Precision medicine in Non-Small Cell Lung Cancer, with a focus on *KRAS* mutations

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I dedicate this thesis to the memory of my beloved grandmother, with gratitude for all she gave me. Laila "Mållis" Äng (1940-2020).

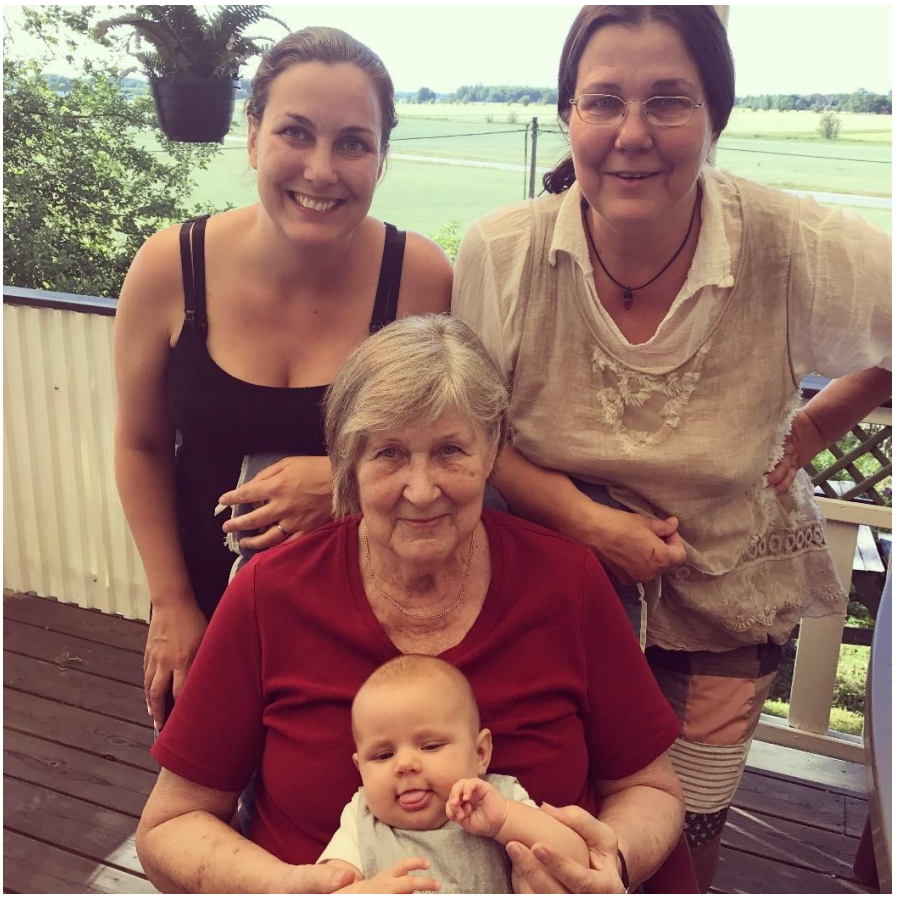

Photo taken by Linus Eklund the summer of 2017capturing four generations.

Now this is not the end. It is not even the beginning of the end.
But it is, perhaps, the end of the beginning.

-Winston S. Churchill

Precision medicine in Non-Small Cell Lung Cancer, with a focus on *KRAS* mutations

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ABSTRACT

KRAS mutations - the most common oncogenic drivers in Non-Small Cell Lung Cancer (NSCLC) accounting for around 35% of patients - were historically linked to poor prognosis. With immune checkpoint blockade (ICB), this may be changing. There is a need to clarify the prognostic and predictive roles of *KRAS* and discover ICB response biomarkers to refine treatment and develop new therapies for *KRAS*-mutant NSCLC.

The aim of this thesis was to broadly investigate the overall prognostic and predictive impact of *KRAS* mutations on currently available treatments, explore new prognostic biomarkers for ICB treatment, study the impact of aging on *KRAS* mutated lung cancer progression and explore novel treatment vulnerabilities.

Our findings from multicenter retrospective studies show that having *KRAS* mutations is a negative prognostic factor overall and for chemotherapy treatment in the metastatic setting (stage IV). However, having a *KRAS* mutation and a high (≥50%) PD-L1 expression is a positive prognostic factor for ICB monotherapy. We have also elucidated that all *KRAS* mutations are not the same, as *KRAS* G12D seems to respond less well than *KRAS* G12C or G12V to ICB. In locally advanced (stage III) NSCLC treated with combined chemoradiotherapy, the negative prognostic impact of *KRAS* mutations can be reversed with the implementation of adjuvant ICB treatment. In contrast to stage III and stage IV disease we found that in early-stage resectable NSCLC (stage I-II), *KRAS* status – alone or combined with tumor size – did not affect overall survival. In the prospectively included BIOLUNG cohort we identified *KRAS+LRP1B* mutation (and *KRAS+LRP1B+TP53*) as a potential predictive biomarker for ICB benefit. Finally, aging epigenetically induces *ATF4* in *KRAS* mutated adenocarcinoma, driving EMT, anoikis resistance, and glutamine-dependent metabolic plasticity for metastasis in preclinical models, and higher *ATF4* correlated with advanced stage and poorer survival in patients. This finding has identified new potential therapeutic targets.

In conclusion, findings in this thesis add to the understanding of the prognostic and predictive impact of *KRAS* mutations and that it is context dependent. Potential new predictive biomarkers for ICB treatment have been identified and provided important biological insight including identifying novel therapeutic targets.

Keywords: Non-Small Cell Lung Cancer, *KRAS*-mutations, precision medicine

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SAMMANFATTNING PÅ SVENSKA

Lungcancer är en av de vanligaste och dödligaste cancerformerna i världen. Cancer uppstår när celler böjar växa okontrollerat och det bildas tumörer. Oftast orsakas cancer pga. att det blivit skador på cellens arvsmassa, så kallat DNA. Den vanligaste orsaken till skada är rökning. Hos ungefär en tredjedel av patienterna orsakar skadan förändringar i genen *KRAS*, en mutation, så att det kontinuerligt signaleras till cellen att växa och dela på sig. Tidigare kopplades mutationer i *KRAS* till sämre prognos, men införandet av immunterapi (en ny sorts cancerbehandling) kan ha ändrat bilden. För att ge rätt behandling till rätt patient behöver vi förstå när *KRAS* betyder något för prognosen och om det finns biomarkörer som förutser vem som har nytta av behandling med immunterapi.

Syftet med avhandlingen var att brett undersöka hur *KRAS* påverkar utfallet av dagens behandlingar, att hitta nya biomarkörer för immunterapi, att studera hur åldrande påverkar sjukdomsförloppet vid lungcancer samt att identifiera nya svagheter som kan utnyttjas i behandling.

Vi fann att *KRAS*-mutationer är kopplad till sämre överlevnad vid cellgiftsbehandling vid spridd sjukdom (stadium IV). Samtidigt fann vi att patienter med *KRAS*-mutation och hög nivå av biomarkören PD-L1 (≥50 %) har bättre prognos vid immunterapi som ensam behandling. Alla *KRAS*-mutationer beter sig inte likadant: varianten G12D svarade sämre på immunterapi än G12C och G12V. Vid lokalt avancerad sjukdom (stadium III) kunde den negativa effekten av *KRAS* vändas när immunterapi lades till efter kemo-radioterapi. I tidig, operabel sjukdom (stadium I–II) påverkade *KRAS*-status – ensamt eller tillsammans med tumörstorlek – inte överlevnaden.

Vi har även identifierat att kombinationen av mutation i *KRAS* och *LRP1B* är en möjliga prediktiv biomarkör för nytta av immunterapibehandling. Vi visar också i en åldrande musmodell att *ATF4* överuttrycks i *KRAS*-muterad lungcancer, även hos människor, vilket driver tumörcellers rörlighet och överlevnad samt ett ökat beroende av glutamin – faktorer som hänger ihop med spridning och sämre överlevnad. Detta pekar ut nya behandlingsmöjligheter.

Nyckelord: Icke-småcellig lungcancer, *KRAS*-mutationer, immunterapi, biomarkörer, precisionsmedicin.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. ***KRAS* Mutations Impact Clinical Outcome in Metastatic Non-Small Cell Lung Cancer**

Eklund EA, Wiel C, Fagman H, Akyürek LM, Raghavan S, Nyman J, Hallqvist A, Sayin VI.

Cancers (Basel). 2022; DOI: 0.3390/cancers14092063.

II. **Assessing the prognostic value of *KRAS* mutation combined with tumor size in stage I-II non-small cell lung cancer: a retrospective analysis**

Eklund EA, Mourad A, Wiel C, Sayin SI, Fagman H, Hallqvist A, Sayin VI.

Front Oncol. 2024; DOI: 10.3389/fonc.2024.1396285.

III. **Comprehensive genetic variant analysis reveals combination of *KRAS* and *LRP1B* as a predictive biomarker of response to immunotherapy in patients with non-small cell lung cancer**

Eklund EA[#], Svensson J[#], Näslund LS, Yhr M, Sayin SI, Wiel C, Akyürek LM, Torstensson P, Sayin VI, Hallqvist A, Raghavan S, Rohlin A.J

[#]Equal Contribution

Exp Clin Cancer Res. 2025; DOI: 10.1186/s13046-025-03342-6.

IV. **Equalizing prognostic disparities in *KRAS*-mutated stage III NSCLC patients: addition of durvalumab to combined chemoradiotherapy improves survival**

Eklund EA, Orgard M, Wallin D, Sayin SI, Fagman H, Isaksson J, Raghavan S, Akyürek LM, Nyman J, Wiel C, Hallqvist A, Sayin VI

Lung Cancer. 2025; DOI: 10.1016/j.lungcan.2025.108573.

V. **Monotherapy With Immune Checkpoint Blockade Improves Survival Outcomes in *KRAS*-Mutant but Not *KRAS* Wild-Type Metastatic Lung Adenocarcinoma: Validation From an Extended Swedish Cohort**

Eklund EA, Sayin SI, Jonsson JS, van Renswoude H, Nyman J, Hallqvist A, Wiel C, Sayin VI

JTO Clin Res Rep. 2025; DOI: 10.1016/j.jtocrr.2025.100880.

VI. **Aging promotes metastasis of lung cancer through epigenetic activation of the integrated stress response**

Patel AAH, Dzanan JJ[#], Ali KX[#], **Eklund EA**[#], Alvarez SW, Raj D, Dankis M, Altinönder I, Schwarz M, Bedel E, Gul N, Zowalaty AEE, Jonasson E, Albatrok H, Le Gal K, Bossowski J, Pillai R, Micke P, Botling J, Akyürek L, Angeletti D, Sayin SI, Olofsson Bagge R, Härtlova A, Papagiannakopolus T, Ståhlberg A, Hallqvist A, Wiel C, Sayin VI

[#]Equal Contribution

Manuscript submitted.

Additional publications, not part of this thesis:

i. **Genomic profiling of the transcription factor Zfp148 and its impact on the p53 pathway**
Zou ZV, Gul N, Lindberg, Bokhari AA, **Eklund EA**, Garellick V, Patel AAH, Dzanan JJ, BO, Le Gal KG, Johansson I, Tivesten Å, Forssell-Aronsson E, Bergö MO, Staffas A, Larsson E, Sayin VI, Lindahl P
Sci Rep. 2020; DOI: 10.1038/s41598-020-70824-2.

ii. **Combinatory analysis of immune cell subsets and tumor-specific genetic variants predict clinical response to PD-1 blockade in patients with non-small cell lung cancer**
Dutta N, Rohlin A, **Eklund EA**, Magnusson MK, Nilsson F, Akyürek LM, Torstensson P, Sayin VI, Lundgren A, Hallqvist A, Raghavan S.
Front Oncol. 2023; DOI:10.3389/fonc.2022.1073457.

iii. **Excessive copper impairs intrahepatocyte trafficking and secretion of selenoprotein P**
Schwarz M, Meyer CE, Löser A, Lossow K, Hackler J, Ott C, Jäger S, Mohr I, **Eklund EA**, Patel AAH, Gul N, Alvarez S, Altinonder I, Wiel C, Maares M, Haase H, Härtlova A, Grune T, Schulze MB, Schwerdtle T, Merle U, Zischka H, Sayin VI, Schomburg L, Kipp AP
Nat Commun. 2023 Jun; DOI: 10.1038/s41467-023-39245-3.

iv. **Pulmonary Adenocarcinoma In Situ and Minimally Invasive Adenocarcinomas in European Patients Have Less *KRAS* and More *EGFR* Mutations Compared to Advanced Adenocarcinomas**
Petterson J, Mustafa D, Bandaru S, **Eklund EÄ**, Hallqvist A, Sayin VI, Gagné A, Fagman H, Akyürek LM
Int J Mol Sci. 2024; DOI: 10.3390/ijms25052959.

v. **Distinct metastatic organotropism shapes prognosis in lung adenocarcinoma with brain metastasis**
Sayin SI, **Eklund EA**, Ali KX, Dzanan JJ, Xylander M, Dankis M, Lindahl P, Sayin VI, Hallqvist A, Wiel C
Front Oncol. 2025; DOI: 10.3389/fonc.2025.1569517.

vi. **High baseline PD-1+ CD8 T Cells and TIGIT+ CD8 T Cells in circulation associated with response to PD-1 blockade in patients with non-small cell lung cancer**
Dutta N, Svensson J, Saad G-A, Mello M, **Eklund EA**, Altinönder I, Torstensson P, Sayin VI, Rohlin A, Luche H, Hallqvist A, Raghavan S
Cancer Immunol Immunother. 2025; DOI: 10.1007/s00262-025-04086-0

vii. **BRAF inhibition increases TGFβ2 production and stimulates metastasis in mice with endogenous BRAFV600E-induced hepatocellular carcinoma**
Cisowski J*[#], Zowalaty AEE*, Sayin SI *, Czarnota P*, Gromowski T, **Eklund EA**, Kashif M, Patel AAH, Molinaro A, Lindahl P, Wiel C, Sayin VI[#] and Bergo M[#]
PNAS Accepted 251001

CONTENTS

ABBREVIATIONS

ALK	Anaplastic Lymphoma Kinase
ASNS	Asparagine Synthetase
ATF4	Activating Transcription Factor 4
BRAF	B-RAF proto-oncogene
cCRT	Concurrent Chemoradiotherapy
CNS	Central Nervous System
CT	Computer Tomography
CTLA-4	Cytotoxic T-Lymphocyte Associated protein 4
DNA	Deoxyribonucleic Acid
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial-Mesenchymal Transition
ERBB2	Erb-B2 Receptor Tyrosine Kinase 2
GDP	Guanosine Diphosphate
GEMM	Genetically Engineered Mouse Models
GTP	Guanosine Triphosphate
ICB	Immune Checkpoint Blockade
IHC	Immunohistochemistry
IRS	Integrated Stress Response
KEAP1	Kelch-Like ECH-Associated Protein 1

KRAS	Kirsten Rat Sarcoma
LKBI1	Liver Kinase B1
LRP1B	Low-Density Lipoprotein Receptor Related Protein 1B
LUAD	Lung Adeno Carcinoma
MET	MET Proto- Oncogene
MRI	Magnetic Resonance Imaging
NOS	Not Otherwise Specified
NSCLC	Non-small Cell Lung Cancer
NTRK	Neurotrophic Tyrosine Receptor Kinas
OS	Overall Survival
PD-L1	Programmed Death-Ligand 1
PDX	Patient Derived Xenograft
PET-CT	Positron Emission Tomography-Computed Tomography
PFS	Progression Free Survival
PS	Performance Status
RET	RET Proto-Oncogene
RNA	Ribonucleic Acid
ROS1	ROS Proto-Oncogene 1
SBRT	Stereotactic Body Radiation Therapy
SCLC	Small Cell Lung Cancer
STK11	Serine/Threonine Kinase 11
TCA	Tricarboxylic Acid

TKI	Tyrosine Kinase Inhibitor
TNM	Tumor Nodes Metastasis
TP53	Tumor Protein 53
TPS	Tumor Proportional Score

1 INTRODUCTION

The blueprint of the body is in our DNA. It contains information about how the different cells should be built and what function they will have. The blueprint has a rigorous protection system to withhold its integrity. However, both intrinsic factors such as inherited changes (genetic predisposition), extrinsic factors such as external exposures (chemicals, smoking, diet) and aging can compromise the protection system. When that happens in a cell the blueprint changes and with enough changes the cell can escape the protection system and give rise to cancer.

Cancer is a complex group of diseases with more than 200 distinct types. They are usually classified by their location in the body (e.g. prostate cancer, breast cancer and lung cancer) or by the cell of origin carcinomas, sarcomas or leukemias. Advances in research have significantly enhanced our understanding of cancer biology, improved diagnostic methods and development of more effective therapeutic strategies, such as targeted treatments and immunotherapies. These advances have given rise to increased and earlier detection and together with more effective treatments, the number of people that are diagnosed and living with cancer has increased. However, cancer is still a leading cause of death worldwide, accounting for nearly one in six deaths [1]. There is a need for further understanding of cancer development, progression and treatment.

1.1 TUMORIGENESIS

Our DNA codes for proteins which are the building blocks and effectors in our cells. Normally a cell should only grow and divide under well controlled situations, usually initiated by internal or external signaling. If growth occurs without accurate signaling internal control functions will put the cell in growth arrest or apoptosis (controlled cell death). Cancer arises when a normal cell escapes the protection systems that control what the cell should do, how it will grow, and its capabilities to divide, migrate and metastasize. These “Hallmarks of Cancer” were described in 2000 by Hanahan and Weinberg, identifying six essential traits shared by most human cancers. These hallmarks are: sustained proliferative signaling, evasion of growth suppressors, replicative immortality, resisting cell death, inducing angiogenesis and activation of invasion and metastasis [2]. The hallmarks summarize different ways to change writing and reading of DNA, avoiding our intrinsic protection and immune system,

enabling cancer cells to grow, alter metabolism, divide and move to other parts of the body.

These hallmarks are often induced by changes in the DNA called mutations and can be either hereditary or non-hereditary (somatic). If you have hereditary mutations, they will be present in all cells and the likelihood that cancer will occur is higher since fewer somatic mutations are needed. A somatic mutation will start in one cell and can be caused by multiple factors such as:

- Replicative errors, mistakes made when cells divide
- Environmental factors such as smoking and UV radiation
- Spontaneous changes caused by chemical reactions inside the cell

The most common types of changes are point mutations (substitutions, insertions, deletions, frameshifts), structural variants (duplications, translocations, inversions), or larger-scale chromosomal abnormalities like aneuploidy (Figure 1). Epigenetic factors, controlling which parts of the DNA are accessible by acetylation or methylation, can also be altered leading to transcriptional changes (Figure 2).

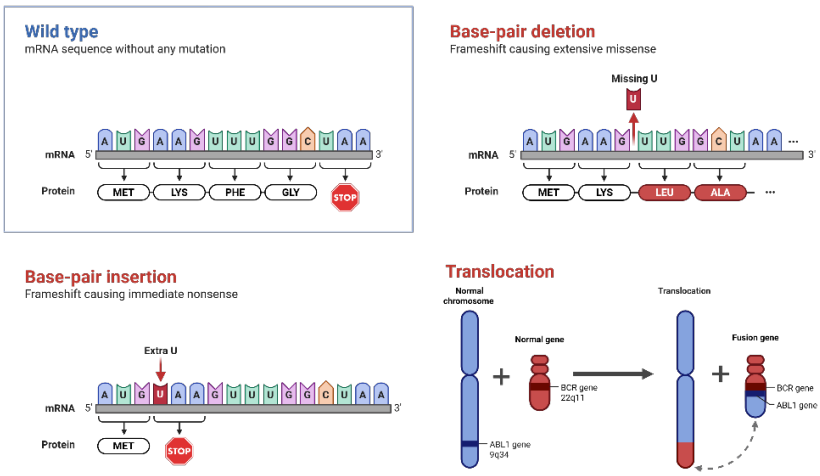

Figure 1: Overview of mutation types.

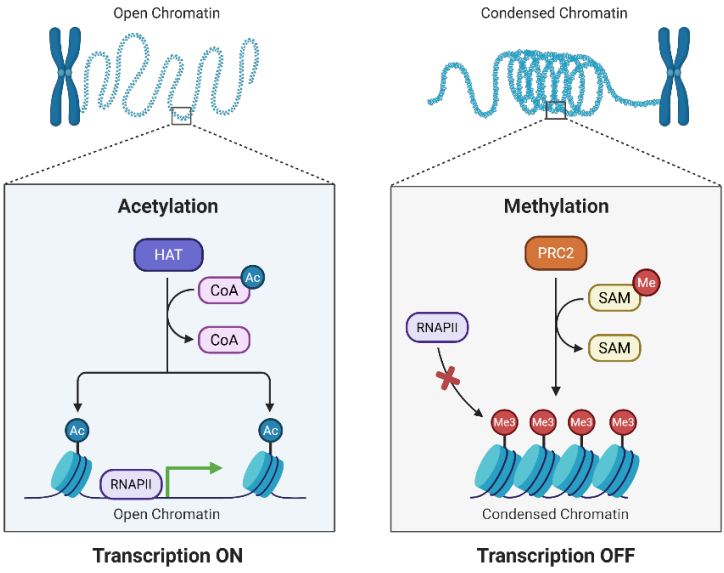

Figure 2: Overview of open and closed chromatin.

These changes can modify gene transcription, leading to increased protein production, loss of protein expression, or changes in protein function. When a change activates genes involved in proliferation and differentiation, they are called oncogenes. Mutations in oncogenes can induce uncontrolled proliferation. However, this effect can be counteracted by functional suppressors, genes involved in the self-regulation of cells, such as *TP53*. Therefor mutations leading to silencing or reduced function of tumor suppressors are often needed to cause malignant transformation.

In the last decades the roll of the immune system in cancer has been increasingly understood. If the intrinsic control fails, immune cells can detect and destroy abnormal cells, which are important for cancer cells to avoid. One way for the cancer cells to avoid elimination by the immune system is to express programmed death-ligand 1 (PD-L1) on the surface which signals "normal cell" to the immune cells carrying the programmed death receptor 1 (PD1). Further, the understanding of how the tumor microenvironment and altered cellular metabolism in tumors are involved in cancer progression and treatment response has rapidly emerged. These traits are included in the expanded hallmarks of cancer [3, 4] (Figure 3).

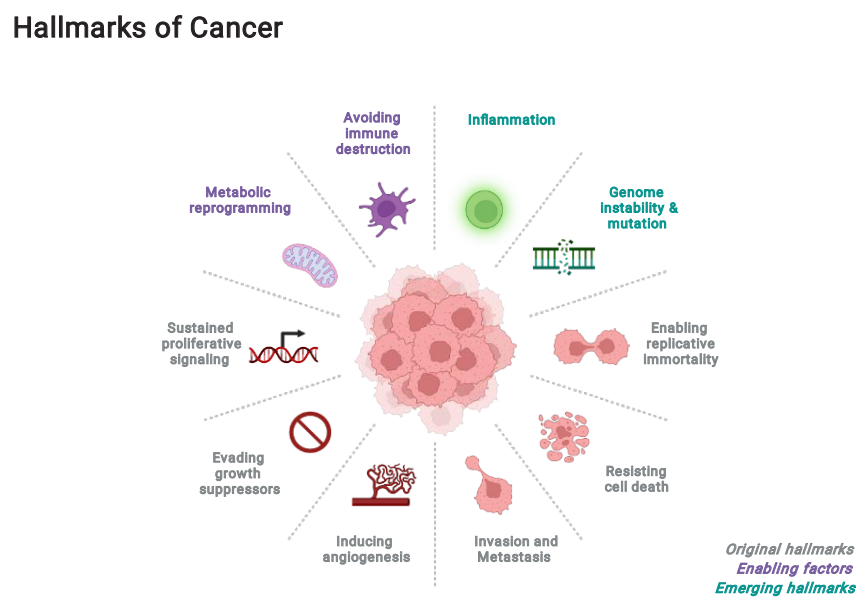

Figure 3: Hallmarks of cancer based on Hanahan and Winberg [2-4].

1.2 CANCER AND THE IMMUNE SYSTEM

The interaction between cancer and the immune system is multifaceted, reflecting both protective and tumor-promoting influences. The concept of immune surveillance, first proposed decades ago, describes the capacity of the immune system to recognize and eliminate malignant cells before they develop into clinically detectable tumors [5]. Cytotoxic $CD8^+$ T cells, natural killer (NK) cells, and dendritic cells play central roles in this process, detecting tumor-associated antigens and initiating targeted cytotoxic responses [6, 7].

Tumor progression, however, often involves the acquisition of immune-evasive mechanisms. Cancers can downregulate antigen presentation, most notably by reducing MHC class I expression, thereby evading recognition by T cells [8]. In addition, tumors remodel their microenvironment to suppress immune activity through the recruitment of regulatory T cells (Tregs), tumor-associated macrophages, and myeloid-derived suppressor cells (MDSCs), while secreting immunosuppressive cytokines such as TGF-β and IL-10 [9].

A central axis of immune suppression is mediated by immune checkpoint pathways, which normally serve to maintain immune homeostasis and prevent autoimmunity. In cancer, tumor expression of PD-L1 engages PD-1 on T cells, leading to functional exhaustion and impaired cytotoxic activity [10]. Similarly, CTLA-4 signaling restrains early stages of T-cell priming, limiting effective antitumor immunity [11] (Figure 4).

This immune landscape has been conceptualized in terms of "hot" and "cold" tumors. *Hot tumors* are characterized by high levels of immune infiltration, abundant neoantigen presentation, and an inflamed microenvironment. These tumors are generally more responsive to immune checkpoint blockade, as they harbor pre-existing antitumor immunity that can be reinvigorated. By contrast, *cold tumors* display sparse T-cell infiltration, low immunogenicity, and strong immunosuppressive signaling [12] (Figure 5).

Together, these processes illustrate cancer immunoediting, where the immune system first eliminates susceptible clones, then constrains tumor outgrowth in an equilibrium phase, but eventually allows the escape of resistant variants that establish progressive disease [13].

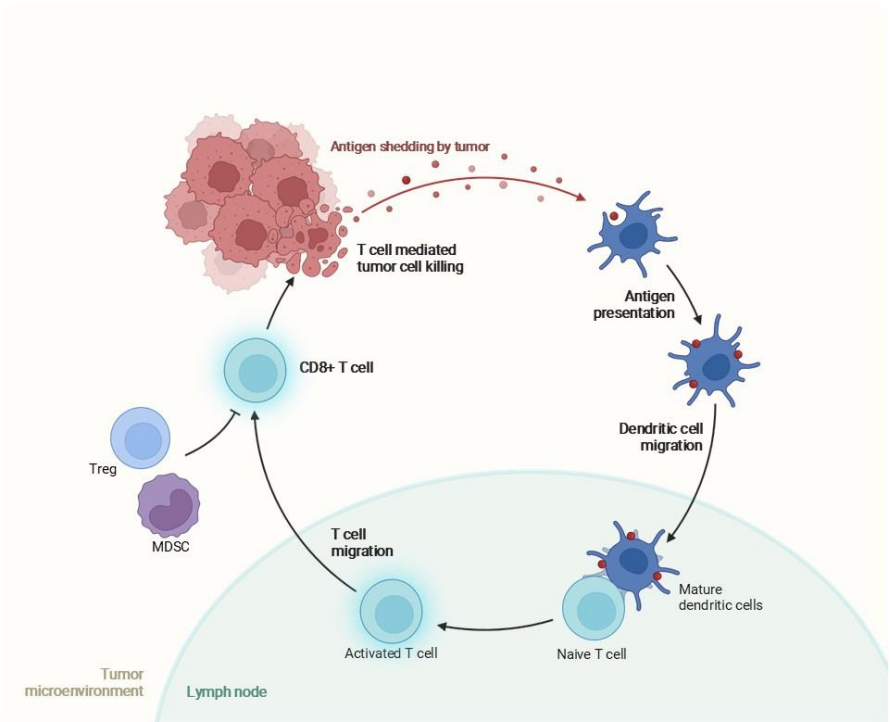

Figure 4: Schematic illustration of the priming and activation of tumor-specific cytotoxic CD8+ T cell.

1.2.1 IMMUNOTHERAPY

Recognition of these immune escape strategies has laid the foundation for immunotherapy, particularly immune checkpoint blockade (ICB). By targeting PD-1/PD-L1 and CTLA-4 pathways, ICB removes inhibitory signals and restores effector T-cell activity [13, 14]. Unlike chemotherapy or targeted therapies, which act directly on the tumor, ICB harnesses the host immune system, enabling durable responses and long-term survival in subsets of patients with advanced disease [15]. Importantly, the concept of hot versus cold tumors informs clinical outcomes: hot tumors, often with high PD-L1 expression or high tumor mutational burden (TMB), are most likely to benefit from ICB, whereas cold tumors remain a major therapeutic challenge, requiring combination approaches such as chemotherapy, radiotherapy, or novel immunomodulators to induce immune activation [12] (Figure 5). Thus, immunotherapy represents a paradigm shift in oncology, enabling durable

tumor control in subsets of patients across multiple cancer types, including lung cancer, and underscores the importance of biomarker development to identify patients most likely to benefit.

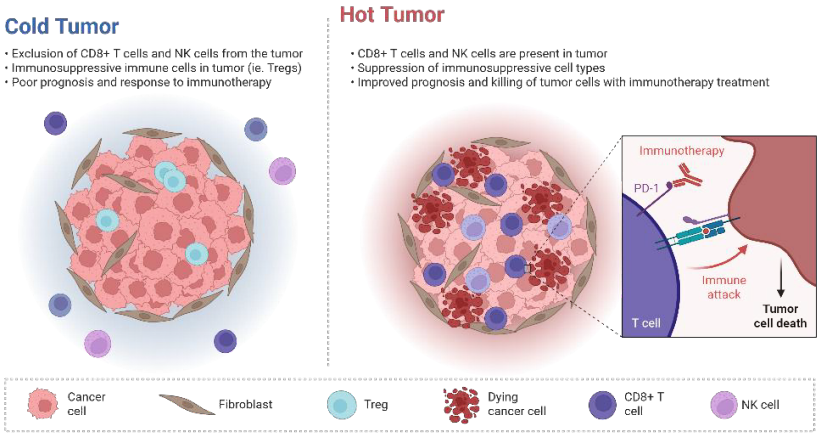

Figure 5: Schematic illustration of cold tumors, hot tumors and immunotherapy.

1.3 AGING AND CANCER

As long as we live, we will age. However, aging is a complex biological process characterized by progressive decline in cellular and organismal function not solely caused by chronological age. During our lives intrinsic and extrinsic factors will cause epigenetic drift, altered metabolism and accumulation of DNA damage leading to increased mutation burden. These changes compromises the control functions and increases the risk of cancer development. Another trait of aging is cellular senescence, a type of growth arrest associated with secretion of pro-inflammatory cytokines which contribute to tissue dysfunction and promote tumor progression. These hallmarks of aging was defined by López-Otín et al [16, 17] (Figure 6).

Cancer is primarily, but not exclusively, a disease of aging with the vast majority of cases being diagnosed after the age of 55 [18]. Cancer and aging share several biological hallmarks, including immune evasion, cellular

senescence, chronic inflammation, and epigenetic alterations [2-4, 16, 17] (Figure 7).

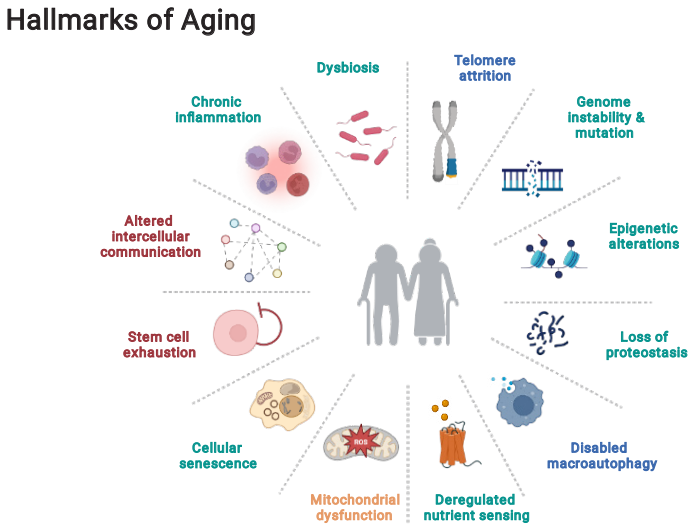

Figure 6: Hallmarks of cancer and aging based on López-Otín et al [16, 17] .

While aging is a risk factor for various types of cancers, lung cancer is a disease in which the incidence increases massively with age [19]. Nonetheless lung cancer has previously only been modeled in young mouse models and the impact of age on tumorigenesis and treatments has not been investigated. This might have implications for clinical translation of preclinical treatment studies since the target patient group for the treatments is mostly old.

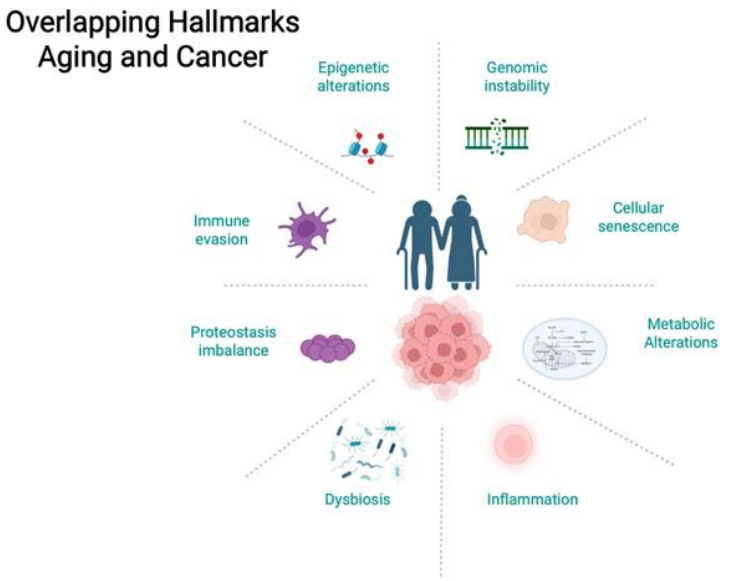

Figure 7: Illustration of the overlapping hallmarks of aging and cancer based on Hanahan et al and López-Otín et al [2-4, 16, 17].

1.4 LUNG CANCER

Lung cancer originates from the different cell types that make up the lung, most commonly epithelial cells lining the airways. When these cells acquire genetic and epigenetic changes that disrupt normal growth control, they may begin to divide uncontrollably, eventually forming malignant tumors within the lung tissue.

1.4.1 EPIDEMIOLOGY

Lung cancer remains one of the most devastating malignancies worldwide, both in terms of incidence and mortality. Globally, it is the 2nd most common type of cancer with an incidence of around 2.5 million, and importantly, the leading cause of cancer-related mortality, responsible for an estimated 1.8 million deaths annually [1, 20]. Lung cancer is the 6th most common form of cancer in Sweden with an incidence of around 4600 cases per year. Since most cases of lung cancer are diagnosed in an advanced stage, often when curative

treatment is no longer feasible, is the leading cause of cancer related death also in Sweden [21]. Lung cancer is generally rare before age 40, and incidence rises with age. Globally the median age at diagnosis is approximately 70 years with ranges from 61-76 in different populations [19, 21]. In Sweden it is slightly more common in women than men which differs from the global trend [21].

The etiology of lung cancer is multifactorial, with tobacco smoke being the principal risk factor. Approximately 85% of cases are attributable to active smoking, with risk correlating to both the duration and intensity of exposure [22-24]. However, other important contributors include exposure to environmental carcinogens such as radon gas, asbestos, and airborne pollutants like PM2.5 [23, 25-28]. Genetic predisposition, prior lung disease (including chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis), and occupational hazards also play significant roles for the susceptibility to lung cancer [23, 29].

1.4.2 SYMPTOMS

The symptoms of lung cancer are often non-specific and easily mistaken for benign respiratory conditions. While some patients may remain asymptomatic in the early phases of the disease, others develop symptoms that reflect either local tumor growth, systemic illness or paraneoplastic phenomena. The most reported symptom is cough. Other symptoms such as hemoptysis, shortness of breath, chest pain, recurrent lower respiratory tract infections, unintentional weight loss, fatigue, and anorexia are generally associated with more advanced disease and often accompany widespread metastasis [30].

1.4.3 DIAGNOSTICS

The first step to a diagnosis is generally radiological imaging, which could be done due to symptoms from the airways or to assess something unrelated such as a chest trauma, referred to as incidentaloma. When a cancer lesion is suspected, a tissue biopsy is performed in order to get a histopathological diagnosis. There are two major histological subtypes of lung cancer. Small cell lung cancer (SCLC) constitutes about 15% and non-small cell lung cancer (NSCLC) about 80% of all cases [31].

NSCLC is further separated into squamous carcinoma and non-squamous carcinoma with adenocarcinoma being the most common type. When no

typical histopathological traits are detected in a non-small cell lung cancer it is called not otherwise specified (NOS). When the histopathological diagnosis is secured, the next step is further molecular and immunohistochemical testing. Genomic profiling of tumor samples allows for the identification of actionable mutations in oncogenes such as *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *RET*, *NTRK*, *ERBB2* and *KRAS* [32, 33] (Figure 8). These mutations are more common in adenocarcinomas and varies in frequencies depending on populations, i.e. *EGFR* mutations are much more common in Asia. However, not all NSCLC tumors have a known driver mutation. Immunohistochemical evaluation of PD-L1 expression, evaluated as tumor proportional score (TPS), provides prognostic and predictive information, especially in relation to ICB [34]. When tissue is insufficient or inaccessible, liquid biopsy techniques that analyze circulating tumor DNA offer a non-invasive method of detecting mutations and monitoring resistance mechanisms[33, 35]. Lately the importance of co-occurrent alterations in tumor suppressor genes has been shown for example in *TP53*, *LKB1*/*STK11* and *KEAP1*, but this information is not yet implemented in clinical praxis [36-42]. The histopathological information together with staging of the disease is crucial for treatment decision further down the line.

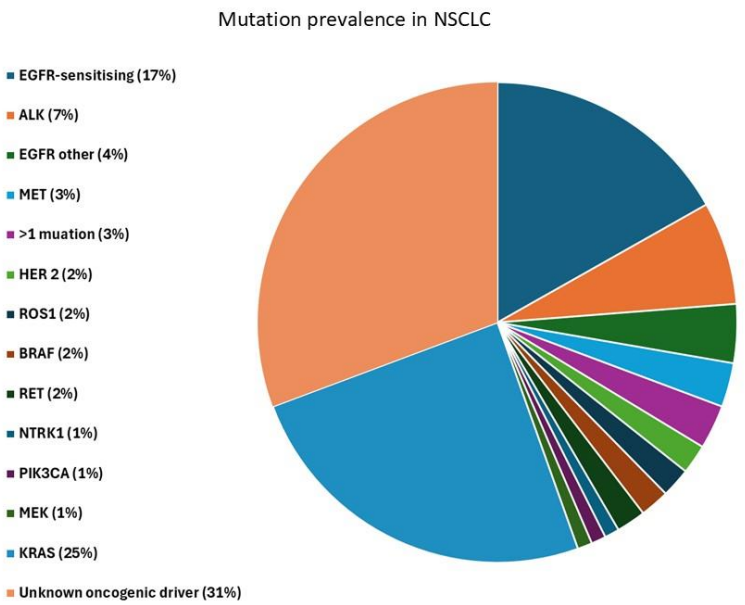

Figure 8: Distribution of diver mutations in NSCLC adapted from Hisch et. al.[33].

1.4.4 STAGING

Accurate staging of lung cancer is the most important prognostic factor and the foundation for planning the treatment. The tumor–node–metastasis (TNM) is used to classify disease extent based on imaging and biopsy findings [43, 44]. T describes the size, extent and growth of the primary tumor. N stands for local lymph node involvement, their location and number. M gives information about distant metastasis (Table 1). The TNM classification is further grouped into stages [43, 44] (Table 2).

Computer tomography (CT) of the thorax and abdomen together with PET-CT are the most common imaging modalities. Brain magnetic resonance imaging (MRI) is performed to assess intracranial metastases, particularly in patients with known driver mutations or only a localized tumor in the lung at the initial imaging [45, 46]. If there are suspected enlarged lymph nodes further biopsies may be pursued, to determine whether they are malignant or not. In short, stage I and II are localized disease that could include minor lymph node involvement, stage III cases are locally advanced and stage IV cases show distant metastasis.

Staging combined with molecular diagnostics have dramatically improved the precision and personalization of lung cancer care.

***Table 1**: TNM Definitions for lung cancer in the 8th edition [43, 44].*

T: Primary Tumor	
Tx	Primary tumor cannot be assessed or tumor proven by presence of malignant cells in sputum or bronchial washings but not visualized by imaging or bronchoscopy
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor ≤3 cm in greatest dimension surrounded by lung or visceral pleura without bronchoscopic evidence of invasion more proximal than the lobar bronchus (i.e. not in the main bronchus)
T1a(mi)	Minimally invasive adenocarcinoma
T1a	Tumor ≤1 cm in greatest dimension
T1b	Tumor >1 cm but ≤2 cm in greatest dimension
T1c	Tumor >2 cm but ≤3 cm in greatest dimension
T2	Tumor >3 cm but ≤5 cm or tumor with any of the following features. – involves main bronchus regardless of distance from the carina but without involvement of the carina. –Invades visceral pleura – Associated with atelectasis or obstructive pneumonitis that extends to the hilar region, involving part or all of the lung
T2a	Tumor >3 cm but ≤4 cm in greatest dimension
T2b	Tumor >4 cm but ≤5 cm in greatest dimension
T3	Tumor >5 cm but ≤7 cm in greatest dimension or associated with separate tumor nodule(s) in the same lobe as the primary tumor or directly invades any of the following structures: chest wall (including the parietal pleura and superior sulcus tumors), phrenic nerve, parietal pericardium
T4	Tumor >7 cm in greatest dimension or associated with separate tumor nodule(s) in a different ipsilateral lobe than that of the primary tumor or invades any of the following structures: diaphragm, mediastinum, heart, great vessels, trachea, recurrent laryngeal nerve, oesophagus, vertebral body, and carina
N: Regional lymph node involvment	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)
M: Distant metastasis	
M0	No distant metastasis
M1	Distant metastasis present
M1a	Separate tumor nodule(s) in a contralateral lobe; tumor with pleural or pericardial nodul(s) or malignant pleural or pericardial effusion
M1b	Single extrathoracic metastasis
M1c	M1c Multiple extrathoracic metastases in one or more organ

***Table 2**: Lung Cancer stage grouping in the 8th edition TNM [43, 44].*

T/M	Label	N0	N1	N2	N3
T1	T1a	IA1	IIB	IIIA	IIIB
	T1b	IA2	IIB	IIIA	IIIB
	T1c	IA3	IIB	IIIA	IIIB
T2	T2a	IB	IIB	IIIA	IIIB
	T2b	IIA	IIB	IIIA	IIIB
T3	T3	IIB	IIIA	IIIB	IIIC
T4	T4	IIIA	IIIA	IIIB	IIIC
M1	M1a	IVA	IVA	IVA	IVA
	M1b	IVA	IVA	IVA	IVA
	M1c	IVB	IVB	IVB	IVB

1.5 TREATMENT

Multiple types of treatments are available for patients with lung cancer. The patient's co-morbidities and performance status, assessed using the ECOG scale, predict the ability to withstand the offered therapy [47].

The treatment types available today in the management of NSCLC are surgery, radiotherapy, targeted treatments, ICB and chemotherapy [45, 46] (Figure 9). These modalities can be given alone or in combinations depending on stage, performance status, co-morbidities, molecular findings and PD-L1 status.

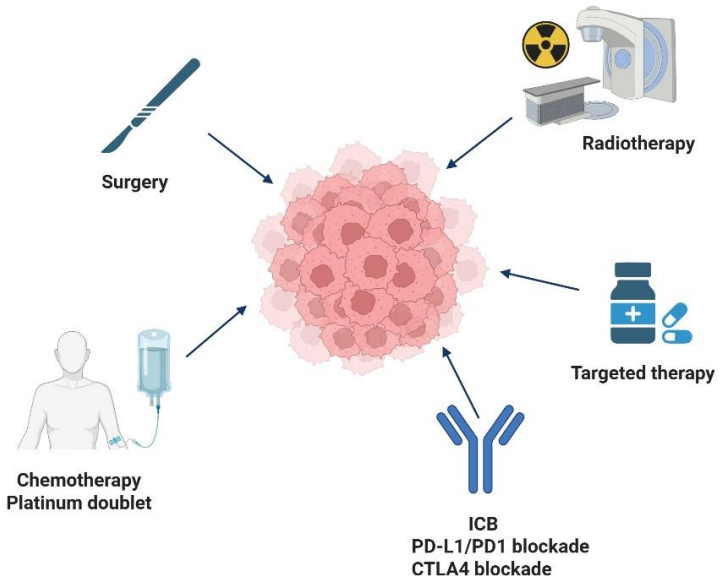

Figure 9: Illustrative summary of available treatments for NSCLC.

1.5.1 EARLY STAGE

In early-stage NSCLC (Stage I and II), surgical resection remains the cornerstone of curative treatment. Lobectomy, with or without mediastinal lymph node dissection, is considered the standard of care and leads to the best outcomes [48, 49]. For patients who are not surgical candidates due to medical comorbidities or poor pulmonary reserve, stereotactic body radiotherapy (SBRT) offers an effective non-invasive alternative. SBRT delivers high-dose radiation in a limited number of fractions providing local control, particularly in peripheral tumors [50, 51]. For tumors with high-risk features, such as large size, vascular invasion, or nodal involvement, adjuvant platinum-based chemotherapy is recommended to reduce the risk of recurrence [52]. The adjuvant treatment, after surgery, is generally a combination of cisplatin or carboplatin with another third-generation chemotherapy (platinum doublet) that improves 5-year overall survival (OS) with approximately around 5% [53]. Recently two phase III randomized controlled trials (RCT) showed significantly improved disease-free survival with an ICB after platinum doublet treatment and now the ICB atezolizumab is approved for stage II-III patients with at least 50% PD-L1 expression, without *EGFR* or *ALK* mutations, for up to one year [54, 55]. Neoadjuvant ICB treatment is currently under

implementation [56, 57]. For patients stage IB-IIIA patients with *EGFR* mutation with exon 19 deletion or *EGFR* L858R mutation, or stage II-IIIA patients with *ALK*-fusion oncogenes, adjuvant tyrosine kinase inhibitor therapy is available for up to three and two years respectively [58, 59].

1.5.2 LOCALLY ADVANCED

Management of locally advanced NSCLC (Stage III) is complex and generally requires a multimodal approach. For patients with resectable disease and adequate functional status, surgery or a combination of neoadjuvant/adjuvant chemotherapy (or chemoradiotherapy) followed by surgery may be considered. In unresectable cases, being the majority, concurrent chemoradiotherapy (cCRT) remains the standard [60, 61]. More recently, consolidation immunotherapy with durvalumab, a PD-L1 inhibitor, has been shown to significantly improve progression-free and overall survival following chemoradiotherapy, thereby becoming a new standard of care for eligible patients with PD-L1 positivity [62, 63]. More recently, adjuvant treatment with the tyrosine kinase inhibitor osimertinib was approved as the first targeted treatment for stage III NSCLC in adjuvant setting after cCRT for patients harboring *EGFR* mutation with exon 19 deletion or L858R [64].

1.5.3 METASTATIC

In metastatic or advanced NSCLC (Stage IV), treatment selection is highly dependent on molecular profiling. Patients harboring actionable mutations such as *EGFR*, *ALK*, *ROS1*, *BRAF V600E*, *RET*, *MET exon 14 skipping*, and *NTRK fusions* are typically started on first-line targeted therapies, which have demonstrated superior efficacy compared to traditional chemotherapy [46]. For instance, *EGFR*-mutant tumors, with exon 19 deletion and L858R mutations, are treated with third-generation tyrosine kinase inhibitors such as osimertinib, which is associated with prolonged survival and better central nervous system (CNS) penetration compared to earlier agents [65]. Similarly, *ALK*-rearranged tumors are treated with next-generation inhibitors like alectinib or lorlatinib, which have demonstrated strong activity even in the presence of brain metastases [66]. Patients with *KRAS G12C* mutations, a subset previously considered untargetable, can now be offered selective inhibitors such as sotorasib in second line treatment, which have shown promising clinical efficacy [67].

For patients without actionable mutations, or whose tumors expressing high levels of PD-L1, ICB has revolutionized the therapeutic landscape [45].

Monotherapy with pembrolizumab, atezolizumab or cemiplimab, has shown significant improvement in both progression-free and overall survival compared to chemotherapy in patients with PD-L1 expression ≥50% [68-72]. In cases with lower or negative PD-L1 expression, regimens incorporating a combination of immunotherapy and chemotherapy have shown benefit [73-76]. Pembrolizumab, atezolizumab or cemiplimab in combination with platinum-doublet chemotherapy or nivolumab and ipilimumab with chemotherapy are now routinely used [34, 77].

Despite these advancements, acquired resistance to both targeted therapies and immunotherapy remains a major clinical challenge. Mechanisms of resistance include secondary mutations in the target gene (e.g., *EGFR T790M* or *ALK G1202R*), activation of bypass signaling pathways, histological transformation, and immunologic evasion [78-82]. Addressing resistance requires repeated biopsies, either via tissue or liquid biopsy, and often necessitates switching to next-line therapies or enrolling in clinical trials exploring novel agents and combinations.

1.5.4 SUMMARY

Lung cancer is not a single disease entity but rather a group of distinct histopathological and molecular subtypes, each with its own biology and clinical course. The treatment of lung cancer has evolved from a stage-based, one-size-fits-all approach to a personalized strategy that incorporates detailed molecular and immunological profiling. Surgical resection, radiation, and chemotherapy remain foundational, but the integration of targeted therapies and ICB has substantially improved the prognosis for many patients. However, the prognosis of NSCLC is highly variable, influenced by factors such as stage, molecular subtype, and treatment response. The five-year survival rates range from approximately 70–90% in stage I disease to less than 20% in metastatic cases. Nevertheless, the development of precision medicine and the expansion of therapeutic options have begun to improve these outcomes in meaningful ways. Ongoing research into resistance mechanisms, novel targets, and biomarker refinement continues to shape the future of lung cancer management.

Resistance mechanisms may be intrinsic, such as the presence of co-occurring mutations (e.g., *TP53*, *STK11*, *KEAP1*) that dampen response to therapy, or acquired, such as secondary mutations in *EGFR* (e.g., T790M) or histologic transformation. Addressing resistance requires ongoing surveillance, often

through repeat biopsies or liquid biopsy techniques, and consideration of next-generation inhibitors or clinical trial enrollment.

Looking forward, the integration of technologies such as artificial intelligence for radiogenomic prediction, neoantigen-based vaccine development, and novel combination regimens will likely further refine our approach to NSCLC. Continued investment in biomarker discovery, longitudinal patient monitoring, and resistance mechanism elucidation will be essential to achieving more durable responses and potential cures.

1.6 PRECISION MEDICINE IN NSCLC

At its core, precision medicine reflects a paradigm shift from "one-size-fits-all" care to biology-informed, context-aware, and continuously adaptive medicine, with the ultimate aim of improving effectiveness, minimizing harm, and ensuring that the right intervention is offered to the right patient at the right time. With the rapid introduction of new treatments there is an urgent need for reliable predictive and prognostic biomarkers (Figure 10).

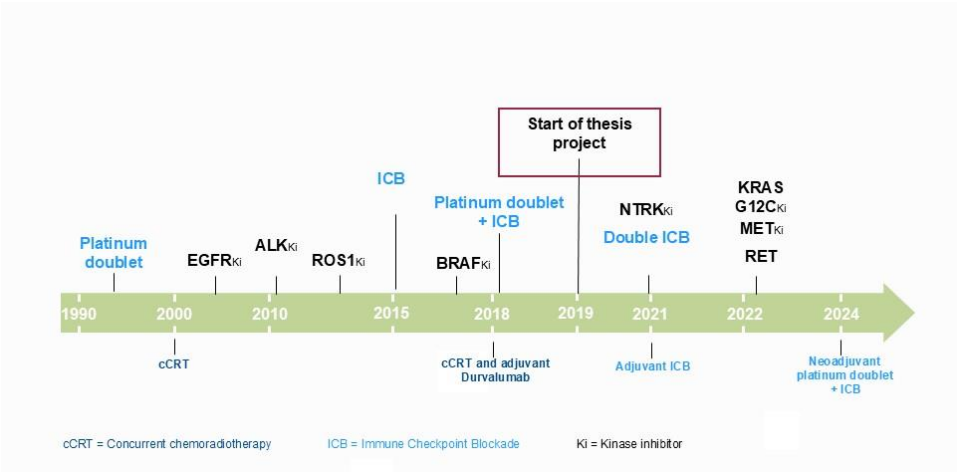

Figure 10: Overview timeline of introduction of new treatments for NSCLC

1.6.1 BIOMARKERS

Historically, surgery, chemotherapy and radiotherapy have been the standard treatment options for NSCLC. The first biomarker was the histology subgrouping showing a better outcome with pemetrexed in adenocarcinoma [83]. With the development of genomic sequencing and the discovery of driver mutations, more biomarkers became available. However, biomarkers are not static and are highly context-dependent. With the development of targeted therapies, harboring for example *EGFR* exon 19 deletion or *EGFR* L858R mutations has become a predictive biomarker for targeted treatment and positive prognostic biomarkers. A broader understanding of the immune system's involvement in cancer led to the development of ICB therapies, which instead of a direct cytotoxic approach, are hindering malignant cells to hide from the immune system enabling immune cells to find and kill the cancer. The first available ICB, an inhibitor of cytotoxic T-lymphocyte associated protein 4 (CTLA-4), did not have a biomarker for prediction of treatment efficacy and the response rate was low. PD1/PD-L1 inhibitors were the next type of ICB developed and a high PD-L1 expression on the tumor cells is the most used treatment predictive marker in Swedish clinical praxis today [34]. However, *EGFR* mutated tumors with a high expression of PD-L1 show a very poor, if any, response to ICB [84]. Also, a high PD-L1 expression does not guarantee ICB response and some patients lacking expression still respond well [73, 85-89]. Other biomarkers for immunotherapy are TMB and currently tumor infiltrating lymphocytes are investigated [90-94]. Hence, treatment selection for NSCLC is complex and multifaceted.

1.7 KRAS MUTATED LUNG CANCER

Among its oncogenic drivers, mutations in the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) are the most common, present in 25–30% of lung adenocarcinomas (LUAD) and around 3–5% of squamous cell carcinomas. The *KRAS* gene, located on chromosome 12p12.1, encodes a 21-kDa guanosine triphosphate (GTP)-binding protein belonging to the RAS family of small GTPases. Under physiological conditions, KRAS cycles between an inactive guanosine diphosphate (GDP)-bound and an active GTP-bound state, thereby acting as a molecular switch that regulates signal transduction from

activated receptor tyrosine kinases (RTKs) to multiple downstream effector pathways (Figure 11).

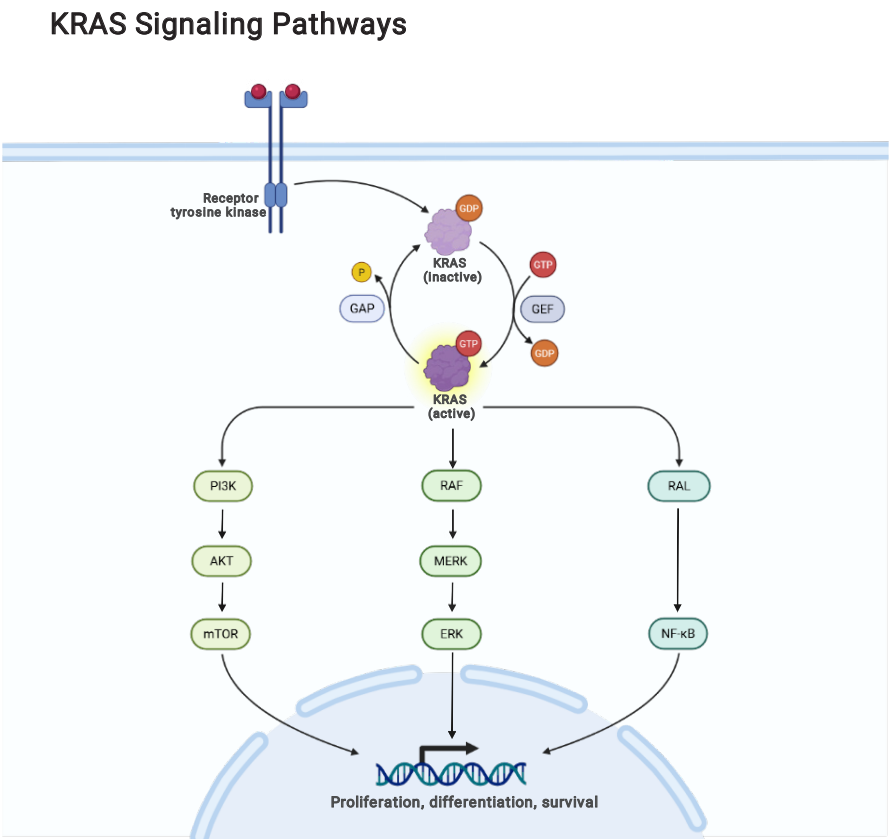

Figure 11: KRAS activation and signaling pathway.

The majority of oncogenic *KRAS* mutations in NSCLC occur at codon 12 (exon 2), with less frequent alterations in codon 13 (exon 2) and codon 61 (exon 3). Among these, *KRAS* G12C is the most prevalent variant, representing about 40% of *KRAS* mutations and detected in ~13% of all adenocarcinomas. Other recurrent mutations include G12V and G12D, while G13D and Q61H are less prevalent (Figure 12). These variants differ in their epidemiological associations and biological behaviors: G12C and G12V are strongly linked to tobacco exposure, whereas G12D occurs more often in never-smokers and has been associated with mucinous adenocarcinomas.

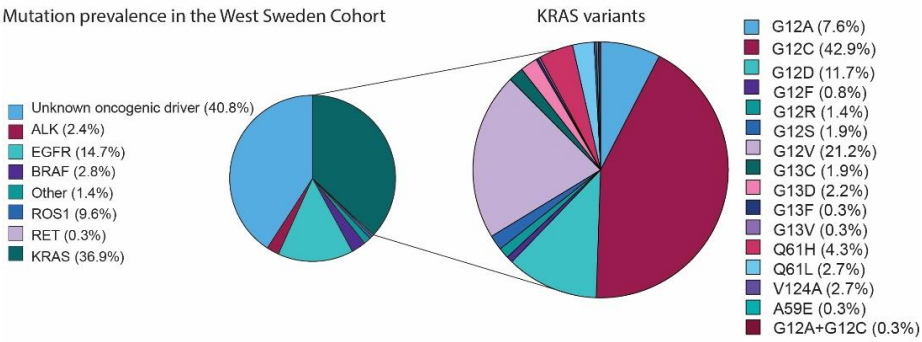

Figure 12: Frequencies of KRAS variants from the West Sweden Cohort patients diagnosed 2016-2018.

Historically, *KRAS* mutations in NSCLC were associated with poor prognosis and resistance to standard therapies, leading to their classification as undruggable [95, 96]. Genetic heterogeneity adds further complexity, with frequent co-alterations in *TP53*, *STK11*, and *KEAP1* defining distinct biological subsets that influence immunogenicity, metastatic patterns, and therapeutic responsiveness [36, 37, 97-102]. Recent advances have challenged this dogma, most notably with the development of covalent inhibitors targeting the cysteine residue created by the *KRAS* G12C substitution (Figure 13). Agents such as sotorasib and adagrasib have demonstrated clinically meaningful responses in previously treated NSCLC, representing a breakthrough in targeted therapy [103, 104]. Nevertheless, resistance mechanisms, both on-target and through bypass signaling, emerge rapidly and limit the durability of the treatment effect [105].

Immunotherapy has also reshaped treatment, with *KRAS*-mutant tumors often exhibiting high tumor mutational burden and variable PD-L1 expression. While subsets with concomitant TP53 mutations may respond favorably, co-mutations in *STK11* or *KEAP1* are frequently associated with resistance to immunotherapy [98, 106].

Despite these therapeutic advances, effective options for cases harboring non-G12C *KRAS* variants remain limited, and the biological diversity of *KRAS*-mutant NSCLC continues to challenge durable disease control. Ongoing

research into variant-specific signaling, co-mutation biology, and rational therapeutic combinations is therefore critical to advance precision oncology in NSCLC.

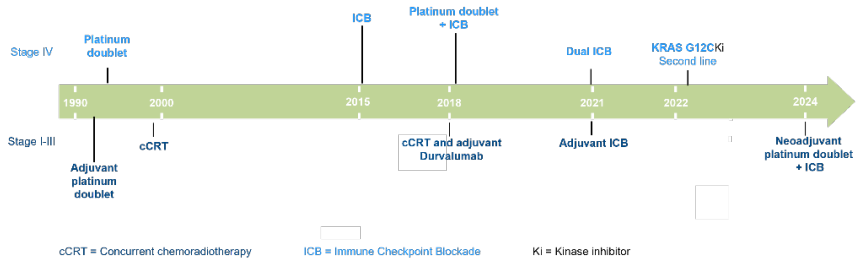

Figure 13: Overview timeline of introduction of new treatments for KRAS-mutated NSCLC

1.8 STUDYING LUNG CANCER

1.8.1 PRECLINICAL MODELS

Preclinical models play a crucial role in understanding cancer progression and evaluating potential therapeutic interventions before clinical application. These models provide insights regarding tumor biology, drug efficacy, and resistance mechanisms. The most commonly used preclinical models for NSCLC research include cell lines, patient-derived organoids, genetically engineered mouse models (GEMMs), and patient-derived xenografts (PDX). In vitro models, such as 2D and 3D cultures of cancer cell lines, are widely used for high-throughput drug screening and mechanistic studies.

Animal models, particularly GEMMs and PDX models, allow for a more physiologically relevant assessment of tumor growth and drug response. GEMMs facilitate the study of specific genetic alterations, such as KRAS mutations, in an immune-competent setting, while PDX models retain the genetic and histopathological features of patient tumors, making them valuable for development of personalized therapies.

By integrating various preclinical models, researchers can gain a comprehensive understanding of NSCLC pathogenesis and progression mechanisms, and identify effective therapeutic strategies tailored to specific genetic and molecular tumor profiles.

1.8.2 CLINICAL STUDIES

Clinical research is essential in advancing our understanding of disease progression and evaluating novel therapeutic strategies. Clinical studies are broadly categorized into prospective and retrospective designs, each with its strengths and limitations.

Prospective clinical studies are designed to follow a cohort of individuals over time, collecting data as events unfold. These studies are often conducted to investigate disease progression or the efficacy of new treatments. In contrast, retrospective clinical studies analyze existing data from past medical records, patient registries, or previously conducted trials to identify patterns and correlations.

While prospective studies offer higher-quality evidence by actively collecting data over time through predetermined protocols, retrospective studies provide a more practical and efficient approach for generating hypotheses and exploring associations. In NSCLC research, both study designs play a crucial role: prospective trials are essential for validating new therapies, while retrospective analyses help identify prognostic factors and assess real-world treatment outcomes. Integrating insights from both approaches enhances the robustness of clinical research and accelerates the development of precision medicine strategies.

1.8.3 THE SWEDISH LUNG CANCER REGISTRY

The Swedish Lung Cancer Registry (NLCR), founded in 2002, collects standardized nationwide data on invasive primary lung cancer to monitor and improve care [107]. It records diagnostics, staging, treatments, key time intervals, and whether cases are discussed at MDT meetings. Coverage exceeds 90% of lung cancer cases, supported by mandatory reporting and broad clinical participation. The NLCR links seamlessly with the Swedish Cancer Registry, National Patient Registry, and Total Population Registry, enabling long-term follow-up, mortality tracking, and real-world evaluations of treatment and healthcare performance. Limitations include exclusion of in situ and autopsy-only cases and occasional reporting delays, mitigated by ongoing validation [107]. Overall, the NLCR is a robust platform for quality assurance, research, and policy in Swedish lung cancer care.

2 AIM

This thesis aimed to generate a comprehensive understanding of the biological and clinical relevance of *KRAS* mutations in lung cancer by integrating prognostic, predictive, and translational perspectives. The overall aim was to broadly investigate the prognostic and predictive impact of *KRAS* mutations on currently available treatments, explore new prognostic biomarkers for ICB treatment, study the impact of aging on *KRAS* mutated lung cancer progression and explore novel treatment vulnerabilities. To achieve this, we have used a broad range of methods to investigate the clinical and preclinical implications of *KRAS* mutations including retrospective studies, prospectively enrolled patient cohorts, and translational analyses bridging clinical and laboratory data. The specific aims of the papers were to:

Paper I: Investigate the impact of *KRAS* mutations on overall survival and the association to specific treatment regimens in stage IV NSCLC.

Paper II: Evaluate whether *KRAS* mutations combined with tumor size represent an independent adverse prognostic factor in stage I–II NSCLC.

Paper III: Search for new potential predictive biomarkers of treatment response to ICB containing regimes in NSCLC.

Paper IV: Assess survival outcomes in *KRAS* mutated vs *KRAS* wildtype stage III NSCLC patients receiving cCRT before and after the introduction of durvalumab.

Paper V: Decipher the impact of *KRAS* mutations in response to distinct immunotherapy treatment regimes.

Paper VI: Define the impact of physiological aging on *KRAS*-mutated lung cancer progression and metastasis and develop novel therapies for NSCLC targeting this mechanism.

3 METHODS

This section contains a summary of the patient cohorts, and the clinical and pre-clinical methods used throughout this thesis. Further detailed method sections can be found in the attached papers and manuscripts.

3.1 PATIENT COHORTS

Two main patient cohorts have been used in this thesis:

- The "West Sweden cohort", a retrospective cohort comprising all NSCLC patients molecularly assessed with next generation sequencing at the Pathology department at Sahlgrenska University Hospital 2016-2021.

- The BIOLUNG cohort with prospective inclusion of NSCLC patients receiving immunotherapy.

3.1.1 THE WEST SWEDEN COHORT

This patient cohort was selected through molecular pathology assessment at Sahlgrenska University Hospital. It includes all consecutive NSCLC patients who have undergone molecular assessments from 2016-2021 in west Sweden as most of the regional hospitals send the samples there. For part of the period the Skaraborg regional hospital did their molecular assessment elsewhere. The information has been collected in two rounds, 2016-2018 and 2019-2021. The 2016-2018 cohort was included in paper I, II and VI where as both of the cohorts (2016-2021) were merged for paper IV and V. Approval from the Swedish Ethical Review Authority was obtained for 2016-2018 cohort (Dnr 2019-04771) and an amendment was approved for the 2019-2021 cohort (Dnr 2021-04987).

3.1.2 THE BIOLUNG COHORT

The BIOLUNG cohort is a prospective bicentric study consecutively including patients receiving immunotherapy containing treatment at Sahlgrenska University Hospital and Skaraborg regional hospital with stage III or stage IV disease at inclusion, age≥18 years, and receiving ICB in any line setting

according to standard practice. Patients with treatment for newly diagnosed, recurrent and progressive disease were included. A single dose of ICB containing treatment was deemed to be sufficient. The patients included in paper III were recruited between April 2019 and October 2021 and data cut of was 28th of February 2023. The study was approved by the Regional Ethics Review Board in Gothenburg, Sweden (Permit number 953/18), and all participating patients signed an informed consent.

3.2 CLINICAL DATA

Patient demographics (including age, gender, Eastern Cooperative Oncology Group (ECOG) performance status and smoking history), cancer stage, pathological details (histology, PD-L1, mutation status including *KRAS* mutational status and subtype), primary tumor size at diagnosis (paper II and VI), treatment and outcome data were retrospectively collected from patient charts (paper I-VI) and the Swedish Lung Cancer Registry (paper I, II, IV-VI).

3.2.1 CLINICAL OUTCOME

The clinical outcomes assessed were overall survival (OS), progression free survival (PFS) and response.

OS was calculated from date of diagnosis (paper I) or date of first treatment to death from any cause (paper II-V). PFS was defined as the time interval from the date of first treatment to disease progression or death (paper III-V).

The clinical response to treatment (paper III) was determined based on the CT-scan results obtained every third month, in line with the immune-related Response Evaluation Criteria in Solid Tumors (irRECIST) algorithm, assessed by an oncologist according to clinical judgment. The clinical response was divided into complete response, partial response, stable disease, and progressive disease. Responders were defined as patients who did not have progressive disease at nine months (third assessment) after the start of ICB therapy.

3.3 PD-L1 EXPRESSION

During routine diagnostics, PD-L1 expression was determined using the PD-L1 IHC 28-8 pharmDx assay and reported as tumor proportion score (TPS)—the fraction of tumor cells showing membranous staining (negative <1%, low 1–49% or high ≥50%) by lung pathologist (paper I,III-V).

3.4 STATISTICS

We summarized clinical characteristics descriptively and performed univariate analyses. We estimated OS and PFS using Kaplan–Meier curves and compared groups with log-rank tests. To adjust for potential confounding factors, we built multivariable Cox models. Median follow-up was calculated by the reverse Kaplan–Meier method. Statistical significance was set at two-sided $p<0.05$ without multiple-comparison adjustment. Analyses were conducted in IBM SPSS Statistics 27 and GraphPad Prism 9.

3.5 MOUSE MODELS OF CANCER

Approximately 99% of the mouse genes have a human homologue and since nearly half a century ago modeling of cancer in mice has been a tool to understand the impact of oncogenes and tumor suppressor genes in the development of different malignancies [108]. Introduction of mutations commonly detected in human cancers into the mouse generally leads to the initiation and progression of tumors with biological similarities to the human counterparts. Thus, mouse models provide a possibility to study complex biology in a controlled environment minimizing external confounding factors.

3.5.1 THE CRE-LOXP SYSTEM

The mouse model used in this thesis (paper VI) harbors the Cre-*loxP* system for conditional activation or deactivation of the targeted genes *Kras* and *Tp53*.

Cre is a DNA-cutting enzyme from bacteriophage P1 that recognizes short DNA sequences called *loxP* sites. A *loxP* site is 34 base pairs long: two identical 13-bp inverted repeats flanking an 8-base pairs (bp) spacer that gives the site its direction. When a DNA segment is “floxed” (flanked by two *loxP* sites), Cre binds both sites and recombines them. If the *loxP* sites point the same way, the floxed segment is cut out and forms a small circular piece of DNA, leaving a single *loxP* behind in the genome.

By engineering *loxP* sites into specific places in mammalian genomes, genes can be deleted only when and where Cre is present—making gene control conditional in time and cell type. Cre expression can be provided transiently (e.g., by delivering a Cre-expressing plasmid or virus) or built into the animal under a tissue-specific or inducible promoter.

In this thesis (Paper VI), Cre was delivered to the lung epithelium of mice using an adenoviral vector administered by inhalation in a calcium phosphate precipitate. This approach activates Cre in the target tissue, excising the floxed DNA segment and enabling precise, conditional gene modification in vivo. The Cre–*loxP* system is now a standard toolkit for controlled gene editing in animal models.

3.5.2 *KRAS*$^{LSL\text{-}G12D/+}$ *TRP53*$^{FLOX/FLOX}$ MICE

For controlled induction of tumors in the lung, the *Kras*$^{LSL\text{-}G12D/+}$ *Trp53*$^{flox/flox}$ model (KP) was used. The mice carries a *Kras* allele with a *LoxP* flanked STOP cassette (LSL) followed by an activating *Kras*^{G12D} mutation. Additionally, *Trp53* is flanked by *LoxP* on both alleles. Without Cre expression there will only be production of wild type *KRAS* and normal expression of *Tp53*. However, with Cre expression, the STOP cassette is cleaved and *Kras*^{G12D} will be expressed and *Trp53* will be deleted. This causes to increased cell proliferation and progression in target cells, leading to the formation of lung tumors. To study the impact of aging on lung cancer progression and metastasis, lung tumors were simultaneously induced in mice at 2-3 months defined as young (KP-Y) equivalent to early adulthood in humans and 18-19 months defined as old (KP-O) equivalent to human age around 65 years.

3.5.3 NXG IMMUNODEFICIENT MICE

To investigate the ability for cancer cells to survive, grow and metastasize outside of their primary habitat we used NXG mice (NOD-Prkdcscid - IL2rgTm1 484 / Rj) (paper VI). The NXG Xenograft Gamma strain is a mouse strain rendered immunodeficient due to two mutations:

- The Prkdc^{scid} mutation, commonly known as “SCID” for “Severe Combined Immunodeficiency” that induces immune deficiency by blocking the development of T and B cells.
- A *IL2rgTm1* knockout mutation (called γc), deleting the gene encoding the common c gamma chain present in interleukins (IL-2, IL-4, IL-7, IL-9 and IL-15). This gene is necessary for normal

differentiation and function of numerous hematopoietic cells with a full impact on the development of natural killer cells (NK).

The combination of these two mutations Prkdc^{scid} and IL2rgTm1 in this strain induces a severe immunodeficiency with absence of T, B and NK lymphocyte compartments, preventing recognition and destruction of cancer cells by the immune system [109].

Cell suspension from primary mouse lung tumors was either subcutaneously injected into the lower flanks or into the lateral tail vein.

3.6 MOLECULAR DATA

3.6.1 DNA SEQUENCING AND MOLECULAR DIAGNOSTICS

Patients in the "West Sweden cohort" (paper I-VI) underwent NGS on DNA from formaline-fixed paraffin embedded formalin-fixed paraffin-embedded (FFPE) blocks or cytological smears using the Ion AmpliSeq™ Colon and Lung Cancer Panel v2 from Thermo Fisher Scientific until 2019. Thereafter the Thermo Fisher OncomineTM Focus Assay was used, assessing hotspot mutations in *EGFR*, *BRAF*, *KRAS* and *NRAS*. Until June 2017, *ALK*-fusions were assessed with immunohistochemistry (IHC), and with fluorescence in situ hybridization (FISH) if positive or inconclusive IHC; *ROS1* was analyzed upon request using FISH. Thereafter, *ALK*, *ROS1* and *RET* fusions were assessed on RNA level using the Oncomine Solid Tumor Fusion Panel from Thermo Fisher Scientific. All analyses were done as a part of the diagnostic workup process at the Department of Clinical Pathology at Sahlgrenska University Hospital.

The BIOLUNG cohort was additionally assessed with the INVIEW Oncoprofiling Panel (Oncopanel All in One v2.8, Eurofins Genomics (Europe Sequencing GmbH, Germany)) (paper III). All steps, including extraction of DNA from blood and FFPE samples, quantification, library preparation (Agilent Technologies, Santa Clara, CA, USA) and sequencing, were performed at Eurofins Genomics using in-house protocols and sequencing on the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA)

3.6.2 RNA SEQUENCING

RNA sequencing was employed from primary tumor cultures to identify differentially expressed genes comparing KP-O and KP-Y mice. It was conducted at the Genomics Core Facility at the University of Gothenburg on a NextSeq 500 instrument (Illumina) using the NextSeq 500/500 703 High Output Kit v2.5 and paired-end sequencing (paper VI). Functional annotation of genes by Gene Ontology (GO) and pathway analysis by KEGG were done using DAVID [110, 111].

3.6.3 ATAC-SEQUENCING

To examine differences in chromatin accessibility between KP-O and KP-Y primary tumor cultures, we performed unbiased Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) on Illumina HiSeq, performed at GENEWIZ Azenta Life Sciences (Germany). Genomic Regions of Enrichment of Annotations Tool (GREAT) analysis was done to identify the enriched pathways from ATAC sequencing (paper VI).

3.7 HISTOLOGY ANALYSES

Histology analyses were conducted in paper VI. Tissue from mouse lungs and other organs such as kidneys, heart and lymph nodes were paraffin embedded, sliced into 5µm sections and stained with hematoxylin/eosin to assess tumor burden and metastasis incidence. BioPix iQ software (v 2.1.4) was used to quantify tumor burden defined as percent tumor area per lung area.

3.7.1 IMMUNOHISTOCHEMISTRY

Immunohistochemistry was used to visualize tissue expression of proteins in tumor samples.

Mouse lung sections and human lung tissue microarray (TMA) constructed from FFPE tumor tissue were deparaffinized followed by epitope retrieval and blocking with H2O2 for endogenous peroxidase activity. Thereafter sections were incubated with primary antibodies targeting ATF4 protein expression and expression of ATF4 target proteins such as ASNS and SLC7A11. Total number of positively stained tumor cells were counted and normalized either to the tumor area or the total number of cells (paper VI).

3.8 CELL ASSAYS

To investigate differences between KP-Y and KP-O, primary tumor cells were isolated from lung tumors of respective mouse groups (paper VI).

3.8.1 PROLIFERATION AND VIABILITY ASSAYS

We evaluated proliferative capacity using population-doubling assays in which cells were seeded in triplicate in six-well plates, counted at three-day intervals, and reseeded at the original density for 12–15 days. To assess drug effects on viability, cells were plated in white, opaque, clear-bottom 96-well plates, treated 24 hours after seeding at the indicated concentrations, and analyzed 72 hours later with CellTiter-Glo 2.0.

3.8.2 3D CULTURES AND ANOIKIS RESISTANCE ASSAY

Anoikis resistance was evaluated by seeding cells in ultra–low-attachment 96-well plates and, in parallel, in standard adherent 96-well plates. Viability was measured 48 hours after seeding. Values from ultra–low-attachment wells were normalized to adherent controls measured 16 hours post-seeding.

3.8.3 GENE EDITING

The different types of gene editing of *Atf4* in primary cells were used as proof of concept:

- knockout rendering the gene to be permanently silenced
- knockdown inducing transient silencing
- overexpression inducing and increasing transcription

For overexpression the cells were given an extra copy of the *Atf4* gene, complimentary DNA with a strong promoter, by stable lentiviral vector transduction with puromycin selection. Gene knockout was performed using CRISPR. The expression of target proteins in CRISPR-knockout and overexpression experiments were evaluated by western blotting 3–5 days after selection.

For studying acute depletion of Atf4, lenti-vectors with doxycycline inducible short hairpin RNA (shRNA) targeting the *Atf4* transcript was produced. Single vectors were transduced into cells and selected with 2µg/ml puromycin for two

days. Knockdown of *Atf4* was verified by western blot analysis following 72 hours of treatment with 1 μg/mL doxycycline.

3.8.4 WESTERN BLOTTING

To visualize and quantify differences in target protein levels, proteins was isolated by using the 2x Laemmli Buffer supplemented with β-mercaptoethanol. Samples were subsequently heated at 95°C for 10 min. Proteins were separated on 4–20% Mini-PROTEAN TGX Stain-Free gel and then transferred onto a 0.2 μM nitrocellulose membrane, incubated with specific primary antibodies. Protein bands were detected using Clarity Western ECL substrate with the Amersham ImageQuant 800 Western blot imaging systems.

3.8.5 METABOLIC ASSAYS

To assess alterations of cellular metabolism Gas Chromatography/Mass Spectrometry (GC/MS) analysis of polar metabolites and stable isotope tracing was conducted. Isotope tracing with D-Glucose was performed to investigate carbon flow through the TCA-cycle. Stable isotope tracing with L-glutamine was performed to investigate the flow of glutamine in the TCA-cycle.

3.9 ONLINE DATASETS

Publicly available clinical and cancer genomics data was used in paper III and VI.

In paper III we extracted data from cBioPortal, including studies where immunotherapy was used as a first- or second-line treatment with PFS data and with mutational status data available for *KRAS*, *TP53*, and *LRP1B* [112, 113]. These data were merged with our dataset, referred to as merged PFS.

For paper VI publicly available clinical and cancer genomics data was obtained from cBioPortal for Cancer Genomics, an online tool available at http://www.cbioportal.org/, and from DepMap portal available at https://depmap.org/portal. Normalized mRNA expression values and age at diagnosis of LUAD patients and cancer cell lines were collected from The Cancer Genome Atlas (TCGA-LUAD) dataset (https://www.cancer.gov/tcga), and Cancer Cell Encyclopedia (CCLE) dataset respectively. Information regarding LUAD organotropism was obtained from MSK-IMPACT data. Patients were divided into two groups according to age at diagnosis and

compared for *KRAS* gene alteration frequencies. Population doublings time and age of cell lines were retrieved from the database https://www.cellosaurus.org

4 RESULTS

This chapter presents an overview of the results, for more details see attached papers at the end of the thesis.

4.1 PAPER I

***KRAS* Mutations Impact Clinical Outcome in Metastatic Non-Small Cell Lung Cancer. Cancers 2022.**

The overarching aim of this study was to determine whether *KRAS* mutational status carried prognostic value in stage IV disease and for first-line treatment choice between platinum doublet chemotherapy (PT) and ICB. We conducted a real-world, multicenter retrospective study of patients with metastatic (stage IV) NSCLC diagnosed and molecularly profiled in the Region Västra Götaland (West Sweden) between 2016 and 2018. In total, 580 consecutive stage IV patients were included; clinical variables, histology, smoking history, ECOG performance status, metastatic burden, first-line therapy, and outcomes were abstracted from charts and the Swedish Lung Cancer Registry, with molecular testing performed as part of routine diagnostics (targeted NGS for key drivers and PD-L1 assessment by IHC). Within this population, 35.5% harbored *KRAS* mutations. First-line treatments comprised PT (n=195) or ICB (n=37), reflecting the practice patterns during the study period; a subset with known PD-L1 status (n=261) was available for biomarker-stratified analyses.

In the full cohort, detection of a *KRAS* mutation was associated with shorter overall survival and remained an independent adverse factor after multivariable analysis. Among patients treated with PT, *KRAS* mutation predicted inferior survival compared to *KRAS* wild-type disease. By contrast, in patients treated with first-line ICB as monotherapy, *KRAS*-mutant tumors were associated with markedly longer survival than *KRAS* wild-type tumors (median 23 vs. 6 months), and *KRAS* status remained independently associated with better outcomes on ICB in multivariable models.

Taken together, this regional, population-based cohort indicates that *KRAS* mutations in stage IV NSCLC have dual clinical relevance: they are linked to poorer overall survival and worse outcomes with platinum-based chemotherapy, yet they identify patients more likely to benefit from immune checkpoint inhibition. We conclude that incorporating *KRAS* status alongside

PD-L1 in routine workups might sharpen first-line therapeutic decision-making for advanced NSCLC.

4.2 PAPER II

Assessing the prognostic value of *KRAS* mutation combined with tumor size in stage I-II non-small cell lung cancer: a retrospective analysis. **Front Oncol 2024.**

Early-stage NSCLC is primarily risk-stratified by TNM stage with extra focus on primary tumor size**,** which correlates with outcome. At the same time, the prognostic role of *KRAS* mutations in stage I–II disease remains unknown**,** with prior studies reporting mixed results and current guidelines not recommending routine *KRAS* testing for resectable tumors. We therefore asked whether combining *KRAS* mutational status with tumor size adds prognostic information beyond either factor alone in stage I–II NSCLC.

To investigate this question we conducted a multicenter, retrospective cohort study of 310 consecutive stage I–II NSCLC patients diagnosed and molecularly profiled in the Region Västra Götaland (West Sweden) between 2016 and 2018; 37% harbored a *KRAS* mutation, the majority had ECOG 0–1, adenocarcinoma histology, and underwent surgical resection, with a median follow-up time of 63 months**.** Primary outcomes were OS and risk of death (hazard ratio), assessed overall and in analyses stratified by stage and T-category.

First, we confirmed stage and tumor size as prognostic in this early-stage cohort. Second, *KRAS* mutational status did not significantly affect OS in the overall stage I–II population or within stage- or T-stratified analyses. Third, combining *KRAS* status with tumor size did not modify risk of death, there was no interaction suggesting that *KRAS* mutational status has influence on the prognostic effect of primary tumor size. Fourth, in exploratory comparisons of how size was measured, larger pathologic (resection specimen) tumor size—but not pre-treatment CT size—was associated with higher risk of death**,** and *KRAS*-mutant tumors were smaller on CT at diagnosis although this difference was not confirmed using pathologic measurement. Together, these results indicate that in stage I–II disease, traditional anatomic factors dominate prognosis**,** while *KRAS* status alone, or in combination with size, does not add clinically meaningful prognostic information**.**

In conclusion, this real-world, population-based analysis supports the primacy of pathologic tumor size and stage for risk assessment in resectable NSCLC stage I–II disease. And there was no impact on prognosis with *KRAS* mutations in combination with primary tumor size.

4.3 PAPER III

Comprehensive genetic variant analysis reveals combination of *KRAS* and *LRP1B* as a predictive biomarker of response to immunotherapy in patients with non-small cell lung cancer. Exp Clin Cancer Res 2025.

Although ICB has reshaped the treatment of advanced NSCLC, only a minority of the patients benefit from the treatment. PD-L1 expression is the current clinical standard but is an imperfect predictor, and the utility of tumor mutational burden remains contested. This creates a need for additional, genomically anchored predictors – either single genes or co-mutation patterns – that can refine selection for ICB treatment.

In this prospective, bicentre clinical study of 49 patients with stage III–IV NSCLC treated with ICB, we profiled tumors using a 597-gene NGS panel and applied an in-house variant-interpretation framework integrating multiple databases and prediction tools. We evaluated OS and PFS in relation to candidate variants considered alone and in combination, and extended PFS analyses by merging with external cBioPortal cohorts for validation ("merged PFS").

The primary aim was to identify and predict pathogenicity of tumor genetic variants—individually or as co-variants—that predict clinical benefit from ICB. As single biomarkers, variants in *LRP1B* were associated with significantly longer OS in the prospective cohort and prolonged PFS in the merged dataset and remained independently predictive in multivariable analyses. *KRAS* pathogenic variants correlated with longer OS in univariate analyses but were not independently predictive; *TP53* variants did not affect OS or PFS in the local cohort but were linked to improved merged PFS. Most notably, co-mutation patterns strengthened the prediction: *KRAS*+*LRP1B* co-variants were independently associated with superior OS and with longer merged PFS, and the triple combination *KRAS*+*LRP1B*+*TP53* variants was associated with markedly favorable outcomes (all six patients alive at last follow-up; merged PFS benefit and independent effect in multivariable models). Incorporating PD-L1 further refined the risk assessment: patients with *KRAS*+*LRP1B* variants and PD-L1 positivity– especially PD-L1 ≥50%–

had not-reached medians for OS and PFS, with independent associations on multivariable analysis.

Our findings indicate that composite genomic signatures– particularly co-occurring variants in *KRAS* and *LRP1B*, and potentially the triad of *KRAS*, *LRP1B* and *TP53* variants– offer predictive value for ICB outcomes beyond single-gene markers or PD-L1 alone. These findings support routine inclusion of *LRP1B* in targeted panels and motivate larger, confirmatory cohorts and functional studies to clarify biological mechanisms and optimize biomarker-guided immunotherapy in NSCLC.

4.4 PAPER IV

Equalizing prognostic disparities in *KRAS*-mutated stage III NSCLC patients: addition of durvalumab to combined chemoradiotherapy improves survival. Lung Cancer 2025.

We previously described *KRAS* mutations to be linked to poorer outcomes when treated with chemotherapy and improved prognosis on monotherapy ICB in metastatic disease. However, there are few studies investigating the prognostic impact on *KRAS* mutations after cCRT in stage III disease. With the implementation of adjuvant PD-L1 blockade (durvalumab) there is further interest in investigating the prognostic impact of *KRAS* mutations. In this study we asked whether *KRAS* status influenced outcomes of patients receiving cCRT before and after the introduction of consolidation durvalumab. In a real-world, multicenter cohort from western Sweden (2016–2021), 145 unresectable stage III NSCLC patients treated with curative-intent cCRT and molecular profiles were analyzed; 32% (46/145) harbored activating *KRAS* mutations. *KRAS*-mutant patients had significantly worse overall survival (median 25 vs 46 months) and progression-free survival (9 vs 16 months) than *KRAS* wild-type patients, and *KRAS* status remained independently prognostic on multivariable analysis. Among patients treated with cCRT alone, detection of a *KRAS* mutation again predicted shorter OS and PFS. However, in the subgroup that received consolidation durvalumab after cCRT, no differences in survival between patients with *KRAS*-mutant and *KRAS* wild-type tumors were seen, indicating that adjuvant durvalumab ameliorated the negative impact of the *KRAS* mutation.

In conclusion, our findings provide evidence that *KRAS* mutation confers inferior prognosis after cCRT in stage III NSCLC. However, consolidation

durvalumab removes the negative prognostic factor for *KRAS*-mutated patients.

4.5 PAPER V

Monotherapy With Immune Checkpoint Blockade Improves Survival Outcomes in *KRAS*-Mutant but Not *KRAS* Wild-Type Metastatic Lung Adenocarcinoma: Validation from an Extended Swedish Cohort. JTO Clin Res Rep 2025.

To follow up our previous findings (paper I) we conducted an expanded, real-world cohort study with long follow-up and focus on LUAD. In this retrospective multicenter series of 424 stage IV LUAD patients treated in western Sweden (2016-2021), ~40% harbored *KRAS* mutations. *KRAS*-mutant patients experienced clear survival gains with ICB monotherapy compared with platinum doublet chemotherapy (overall survival 16 vs 8 months; progression-free survival 8 vs 5 months), and monotherapy remained independently beneficial on multivariable analysis (HR ≈0.53). In contrast, *KRAS* wild-type patients experienced no advantage from monotherapy, though both *KRAS*-mutant and wild-type cohorts benefited from chemo-immunotherapy. Subtype analyses indicated that *KRAS* G12C and G12V – though not G12D – were independently associated with improved outcomes on ICB-containing treatment.

In conclusion, *KRAS* mutations, particularly G12C/G12V, function as a predictive biomarker for selecting ICB monotherapy in stage IV LUAD, while *KRAS* wildtype disease may warrant chemo-immunotherapy instead.

4.6 PAPER VI

Aging promotes metastasis of lung cancer through epigenetic activation of the integrated stress response. Manuscript submitted.

Physiological aging shapes LUAD in ways that may not be captured by studies in young models. In this study we aim to define the impact of physiological aging on lung cancer progression and metastasis. In *Kras/Trp53* GEMMs induced at 2–3 vs 18–19 months, older mice developed smaller, less proliferative primary tumors yet markedly higher rates of nodal and distant

metastasis. Tumor cultures from aged mice showed epithelial-mesenchymal transition (EMT) activation, anoikis resistance, and enhanced metastatic seeding in multiple in vivo assays. Multi-omics profiling revealed broad chromatin accessibility changes with enrichment of unfolded protein response/integrated stress response (UPR/ISR) programs and strong induction of *Atf4*, a key effector of UPR; pharmacologic ISR inhibition or genetic *Atf4* loss curtailed EMT features, spheroid survival, and metastatic burden, while *Atf4* overexpression in "young" tumor cells was sufficient to increase EMT, anoikis resistance, and metastasis. Mechanistically, we show that *Atf4* rewired metabolism toward aerobic glycolysis and glutamine anaplerosis; aged tumors became selectively sensitive to glutaminase blockade (e.g., CB-839), which suppressed metastasis without shrinking primary tumors in vivo, and this sensitivity required *Atf4*. Clinically, in regional real-world cohorts, older patients with *KRAS*-mutant NSCLC more often presented with advanced/metastatic disease despite smaller primary tumors, and tumors from older patients expressed more *ATF4*; across external datasets, high *ATF4* was associated with worse survival in advanced stages. Together, the results identify epigenetically driven *ATF4* induction as a central, age-amplified mechanism that promotes metastatic fitness and creates a glutamine-metabolism dependency that can be therapeutically exploited.

We conclude that aging is not merely a background variable but an active modifier of tumor cell state and metastatic behavior. By linking age-associated chromatin changes to *ATF4*-driven metabolic plasticity and EMT, our study provides a mechanistic rationale for testing *ATF4*/ISR modulators or glutamine-axis inhibitors as adjuvant strategies to reduce dissemination in older LUAD patients, particularly those with *KRAS* mutations.

5 DISCUSSION

The prognostic impact of *KRAS* mutations, the most frequent oncogenic driver in NSCLC, has been debated. And with the rapid development of new treatments there is a need to continuously evaluate prognostic and predictive biomarkers. In this thesis we have broadly investigated the prognostic and predictive impact of harboring *KRAS* mutations as well as co-mutations in the era of ICB treatments. Additionally, we have explored the impact of aging on lung cancer progression and metastasis where our findings have identified new mechanisms and novel treatment vulnerabilities in *KRAS* mutated LUAD.

5.1 ASSESSING *KRAS* MUTATIONS IN NSCLC

The prognostic and predictive value of *KRAS* mutations has been unclear in previous literature. We have conducted retrospective real world population studies and a prospective cohort study to add knowledge on the impact of *KRAS* mutations in NSCLC. We show that its impact is context-dependent—shaped by disease stage, treatment modality, and co-mutations.

We found no impact of *KRAS* mutations on OS in stage I-II disease, alone or in combination with primary tumor size (paper II). Previous studies have been inconclusive regarding the impact on prognosis of *KRAS* mutations [114-118]. However, the reliance on OS as the primary outcome measure in this study may be considered a limitation, as it captures mortality from all causes rather than cancer-specific effects. Alternative endpoints, such as recurrence-free survival or lung cancer–specific survival, would likely provide a more precise assessment of disease-related outcomes. Furthermore, the cohort studied was relatively small. The impact of *KRAS* mutations and new prognostic combinations need further investigation.

In stage III and IV disease *KRAS* mutations are poor prognostic factors when receiving cCRT or chemotherapy (paper I, IV &V). However, when receiving adjuvant durvalumab treatment the adverse prognostic effect is mitigated in stage III disease and this result is complimentary to Barsouk et. al showing the same effects on PFS [119]. In stage IV disease *KRAS* mutations became a positive prognostic factor for ICB monotherapy, and we further described that the subtype of *KRAS* mutation also impacts the prognostic and predictive

impact on ICB therapy in LUAD. *KRAS* G12C and G12V aligned with improved outcomes on ICB-containing regimens, whereas G12D mutations did not, arguing for subtype-aware therapeutic choices. Together, these data support a practical first-line algorithm in advanced LUAD: consider ICB monotherapy for *KRAS*-mutant (especially G12C/V) tumors with high PD-L1 expression. However, these results alone are not going to change treatment recommendations for lung cancer: there is further need for pooled analysis and prospective studies. This is especially true in light of emerging evidence of co-mutations in tumor suppressor genes *STK11*, *KEAP1* and *TP53* as predictors for response to ICB treatment. Mutations in *STK11* and *KEAP1* have been associated with unfavorable treatment responses, whereas *TP53* mutations appear to confer a more favorable effect [102, 106, 120, 121]. Co-mutations could be responsible for the different reported outcomes regarding *KRAS*-mutations.

To further investigate predictive biomarkers for ICB treatment we prospectively studied patients receiving ICB containing treatment (paper III). We aimed to develop variant classification to predict the pathogenicity of newly identified variants and to investigate the impact of single variants and combinations of variants on the clinical outcome of ICB treatment. In this study *KRAS*-mutations were associated with a better OS but not PFS. Further, we identified variants in the tumor suppressor gene *LRP1B*, to be a promising predictor for ICB treatment response which has previously been described in multiple cancer types to have a prognostic impact [122-124]. However, we are the first to report that the combination of *KRAS* and *LRP1B* variants strongly correlates to better treatment outcome and is a better predictive biomarker than PD-L1 expression for ICB treatment in lung cancer. We also show that the combination of *KRAS* and *LRP1B* variants together with high PD-L1 expression or the triple *KRAS*, *LRP1B* and *TP53* combination was even more favorable. This study emphasizes the need for refinement in the interpretation of the pathogenic impact of variants in less known genes as well as the limitations of using pan cancer genomic panels since the ones largely used today (MSK-impact, Foundation one etc.) does not include *LRP1B*. The *LRP1B* gene is involved in processes such as cell proliferation and migration and mutations have been associated with elevated expression of immune-related genes and increased immune cell infiltration in LUAD patients treated with ICB [125-130]. However, there is a further need to investigate the mechanistic connections to ICB treatment both for *LRP1B* alone as well as in combination with *KRAS* mutations. Finally, our findings need to be assessed in lager cohorts with less heterogeneity in prior treatment.

In paper VI the impact of aging on tumor progression and metastasis is explored. Previous mechanistic studies have been conducted on young mice and this could be a reason for poor translational outcomes when applied to humans. In this study we provide novel insights into how physiological aging influences lung cancer progression, highlighting a paradoxical shift where primary tumor growth is attenuated while metastatic dissemination is enhanced. We demonstrate that aging-associated epigenetic reprogramming drives induction of *Atf4*, a central effector of the integrated stress response. *Atf4* upregulation results in metabolic rewiring, epithelial–mesenchymal transition, and anoikis resistance, collectively promoting metastasis despite reduced primary tumor burden. A key strength of this study lies in its integration of mechanistic animal models with clinical datasets, providing translational relevance. The consistent observation that elderly NSCLC patients, particularly those with *KRAS* mutations, present with smaller primary tumors yet higher metastatic incidence closely mirrors the preclinical findings. We established aging not merely as a background variable but as an active determinant of lung cancer biology. Importantly, high *ATF4* expression in patient tumors correlated with poor survival, underscoring its potential as a prognostic biomarker and therapeutic target. The results also reveal that aging-driven *Atf4* induction confers a metabolic plasticity that sensitizes tumor cells to glutaminase inhibition. Pharmacological targeting of glutamine metabolism, particularly with CB-839, effectively suppressed metastasis in vivo without significantly affecting primary tumor growth. This suggests that interventions tailored to exploit metabolic dependencies in aged tumors could represent promising adjuvant strategies for elderly NSCLC patients, a population underrepresented in clinical trials.

Collectively, the work elevates aging from a background variable to a central determinant of lung cancer progression and supports integrating aging biology and *ATF4*-centered strategies into precision therapy design.

6 CONCLUSION

In this thesis we have broadly explored the impact of *KRAS* mutations on treatment outcome and biology in lung cancer and as a predictive biomarker.

Paper I: Our findings indicate that *KRAS* mutations in conjunction with high PD-L1 expression is a promising biomarker for favorable response to ICB monotherapy, while simultaneously representing a negative prognostic factor in the context of chemotherapy.

Paper II: *KRAS* mutations did not influence overall survival in early-stage lung cancer, and no significant difference in mortality risk was observed when integrating *KRAS* mutational status with primary tumor size.

Paper III: We identified co-variants in *KRAS* and *LRP1B* as potential predictive biomarkers for ICB treatment response.

Paper IV: We demonstrated that the addition of durvalumab following cCRT in stage III NSCLC patients improved outcomes in the *KRAS*-mutant subgroup, ameliorating the negative prognostic impact of *KRAS* mutations.

Paper V: We showed that in LUAD, *KRAS* mutations, particularly G12C and G12V, appear to predict substantial, durable benefit from ICB monotherapy whereas patients without *KRAS* mutations seem to derive limited benefit from ICB alone.

Paper VI: We identified aging-driven *ATF4* induction to drive metastasis, induce metabolic plasticity and sensitize tumor cells to glutaminase inhibition.

7 ETHICAL CONSIDERATIONS

Approval from the Swedish Ethical Review Authority was obtained prior to the commencement of all the studies on the "West Sweden Cohort". For paper I, II, IV-VI Dnr 2019- 04771 and 2021-04987, no informed consent was required according to the Swedish Ethical Review Authority. The majority of patients was deceased, only disease specific variables was collected by a strictly limited number of people, and the data was analyzed and presented in a grouped pseudonymized form to avoid any identification of individuals. Overall lung cancer is still a disease with poor prognosis and there is a clear public interest in research that can help improve the outcomes for future patients and our research was conducted with a minimal risk for the included patients.

The BIOLUNG study was approved by the Regional Ethics Review Board in Gothenburg, Sweden (Permit number 953/18), all participating patients signed an informed consent and no payment or other compensation was offered for participating in the study. All data was presented in a de-identified form.

All mouse experiments were approved by the Research Animal Ethics Committee in Gothenburg (2071/19; 2077/19 and 6057/24). All animal experiments was conducted in adherence to the principals of 3Rs (Replacement, Reduction and Refinement) to the greatest extent possible, minimizing the number of animals used. Animal experiments to investigate tumor progression, metastasis and to evaluate new potential treatments is currently a necessity before going into human clinical trials as no equivalent alternatives exist. The medical benefit of our research therefore outweighs the risks.

8 FUTURE PERSPECTIVES

Precision medicine in oncology has been a central topic for over a decade: the right treatment for the right patient. Lung cancer is one of the diagnoses where it has come the furthest due to the continuous discovery of new oncogenic drivers and development of drugs targeting them. *KRAS* mutations have historically been seen as non-targetable. The first targeted treatment for a subgroup, *KRAS* G12C, was approved in 2022 and is not part of this thesis. However, precision medicine reaches beyond specific driver mutations with the rapid mapping of the tumor microenvironment, the impact co-alterations in e.g. tumor suppressor genes, aging and metabolic rewiring influencing the treatment outcomes.

Lung cancer can no longer only be divided into small cell lung cancer or non-small cell lung cancer and NSCLC can no longer only be divided into squamous cell carcinoma and adenocarcinoma. Likewise, adenocarcinoma cannot only be divided into driving oncogene groups as all mutations are not the same (i.e. *EGFR* or *KRAS*). Thus, the treatment algorithms for NSCLC will continue to expand with layers of complexity.

This thesis has shed light on the fact that the introduction of ICB treatment has improved the outcome for *KRAS* mutated patients in advanced stages. Further, *KRAS* mutations in combination with high PD-L1 expression might be predictive for response to monotherapy ICB, especially for *KRAS* G12C and *KRAS* G12V. On the other hand *KRAS* G12D mutated patients might be less responsive and in need of more comprehensive treatment strategies. This is however a simplified assumption as we have also shown that co-variants in *LRP1B* and *TP53* are potential predictive factors together with *KRAS*-mutations. PD-L1 expression alone is a poor biomarker for ICB response. Perhaps in awaiting the implementation of broader molecular assessment of NSCLC in clinical praxis the combination of *KRAS* G12C/V and high PD-L1 could be a biomarker for selecting patients for monotherapy with ICB. Therefore, larger studies are in the pipeline to address this further.

It is also important to consider the phenotype, as all DNA alterations will not affect the protein function. In the Biolung cohort there were two patients with co-current *LRP1B* and *KEAP1* variants where one was a non-responder and the other a responder. For the responder the *KEAP1* variant was localized on the last nucleotide of the last exon in the main transcript and therefore might not have affected the protein. This emphasizes the importance of functional

assessments of genomic variants and perhaps RNA sequencing and/or proteomics as future tools in clinical praxis.

Last but not the least, have we found a way to prevent metastatic disease after resection of lung cancer with elevated *ATF4* expression? As about 50% of resected patients have recurrent disease within three years, this is a pressing question. Could the glutamine dependency of these tumors be targeted in combination with current treatments in advanced NSCLC? Would that give rise to additive effects? If we could inhibit further metastatic development, there would be a possibility that local treatment of primary metastases could be more successful also in advanced stages. Perhaps in the future we will have a new molecular subgroup named *ATF4* overexpressed NSCLC. However, this needs to be investigated further both in mice and humans.

Overall, there is still hope for a more precise precision medicine approach for lung cancer patients with *KRAS* mutations in the future.

9 USE OF GENERATIVE AI

Generative artificial intelligence (AI) tools, including ChatGPT (OpenAI, GPT-5 and Perplexity), were used during the preparation of this thesis frame to support the writing and editing process. Specifically, AI assistance was applied to:

- generate preliminary drafts of certain sections to aid in text structuring and flow
- improve clarity, coherence, and academic tone
- refine grammar, phrasing, and readability,
- summarize or rephrase background information for improved accessibility
- assist in finding references

All AI-generated text and reference suggestions were critically reviewed, verified, and edited by me. I have ensured that all referenced works were checked against original sources, and that only accurate and verifiable citations were included. Generative AI tools were not used to generate or analyze data, interpret results, or draw scientific conclusions. All intellectual content, data interpretation, and scientific reasoning are my own and I take full responsibility for the content.

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REFERENCES

1. *World Health Organization, International Agency for Research on Cancer. Globocan 2022: Lung Cancer. International Agency for Research on Cancer. Available at http://gco.iarc.fr/today/data/factsheets/cancers/15-Lung-fact-sheet.pdf. Accessed: September 12, 2025.*
2. Hanahan, D. and R.A. Weinberg, *The hallmarks of cancer.* Cell, 2000. **100**(1): p. 57-70.
3. Hanahan, D., *Hallmarks of Cancer: New Dimensions.* Cancer Discov, 2022. **12**(1): p. 31-46.
4. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.
5. Dunn, G.P., L.J. Old, and R.D. Schreiber, *The immunobiology of cancer immunosurveillance and immunoediting.* Immunity, 2004. **21**(2): p. 137-48.
6. Vesely, M.D., et al., *Natural innate and adaptive immunity to cancer.* Annu Rev Immunol, 2011. **29**: p. 235-71.
7. Chen, D.S. and I. Mellman, *Oncology meets immunology: the cancer-immunity cycle.* Immunity, 2013. **39**(1): p. 1-10.
8. Garrido, F., et al., *The urgent need to recover MHC class I in cancers for effective immunotherapy.* Curr Opin Immunol, 2016. **39**: p. 44-51.
9. Gajewski, T.F., H. Schreiber, and Y.X. Fu, *Innate and adaptive immune cells in the tumor microenvironment.* Nat Immunol, 2013. **14**(10): p. 1014-22.
10. Pardoll, D.M., *The blockade of immune checkpoints in cancer immunotherapy.* Nat Rev Cancer, 2012. **12**(4): p. 252-64.
11. Wei, S.C., C.R. Duffy, and J.P. Allison, *Fundamental Mechanisms of Immune Checkpoint Blockade Therapy.* Cancer Discov, 2018. **8**(9): p. 1069-1086.
12. Wu, B., et al., *Cold and hot tumors: from molecular mechanisms to targeted therapy.* Signal Transduction and Targeted Therapy, 2024. **9**(1): p. 274.
13. Schreiber, R.D., L.J. Old, and M.J. Smyth, *Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion.* Science, 2011. **331**(6024): p. 1565-70.
14. Sharma, P. and J.P. Allison, *Dissecting the mechanisms of immune checkpoint therapy.* Nat Rev Immunol, 2020. **20**(2): p. 75-76.
15. Ribas, A. and J.D. Wolchok, *Cancer immunotherapy using checkpoint blockade.* Science, 2018. **359**(6382): p. 1350-1355.
16. López-Otín, C., et al., *The hallmarks of aging.* Cell, 2013. **153**(6): p. 1194-217.

17. López-Otín, C., et al., *Hallmarks of aging: An expanding universe.* Cell, 2023. **186**(2): p. 243-278.
18. https://www.cancer.gov/about-cancer/causes-prevention/risk/age. *Nantional Cancer Institute Age and Cancer Risk*. [cited 2025 20251010].
19. https://seer.cancer.gov/statfacts/html/lungb.html. *National Cancer Institute Cancer Stat Facts: Lung and Bronchus Cancer*. 2025 [cited 2025 20251010].
20. Sung, H., et al., *Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries.* CA Cancer J Clin, 2021. **71**(3): p. 209-249.
21. https://cancercentrum.se/diagnosbehandling/cancerdiagnoser/lungaochlungsack/statistik.8135.html. *RCC Cancercentrum Statistik lungcancer*. 2025 [cited 2025 20201010].
22. Alberg, A.J., M.V. Brock, and J.M. Samet, *Epidemiology of lung cancer: looking to the future.* J Clin Oncol, 2005. **23**(14): p. 3175-85.
23. Barta, J.A., C.A. Powell, and J.P. Wisnivesky, *Global Epidemiology of Lung Cancer.* Ann Glob Health, 2019. **85**(1).
24. Lee, P.N., B.A. Forey, and K.J. Coombs, *Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer.* BMC Cancer, 2012. **12**: p. 385.
25. Hamra, G.B., et al., *Outdoor particulate matter exposure and lung cancer: a systematic review and meta-analysis.* Environ Health Perspect, 2014. **122**(9): p. 906-11.
26. Fajersztajn, L., et al., *Air pollution: a potentially modifiable risk factor for lung cancer.* Nat Rev Cancer, 2013. **13**(9): p. 674-8.
27. Nielsen, L.S., et al., *Occupational asbestos exposure and lung cancer--a systematic review of the literature.* Arch Environ Occup Health, 2014. **69**(4): p. 191-206.
28. Darby, S., et al., *Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies.* Bmj, 2005. **330**(7485): p. 223.
29. De Matteis, S., et al., *Impact of occupational carcinogens on lung cancer risk in a general population.* International Journal of Epidemiology, 2014. **42**(6): p. 1902-1902.
30. Rivera, M.P. and A.C. Mehta, *Initial diagnosis of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition).* Chest, 2007. **132**(3 Suppl): p. 131s-148s.
31. Nicholson, A.G., et al., *The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015.* J Thorac Oncol, 2022. **17**(3): p. 362-387.

32. Herbst, R.S., D. Morgensztern, and C. Boshoff, *The biology and management of non-small cell lung cancer.* Nature, 2018. **553**(7689): p. 446-454.
33. Hirsch, F.R., et al., *Lung cancer: current therapies and new targeted treatments.* Lancet, 2017. **389**(10066): p. 299-311.
34. *Regionala Cancercentrum. Nationellt vårdprogram lungcancer- Avaliable from: https://cancercentrum.se/samverkan/cancerdiagnoser/lunga-och-lungsack/vardprogram/. Acessed 12 september 2025*.
35. van der Leest, P., et al., *Detection of actionable mutations in circulating tumor DNA for non-small cell lung cancer patients.* Communications Medicine, 2025. **5**(1): p. 204.
36. Galan-Cobo, A., et al., *LKB1 and KEAP1/NRF2 Pathways Cooperatively Promote Metabolic Reprogramming with Enhanced Glutamine Dependence in KRAS-Mutant Lung Adenocarcinoma.* Cancer Res, 2019. **79**(13): p. 3251-3267.
37. Goeman, F., et al., *Mutations in the KEAP1-NFE2L2 Pathway Define a Molecular Subset of Rapidly Progressing Lung Adenocarcinoma.* J Thorac Oncol, 2019. **14**(11): p. 1924-1934.
38. Jeong, Y., et al., *Role of KEAP1/NFE2L2 Mutations in the Chemotherapeutic Response of Patients with Non-Small Cell Lung Cancer.* Clin Cancer Res, 2020. **26**(1): p. 274-281.
39. Jeong, Y., et al., *Role of KEAP1/NRF2 and TP53 Mutations in Lung Squamous Cell Carcinoma Development and Radiation Resistance.* Cancer Discov, 2017. **7**(1): p. 86-101.
40. Sayin, V.I., et al., *Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer.* Elife, 2017. **6**.
41. Scalera, S., et al., *KEAP1 and TP53 frame genomic, evolutionary and immunological subtypes of lung adenocarcinoma with different sensitivity to immunotherapy.* J Thorac Oncol, 2021.
42. Sitthideatphaiboon, P., et al., *STK11/LKB1 Mutations in NSCLC Are Associated with KEAP1/NRF2-Dependent Radiotherapy Resistance Targetable by Glutaminase Inhibition.* Clin Cancer Res, 2021. **27**(6): p. 1720-1733.
43. Detterbeck, F.C., et al., *The Eighth Edition Lung Cancer Stage Classification.* Chest, 2017. **151**(1): p. 193-203.
44. Detterbeck, F.C., *The eighth edition TNM stage classification for lung cancer: What does it mean on main street?* J Thorac Cardiovasc Surg, 2018. **155**(1): p. 356-359.
45. Hendriks, L.E., et al., *Non-oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up.* Ann Oncol, 2023. **34**(4): p. 358-376.

46. Hendriks, L.E., et al., *Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up.* Ann Oncol, 2023. **34**(4): p. 339-357.
47. Oken, M.M., et al., *Toxicity and response criteria of the Eastern Cooperative Oncology Group.* Am J Clin Oncol, 1982. **5**(6): p. 649-55.
48. Rajaram, R., et al., *Recurrence-Free Survival in Patients With Surgically Resected Non-Small Cell Lung Cancer: A Systematic Literature Review and Meta-Analysis.* Chest, 2024. **165**(5): p. 1260-1270.
49. Remon, J., J.C. Soria, and S. Peters, *Early and locally advanced non-small-cell lung cancer: an update of the ESMO Clinical Practice Guidelines focusing on diagnosis, staging, systemic and local therapy.* Ann Oncol, 2021. **32**(12): p. 1637-1642.
50. Chang, J.Y., et al., *Stereotactic ablative radiotherapy for operable stage I non-small-cell lung cancer (revised STARS): long-term results of a single-arm, prospective trial with prespecified comparison to surgery.* Lancet Oncol, 2021. **22**(10): p. 1448-1457.
51. Chang, J.Y., et al., *Stereotactic ablative radiotherapy versus lobectomy for operable stage I non-small-cell lung cancer: a pooled analysis of two randomised trials.* Lancet Oncol, 2015. **16**(6): p. 630-7.
52. Arriagada, R., et al., *Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data.* Lancet, 2010. **375**(9722): p. 1267-77.
53. Arriagada, R., et al., *Long-term results of the international adjuvant lung cancer trial evaluating adjuvant Cisplatin-based chemotherapy in resected lung cancer.* J Clin Oncol, 2010. **28**(1): p. 35-42.
54. Felip, E., et al., *Overall survival with adjuvant atezolizumab after chemotherapy in resected stage II-IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase III trial.* Ann Oncol, 2023. **34**(10): p. 907-919.
55. O'Brien, M., et al., *Pembrolizumab versus placebo as adjuvant therapy for completely resected stage IB-IIIA non-small-cell lung cancer (PEARLS/KEYNOTE-091): an interim analysis of a randomised, triple-blind, phase 3 trial.* Lancet Oncol, 2022. **23**(10): p. 1274-1286.
56. Forde, P.M., et al., *Neoadjuvant Nivolumab plus Chemotherapy in Resectable Lung Cancer.* N Engl J Med, 2022. **386**(21): p. 1973-1985.
57. Li, M.S.C., K.K.S. Mok, and T.S.K. Mok, *Developments in targeted therapy & immunotherapy-how non-small cell lung cancer management will change in the next decade: a narrative review.* Ann Transl Med, 2023. **11**(10): p. 358.

58. Tsuboi, M., et al., *Overall Survival with Osimertinib in Resected EGFR-Mutated NSCLC.* N Engl J Med, 2023. **389**(2): p. 137-147.
59. Wu, Y.L., et al., *Alectinib in Resected ALK-Positive Non-Small-Cell Lung Cancer.* N Engl J Med, 2024. **390**(14): p. 1265-1276.
60. Ahn, J.S., et al., *Multinational Randomized Phase III Trial With or Without Consolidation Chemotherapy Using Docetaxel and Cisplatin After Concurrent Chemoradiation in Inoperable Stage III Non-Small-Cell Lung Cancer: KCSG-LU05-04.* J Clin Oncol, 2015. **33**(24): p. 2660-6.
61. Yoon, S.M., T. Shaikh, and M. Hallman, *Therapeutic management options for stage III non-small cell lung cancer.* World J Clin Oncol, 2017. **8**(1): p. 1-20.
62. Antonia, S.J., et al., *Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC.* N Engl J Med, 2018. **379**(24): p. 2342-2350.
63. Spigel, D.R., et al., *Five-Year Survival Outcomes From the PACIFIC Trial: Durvalumab After Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer.* J Clin Oncol, 2022. **40**(12): p. 1301-1311.
64. Lu, S., et al., *Osimertinib after Chemoradiotherapy in Stage III EGFR-Mutated NSCLC.* N Engl J Med, 2024. **391**(7): p. 585-597.
65. Soria, J.C., et al., *Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer.* N Engl J Med, 2018. **378**(2): p. 113-125.
66. Peters, S., et al., *Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer.* N Engl J Med, 2017. **377**(9): p. 829-838.
67. Skoulidis, F., et al., *Sotorasib for Lung Cancers with KRAS p.G12C Mutation.* N Engl J Med, 2021.
68. Reck, M., et al., *Five-Year Outcomes With Pembrolizumab Versus Chemotherapy for Metastatic Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score ≥ 50.* J Clin Oncol, 2021. **39**(21): p. 2339-2349.
69. Reck, M., et al., *Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer.* N Engl J Med, 2016. **375**(19): p. 1823-1833.
70. Herbst, R.S., et al., *Atezolizumab for First-Line Treatment of PD-L1-Selected Patients with NSCLC.* N Engl J Med, 2020. **383**(14): p. 1328-1339.
71. Rittmeyer, A., et al., *Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial.* Lancet, 2017. **389**(10066): p. 255-265.

72. Sezer, A., et al., *Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial.* Lancet, 2021. **397**(10274): p. 592-604.
73. Borghaei, H., et al., *Pembrolizumab plus chemotherapy versus chemotherapy alone in patients with advanced non-small cell lung cancer without tumor PD-L1 expression: A pooled analysis of 3 randomized controlled trials.* Cancer, 2020. **126**(22): p. 4867-4877.
74. Frederickson, A.M., et al., *Pembrolizumab plus chemotherapy for first-line treatment of metastatic nonsquamous non-small-cell lung cancer: a network meta-analysis.* Immunotherapy, 2019. **11**(5): p. 407-428.
75. Gandhi, L., et al., *Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer.* N Engl J Med, 2018. **378**(22): p. 2078-2092.
76. Garassino, M.C., et al., *973MO KEYNOTE-189 5-year update: First-line pembrolizumab (pembro) + pemetrexed (pem) and platinum vs placebo (pbo) + pem and platinum for metastatic nonsquamous NSCLC.* Annals of Oncology, 2022. **33**: p. S992-S993.
77. Han, J., et al., *MEK inhibitors for the treatment of non-small cell lung cancer.* J Hematol Oncol, 2021. **14**(1): p. 1.
78. Fancelli, S., et al., *Chasing the Target: New Phenomena of Resistance to Novel Selective RET Inhibitors in Lung Cancer. Updated Evidence and Future Perspectives.* Cancers (Basel), 2021. **13**(5).
79. Kwack, W.G., S.Y. Shin, and S.H. Lee, *Primary Resistance to Immune Checkpoint Blockade in an STK11/TP53/KRAS-Mutant Lung Adenocarcinoma with High PD-L1 Expression.* Onco Targets Ther, 2020. **13**: p. 8901-8905.
80. Ricciuti, B., et al., *Genomic and Immunophenotypic Landscape of Acquired Resistance to PD-(L)1 Blockade in Non-Small-Cell Lung Cancer.* J Clin Oncol, 2024: p. Jco2300580.
81. Gainor, J.F., et al., *Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in ALK-Rearranged Lung Cancer.* Cancer Discov, 2016. **6**(10): p. 1118-1133.
82. Ricordel, C., et al., *Molecular mechanisms of acquired resistance to third-generation EGFR-TKIs in EGFR T790M-mutant lung cancer.* Ann Oncol, 2018. **29**(suppl_1): p. i28-i37.
83. Scagliotti, G.V., et al., *Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer.* J Clin Oncol, 2008. **26**(21): p. 3543-51.
84. Gainor, J.F., et al., *EGFR Mutations and ALK Rearrangements Are Associated with Low Response Rates to PD-1 Pathway Blockade in*

Non-Small Cell Lung Cancer: A Retrospective Analysis. Clin Cancer Res, 2016. **22**(18): p. 4585-93.
85. Doroshow, D.B., et al., *Programmed Death-Ligand 1 Tumor Proportion Score and Overall Survival From First-Line Pembrolizumab in Patients With Nonsquamous Versus Squamous NSCLC.* J Thorac Oncol, 2021.
86. Reck, M., et al., *Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non-Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater.* J Clin Oncol, 2019. **37**(7): p. 537-546.
87. Shah, M., et al., *Association Between Survival and Very High Versus High PD-L1 Expression in Patients Receiving Pembrolizumab as First-line Treatment for Advanced Non-Small Cell Lung Cancer.* Clin Lung Cancer, 2022. **23**(8): p. 731-736.
88. Wang, A., et al., *The prognostic value of PD-L1 expression for non-small cell lung cancer patients: a meta-analysis.* Eur J Surg Oncol, 2015. **41**(4): p. 450-6.
89. Zhang, B., et al., *Predictive effect of PD-L1 expression for immune checkpoint inhibitor (PD-1/PD-L1 inhibitors) treatment for non-small cell lung cancer: A meta-analysis.* Int Immunopharmacol, 2020. **80**: p. 106214.
90. Jardim, D.L., et al., *The Challenges of Tumor Mutational Burden as an Immunotherapy Biomarker.* Cancer Cell, 2021. **39**(2): p. 154-173.
91. Ricciuti, B., et al., *Association of High Tumor Mutation Burden in Non-Small Cell Lung Cancers With Increased Immune Infiltration and Improved Clinical Outcomes of PD-L1 Blockade Across PD-L1 Expression Levels.* JAMA Oncol, 2022. **8**(8): p. 1160-1168.
92. Shao, M.M., et al., *Tumor mutational burden as a predictive biomarker for non-small cell lung cancer treated with immune checkpoint inhibitors of PD-1/PD-L1.* Clin Transl Oncol, 2024.
93. Zheng, M., *Tumor mutation burden for predicting immune checkpoint blockade response: the more, the better.* J Immunother Cancer, 2022. **10**(1).
94. Presti, D., et al., *Tumor infiltrating lymphocytes (TILs) as a predictive biomarker of response to checkpoint blockers in solid tumors: A systematic review.* Crit Rev Oncol Hematol, 2022. **177**: p. 103773.
95. Addeo, A., G.L. Banna, and A. Friedlaender, *KRAS G12C Mutations in NSCLC: From Target to Resistance.* Cancers (Basel), 2021. **13**(11).
96. Ferrer, I., et al., *KRAS-Mutant non-small cell lung cancer: From biology to therapy.* Lung Cancer, 2018. **124**: p. 53-64.
97. *Comprehensive molecular profiling of lung adenocarcinoma.* Nature, 2014. **511**(7511): p. 543-50.

98. Arbour, K.C., et al., *Effects of Co-occurring Genomic Alterations on Outcomes in Patients with KRAS-Mutant Non-Small Cell Lung Cancer.* Clin Cancer Res, 2018. **24**(2): p. 334-340.
99. Lignitto, L., et al., *Nrf2 Activation Promotes Lung Cancer Metastasis by Inhibiting the Degradation of Bach1.* Cell, 2019. **178**(2): p. 316-329.e18.
100. Romero, R., et al., *Keap1 loss promotes Kras-driven lung cancer and results in dependence on glutaminolysis.* Nat Med, 2017. **23**(11): p. 1362-1368.
101. Skoulidis, F., et al., *Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities.* Cancer Discov, 2015. **5**(8): p. 860-77.
102. West, H.J., et al., *Clinical efficacy of atezolizumab plus bevacizumab and chemotherapy in KRAS-mutated non-small cell lung cancer with STK11, KEAP1, or TP53 comutations: subgroup results from the phase III IMpower150 trial.* J Immunother Cancer, 2022. **10**(2).
103. de Langen, A.J., et al., *Sotorasib versus docetaxel for previously treated non-small-cell lung cancer with KRAS(G12C) mutation: a randomised, open-label, phase 3 trial.* Lancet, 2023. **401**(10378): p. 733-746.
104. Barlesi, F., et al., *Adagrasib versus docetaxel in KRAS(G12C)-mutated non-small-cell lung cancer (KRYSTAL-12): a randomised, open-label, phase 3 trial.* Lancet, 2025. **406**(10503): p. 615-626.
105. Isermann, T., et al., *KRAS inhibitors: resistance drivers and combinatorial strategies.* Trends Cancer, 2025. **11**(2): p. 91-116.
106. Mathiot, L., et al., *Prognostic Impact of TP53 Mutations in Metastatic Nonsquamous Non-small-cell Lung Cancer.* Clin Lung Cancer, 2024. **25**(3): p. 244-253.e2.
107. *The Swedish Lung Cancer Registry Report 2023*. https://The Swedish Lung Cancer Registry Report 2023.se/utvecklingsarbeteutbildning/statistikrapporter/rapporter/rapporter/nationellkvalitetsrapportlungcancerfor2023.10316.html.
108. Waterston, R.H., et al., *Initial sequencing and comparative analysis of the mouse genome.* Nature, 2002. **420**(6915): p. 520-62.
109. Bombil, F., et al., *A promising model of primary human immunization in human-scid mouse.* Immunobiology, 1996. **195**(3): p. 360-75.
110. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.* Nat Protoc, 2009. **4**(1): p. 44-57.
111. Sherman, B.T., et al., *DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update).* Nucleic Acids Res, 2022. **50**(W1): p. W216-w221.

112. Jordan, E.J., et al., *Prospective Comprehensive Molecular Characterization of Lung Adenocarcinomas for Efficient Patient Matching to Approved and Emerging Therapies.* Cancer Discov, 2017. **7**(6): p. 596-609.
113. Sanikini, H., et al., *Body mass index and lung cancer risk: a pooled analysis based on nested case-control studies from four cohort studies.* BMC cancer, 2018. **18**(1): p. 220-220.
114. D'Angelo, S.P., et al., *Distinct clinical course of EGFR-mutant resected lung cancers: results of testing of 1118 surgical specimens and effects of adjuvant gefitinib and erlotinib.* J Thorac Oncol, 2012. **7**(12): p. 1815-1822.
115. Dalvi, T., et al., *Biomarker expression and survival in patients with non-small cell lung cancer receiving adjuvant chemotherapy in Denmark.* PLoS One, 2023. **18**(4): p. e0284037.
116. Gallina, F.T., et al., *KRAS G12C mutation and risk of disease recurrence in stage I surgically resected lung adenocarcinoma.* Lung Cancer, 2023. **181**: p. 107254.
117. Garinet, S., et al., *Updated Prognostic Factors in Localized NSCLC.* Cancers (Basel), 2022. **14**(6).
118. Wahl, S.G.F., et al., *The Prognostic Effect of KRAS Mutations in Non-Small Cell Lung Carcinoma Revisited: A Norwegian Multicentre Study.* Cancers (Basel), 2021. **13**(17).
119. Barsouk, A., et al., *Plunging Into the PACIFIC: Outcomes of Patients With Unresectable KRAS-Mutated Non-Small Cell Lung Cancer Following Definitive Chemoradiation and Durvalumab Consolidation.* Clin Lung Cancer, 2024. **25**(3): p. e161-e171.
120. Hellyer, J.A., et al., *Clinical Implications of KEAP1-NFE2L2 Mutations in NSCLC.* J Thorac Oncol, 2021. **16**(3): p. 395-403.
121. Papillon-Cavanagh, S., et al., *STK11 and KEAP1 mutations as prognostic biomarkers in an observational real-world lung adenocarcinoma cohort.* ESMO Open, 2020. **5**(2).
122. Brown, L.C., et al., *LRP1B mutations are associated with favorable outcomes to immune checkpoint inhibitors across multiple cancer types.* J Immunother Cancer, 2021. **9**(3).
123. Lan, S., et al., *Somatic mutation of LRP1B is associated with tumor mutational burden in patients with lung cancer.* Lung Cancer, 2019. **132**: p. 154-156.
124. Wang, J., et al., *Antithetical impacts of deleterious LRP1B mutations in non-squamous and squamous NSCLCs on predicting benefits from immune checkpoint inhibitor alone or with chemotherapy over chemotherapy alone: retrospective analyses of the POPLAR/OAK and CHOICE-01 trials.* Sci China Life Sci, 2025. **68**(1): p. 249-262.

125. Bhat, S.S., R. Ali, and F.A. Khanday, *Syntrophins entangled in cytoskeletal meshwork: Helping to hold it all together.* Cell Prolif, 2019. **52**(2): p. e12562.
126. Chu, P.Y., et al., *EGF-induced Grb7 recruits and promotes Ras activity essential for the tumorigenicity of Sk-Br3 breast cancer cells.* J Biol Chem, 2010. **285**(38): p. 29279-85.
127. Han, D.C., et al., *EphB1 associates with Grb7 and regulates cell migration.* J Biol Chem, 2002. **277**(47): p. 45655-61.
128. Palmieri, D., et al., *Ran Binding Protein 9 (RanBP9) is a novel mediator of cellular DNA damage response in lung cancer cells.* Oncotarget, 2016. **7**(14): p. 18371-83.
129. Wang, R., et al., *Prognostic Implications of LRP1B and Its Relationship with the Tumor-Infiltrating Immune Cells in Gastric Cancer.* Cancers (Basel), 2023. **15**(24).
130. Xu, F., et al., *Prognostic biomarkers correlated with immune infiltration in non-small cell lung cancer.* FEBS Open Bio, 2023. **13**(1): p. 72-88.