, while in SCLC cells the ligand is expressed homogeneously on the cell surface. (88) When DLL3 binds to Notch receptor on the cell membrane, *in cis*,

Notch1 is relocated to the Golgi apparatus and becomes inactivated. (67) The inactivation of Notch signaling pathway results in neuroendocrine cell proliferation, which is believed to be more sensitive to platinum CT. The Notch signaling pathway inactivation is show in **Figure 4b** (71, 77)

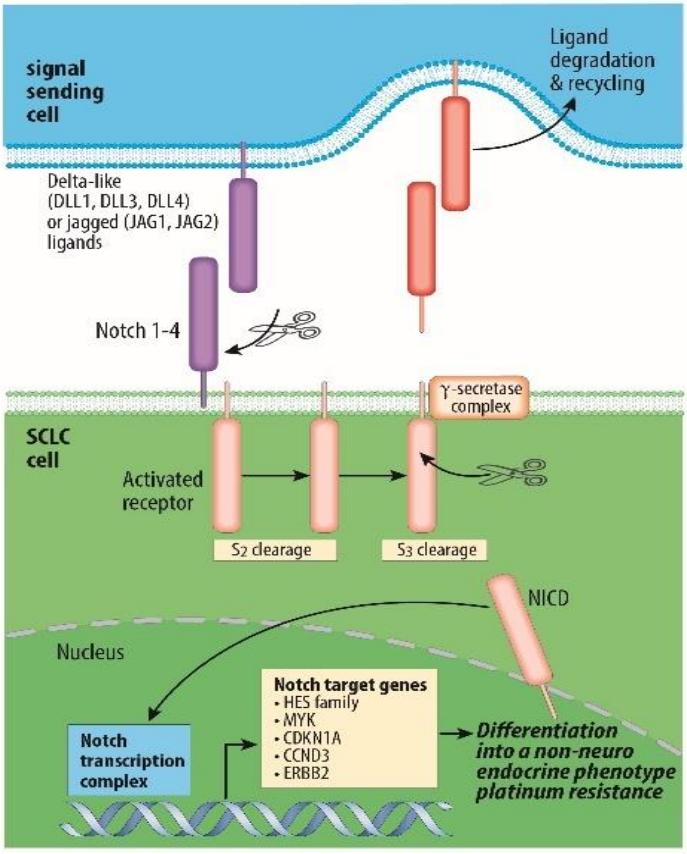

Figure 4a Notch signaling activation.

1. Mindbomb is a protein that ubiquitinates and activates the Notch ligand(s) on the signaling sending cell and then binds to the Notch receptor, *in trans*, on the plasma membrane of the receiving SCLC cell. 2. The S3 cleavage via the proteases, y-secretase, results in releasing the NICD from the transmembrane portion of the Notch receptor and moves the NICD to the nucleus where it activates Notch target genes such as Hes1. 3. The activation of Notch signaling pathway results in a non-neuroendocrine cell proliferation, which is believed to be more resistant to platinum CT.

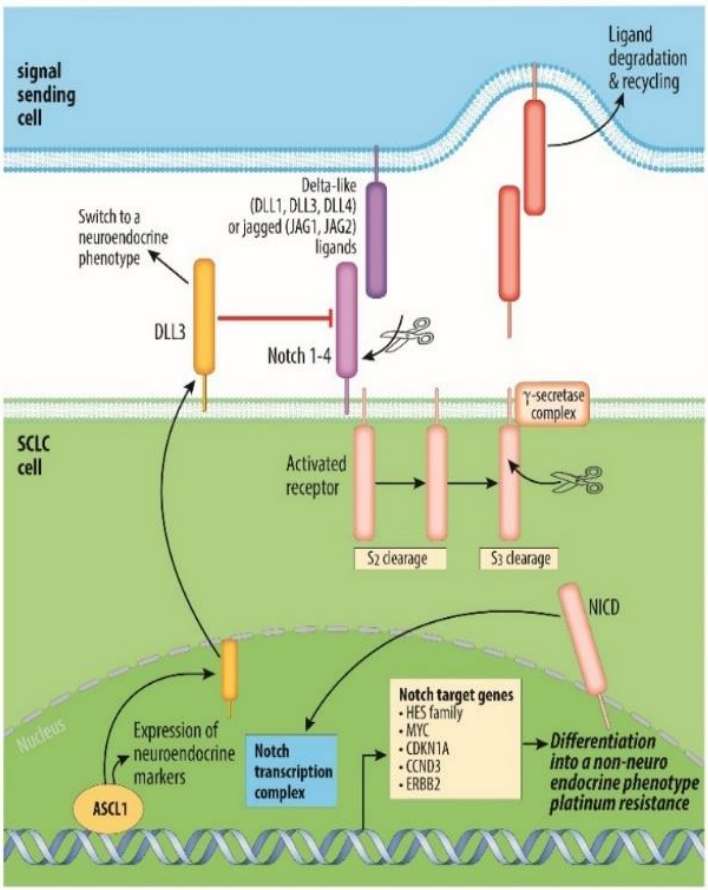

Figure 4b Notch signaling inactivation

1. Ascl1 transcription factor activates the Notch ligand (DLL3). DLL3 moves to the cell membrane where it binds to the Notch receptor, *in cis*, and competes for the same binding site as the sending cell. 2. When DLL3 has successfully, the release of NICD is inhibited and Hes1 is subsequently not expressed. 3. The inactivation of Notch signaling pathway results in a neuroendocrine cell proliferation, which is believed to be more sensitive to platinum CT.

1.11 Treatment of SCLC

There are many different treatment options administered to SCLC patients depending on the stage of the disease and the patient's PS. (89)

1.11.1 Surgery

Surgery is not the main treatment for patients with SCLC as surgical resection of the primary tumor is only indicated for those subjects who present with a solitary pulmonary nodule without mediastinal involvement or distant metastasis. (33) In the IASLC database, only 4% of SCLC cases underwent surgery. (33) There are no randomized clinical trials (RCT) that have compared surgery vs. surgery followed by adjuvant CT or chemo-and radio-therapy (CRT). (90) However, in an observational study, patients receiving adjuvant CT (with or without radiation) had an improved five-year OS compared to those who performed surgery alone (53% vs. 40%, respectively). (91) Today, adjuvant CT is recommended for SCLC patients who have undergone tumor resection. (90, 92) Patients with a lymph nodal involvement may be considered to receive adjuvant CRT. (93)

1.11.2 Concomitant CRT for stage I-III disease

The combination of CRT is the standard of care for patients whose tumors are considered unresectable but may be potentially cured (stage I-III disease). Two meta-analyses have demonstrated that CRT significantly improves survival outcomes compared to CT alone. (94, 95) Administering RT in combination with CT (concomitant) results in better survival outcomes than using RT after CT (sequential). (94, 96) The most frequent start of RT is after the first cycle of CT. (97) The PDCT regimen is the preferred CT administered in the first-line (1st line) setting of stage I-III disease.

There remains uncertainty regarding the optimal radiation dose and fractionation. The most frequently used schedule today is a total RT dose of 45 Gray (Gy) delivered in 30 fractions, twice per day (bid). An RCT from 1999 showed that the twice per day (bid) schedule was superior compared to a once per day schedule (5-year OS of 26% vs. 16%). (98) The main difference between these schedules appears to be the temporary acute toxicity, with an increase in grade III esophagitis when using the bid schedule (27% vs. 11%, $p < 0.001$). (98)

1.11.3 PCI for patients with stage I-III disease treated with CRT

The general recommendation for patients who have responded to CRT is prophylactic cranial irradiation (PCI) in order to prevent the development of brain metastases. (99) Two meta-analyses have evaluated the role of PCI in patients who achieved complete remission after CRT. (99, 100) The results from the first study showed that there was a significant decrease in incidence of brain metastases and mortality with PCI compared to observation with no treatment (hazard radio 0.46; 95% CI 0.38-0.57). (99) A second meta-analysis evaluating twelve RCTs reported similar findings. (100) Today, the most common PCI schedule used in Sweden is 2.5 Gy delivered in 10 fractions, once per day. (19)

1.11.4 Systemic 1st line CT for stage IV disease

Patients who are diagnosed with stage IV disease are generally recommended systemic 1st line treatment with PDCT. (35) The treatment in the 1st line setting had remained unchanged for several decades until the recent introduction of Atezolizumab (anti PD-L1 blockade inhibitor), which is administered together with PDCT. The results from a study, known as the Impower-133, found a two month prolonged median OS in favor of the experimental arm — Atezolizumab together with PDCT rather than PDCT alone (12.2 months vs. 10.2 months, respectively). (101) The combination of PDCT together with Atezolizumab as maintenance therapy became approved by the U.S. Food and Drug Administration (FDA) in 2019. (102)

In multiple RCTs, PDCT appears to be a more effective CT combination than older regimens such as cyclophosphamide, epirubicin, and vincristine. (103, 104) Carboplatin and cisplatin are the two types of platinum-agents that can be used in the PDCT regimen. Carboplatin is typically preferred to cisplatin, because carboplatin has a favorable toxicity profile. (105) (106) However, a meta-analysis found no statistically significant differences in survival outcomes between the two platinum agents. (107) There are also two different kinds of CT

agents used together with platinum; irinotecan and etoposide. (108) Etoposide is more frequently used in the Western world, while irinotecan is preferred in Asian populations. Four RCTs have compared these two agents together with platinum in the 1st line setting. The results showed no significant survival differences, with less hematologic and more gastrointestinal toxicity with irinotecan. (108-111)

1.11.5 Re-challenge with PDCT in 2nd line setting for stage IV patients

Most SCLC patient inevitably progress after receiving PDCT. Previous studies have shown the difficulty in treating these patients beyond the 1st line setting, because of platinum resistance. (112, 113) Relapsed patients survive only 2 to 4 months without second-line (2nd line) therapy. (114, 115) Re-challenge is defined as the administration of the same PDCT regimen provided in the 1st line setting. (116) Patients who have a longer duration without relapse after receiving 1st line PDCT will benefit more from re-challenge than patients with a shorter time before relapse. (116) According to this principle, relapsed patients are defined as either “sensitive” or “refractory” to PDCT. (114) The definitions of these subgroups differ between studies. The most frequently used definitions for “sensitive” and “refractory” relapse are defined as progression-free survival (PFS) of longer or shorter than 90 days after completion of CT, respectively. (114, 117, 118) Patients with “sensitive” relapse are recommended re-challenge with PDCT, while patients with "refractory” relapse should receive another CT regimen. These recommendations are based on several studies. (116, 117, 119, 120)

Other CT agents used in the 2nd line setting include taxanes, vinorelbine, gemcitabine, and temozolomide. These are usually administered as monotherapy and have response rates around 10%-15%. (121-124)

1.11.6 Consolidating RT for stage IV

Patient with stage IV disease, who have a complete response of distant metastasis and residual disease limited to the thorax after 1st line therapy, can be treated with consolidating RT to the thorax. (125, 126) The rationale for this treatment approach is to improve local control of the remaining tumor burden and ultimately prolong the survival. (125, 126) In an RCT, patients receiving 30 Gy to the residual tumor in the thorax had a longer median OS compared to the observational group. (125) The survival curves diverged after 18 months, with a statistically significant longer two-year OS in favor of the RT group, 13% vs. 3%, respectively ($p = 0.004$). (125) Because of this trial, consolidating RT has been used for stage IV patients more frequently during the past years. (125)

1.11.7 PCI for stage IV disease

The rationale of administering PCI in stage IV patients who have responded to 1st line CT is to decrease the risk of developing brain metastasis and thereby improve survival. (127) However, the prognostic impact of PCI in stage IV remains uncertain, as RCTs have shown conflicting results. (127, 128) A phase III trial conducted in Europe (n=286) randomized

patients 1:1, receiving either PCI or observation. (128) A brain MRI was not a requirement prior to PCI for this study, which meant that some patients may have had brain metastasis prior to initializing the therapy. (128) The results showed that patients treated with PCI had a statistically significant decreased risk of developing symptomatic brain metastases after a one-year follow-up (15% in the PCI group vs. 40% in the observational group developed brain metastasis). A Japanese trial (n=224) had a similar design, but patients were required to have performed an MRI of the brain prior to initializing PCI, and the presence of brain metastasis was an exclusion criterion. (127) The results from this study did not show a survival benefit for patients receiving PCI. The general recommendation for stage IV patients is currently to limit the use of PCI to those patients that have a complete response to 1st line CT and have a PS of 0-1. (19)

1.11.8 Systemic 3rd line therapy for stage IV disease

A minor proportion of SCLC patients have a PS which allows them to receive a third-line (3rd line) treatment. For those who are eligible, the survival outcomes are generally poor. (119) The only FDA approved drug in the 3rd line setting is an immunotherapy, nivolumab (anti-programmed cell death receptor 1) antibody, with a response rate of less than 20%. (129) Other treatment options depend on the individual patient's treatment history and may include a single-agent CT if the patient has already progressed on PDCT. (130)

1.11.9 Role of molecular targeted agents in SCLC

Several molecular targeted agents have been tested in phase I and phase II clinical trials for 1st, 2nd, and 3rd line SCLC patients, as well as maintenance after 1st line. (131-133) Some of these agents act broadly on the tyrosine kinase signaling pathways (for example, sunitinib, bevacizumab, and everolimus). The phase II and phase III clinical trials testing these drugs have all shown poor response rates as well as poor tolerability when used alone or in combination with PDCT. (134-137) Furthermore, therapies targeting other signaling pathways including epidermal growth factor receptor (EGFR), PI3K/AKT/mTOR**,** and insulin-growth factor receptor (IGFR), have also demonstrated poor efficacy. (138, 139)

One of the most promising agents developed recently is the so called Rovalpituzumab Tesirine (Rova-T), an antibody-drug conjugate (ADC) composed of a humanized DLL3-specific IgG1 monoclonal antibody, a protease-cleavable linker, and a DNA cross-linking agent pyrrolobenzodiazepine (PDB). (81) Rova-T has been shown to bind DLL3 with high affinity on the cell surface. Internalization to the lysosome occurs rapidly, resulting in the cleavage of the linker and release of the toxin, which subsequently leads to a cell-cycle arrest. (81, 86) In a patient derived xenograft (PDX) model, mice treated with Rova-T had a prolonged response compared to mice treated with platinum CT. PDX tumor models that were resistant to platinum also showed responses to Rova-T. (81)

In a first-in-human, open-label phase I study, 11 (17%) patients receiving Rova-T achieved durable responses and 35 (54%) patients had stable disease (SD). (140) Thirty-nine patients provided tumor samples for analysis of DLL3 expression. Twenty-nine assessable patients

had DLL3-high tumors (> 50% or more tumor cells by IHC). None of the 10 patients with DLL3-low tumors (< 50%) showed a clinical response. Rova-T was found to have unacceptable delayed toxicity in humans, such as pleural effusion. (140) One reason may be that the PBD toxin stays in the systemic circulation because of a premature lysis of the linker attached to the PBD toxin. This subsequently leads to severe toxicity since the DLL3 ligand is not expressed on the cell surface of normal tissues. (141)

Rova-T has been investigated in several RCTs without showing any survival benefit. (142) These results have been disappointing considering the promising antitumoral effects of Rova-T in mouse models. Further studies are needed to modify the molecule, for example by adding an alternative cytotoxic payload, to achieve acceptable tolerability.

The timeline for the development of therapies in SCLC is described in **Figure 5**.

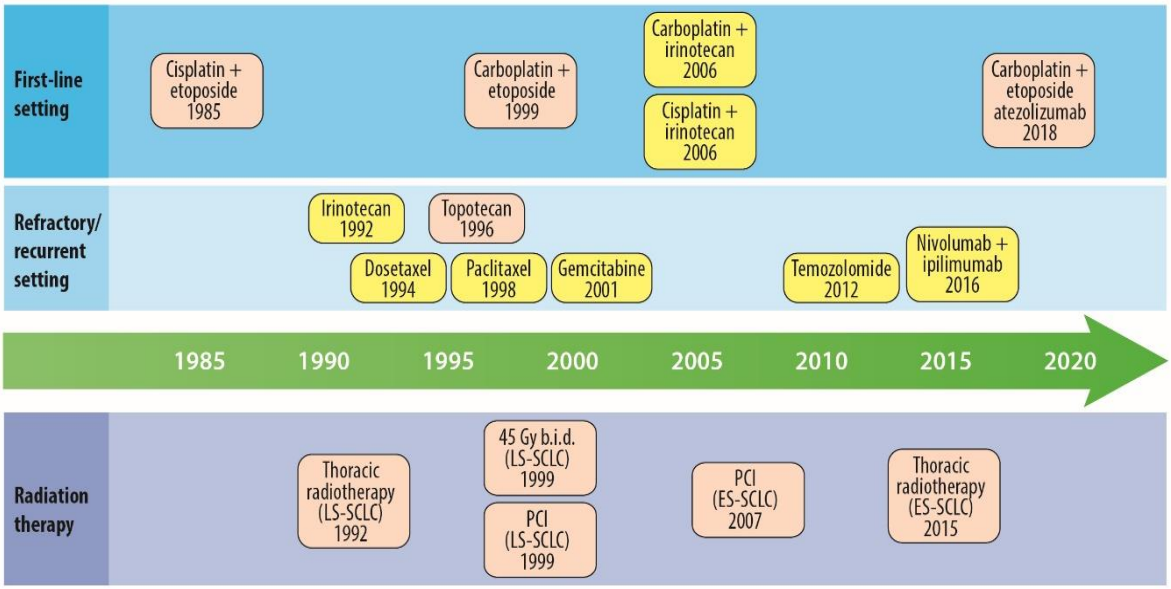

Figure 5 Timeline of treatment options in SCLC from the 1980s to 2020.

The development of treatment options for SCLC with regards to chemotherapy, radiotherapy, immunotherapy and different combinations. Abbreviations; LS- Limited stage, ES- Extensive stage, PCI- Prophylactic brain irradiation, B.i.d- twice per day.

2 Aims of the Thesis

The purpose of the thesis was to expand on the understanding of SCLC; epidemiological trends and treatment patterns, changes to classifying patients and prognostic biomarkers. The specific aims for each paper were;

Paper I was performed to investigate if there was an association between educational status and overall death of Swedish SCLC patients. It also examined if educational level influenced management and lead times.

Paper II aimed to discover if the 8th TNM staging system could provide additional prognostic information compared to the previous 6th and, 7th TNM versions and the older VALSG classification system.

Paper III evaluated the treatment patterns of SCLC patients. Another goal was to investigate the outcome of SCLC patients upon re-challenge with PDCT, and associations between clinical characteristics and survival outcomes.

Paper IV aimed to analyze the prevalence of the expression of Notch1, Hes1, Ascl1, and DLL3, and the potential association of these four biomarkers to sensitivity to PDCT, prognosis, and clinical characteristics of SCLC.

3 Patients, Material, and Methods

3.1 Patient Cohort

All four studies were based on retrospectively collected data from registries and patient files. The data for paper I was obtained from the LcBase, the NLCR, and several other population-based registers in Sweden, and covered SCLC patients diagnosed between 2002 and 2011. For paper II, III, and IV, the patient data was obtained from the NLCR with some clinical characteristics also collected from each individual health record file. For paper II and III, SCLC cases were obtained over an eight year time period, from 2008 to 2016. In paper IV, all patients' samples were retrieved from the Karolinska University Hospital, diagnosed with SCLC between 2008 and 2015. The cohort was highly selected depending on the amount of biopsy material, PDCT administered in 1st line setting, and follow-up data in order to evaluate the specific aims of the study. These strict criteria for paper IV resulted in excluding certain cases. All projects were undertaken with approved ethical and regulatory permissions (EPN 2012/1162-31/4, 2016-8/31), as well as permission from the Stockholm medical biobank (BBK 1693 FUB 2016087).

3.2 Re-classifying patients according to the TNM classification

In paper II, each case was re-classified from the VALSG classification to the different TNM editions (6th, 7th, and 8th) by using all available radiological scans at the time of diagnosis. The radiological scans available were reviewed; including CT scan of the thorax and abdomen, ^{18}F-FDG-PET/CT scan, CT/MRI scan of the brain, and ultrasound of the liver. Radiological scans that were difficult to assess were reviewed together with an experienced radiologist.

3.3 RWD analysis and definitions of treatment patterns and outcomes

Real-world data (RWD) analysis means data collected for the purpose of evaluating the treatment outcomes for patients not treated in clinical trials. RWD analyses can be used to support regulatory decision-making and to guide clinical practice. (143)

In SCLC patients, the survival outcomes have been shown to be worse in a real-world setting compared to RCTs (including the observational group). Thus, a matched comparison analysis showed a survival benefit for patients treated in an RCT compared to a real-world setting. (144, 145) One of the suggested explanations may be that patients enrolled in RCTs are a small and highly selected subgroup, which includes younger subjects who have a better PS compared to patients in clinical practice. (146-148) This highlights the limitations of extrapolating data from RCTs to a real-world setting.

The definitions of survival outcomes (PFS and OS) were important for evaluating the results presented in paper III and IV. In paper III, the PFS was defined as the start of each line of CT until documented progression either clinically, on a radiological scan, or death. The definition for OS was the same for both papers, defined as the interval between the date of initiating each line of CT until death. (149)

However, the definition for platinum sensitivity to 1st line PDCT differed between the papers. In paper III, the "sensitive" subgroup only included patients that relapsed after PDCT and had a PFS of longer than 180 days after start of 1st line, since the aim was to evaluate the cases that were eligible for re-challenge. In paper IV the "sensitive" subgroup included all patients with a PFS of longer than 90 days after completion of PDCT, regardless if relapse occurred, since the aim was to investigate the sensitivity of platinum CT with regards to biomarkers.

3.4 Immunohistochemistry

Immunohistochemistry (IHC) is an established routine diagnostic method that uses antibodies to detect the localization and staining patterns of proteins in a tumor specimen. (150, 151) This diagnostic method is widely available and cost-efficient with a rapid turnaround time, making it the foundation of diagnosing SCLC. (151) In paper IV, the protein expression was determined using IHC on 4-µm-thick formalin-fixed, paraffin-embedded (FFPE) sections. (152) The Notch1, Hes1, and Ascl1 antibodies were applied manually, and several concentrations for each antibody were tested on human SCLC control cases in order to determine the optimal concentration. Two DLL3 antibodies were also tested using western blot and IHC, with poor results. (153) Therefore, when the automated immunostaining instrument (Ventana platform) for the DLL3 antibody became commercially available, it was used for paper IV. (154, 155)

The FFPE sections were de-paraffinized in xylene and rehydrated in alcohol. To visualize the primary antibody binding a secondary antibody was applied. For each case, one hematoxylin-eosin staining was performed and a positive/negative control for each biomarker was performed according to the manufacturer's instruction. (156)

The IHC evaluations was performed by one pathologist (L.K), who was blinded to the clinical data. The number of positive tumor cells was counted under high magnification (x20 and x40). The tumor cells were counted in a quantitative manner and three random and non-overlapping fields (with approximately 100 tumor cells per field, a total of 300 tumor cells per specimen). (157) The scoring of IHC staining were made into four categories according to the number of positive tumor cells stained; 0: No positive cells, 1; ≥1-25% positive cells, 2; ≥26-50 % positive cells, 3; ≥51-75% cells, 4; ≥76% positive cells. The staining intensity was defined as any positivity in the tumor cells of each specimen as previously described. (158, 159)

Of the analyzed markers only DLL3 had a previously established cut-off between low vs. high expression (more or less than 50% of neoplastic cells, respectively). (140) For the other markers; Notch1, Hes1, and Ascl1, a cut-off was established based on sensitivity to PDCT using a dichotomizing score with receiver operating characteristic (ROC) curve analysis. The hypothesis was that Notch1 and Hes1 high expression would be more "refractory" to PDCT, while Ascl1 high expression be more "sensitive" to PDCT. The most statistically significant cutoff for each biomarker was defined as the point on the ROC curve giving the smallest distance to the point; 1-specificity, sensitivity = (0,1). (160)

3.5 Statistical analysis

Two statisticians analyzed the data collected for paper I-IV in the thesis. Established prognostic clinical factors for each paper were included in uni- and multi- variate cox regression analysis using hazard ratio (HR) of 95% confidence interval (CI). Statistical significance was defined as $p< 0.05$. (161) For paper II, III, and IV, the survival models were compiled with the Kaplan-Meier curves and the log-rank test. (162) The correlation between the different biomarkers in paper IV was analyzed using Pearson's correlation coefficient. (163)

Statistical analysis presented in paper II was performed using the JMP software version 5.1.2, while R 3.5 (R Foundation for Statistical Computing, Vienna, Austria) was used in paper I and III. The statistical analyses for paper IV was carried out using SPSS© version 25 (IBM Corp, Armonk, NY, USA).

4 Results and Discussion

4.1 The influence of educational level on outcome of SCLC patients (Paper I)

Paper I aimed to explore a potential association in SCLC patients between educational level and death. This included examining if educational level influenced management, lead times, and prognosis of SCLC patients over a 10-year period. These research topics had not previously been explored in a SCLC cohort.

We identified a large number of patients for this nationwide register-based study (n=4256). The median age at diagnosis was 69 years (Interquartile range of 64-75), 50.3% were women, and the majority of patients were diagnosed with ED (74.4%). Over 50% of cases had low education, while 39% had a medium level of education and 11% of the patients were categorized as having high education.

The study had a number of key findings. With respect to lead time and treatment patterns, there were no significant differences between patients with low, medium, and high educational levels.

However, educational level was found to influence prognosis in certain populations groups. Men with a high educational level had a better prognosis (high versus low: HR 0.84, 95% CI 0.73-0.98). This association was not observed in women. (HR 0.92, 95% CI 0.78-1.09). The reason men with a lower education had a worse prognosis may be explained by the rate of smoking cessation after diagnosis. Men with a low education were more likely to continue smoking post-diagnosis compared to those with high education in a previous study. (164) It is known that continued smoking after being diagnosed with LC reduces the efficacy of oncological treatments and therefore could result in a worse prognosis. (165, 166) This explanation is a hypothesis, since data on smoking cessation could not be obtained from the registries.

High educational level was also a statistically significant prognostic factor in LD patients (HR 0.76, 95 % CI; 0.58-0.98). This was not found in ED patients. Since SCLC often relapse after curative intended treatment, the adherence to follow-up visits and radiological scans are crucial. It could be speculated that lower educated patients may lack knowledge on the importance of follow-up visits, especially if clinical symptoms related to relapse appears.

The underlying causes contributing to differences between educational level remain unclear, but have been discussed in relation to management of the disease and the role of the healthcare system. (23) This study found that socioeconomic differences exist in SCLC patients, which is in accordance with reports on patients with other cancer diagnoses. (20)

In addition to exploring educational level the study investigated other SEI factors, such as cohabitations status. A poorer prognosis was observed in women living alone (HR 1.15, 95% CI; 1.05-1.26), which was not seen in men. This is in contrast to results from other studies

where it was shown that cohabitation status was a more important prognostic factor in men. (167)

The strengths of this study were that almost all SCLC patients from 2002 to 2011 were included in the study and large amounts of patient data for these patients could be analyzed. A limitation was that data on smoking cessation could not be obtained from the registries. Another limitation was that the study only included patients diagnosed until 2011, which limits the extrapolation of data to today's setting, since there is a trend towards patients receiving more consolidating RT and less PCI during the past decade. (36)

4.2 Validation of the 8th TNM classification for SCLC in a retrospective study from Sweden (Paper II)

The applicability of the TNM classification system has previously been studied mainly on small cohorts with stage I and II disease. (168-170) The results from these studies do not extrapolate to a majority of SCLC cases, since they are predominantly diagnosed with stage IV disease. (171) **Paper II** included a large patient cohort (n= 706), with most patients having locally advanced or metastatic disease. The aim of this study was to investigate if the 8th TNM edition can be used to classify SCLC patients and compare its prognostic performance to the older TNM editions and the VALSG definitions.

The results from this study showed that tumor size (T) and nodal involvement (N) were important prognostic subgroups in the 8th TNM edition, with poorer prognosis observed in patients with larger tumors or more extensive nodular involvement. The proportion of SCLC patients with a single metastasis (M1b) was low, as expected, since SCLC usually presents with a larger metastatic burden. Patients with a M1b disease had a better prognosis compared to subjects with pleural dissemination or multiple extrathoracic metastasis, M1a and M1c, respectively. In recent years, the concept of oligometastatic disease has been incorporated into clinical trials for NSCLC patients were survival outcomes have improved in patients treated aggressively with local therapies such as stereotactic body radiation therapy (SBRT) compared to observation. (172) Patients with M1a disease had similar prognosis with M1c, illustrating the difficulty of treating SCLC patients with pleural dissemination.

The proportion of stage I and II patients was low in this study, which makes the results from these subgroups difficult to interpret. In previous reports, stage I and II patients, had a better prognosis compared to advanced stage disease. (173) A large proportion of patients migrated to three new stage subgroups in the 8th TNM edition; IIIC, IVA, and IVB, which illustrates the importance of these subgroups. Patients with stage IIIC disease had a poor prognosis, similar to stage IVA disease. This illustrates the difficulty of effectively treating subjects with CRT that have a large primary tumor (T3-T4) and extensive lymph nodal involvement (N3-disease). (35)

This study showed that the 8th TNM edition was an independent statistically significant prognostic factor for SCLC patients after adjusting for clinical characteristics in the multi-variate analysis. Given that most SCLC patients are diagnosed with locally advanced or

metastatic disease, the 8th TNM edition will not frequently alter the clinical management. Nevertheless, the 8th TNM edition should be incorporated into clinical research since more precise classification of subgroups could influence treatment approaches in the future.

4.3 Treatment patterns and survival outcomes for SCLC patients – a Swedish single center cohort study (Paper III)

Paper III was an RWD analysis of a large patient cohort (n=544) treated at Karolinska University Hospital and diagnosed between 2008-2016. The aim was to evaluate the treatment patterns and survival outcomes from Karolinska University Hospital compared to other studies.

The survival outcomes of patients with ED in paper III were similar to previous studies. (35, 117) However, the survival of patients with locally advanced disease was shorter compared to a large RCT. (174) One explanation for the discrepancy may be that monitoring of patients in a RCT is more intense, with more frequent follow-up visits and radiological scans, which subsequently leads to longer survival. (144) The number of patients that did not show signs of relapse was similar with historical data. (175)

The survival outcomes for 2nd and 3rd line therapies, regardless of initial stage of disease, were very poor, illustrating the difficulty in treating patients after relapse occurs. (115) The main reasons for these results are that resistance mechanisms occurs rapidly in the course of the disease, together with a deteriorating PS. This can also in part explain the failure of molecular targeted agents and immunotherapies in the 2nd and 3rd line setting. (35, 115)

The evidence of using re-challenge with PDCT is based on retrospective studies. (115, 119) In paper III, both LD and ED patients with Sensitive relapse (Sr) after PDCT had a longer median OS compared to the Resistant relapse (Rr) subgroup. Therefore, the length of PFS after 1st line PDCT can be a predictor for prognosis for 2nd line CT in both LD and ED patients. Patients without treatment for a longer time period may have a better PS when relapses occur compared to those with a shorter relapse-free interval. This enables the patient to better tolerate the next line of CT which may result in a longer survival. Another reason could be that the tumor has not developed platinum-resistance yet. (119)

There were no significant differences in survival outcomes between CT regimens used in the monotherapy setting.

Paper III had several limitations, including that data on tumor responses was not collected. Different radiological modalities were used and strict criteria to evaluate the treatment were therefore not feasible to carry out. Furthermore, there was an inconsistency between patients with regards to the timing of assessing the treatment response, with radiological scans performed after two up to five cycles of CT.

4.4 The prognostic implications of Notch1, Hes1, Ascl1, and DLL3 protein expression in SCLC patients receiving platinum-based chemotherapy (Paper IV)

In **Paper IV** the protein expression of Notch1, Hes1, Ascl1, and DLL3 were analyzed in biopsies of SCLC patients and their correlation to PDCT sensitivity as well as prognosis was investigated. These four biomarkers have not, to the best of our knowledge, been studied in the same cohort previously. (158, 176, 177)

The results showed that Hes1, Ascl1, and DLL3 expression was found in most cases, while Notch1 expression was absent in the majority of samples. Both findings were in accordance to previous studies. (71, 154, 158, 177) A positive staining in the nucleus or cytoplasm was observed for Notch1, Hes1 and Ascl1, while DLL3 staining was confined to the plasma membrane. These staining patterns were in accordance with previous reports. (158, 176, 177)

We found that Notch1 expression was an independent prognostic factors in the multi-variate analysis and that patients with Notch1-low tumor expression had a favorable prognosis. Notch1 expression has previously been linked to chemotherapy refractoriness consistent with a potential pro-tumorigenic role, which is in accordance with our results. (77) However, in another study on surgically resected SCLC samples (n=125), low Notch1 expression was an unfavorable prognostic factor. (177) These conflicting results highlights the need to future investigate the role of Notch1 with respect to PDCT sensitivity and prognosis in SCLC patients. Furthermore, a negative association between Notch1 and Ascl1 expression was found. This finding is supported by an earlier study which mechanistically reported that Ascl1 has the ability to reduce Notch1 at both transcription and post-translational level, the latter by protein degradation. (67)

Hes1 expression was positive for most cases in paper IV, which substantiate *in vitro* results from a SCLC cell line where Hes1 expression was found in cell lines with neuroendocrine features. (178) Furthermore, there was no significant association between Hes1 and Notch1 expression, which indicates that Hes1 expression is not solely regulated by Notch1. (76)

A predictive or prognostic role of Ascl1 or DLL3 with regards to PDCT and OS was not observed in this study, in accordance with earlier reports. (154, 176)

The results in paper IV are difficult to compare to previous studies, since this study mainly included stage IV patients while previous reports have investigated patients with surgically resected primary tumors. (35, 154, 176)

In paper IV, a single diagnostic technique (IHC) was performed, which has been the most important diagnostic technique for SCLC diagnosis for decades. (151) However, the rapid development of more extensive testing is ongoing, including whole genome sequencing techniques which is illustrated by the human protein atlas and the proposed human tumor atlas. (179, 180) These growing databases will hopefully result in identifying new signaling pathways that can be targeted with small molecules that have improved efficacy and better toxicity profile compared to today's standard of care. The highly complex biology behind

SCLC is yet to be fully understood, but it is clear that the Notch signaling pathway has an important role in both the development and resistance mechanisms in SCLC. (67, 72) Future studies on ADCs targeting the DLL3 expression are ongoing, which will hopefully generate more effective drugs for SCLC patients. (86)

5 Conclusions

Paper I showed that a lower level of education in men and LD SCLC patients was associated with a poorer prognosis compared to individuals with a high level of education. Future studies need to address these findings in order to minimize the discrepancies in survival outcomes between educational groups in SCLC.

Paper II reiterated that the stage of SCLC is the most important prognostic factor for the disease. The 8th TNM edition provided an improvement in the nomenclature to describe characteristics of the tumor size and metastatic patterns as well as distinguish subgroups in SCLC. Further prospective studies are required to confirm the results of paper II.

Understanding the outcomes and management for 1st, 2nd, and 3rd line CT and RT in **paper III** both informs and reflects clinical practice. The results emphasize the need for improving treatment options in SCLC, in all lines of therapy but especially for the majority of patients who eventually relapses after 1st line therapy.

Paper IV focused on expression analysis of multiple proteins involved in the Notch signaling pathway. Notch1 was found to be a significant prognostic biomarker, which needs to be further validated on larger cohorts. DLL3 expression was positively stained in a majority of cases and this supports the notion that DLL3 continues to be a promising target.

6 Future Perspectives

Despite advancements in understanding basic and clinical research regarding SCLC during the past decade, several questions remain to be answered in order to improve treatment options for this deadly disease.

Strategies to achieve equal management for SCLC patients with different educational levels include more attention from healthcare professionals and a standardized program, which monitors compliance to treatment and offers social support. In order to improve the survival outcomes of SCLC patients, there is also a need to further understand the patterns of smoking cessation after SCLC diagnosis.

The lack of new drugs for SCLC has been unsatisfactory during the past decades. (36) However, a new wave of enthusiasm has emerged in the field. The knowledge regarding survival outcomes and treatment patterns are important when designing clinical trials with the aim of improving the prognosis for SCLC patients.

Enrolling a higher proportion of SCLC patients into clinical trials with obligatory biomarker analysis will be crucial to achieving better survival outcomes. This will require an active role of physicians treating SCLC patients as well as easing of eligibility requirements for clinical trials. SCLC is a very challenging disease to treat; however, my opinion is that the future for this disease seems brighter than its past.

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