Oncogenic variants guiding treatment in thoracic malignancies

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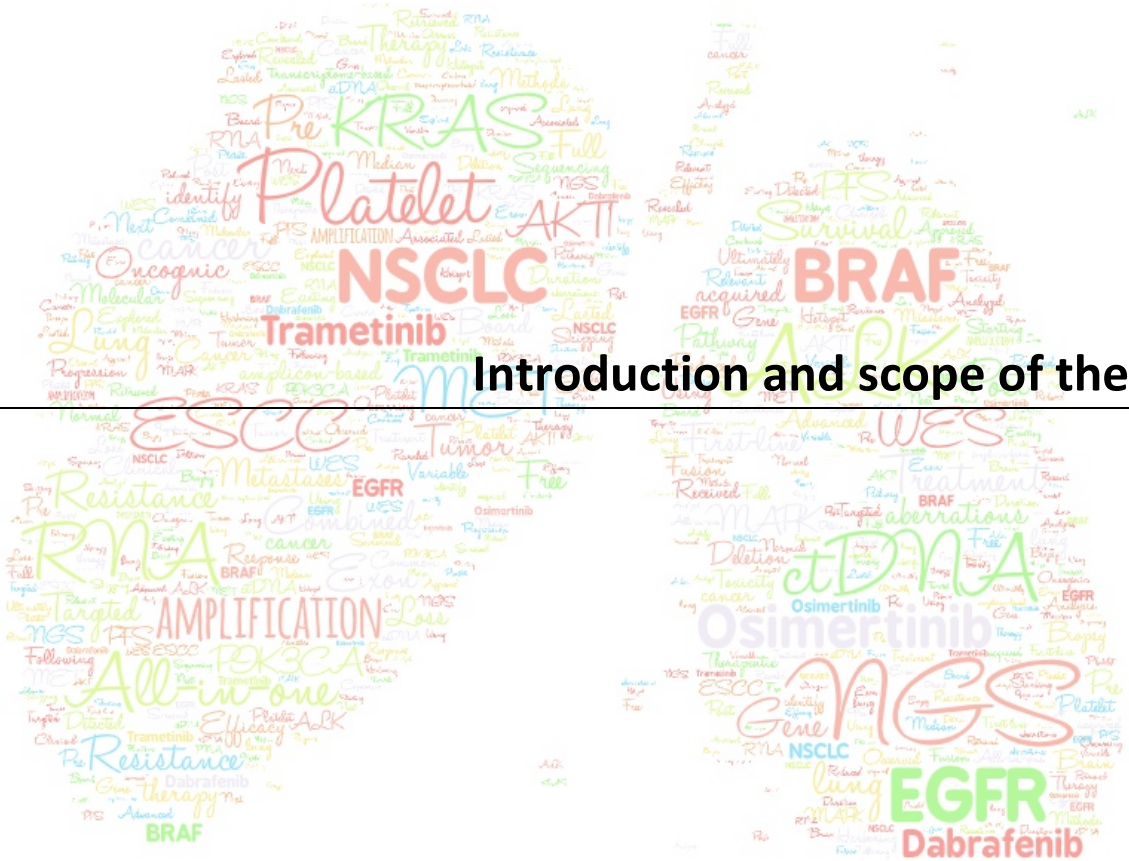
CHAPTER 1



Introduction and scope of thesis

1. Non-small cell lung cancer (NSCLC)

1.1. Epidemiology

Lung cancer ranked first for incidence rate (11.6% of the 18.1 million total cancer cases) and mortality (18.4% of the 9.6 million total cancer deaths) among 36 cancer types in 185 countries irrespective of sex in 2018 [1]. In the United States, the incidence of lung and bronchus cancer was estimated to be the second most common type and ranked first in cancer associated deaths in 2020 with 220,820 new cases and 135,720 deaths [2]. Based on major clinical differences in presentation, metastatic spread, and response to therapy, lung cancer is histologically divided into small cell lung cancer (SCLC) and NSCLC. NSCLC represents about 85% of all lung cancer cases [3]. The common subtypes of NSCLC are adenocarcinoma (approx. 60% of all lung cancer), squamous cell carcinoma (approx. 20% of all lung cancer) and large cell carcinoma (approx. 3% of all lung cancer) [4,5]. Incidence and mortality of lung cancer, especially of SCLC and squamous cell carcinoma, and to a lesser extent adenocarcinoma are highly correlated with cigarette smoking [6,7]. The most common lung cancer subtype in never smokers is adenocarcinoma [8]. The incidence of lung cancer declined following the initiation of comprehensive tobacco control programs in the US, UK and some other countries [9]. The tobacco control program remains at an earlier stage in China and the significance of tobacco smoking control in recent decades have not yet been recognized [10].

1.2 Diagnosis and prognosis overview

The majority of lung cancer patients (approx. 75%) present with symptoms at an advanced stage [11] and are diagnosed with unresectable disease in which systemic treatment interventions are largely palliative and with poor prognosis [11,12]. For early-stage patients, up to 60% eventually die of their disease despite curative resection due to recurrence [13-15]. The majority of the recurrences are distant metastasis or combined local and distant metastasis [13,16]. Lung cancer screening using sensitive screening modalities such as low-dose CT scanning has been recommended to allow diagnosis at an early stage [12,17,18]. Initial diagnosis of lung cancer remains to be based on histological and later also immunohistochemical features. The development of next generation sequencing (NGS) and other high-throughput analyses has enabled routine genetic testing and improved selection of patients who will benefit from specific targeted treatment regimens [19]. These developments led to a substantial improvement in survival. In contrast, limited improvement of survival has been achieved for SCLC patients due to limited targeted treatment regimens [20]. The most recent 5-year survival rate published for NSCLC is 24%, compared to 6% for SCLC (*Lung Cancer - Non-Small Cell: Statistics from American Society of Clinical Oncology approved in 2020*).

1.3 Molecular characteristics and targeted treatment options

Molecular characteristics of NSCLC include mutations in various driver genes (Table 1.1). Tumors with *EGFR, PTEN, ALK, ROS1,* and *RET* alterations are found more commonly in never- or light-smokers, whereas *KRAS* and *BRAF* alterations are more common in smokers [21-24]. Squamous cell carcinomas predominantly have genomic aberrations in tumor suppressor genes. A number of molecular alterations have shown to be effective targets for anticancer therapies in different malignancies [25]. In combination with the implementation

of NGS in clinical oncology, this has enabled stratification of patients for targeted therapy [26,27].

In the past years, treatment decision for metastatic NSCLC patients has changed from general cytotoxic therapy to personalized medicine. Personalized therapy has become increasingly important to improve outcome of NSCLC patients. This makes molecular subtyping of NSCLC patients at diagnosis crucial for selecting the most optimal targeted therapy. Currently, standard therapies are available for *EGFR*, *BRAF*, *ALK, ROS1, MET, NTRK* and *RET*. For part of the aberrations, e.g. *PTEN*, *FGFR1*, *AKT1*, no targeted therapies are available. For others, e.g. *KRAS, ERBB2, PIK3CA, NRAS, NRG1* and *MEK* targeted drugs are under development. NSCLC patients without targetable driver mutations can be treated with immune checkpoint inhibitors (ICI) for cases with expression of programmed death ligand-1 (PD-L1) and for those with low PDL-1 expression ICI is combined with chemotherapy [28] (https://www.esmo.org/Guidelines/Lung-and-Chest-Tumours/Metastatic-Non-Small-Cell-Lung-Cancer). After pathological examination and histological subtyping, current guidelines recommend predictive biomarker testing for all patients with advanced, possible, probable or definite adenocarcinoma [29-32]. Molecular testing is recommended in squamous cell carcinoma only when the patient is a never-, long-time ex- or light-smoker (<15 pack-years) [28]. NGS on circulating tumor DNA (ctDNA) is gradually being implemented for testing of adenocarcinomas to identify targetable oncogenes [31,33,34]. Whether fusion genes identified by NGS require further validation by immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) remains an open question [28]. In practice, having one test for all genomic aberrations would be desirable considering limited tissue availability, shorter turnaround times and costs. A brief guideline for molecular testing for advanced NSCLC at initial diagnosis is presented in Figure 1.1.

Table 1.1. Frequencies of gene alterations and targeted agents for lung cancer [35]

Genes	Frequency		Genomic alterations / fusion partners	Approved drugs and selected agents in development#
	Adenocarcinoma	Squamous-cell carcinoma		
EGFR (epidermal growth factor receptor)	10–40%	0%	L858R, E19 DEL/INS, E709A, G719X, S768I, L861Q, Exon 20 insertions	Gefitinib, erlotinib, afatinib, osimertinib, dacomitinib erlotinib-bevacizumab, erlotinib-ramucirumab (EMA and FDA-approved), icotinib (approved in china)
KRAS (kirsten rat sarcoma viral oncogene homolog)	15–33%	0-3%	codon 12, 13 and 61 mutations	AMG 510# (NCT03600883), MRTX849# (NCT03785249)
MAP2K1/2 or MEK1/2 (mitogen-activated protein kinase kinase 1/2)	<1%	<1%	MEK1: codon 56, 57, 67 and 130 mutations; MEK2: codon 60 and 134 mutations	Trametinib (FDA-approved), binimetinib (approved for melanoma, NCT03915951), cobimetinib (approved for melanoma, NCT02457793)
ALK (fusion) (anaplastic lymphoma kinase)	3–13%	NA	EML4, KIF5B, KLC1, TFG, TPC, DCTN1, SQSTM1, TPR, STRN, HIP1, CLTC, NPM1, BCL11A, BIRC6	Crizotinib, ceritinib, alectinib, brigatinib, lorlatinib (EMA and FDA-approved)
AKT1 (AKT serine/threonine kinase 1)	1%	1%	E17K	MK2206# (NCT01294306), AZD5363# (NCT03310541)
FGFR1 (amp) (fibroblast growth factor receptor 1)	1%	20%	NA	Rogaratinib# (NCT01976741), pemigatinib# (NCT02393248), erdafitinib# (NCT02699606), AZD4547# (NCT02154490), Fulvestrant# (NCT00932152)
RET (rearranged during transfection proto-oncogene)	1–2%	NA	KIF5B, CCDC6, NCOA, TRIM33, CUX, KIAA1468 and others	Selpercatinib, Pralsetinib (FDA-approved), RXDX-105# (NCT03784378)

Genes	Frequency		Genomic alterations / fusion partners	Approved drugs and selected agents in development[#]
	Adenocarcinoma	Squamous-cell carcinoma		
MET (MET proto-oncogene)				
Amplification (de novo)	1–4%	0%	NA	Crizotinib[#] (NCT02499614), capmatinib[#] (NCT02414139), cabozantinib[#] (NCT02132598)
Amplification (EGFR TKI-resistance induced)	10–20%	0%	NA	capmatinib[#] (NCT01610336, NCT02468661, NCT01911507), Tepotinib[#] (NCT01982955)
Exon 14 skipping	3–4%	0%	NA	capmatinib[#] (FDA-approved), Tepotinib[#] (NCT01982955), MGCD265[#] (NCT02544633), crizotinib[#] (NCT04084717)
PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha)				Buparlisib[#] (NCT01297491)
Mutation	4–7%	16%	codon 542, 545, 1047 mutations	alpelisib[#] (NCT02276027)
Amplification	2–9%	30–40%	NA	AZD8186[#] (NCT01884285), AZD2014[#] (NCT02664935)
BRAF (B-Raf proto-oncogene)	2–4%	0%	V600	Dabrafenib+trametinib (EMA and FDA-approved), Encorafenib + Binimetinib[#] (NCT03915951), Vemurafenib[#] (NCT02304809)
NRAS (NRAS proto-oncogene)	<1%	<1%	codon 12, 13 and 61 mutations	NA
ROS1 (c-ros oncogene 1)	1-2%	0%	CD74, EZR, SLC34A2, TPM3, SDC4, LRIG3, FIG, CLTC, KDELR2, CCDC6, TPM3, SDC4	Crizotinib, entrectinib (EMA and FDA-approved), lorlatinib[#] (NCT01970865)

Genes	Frequency		Genomic alterations / fusion partners	Approved drugs and selected agents in development[#]
	Adenocarcinoma	Squamous-cell carcinoma		
NTRK1,2,3 (neurotrophic tyrosine receptor kinase)	0.1–3%	0%	TPM3, SQSTM1, TPR, IRF2BP2, MPRIP, ETV6, CD74	Larotrectinib, entrectinib (FDA-approved), PLX7486[#] (NCT01804530)
NRG1 (neuregulin 1)	0.2%	0.02%	CD74, SDC4, SLC3A2, TNC, MDK, ATP1B1, DIP2B, RBPMS, MRPL13, ROCK1, DPYSL2, PARP8	Afatinib[#] (NCT04410653), MCLA-128[#] (NCT03321981)
ERBB2 or HER2 (erb-b2 receptor tyrosine kinase 2)				Afatinib[#] (NCT02369484, NCT02597946), Trastuzumab[#] (NCT03845270)
Mutation	2–4%	0%	E20 INS	Pyrotinib[#] (NCT02535507)
Amplification	5–10%	0%	NA	AP32788[#] (NCT02716116)
DDR2 (discoidin domain receptor tyrosine kinase 2)	0%	4%	S768R	NA
PTEN (phosphatase and tensin homolog)	2%	8%	R233*	MK-2206[#] (NCT01306045)

FDA=US Food and Drug Administration. EMA=European Medicines Agency. # drugs under investigation in clinical trials.

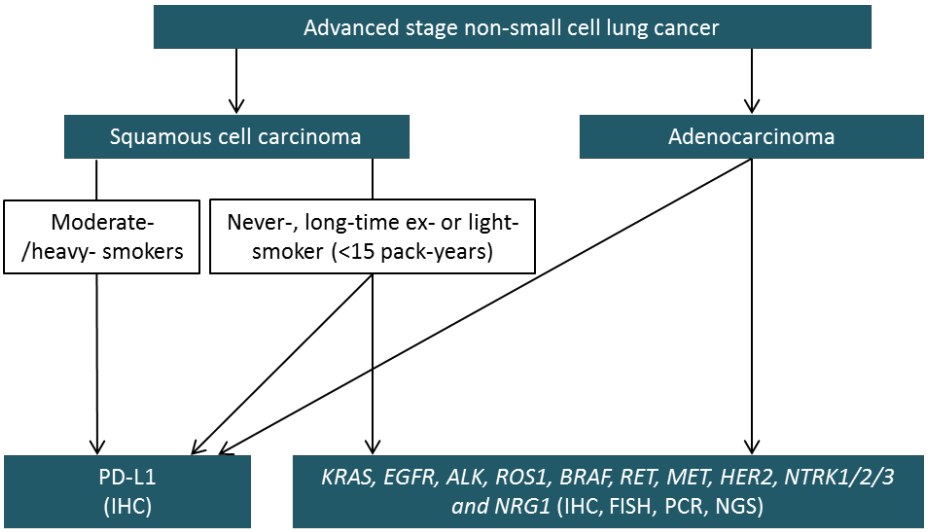


Figure 1.1. Brief overview of molecular testing in advanced NSCLC patients at initial diagnosis. IHC: immunohistochemistry; PCR: polymerase chain reaction; NGS: next generation sequencing; FISH: fluorescence in situ hybridization.

1.4 *EGFR*

Activating *EGFR* mutations occur in 10-20% of Caucasian and up to 60% of Asian patients with advanced NSCLC [36-39]. In-frame deletions of part of exon 19 and the L858R mutation in exon 21 are the most commonly observed aberrations. Together these two mutations account for up to 85% of the activating *EGFR* mutations [40]. Other less common activating *EGFR* mutations include in-frame deletions of part of exon 18, in-frame insertions in exon 19, exon 20 InDels, E709K, S768I, L861Q, multiple mutations leading to an amino acid change at G719, and other mutations within exons 18–25 of the *EGFR* gene [40,41]. These activating mutations were demonstrated to enhance the EGFR tyrosine kinase activity, resulting in constitutive receptor autophosphorylation [42,43]. EGFR tyrosine kinase inhibitors (TKIs) are small molecules that inhibit EGFR autophosphorylation and subsequent receptor activation and signal transduction [43]. Patients with activating *EGFR* mutations are sensitive to 1st generation TKIs such as erlotinib, gefitinib, and the 2nd generation TKIs such as afatinib, dacomitinib, and the 3rd generation TKI osimertinib with response rates up to 83% [44-50]. All these TKIs have been approved for first-line treatment of patients carrying activating EGFR mutations by the FDA [49]. Patients receiving first-line osimertinib have a median response time of 17.2 months versus 8.5 months for patients treated with 1st and 2nd generation TKIs [49]. Treatment with a combination of erlotinib-bevacizumab, and erlotinib-ramucirumab have been approved for first-line treatment as well, with response rates of 69% and 76% respectively [51,52]. Median PFS of treatments with this two-drug combination therapy were 16.0 months and 18.0 months respectively. Activating exon 20 insertions are found in NSCLC in approximately 1.5–2.5% but patients with these mutations did not respond to standard TKIs [53]. At the moment, several studies are performed on poziotinib, TAK-788, high dose osimertinib and afatinib-cetuximab.

1.5 *ALK*

ALK rearrangements leading to ALK fusion genes are present in 3-13% of NSCLC. ALK fusion genes are observed predominantly in adenocarcinoma in young female patients but also occur in males and at various ages. In general, ALK fusion genes are mutually exclusive with *EGFR* and *KRAS* mutations [54-56]. A wide variety of ALK fusion gene partners have been identified and all fusions result in a chimeric product containing the kinase domain of ALK. Echinoderm microtubule-associated protein-like 4 (EML4) is the most prevalent fusion partner. Other less common fusion partners include kinesin family member 5B (KIF5B) and others as shown in Table 1.1 [57]. Crizotinib was the first approved ALK inhibitor (ALKi) for first-line treatment of ALK fusion gene-positive NSCLC patients. In two phase III open-label trials, median progression-free survival (PFS) was significantly longer upon treatment with crizotinib than with chemotherapy (7.7 vs. 3.0 months and 10.9 vs. 7.0 months) [58,59]. Alectinib, ceritinib, brigatinib and lorlatinib have been developed and approved as 2nd and 3rd generation ALKi. Alectinib is now the preferred first-line treatment for ALK fusion gene positive patients because it penetrates the blood-brain barrier, results in a significantly longer PFS as compared to crizotinib and has a less severe toxicity profile [60,61]. Recently, brigatinib and lorlatinib have also been accepted as first-line treatment options for NSCLC with ALK rearrangements [62].

1.6 *BRAF*

BRAF p.(V600E) mutations occur in 1%-2% of NSCLC patients, and account for about half of the *BRAF* mutations in NSCLC [63,64]. This mutation leads to constitutive activation of the mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway [65] and results in activation of the downstream protein kinases MEK1/2 (Figure 1.2). *BRAF* p.(V600) positive NSCLC patients treated with the BRAF inhibitor vemurafenib had a response rate up to 45% [66,67]. Dabrafenib (BRAF inhibitor) treatment resulted in a response in 26/78 (33%) previously treated and 4/6 previously untreated patients [68]. Combining both dabrafenib and trametinib (MEK inhibitor) resulted in a response rate of more than 60% in both first-line and subsequent lines of treatment. This regimen has now been approved for the treatment of NSCLC patients with BRAF V600E mutations [69,70]. Combined treatment with encorafenib and binimitinib is still under investigation in clinical trials.

1.7 *KRAS*

KRAS is the most commonly mutated driver gene of NSCLC with mutations in nearly 30% of the cases [71]. Although trials are ongoing, there is no clinically approved drug yet for this patient group. Early clinical trials using inhibitors against either *KRAS* or upstream and downstream effectors of KRAS, such as ERBB2 and MEK are ongoing [72]. Sotorasib is a small molecule that targets *KRAS* p.(G12C) mutated tumors. A phase I trial demonstrated 32.2% of objective response and 88.1% of disease control to sotorasib in 59 patients with advanced NSCLC harboring the *KRAS* p.(G12C) mutation [73].

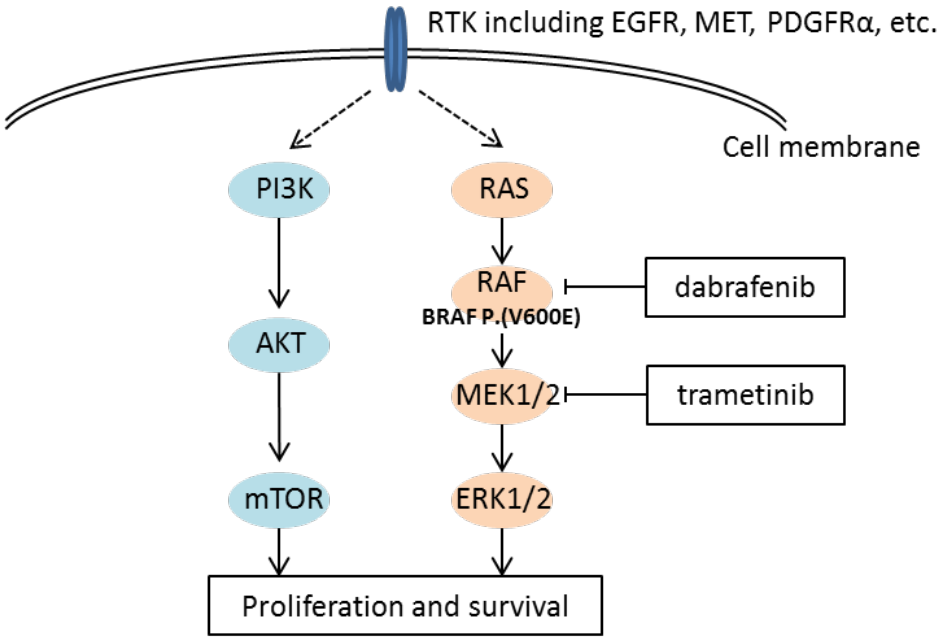


Figure 1.2. Schematic of PI3K and MAPK pathways. The drugs used for the combined targeting of BRAF and MEK as crucial components of the MAPK pathway are shown on the right site.

1.8 Resistance mechanism to targeted treatment

Drug resistance is a strikingly universal feature evolving upon treatment with kinase inhibitors targeting the activated driver proteins. Most malignancies, including lung cancer, evolve as subclones with distinct molecular variants. This is also known as intratumor heterogeneity [74] and contributes to non-responsiveness to treatment which is known as primary/intrinsic resistance. Besides the intrinsic resistance, acquired resistance emerges during the treatment after a good initial response. Resistance mechanisms can be caused by re-activation of the signaling pathway or by activation of a critical parallel signaling pathway(s) caused by alterations in other oncogenic drivers [75]. In this section, I will focus specifically on conventional resistance mechanisms induced by *EGFR* and *BRAF* targeted therapy. Resistance-associated abnormalities involve secondary aberrations preventing binding of the TKI to these genes known as on-target resistance mechanisms or off-target aberrations such as activation of the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) and RAS/RAF/MEK signaling pathways (Figure 1.2) [76].

Pre-existing or treatment-induced on-target *EGFR* variants, activation of bypass pathways, downstream pathway activation and histological transformation have been reported to be responsible for resistance against EGFR-TKI [77]. The *EGFR* p.(T790M) is the most commonly observed resistance-causing mutation to 1st and 2nd generation TKIs [78]. Osimertinib, a 3rd generation EGFR-TKI, has been approved for patients with acquired resistance to 1st or 2nd generation EGFR-TKI due to the occurrence of a T790M mutation [49]. Additional, less common resistance-associated mutations in EGFR include the D761Y, T854A, L747S, C797G/S, L798I, L718Q, L844 V, and L797S and others. Amplification of EGFR has also been demonstrated to be a resistance mechanism for the 3rd generation EGFR-TKI (osimertinib) [79]. In addition, receptor tyrosine kinase (RTK) fusions are reported as actionable resistance mechanisms to EGFR-TKIs [80,81]. Resistance mechanisms

involving activation of bypass or downstream pathways include BRAF mutations [82] and fusions [80,81], and amplifications of *MET* or *ERBB2* and small cell lung cancer transformations [75].

Resistance mechanism to BRAF inhibitor monotherapy have been studied extensively in melanoma. Currently known resistance mechanisms result from re-activation of MAPK, via *BRAF* copy number gains, *BRAF* splice variant, *NRAS* or *MEK1/2* mutations and over-expression of RTKs [83]. The proportion of MAPK re-activation due to combined dabrafenib/trametinib resistance is reported in up to 82% [84]. However, another study showed that resistance mechanisms in melanoma patients treated with mono and combination therapy are independent of MAPK re-activation and involve p21-activated kinases (PAKs) activation [85]. BRAF monotherapy and BRAF / MEK combination therapy induced resistance mechanisms in NSCLC are less well studied thus far. The limited number of reported resistance mechanisms mainly involve the MAPK pathway and PI3K pathways and include *KRAS* p.(G12D/V), or *NRAS* p.(Q61K), *MEK1* p.(K57N), *KRAS* p.(Q61R) and PTEN frameshift mutations [86-90]. These mutations have also been associated with resistance in melanoma.

2. Esophageal squamous cell carcinoma

The incidence of esophageal cancer ranks at the ninth position and it is the sixth most common cause of cancer mortality globally [91]. Histologically, esophageal cancer can be divided to adenocarcinoma and squamous cell carcinoma. Squamous cell carcinoma (ESCC) is the most common histological subtype worldwide, particularly in high-incidence areas of eastern Asia and in eastern and southern Africa [91]. Adenocarcinoma is more predominant in western countries [92]. The diagnosis of esophageal cancer is determined by endoscopy and biopsies for histopathological test. Although there are improvements in endoscopy for early detection, surgical resection, and palliative therapy including immunotherapy, radiotherapy and chemotherapy, the 5-year survival of patients with esophageal cancer is low, mainly due to presentation at middle-late stage of disease at diagnosis [93]. Moreover, recurrence rate of ESCC after esophagectomy was reported to occur in up to 52% of the patients [94]. To increase the survival rates of this patient group, it is of great importance to identify new biomarkers for early detection or as prognostic markers. A potential power approach that could be explored is the analysis of circulating tumor (ct)DNA in ESCC patients.

3. Blood based liquid biopsy

Liquid biopsies have gained much interest as a minimal invasive approach for early detection of cancer, assessing molecular subtype, monitoring therapeutic response and development of resistance and evaluating presence of residual disease after surgery [34]. Commonly used blood based liquid biopsy derived biomolecules, include ctDNA, circulating tumor cells (CTCs), tumor derived circulating extracellular vesicles (EVs), such as exosomes, tumor-educated platelets (TEPs). Potential value of these biomolecules as clinical biomarkers has been shown in different studies [95]. The advantages and disadvantages of liquid and tissue biopsies are summarized in Table 1.2.

Table 1.2. The pros and cons of liquid and tissue biopsies

Approach	Advantages	Disadvantages
Tissue biopsy	Golden standard, allows histological assessment	Invasive, does not capture tumor heterogeneity, not always feasible to obtain, not suitable for follow-up, tumor content might be too low for molecular analysis, artifactual C:G>T:A variants in FFPE DNA
Liquid biopsy	Less invasive, might capture tumor heterogeneity, suitable for monitoring of treatment response and development of resistance	Lack of standard protocols, low tumor content which might lead to high false-negative rates

In healthy individuals, circulating cfDNA is derived from dying normal cells, elevated levels are observed upon exercise or in subjects with fever. In cancer patients the cfDNA also contains variable amounts of ctDNA originating from tumor cells. The main approaches to identify mutations in cfDNA are ddPCR and targeted NGS. The ddPCR was shown to be highly sensitive and allowed detection of mutations with variant allele frequency as low as 0.1% [96]. Targeted NGS of ctDNA allows reliable detection of mutations occurring at a minimal frequency of 0.2% or as shown by some studies even at lower frequencies [97-99]. The use of cfDNA to identify cancer driver or resistance associated mutations has been approved as a medical diagnostic for lung cancer and colorectal cancer by the FDA in 2016 [100]. However, clinical applications for other tumor types still awaits further studies and standardized protocols are required before it can be implemented in a clinical setting.

Exosomes (30-100nm) and microvesicles (100–1000 nm) are both extracellular vesicles containing proteins, nucleic acids and lipids, but differ in size [101]. Tumor cells can release EVs for intercellular communication at local and more distant locations [102]. Platelets capture EV-derived RNAs [103-105] and RNA-seq analysis has indicated that these platelets carry a tumor specific RNA profile [106-108]. This can in theory help to determine the type of cancers even in the absence of representative tissue biopsies. However, the use of these specific signatures has not been implemented in a clinical setting. Analysis of TEPs in ALK-positive lung cancer patients has shown presence of EML4–ALK fusion transcripts [104]. Monitoring of the number of EML4–ALK transcripts in subsequently collected platelet samples may thus be used to monitor effectiveness of ALK inhibitors in NSCLC patients. Moreover, in a single case, the EML4-ALK fusion gene transcript has been detected in platelets two months prior to radiographic disease progression [104]. This suggests that platelet-derived RNA might be used as a potential biomarker to monitor progression. The value of cfDNA, TEPs and/or EVs and their limitations are summarized in Table 1.3.

CTCs are a subset of tumor cells found in the blood of patients as either single migratory cell or multicellular clusters. The CellSearch® CTCs capture system is now an FDA-cleared platform to measure progression of metastatic prostate, breast and colon cancers [109]. Different techniques, such as CellSearch, ISET, Adna test, have been used for isolation CTCs and enumeration in early and advanced NSCLC cohorts to explore the potential of CTCs as early diagnosis or prognosis biomarker [110,111]. However, the results obtained thus far are discrepant and need further validation studies before they can be implemented in a clinical setting.

Table 1.3 Comparison of cell free DNA and tumor educated platelets/ Extra cellular vesicles

Source	Analysis capability	Limitations
Cell free DNA	Mutations, amplifications, methylation patterns	Need of specialized blood tubes to prevent lysis of white blood cells, not all patients contain sufficient amounts of cfDNA, high amount of plasma required for MRD detection.
Tumor-educated platelets	Mutations, fusion gene transcripts, RNA splice variants	Contamination by peripheral blood mononuclear cells, more complex to isolate
Extra cellular vesicles		Contamination by peripheral blood mononuclear cells, more complex to isolate with limited amount

4. NGS and droplet digital PCR (ddPCR)

DNA-based NGS is a high throughput sequencing approach that allows for parallel analysis of a limited number of samples for a specific gene panel (targeted sequencing), the entire genome (whole genome sequencing, WGS) or its coding part (whole exome sequencing, WES) within a few days. Without question, NGS has greatly contributed to the characterization of the oncogenome and has resulted in identification of novel targets for cancer therapy. With the development of tailored therapies for patients with specific gene variants, the FDA has finalized a guide for monitoring the development, safety, and efficaciousness of NGS-testing in clinical use [112].

In multiple cancer subtypes amplifications of specific oncogenes have been demonstrated to act as drivers of tumorigenesis. Moreover, amplifications have been implemented as biomarkers to stratify patients sensitive to specific inhibitors, such as *EGFR* amplifications in gliomas [113] or *ERBB2* amplifications in mamma carcinoma [114]. As both mutations and amplifications were demonstrated to be critical biomarkers to select the most optimal targeted treatment compounds, NGS-based approaches are likely to present the most robust analytic approaches to analyze a comprehensive set of aberrations even with limited tissue specimens.

DdPCR is a preferred approach for the detection of low-abundance variants in a predominant background of wild type targets or for detection of variants in samples with limited DNA input such as cfDNA. It provides an ultrasensitive and absolute quantification method and is relatively cheap to perform [115]. Most ddPCR applications in the field of cancer diagnostics are focused on liquid biopsies. It has become an important diagnostic test for the examination of genetic alterations including single nucleotide variant (SNVs), insertion/deletion variants (indels), and gene rearrangements in different kinds of clinical samples [115,116].

5. Scope of the thesis

Cancers are genetically heterogeneous in nature. This heterogeneity contributes to tumor evolution and results in subclonal dynamics. The aim of this thesis was to explore the value of tumor cell specific biomarkers and to investigate intrinsic and acquired resistance mechanisms.

In chapter 2, we explored the possibility of using our customized all-in-one transcriptome-based assay to identify therapy-guiding genomic aberrations (both mutations and fusion genes) in NSCLC patients. In chapters 3 and 7, we focused on the use of blood based liquid biopsies as biomarkers to identify tumor specific variants to guide treatment and as a tool to monitor treatment response over time. In chapter 3, we tested the feasibility of using platelet derived RNA to monitor mutations using our customized NGS panel (the same panel as used in chapter 2) and ddPCR. In chapter 7, we tested the presence of ctDNA before and after surgery in ESCC patients. In chapter 4, we analyzed targeted NGS data from molecular diagnostic tests of pre- dabrafenib/trametinib treatment samples of BRAF p.(V600E) NSCLC patients with a short and long response duration and a limited number of paired pre- and post-treatment samples. Our aim was to investigate whether mutations occurring concurrent with *BRAF* p.(V600E) correlate with PFS time and to identify acquired resistance mechanisms. In chapter 5, we present two *EGFR* mutant NSCLC patients with osimertinib induced *BRAF* p.(V600E) mutations as a resistance mechanism. We evaluated tumor response to combined EGFR-TKI and BRAF/MEK inhibitor therapy. In chapter 6, we re-analyzed targeted sequencing results from routine molecular diagnostic tests and developed an approach using these NGS data to estimate *EGFR* copy number gains. Moreover, we investigated the clinical value of the *EGFR* copy number gain determined by the targeted NGS. A summary of our main findings and future perspectives are presented in chapter 8.

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