Cell-free DNA in Non-Small Cell Lung Cancer

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Tiivistelmä – Referat – Abstract

Lung cancer caused the most cancer related deaths world-wide in 2018 and despite extensive research the prognosis of a lung cancer patient remains generally poor. Lung cancer is divided into different histological subtypes the two main types being non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Currently lung cancer is diagnosed with radiological imaging and tissue biopsies. Generally, curative treatment can be achieved only by surgical treatment of early-stage NSCLC. Only 20–25% of NSCLC are eligible for curative intent surgery. Furthermore, 30–55% of these patients have a fatal recurrence of lung cancer.

Cell-free DNA (cfDNA) has gained interest in the field of oncology. Generally, cfDNA refers to all the DNA in the body that is free from cellular confinement. Circulating tumor DNA (ctDNA) is cfDNA that originates from cancer cells. It has potential to be a minimally invasive method used in various parts of cancer management including early detection, diagnosis, treatment, monitoring the response for treatment and identification of drug resistance.

While the use of cfDNA still lacks clinical trials to be widely used in a clinical setting, it is highly possible that cfDNA analysis establishes a central role in the future in the oncological field.

Avainsanat – Nyckelord – Keywords

Non-small cell lung cancer, NSCLC, Cell-free DNA, cfDNA, Circulating tumor DNA, ctDNA

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Tiivistelmä – Referat – Abstract

Keuhkosyöpä aiheutti vuonna 2018 eniten syöpäkuolemia. Laaja-alaisesta tutkimuksesta huolimatta keuhkosyöpäpotilaiden ennuste on edelleen keskimäärin huono. Keuhkosyöpä jaetaan erilaisiin histologisiin alatyyppeihin. Kaksi pääalatyyppiä ovat ei-pienisoluinen keuhkosyöpä ja pienisoluinen keuhkosyöpä. Nykyään keuhkosyöpädiagnostiikan kulmakivinä ovat kuvantamistutkimukset ja kudosnäytteen ottaminen. Parantavaan eli kuratiiviseen hoitotulokseen päästään useimmiten vain kirurgisella hoitolinjalla paikallisessa varhaisen vaiheen ei-pienisoluisessa keuhkosyövässä. Vain 20–25% ei-pienisoluisista keuhkosyövistä soveltuvat kuratiiviseen kirurgiseen hoitoon. Lisäksi 30–55%:lla kuratiivisen kirurgisen hoidon saaneista potilaista syöpä uusiutuu.

Soluvapaa DNA (cfDNA) on solun ulkopuolista DNA:ta, jota vapautuu kaikista kehon soluista ruumiin nesteisiin, kuten verenkiertoon. Kun cfDNA on peräisin syöpäsoluista, kutsutaan sitä kiertäväksi kasvain-DNA:ksi (ctDNA). Viime vuosina ctDNA:n käyttö syöpämarkkerina on herättänyt kiinnostusta, koska sitä voisi käyttää keuhkosyöpäpotilaan hoitoketjun eri vaiheissa, kuten tuumorin aikaisemmassa havaitsemisessa, diagnostiikassa, yksilöllisen hoitovalinnan tukena, hoitovasteen seurannassa ja syövän uusiutumisen toteamisessa. Soluvapaa DNA -näytteitä voidaan ottaa nestebiopsioiden muodossa esimerkiksi verestä tai pleuranesteestä. Yksi nestebiopsian eduista on se, että se on nykyään usein käytettyyn radiologisesti ohjattuun kudosbiopsiaan verrattuna vähemmän kajoavampi ja mahdollistaa taudin seurannan. Nestebiopsia voisi tulevaisuudessa ainakin osittain korvata kudosnäytteen ottamisen. Vaikka cfDNA:n käyttö kliinisessä työssä vaatii vielä lisää tutkimuksia, on hyvin mahdollista, että cfDNA:sta tulee tulevaisuudessa keskeinen osa onkologisia hoitoja.

Avainsanat – Nyckelord – Keywords

Ei-pienisoluinen keuhkosyöpä, Soluvapaa DNA, cfDNA, Kiertävä kasvain-DNA, ctDNA

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1 Introduction

Cancer is one of the leading causes of death worldwide and lung cancer is one of the most frequent malignancies with over 2 million new cases in 2018. In addition, of all the cancers lung cancer causes the most deaths by a large margin: in 2018 lung cancer caused almost 1,8 million deaths while colorectal cancer, which causes the second highest amount of cancer related deaths, caused around 862 000 deaths.[1] In Finland, around 2700 patients were diagnosed with lung cancer in 2018.[2] Lung cancer is divided into different histological types. The two main types are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts over 80 % of lung cancers.[3] Unfortunately, lung cancer is frequently diagnosed when the cancer is already advanced or locally advanced and only 20-25 % of NSCLC patients are eligible for curative surgery.[4,5] Furthermore, 30-55% of NSCLC patients treated with a curative intent surgery have a fatal recurrence of lung cancer.[6] This would suggest that better means of identifying a minimal residual disease (MRD) would be highly beneficial in the curative treatment of NSCLC.

Cell-free DNA (cfDNA) has been gaining interest as a marker in the field of oncology. As the name suggests, cfDNA refers to all the DNA in the body that is free from cellular confinement.[7] When the cfDNA originates from cancer cells, it is called circulating tumor DNA (ctDNA).[8] It is a novel technique in the oncologic field and requires more research to be widely used in a clinical setting, but it has the potential to be very useful in various parts of cancer management. This could include for example early detection, diagnosis, treatment, monitoring the response for treatment and identification of drug resistance.[9]

This thesis focuses on NSCLC, its surgical treatment and the current and potential future use of cfDNA in the management of NSCLC. Moreover, it will introduce a new upcoming study “Circulating tumor DNA analyses in surgically treated NSCLC” which will be conducted by a research groups led by professor Marjukka Myllärniemi and docent Ilkka Ilonen.

2 Non-Small Cell Lung Cancer

2.1 Epidemiology in Finland

According to the Finnish Cancer Registry lung and trachea cancer is the third most commonly diagnosed cancer in both women and men in Finland.[2] In 2018 the incidence of lung and trachea cancer for women and men was 1035 and 1710, respectively. In addition, it causes the most cancer related deaths among men and the second most deaths among women. For men, both incidence and mortality has been decreasing since the 1970s while for women both have been increasing steadily. Still, the incidence for men is almost double compared to women. Figure 1 presents the incidence and figure 2 the mortality for the most frequently diagnosed cancers in Finland in 2018 for both men and women according to the Finnish Cancer Registry.

Figure 1. Diagnosed cancer cases in 2018 for A) women and B) men.[2]

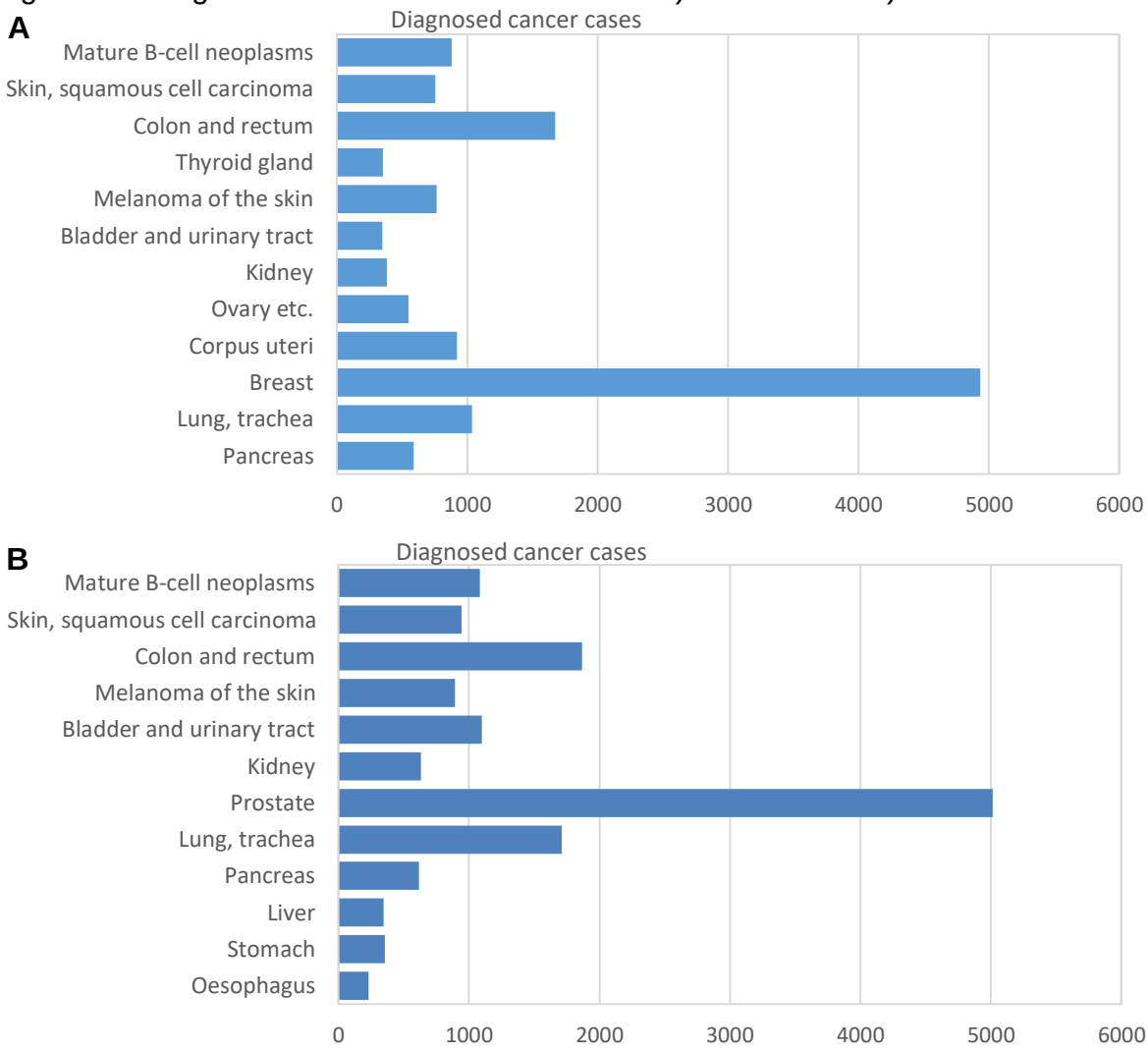
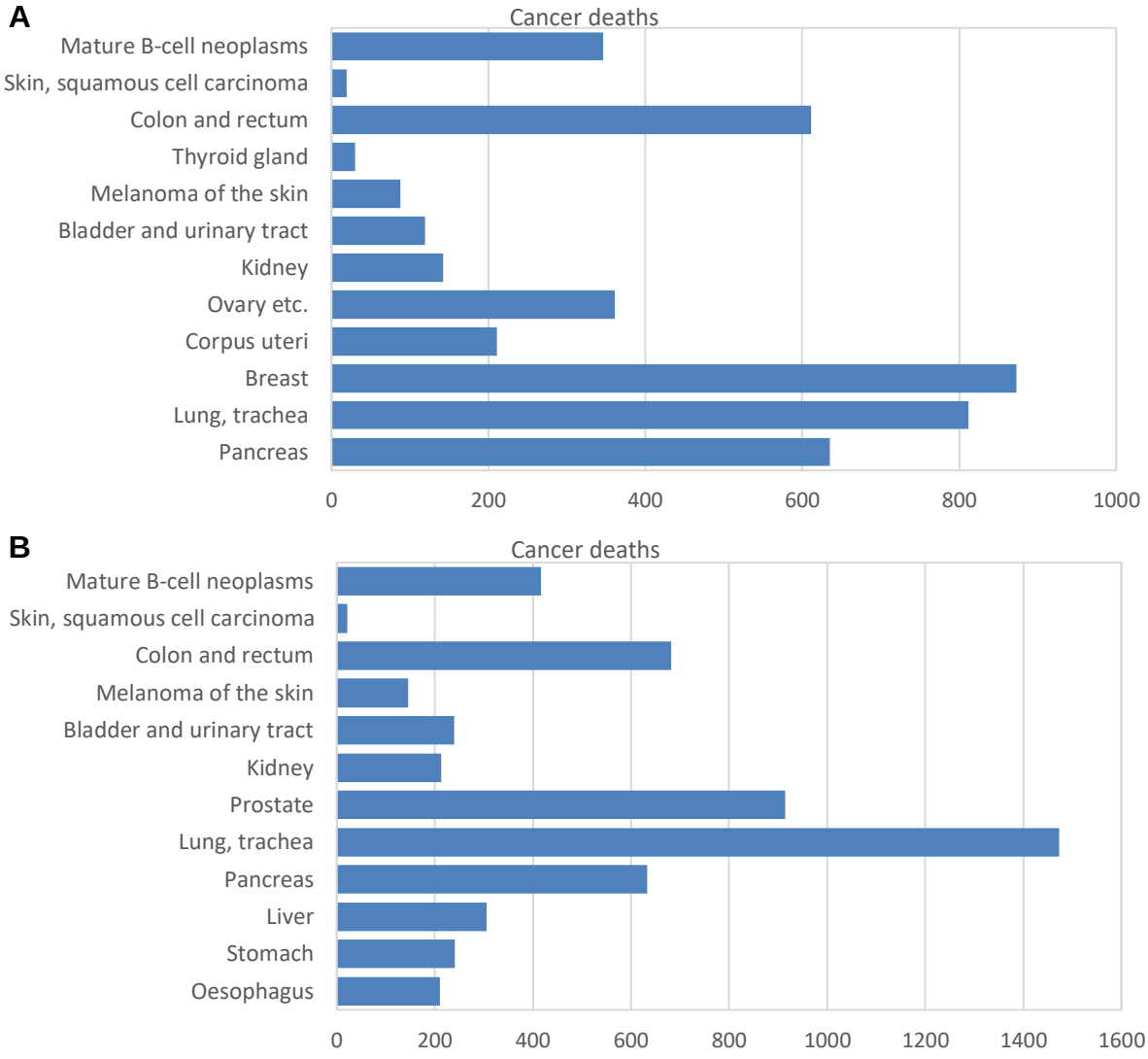


Figure 2. Cancer related deaths in Finland in 2018 for A) women and B) men.[2]



2.2 Etiology and risk Factors

Smoking is the most important risk factor for lung cancer. It is estimated that around 85-90 % of lung cancers are caused by smoking.[3] The risk of lung cancer for smokers is around 20-fold compared to never-smokers.[10] The risk depends on the starting age of smoking, years of smoking and the number of cigarettes smoked per day. The age of smoking cessation can increase life expectancy greatly. Cessation at age 60 can gain about 3 years of life expectancy while cessation at age 30 can gain about 10 years.[11] A meta-analysis comprising data from 99 cohort studies[12] did not detect a difference in the risk of smoking-related cancer between men and women. SCLC is particularly associated with smoking and in a study that analyzed nearly 4800 SCLC patients only 2,5 % of the patients

were never-smokers.[13] In addition, second hand smoking is also a risk factor: exposure to second hand smoking in the childhood increases the risk of lung cancer up to 3,6-fold.[14] Other risk factors include exposure to asbestos, alcohol, certain diets and food supplements, air pollution and genetic factors.[15] For example, mutations in the p53 germline increases the risk of lung cancer. Moreover, for carriers of this mutation smoking further increases the risk of lung cancer 3,16-fold compared to non-smokers carrying the mutation.[16] Generally smoking intensifies the risk of lung cancers caused by environmental factors.[3] For example, the exposure to asbestos seems to rather have a multiplicative effect than an additive effect on the risk of lung cancer when combined with smoking.[17] Asbestos alone increases the risk of lung cancer 2-10-fold but combined with cigarette smoking the risk is increased up to 50-fold.[18] Additionally, smoking electronic-cigarettes also seems to increase the risk of lung cancer, however this is yet to be studied in more depth.[19] Contrarily, exercise and physical activity and a proper diet may decrease the risk of lung cancer.[15]

2.3 Classification of non-small cell lung cancer

The two main types of lung cancer are non-small cell lung cancer (NSCLC) (80–85% of the cases) and small cell lung cancer (SCLC) (15–20%). SCLC is a neuroendocrine carcinoma and generally has a higher degree of malignancy than NSCLC. Lung cancers are further divided into sub-types according to histology and growth patterns. NSCLC is typically divided into adenocarcinomas, squamous cell carcinomas and large cell carcinomas.[3] The significance of subtyping has been increasing because of its increasing relevance in choosing the correct treatment. Therefore, the WHO 2015 classification extended the classification of NSCLC with two more rarer types: adenosquamous cell carcinoma and sarcomatoid carcinoma.[20] The classification of NSCLC is summarized in table 1.

Adenocarcinoma is the most common sub-type with around 40% of all lung cancers.[21] The incidence of adenocarcinoma has been on the rise in the West.[3] It commonly forms in the peripheric parts of the lung and it was formerly known as the bronchoalveolar cell carcinoma. [3,22,23] Histologically there is much variation within adenocarcinoma, and it is classically divided into preinvasive, minimally

invasive and invasive adenocarcinomas. Preinvasive lesions include atypical adenomatous hyperplasia (AAH) and adenocarcinoma in situ (AIS). AAH presents itself as a small ground-glass nodule that measures less than 5 mm and AIS is a small tumor measuring 3 cm or less. Minimally invasive tumors are further divided into nonmucinous, mucinous and mixed mucinous/nonmucinous and they are small (3 cm or less) tumors and have an invasion of a maximum of 5 mm. Invasive carcinoma is divided according to their growth pattern into five categories which are lepidic, acinar, papillary, micropapillary and solid adenocarcinoma. A tumor may include components of several of these histological types and therefore the adenocarcinoma is classified according to the predominant histological component. For an adenocarcinoma to be classified as invasive an invasion of more than 5 mm must be present. Of all surgically resected lung cancers about 70-90% are invasive adenocarcinomas.[24] The growth pattern has prognostic value. A lepidic adenocarcinoma has the best prognosis while micropapillary and solid growth patterns are associated with a more aggressive cancer type.[25] When immunohistochemically identifying adenocarcinomas particularly useful markers are thyroid transcription factor-1 (TTF-1) and napsin A. TTF-1 has been shown to be expressed in over 80 % of adenocarcinomas, while napsin A seems to be a specific marker for lung adenocarcinomas.[26–28]

Squamous cell carcinoma (SCC) is the second most common type of NSCLC accounting for around 25-30 % of all lung cancers.[21] SCC typically forms centrally in the lung but can also occur in the peripheric portion. It's often associated with smoking and upon growing larger SCC has a tendency of displaying necrosis and forming cavities.[3,22] According to the 2015 WHO classification of lung tumors SCC is currently divided into three subtypes: keratinizing, nonkeratinizing and basaloid squamous cell carcinoma. SCC tumor can express features of all three subtypes. If the tumor shows any keratinization the SCC is classified as keratinizing unless the basaloid component is more than 50% in which case the tumor is classified as basaloid regardless of any amount of keratinization present.[20] The classification of SCC doesn't seem to have prognostic significance however there are some studies that suggest a poorer prognosis for basaloid

SCC.[29,30] In addition to these invasive subtypes, SCC can also be classified as a preinvasive squamous cell carcinoma in situ.[20] Immunohistochemically the profile of SCC is very consistent with negative TTF-1 and diffuse p63, 34βE12, CK5/6.[26] An American study consisting of 318 patients with lung cancer (81 with SCC and 237 with adenocarcinoma) reported that the immunohistochemical marker p40 would be highly specific for SCC with a sensitivity of 100% and a specificity of 98%.[31]

Large cell carcinoma (LCC) is a rarer type of NSCLC with around 3% of the lung cancers.[32] LCC cannot be diagnosed based on small biopsies or cytology and should therefore only be diagnosed in tumors that are surgically resected.[20] Most LCCs form in the peripheral portion of the lung. The incidence has dropped because of the new more precise classification of cancers and many cases that used to be classified as LCC are now classified as adenocarcinoma or SCC.[21]

Adenosquamous carcinoma is also a relatively rare type of NSCLC with less than 4% of all lung cancers.[33] To be classified as adenosquamous the tumor must contain components of both adenocarcinoma and SCC. Each of the components must make up at least 10 % of the tumor.[20]

The last type of NSCLC is sarcomatoid carcinoma which can be further divided into carcinosarcoma, pleomorphic carcinoma, giant cell, spindle cell and pulmonary blastoma. Sarcomatoid carcinomas are rare and make up less than 1% of lung cancers. This subtype can generally not be diagnosed based on small biopsies or cytology and it is frequently linked with a poor prognosis.[20]

Table 1. Summarization of the classification of non-small cell lung cancer

NSCLC type	Frequency of all lung cancers	Subtypes
Adenocarcinoma	40%	preinvasive atypical adenomatous hyperplasia adenocarcinoma in situ minimally invasive nonmucinous mucinous mixed mucinous/nonmucinous invasive lepidic acinar papillary micropapillary solid
Squamous cell carcinoma	25-30%	keratinizing nonkeratinizing basaloid
Large cell carcinoma	3%	
Adenosquamous cell carcinoma	<4%	
Sarcomartoid carcinoma	<1 %	Pleomorphic Spindle cell Giant cell Carcinosarcoma Pulmonary blastoma

Note: Modified from The 2015 World Health Organization classification of Lung Tumors

2.4 Diagnosis

The diagnosis of lung cancer is based on the symptoms, physical exam, medical history, radiological findings and biopsy. Lung cancer can present with a wide range of symptoms, which are however unfortunately largely non-specific. The symptoms may include for example persistent coughing, hemoptysis, shortness of breath, wheezing, recurring pneumonias and hoarseness.[3] In addition, a recent review reported cachexia to be common in patients with both advanced and early-stage NSCLC.[34] One of the problems with lung cancer is that it can be

asymptomatic for a long time and once symptoms occur the lung cancer may have been present for years, which is why lung cancer is frequently diagnosed when the cancer is already advanced or locally advanced. In addition, the non-specific symptoms may at first lead to misdiagnosis.[5]

A chest x-ray should be performed on a patient suffering from any of the previously mentioned symptoms. A suspicion of lung cancer may arise due to an abnormal chest x-ray however a normal x-ray does not rule out a lung tumor.[35] A lung cancer diagnosis cannot be made only based on symptoms and an abnormal chest x-ray, but more detailed diagnostic imaging procedures are needed. Other imaging techniques may include computed tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography scan (PET) and PET-CT. CT of the lung and upper abdomen is the primary imaging technique when assessing the size, location and spread of the tumor. A PET-CT scan offers further information when investigating the spread of the cancer in more detail. If suspicion of a brain metastasis arises a brain MRI is indicated.[3]

A physical examination or radiological findings alone are generally not enough to set a precise lung cancer diagnosis, but histopathological investigations are needed. The safest and least invasive diagnosing method is to be preferred depending on the patient's state of health and the findings.[36] If the patient has accessible pleural effusion, repeated pleural fluid cytology is an important diagnostic method with a sensitivity of 72%. Nevertheless, sometimes differential diagnosis between more precise histological subtypes is not possible and a negative result does not rule out lung cancer and further testing is needed.[3,36] If the patient has a central lesion, bronchoscopy can be used to take a biopsy and cytological samples (e.g. brush samples, bronchoalveolar lavage and washing samples). The sensitivity of bronchoscopy for central lesions is 88%. However, for small peripheral lesions with a diameter smaller than 2 cm this method has a sensitivity of 34%. This means that for peripheral lesions the diagnostic value of bronchoscopy decreases, and another method should be considered. A CT or ultrasound guided needle biopsy is an important diagnostic method with a high sensitivity of 90% but a higher risk of pneumothorax when compared to bronchoscopic methods. A thoracoscopic (e.g. video-assisted thoracoscopy

(VATS)) biopsy has the highest diagnostic value with a sensitivity of 95-97%. However, it is also the most invasive of the mentioned methods.[36]

There are also other diagnostic methods including for example taking lymph node samples during a mediastinoscopy or with the help of endobronchial ultrasound. This can be useful when assessing the possible spread of the lung cancer into the lymph nodes of the mediastinum. As a primary or complementary diagnostic method in a metastatic lung cancer biopsies can also be taken straight from the metastasis.[3]

Differentiating between NSCLC and SCLC has been shown to be accurate, but NSCLC forms a heterogenous group of tumors and the diagnosis of NSCLC is not enough anymore. When diagnosing lung cancer, it is absolutely necessary to obtain a sufficient amount of tissue to make a histologically accurate diagnosis (e.g. adenocarcinoma or SCC) and if possible, to determine the mutation status and other predictive biomarkers such as PD-L1. The most important decision by the clinician is to evaluate the location and method of the diagnostic sample. Often obtaining enough tissue is not possible, which leaves minimally invasive methods as the primary mean of diagnosis.[36]

2.5 Staging

Staging of lung cancer is a fundamental tool in assessing the prognosis and planning the therapy for patients suffering from lung cancer. It helps in standardization of treatments and minimizes miscommunication when discussing a particular cancer patient. The primary aim of cancer staging is to be a system used worldwide that provides consistent information about the anatomic extent of the cancer. The TNM is one of the staging systems used worldwide. T-category stands for primary tumor and describes its size and possible invasion of nearby tissue, N-category describes the involvement of lymph nodes and M-category describes the distant metastases. The definitions for the TNM staging of lung cancer according to the AJCC/UICC eight edition lung cancer stage classification published in 2017 are presented in table 2. Additionally, based on the TNM classification, each cancer can be placed into a stage group.[37] These stage groups are presented in table 3. The stage groups have also prognostic value, stage IA1 having the best survival rate (90-92% 5-year survival) and IVB the worst

(0% 5-year survival).[37,38] Furthermore, the context of the stage classification can be specified. For example, clinical TNM (cTNM) refers to the classification prior any treatment based on e.g. radiological imaging, while pathologic (pTNM) refers to the classification after resection based on pathologic findings.[37] Table 4 presents the 5-year survival rates for each clinical and pathologic stage group.

Table 2. The TNM staging system[37]

T (Primary Tumor)	**Description**
T0	No primary tumor
Tis	Carcinoma in situ (Squamous or Adenocarcinoma)
T1	Tumor ≤3 cm,
T1a(mi)	Minimally Invasive Adenocarcinoma
T1a	Superficial spreading tumor in central airways[a]
T1a	Tumor ≤1cm
T1b	Tumor >1 but ≤2 cm
T1c	Tumor >2 but ≤3 cm
T2	Tumor >3 but ≤5 cm or tumor involving: visceral pleura[b], main bronchus (not carina), atelectasis to hilum[b]
T2a	Tumor >3 but ≤4 cm
T2b	Tumor >4 cm but ≤5 cm
T3	Tumor >5 but ≤7 cm or invading chest wall, pericardium, phrenic nerve or separate tumor nodule(s) in the same lobe
T4	Tumor >7 cm or or tumor invading: mediastinum, diaphragm, heart, great vessels, recurrent laryngeal nerve, carina, trachea, esophagus, spine; or tumor nodule(s) in different ipsilateral lobe
N (Regional Lymph Nodes)	
N0	No regional node metastasis
N1	Metastasis in ipsilateral pulmonary or hilar nodes
N2	Metastasis in ipsilateral mediastinal/subcarinal nodes
N3	Metastasis in contralateral mediastinal/hilar, or supraclavicular nodes
M (Distant Metastasis)	
M0	No distant metastasis
M1a	Malignant pleural/pericardial effusion[c] or pleural/pericardial nodules or separate tumor nodule(s) in contralateral lobe;
M1b	Single extrathoracic metastasis
M1c	Multiple extrathoracic metastases (1 or >1 organ)

Tx, Nx: T or N status not able to be assessed
[a]Superficial spreading tumor of any sice but confined to the tracheal or bronchial wall
[b]Such tumors are classified as T2a if >3≤4 cm, T2b if >4≤5 cm
[c]Pleural effusions are excluded that are cytologically negative, non-bloody, transudative, and clinically judged not to be due to cancer

Table 3. Stage groups of lung cancer[37]

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

T = Tumor, N = node, M = metastasis

Table 4. 5-year survival for each stage group[38]

Stage group	IA1	IA2	IA3	IB	IIA	IIB	IIIA	IIIB	IIIC	IVA	IVB
Clinical	92%	83%	77%	68%	60%	53%	36%	26%	13%	10%	0%
Pathologic	90%	85%	80%	73%	65%	56%	41%	24%	12%	-	-

2.6 Treatment of non-small cell lung cancer

The treatment for non-small cell lung cancer depends on the stage of the disease, the histological subtype and the patient's general state of health. Additionally, it can depend on the molecular profile of the tumor. Surgery is the recommended treatment for early stage (stage I and II) NSCLC and if the general health of the patient doesn't allow surgery, radiation therapy can be used instead. The general state of health of the patient is commonly evaluated with the WHO performance status classification from 0 to 4, 0 being the best and 4 the worst.[3] In some instances adjuvant chemotherapy is considered after surgery. Patients with stage III lung cancer is an extremely diverse group. Patients suffering from stage IIIA lung cancer that are treated with curative intent, chemoradiotherapy should be combined with surgical treatment. Benefits of using additionally chemotherapy or targeted treatment as neoadjuvant or adjuvant therapy remains uncertain and further studies are needed.[39] Otherwise stage III lung cancer can be treated with a combination of chemotherapy and radiation therapy.[3]

For stage IV cancer patients with a good performance status (0-1), generally the first-line therapy is chemotherapy with a combination of a platinum-based medication (cisplatin or carboplatin) and a third-generation drug (vinorelbine, gemcitabine or taxanes). Furthermore, for performance status 2 patients a platinum-based combination should be preferred to single-agent chemotherapy with a third-generation drug. Although, a single-agent therapy is also an option for these patients and should especially be used in elderly patients with a performance status of 2.[40]

Due to the advances in the field of oncological personalized treatment it is now possible to use target therapy as treatment for some lung cancer patients. Lung tumors are searched for driver mutations and for spread lung cancer expressing e.g. EGFR mutations or anaplastic lymphoma kinase (ALK) rearrangements can be treated with EGFR TKIs or ALK inhibitors respectively instead of traditional chemotherapy.[20, 39] Especially EGFR TKI:s have consistently shown superior overall response rates, progression-free survival and quality of life in patients with a confirmed EGFR driver mutation when compared to chemotherapy.[41]

As mentioned before, the treatment of lung cancer depends on multiple factors and the treatment must be tailored to the needs and preferences of each individual patient.[39] This thesis will focus primarily on the surgical treatment of NSCLC and it will be discussed in more detail in the following chapter.

2.7 Surgical treatment of non-small cell lung cancer

Surgery is the first line treatment option in stage I and II lung cancer for operable patients.[3] If the patient with early stage lung cancer is unfit for surgery, curative radiotherapy should be offered instead.[42] Operative treatment is also recommended in stage IIIA cancer in some cases if r0 resection is possible.[43,44] Patients suffering from stage IIIA lung cancer is a heterogenous group and the treatment of each patient ought to be planned in a multidisciplinary team[39,44]. The aim of the surgery is to remove the part of the lung containing the tumor with an adequate margin of healthy tissue. In addition, it is recommended to remove or at least take samples from the lymph nodes of the N1 and N2 areas.[4]

Before the surgery the patient must undergo preoperative cardiopulmonary function testing. Spirometry and diffusion capacity are fundamental when assessing the current and calculating the predicted postoperative pulmonary function (PPF) of the patient.[39] The predicted PPF can generally be calculated with the amount of resected segments. The lung consists of 18 segments. When n segments are resected the postoperative function is approximately ((18-n)/18) % of the preoperative figure.[4] If the predicted postoperative values of forced expiratory volume in 1 second (FEV1) and diffusing capacity for carbon monoxide (DLCO) are over 60%, can the PPF be considered sufficient and no more pulmonary testing is needed.[4,39] To be eligible for lung surgery the predicted postoperative FEV1 and DLCO must be at least 30%.[4] If either FEV1 or DLCO is under 60% but over 30% an exercise test e.g. stair climb is recommended.[39] In general, climbing stairs for four floors without a decline in oxygen saturation levels indicates an eligibility for a surgery up to pneumonectomy while climbing two floors suggests that a lobectomy can be considered.[4] If the patient receives neoadjuvant therapy, it is recommended that the pulmonary function tests are repeated after the therapy prior surgery.[39]

In lung cancer surgery the possible operational treatment options include mainly lobectomy, segmentectomy, wedge resection, pneumonectomy and sleeve lobectomy.[4,42] Lobectomy, the removal of one lobe of the lung, is the first line option for tumors with a size of at least 2 cm and that seems solid on CT.[42] Furthermore, in the treatment of SCC, lobectomy has been reported to be superior to both wedge resection and segmentectomy for smaller tumors as well. Segmentectomy is the removal of a segment of the lung while wedge resection is a nonanatomic resection of a part of the lung. For adenocarcinoma segmentectomy has shown equal survival rates as lobectomy for tumors smaller than 2 cm while wedge resection has been reported inferior to lobectomy.[45] Nevertheless, the recurrence rate for stage IA NSCLC tumors treated with sublobar resections (wedge resection or segmentectomy) seems to be 39% higher when compared to patients that have undergone lobectomy.[46] Segmentectomy or wedge resection can be considered instead of lobectomy if the patient is non-eligible for a lobectomy e.g. due to inadequate pulmonary

functions or other risk factors. Pneumonectomy is the removal of a lung and sleeve lobectomy is a procedure where in addition to removing a lobe of the lung, a part of the bronchi is removed as well. Pneumectomy or sleeve lobectomy should be considered in central tumors and/or if there is an invasion of the central bronchus or vasculature.[47] A meta-analysis published in 2012 containing nineteen studies and a total of 3878 patients reported that sleeve lobectomy should be preferred to pneumectomy as it showed a lower mortality and better long-term survival.[48] Pneumonectomy should however be considered if it is estimated that sleeve resection doesn't reach the desired oncological result.[47]

Operational treatment for lung cancer can be performed either as minimally invasive surgery or open surgery. Minimally invasive surgery has been gaining in popularity during the last decades and its techniques include video assisted thoracoscopic surgery (VATS) and robotic-assisted thoracoscopic surgery (RATS).[4,49] In VATS the surgery is performed by inserting a camera and the instruments through small incisions in the chest wall. RATS has a similar idea than VATS, but the surgeon operates through a robot. The surgeon controls the robot through a console and is not located bedside unlike in VATS. Open surgery is done with thoracotomy where a bigger incision is made into the chest to gain access to the lung. VATS has been shown to be a feasible option to open thoracotomy.[50] A meta-analysis containing data from 21 studies suggested that patients with early stage NSCLC treated with VATS had a lower recurrence rate and better 5-year mortality rate when compared to open surgery.[51] Additionally, minimally invasive surgery compared to open surgery is associated with lower complication rate (16,4% vs 31,2%), shorter chest tube duration (4,2 days vs 5,7 days), shorter hospital length of stay (8,3 days vs 13,3 days), which suggests faster recovery times.[52] Open surgery remains a good option when the lung tumor is large, is located near the hilum of the lung or requires a chest wall resection.[4]

The first VATS was performed in 1991 while the first RATS was reported in 2002.[53,54] The advantages of RATS are often associated with the minimization of hand tremor, broader movement of the instruments and better ergonomics.[49,55] This comes with the cost of loss of sensation of tissue manipulation. According to a meta-analysis conducted by Ye et al.[56] in 2015, VATS and RATS show

similar results for perioperative morbidity and mortality. Furthermore, another meta-analysis containing the data from 7438 patients show equal results for RATS and VATS regarding postoperative complications, operation time, duration of hospitalization, days to tube removal, retrieved lymph node and retrieved lymph node stations.[57] However, the 30-day mortality and conversion rate to open surgery was significantly lower for RATS.[57] There is a lack of studies comparing the long-term results of the two methods, but RATS seems to be a feasible alternative to VATS in minimally invasive surgery in the treatment of lung cancer.

2.8 Follow-up and surveillance

NSCLC patients who are treated with a curative intent should be surveilled after the treatment for complications, a possible treatable relapse and a possible second primary lung cancer. A follow-up visit should be scheduled every six months for two years after the treatment. The visit should include a medical history and a physical examination. In addition, a chest CT scan should be performed at least annually for the first two years after the treatment. After this an annual follow-up visit with a medical history, a physical examination and a chest CT scan is recommended. Furthermore, smoking cessation is fundamental part of curative treatment because it is associated with better treatment outcomes. The persistence of smoking after lung cancer surgery is linked to higher risk of recurrence and metastasis[58]. In addition, perioperative smoking is linked to a slower wound healing rate and a higher risk of infections in the surgery.[59] Therefore support for smoking cessation should be offered.[39,42]

3 Cell-Free DNA

3.1 Cell-Free DNA and Circulating Tumor DNA

Nowadays the treatment of lung cancer with targeted therapies e.g. with EGFR TKIs and ALK inhibitors is possible due to the advances in the field of personalized medicine.[20,39] The use of these drugs, however, requires the molecular profiling of the tumor[60]. Tissue biopsies are currently one of the most important tools in lung cancer diagnosis.[3] However, because it is invasive, it poses an increased risk for complications.[61] In addition, the location of the tumor may not allow for a tissue biopsy. The biopsy can also turn out to be inadequate e.g. due to the necrosis of the tumor or other factors in the biopsy taking process.[8] Tumors can also be widely heterogeneous and thus a tissue biopsy may not represent the complete molecular profile of the tumor.[62]

To tackle these issues the interest towards cell-free DNA (cfDNA) has been increasing in the field of oncology.[61] Generally, cfDNA refers to all the DNA in the body that is free from cellular confinement and it was first reported in 1948 by Mandel and Metais.[7,63] The size of most cfDNA fragments range between 80 to 200 base pairs.[64] It is released into the bloodstream mainly as a result of cell death of hematopoietic cells.[65] However it can also derive from other cells among other things due to physiological or external damage e.g. myocardial infarction, exercise or trauma.[66–68] When the cfDNA originates from cancer cells, it is called circulating tumor DNA (ctDNA).[8] In the future, ctDNA could have a use in multiple aspects of cancer management including screening, early detection, diagnosis, treatment, monitoring the response for treatment and identification of drug resistance.[9]

The information from the cfDNA can be obtained through liquid biopsies. The term liquid biopsy refers to the analysis of e.g. cfDNA or proteins in body fluids. In addition to the bloodstream, cfDNA can also be found in other body fluids like pleural fluid and urine.[69,70] Liquid biopsies have several advantages over tissue biopsies: they are more convenient, minimally invasive and can be used for serial testing.[61]

Generally, with ctDNA analysis it is possible to obtain quantitative and qualitative information. In cancer management, quantitative information could have a role in disease staging and assessing treatment response. Contrarily, qualitative information could be used in the molecular profiling of the tumor and therefore for example treatment selection. For example, a specific EGFR mutation, T790M, is routinely looked from the bloodstream if a resistance to TKI is suspected. To identify sequence alterations mainly techniques based on PCR- and Next-Generation Sequencing (NGS) are used.[71]

3.2 Potential use of cfDNA

As previously stated, cfDNA or more specifically ctDNA could be potentially useful in several parts of cancer management. Studies have shown that ctDNA can be detectable in around 50% of stage I cancers.[72,73] This would suggest that ctDNA could be used in early detection of cancer when the cancer can often be curatively treated with surgery.[72] This has also grown interest towards the possibility of using ctDNA for screening asymptomatic patients.[61] Furthermore, ctDNA could be used in cancer staging for it has shown to correlate with the stage of the cancer.[72] In the future, treatment selection could be based on the information acquired about the mutations, translocations, deletions and amplifications detected in ctDNA. In addition, this information may be used in the monitoring of treatment response and the potential development of drug resistance.[71] This would be especially important since it is believed that drug resistance is the reason for the failure of the treatment in over 90% of patients with advanced cancer.[74] For example, Ahlborn et al[75] studied BRAF V600E mutation positive non-melanoma cancer patients treated with BRAF inhibitor combination therapy. They reported that cfDNA can be used to monitor drug response and the developed drug resistance was detectable with cfDNA five weeks prior to radiological evidence. The early detection of drug resistance could allow faster modifications in the treatment. A study conducted by Misale et al showed that the drug resistance in patients with colorectal cancer treated with cetuximab was detectable up to 10 months before radiological evidence of progression.[76] Furthermore, they suggested early intervention with an additional biological drug delayed or even reversed the drug resistance.

Moreover, the nature of liquid biopsies, being minimally invasive and having minimal radiation burden compared to tissue biopsies, is suited for longitudinal monitoring of a disease.[61] It has also been reported that cfDNA has a short half-life ranging from 4 minutes to just under three hours which further enables the monitoring of the cancer in real time.[73,77]

Despite the great potential of cfDNA and ctDNA, so far there is however little evidence to support their clinical use in the current management of early and late-stage cancer due to the lack of clinical trials. There are several challenges to the use of ctDNA that need to be solved. For example, even though the potential of capturing the whole genomic information of a heterogeneous cancer is often seen as an advantage of ctDNA over tissue biopsies, this also poses a challenge. In an advanced cancer, the information about a mutation in a ctDNA sample may originate from any site of metastasis which may give clinically irrelevant information that may lead to a therapy without durable responses.[61] This kind of situation could turn out expensive and possibly harmful for the patient. Nevertheless, there is a great possibility that the analysis cfDNA and ctDNA will have a significant role in the future of the oncologic field. For example, there is currently already a FDA approved cfDNA assay “The Cobas EGFR Mutation Test v2” available for clinical use to identify EGFR mutations in NSCLC patients.[78]

3.3 Current state and future prospects in the treatment of non-small cell lung cancer

Due to the advances in personalized medicine, targeted therapy has achieved a significant role in the treatment of NSCLC.[20,39] This is one of the reasons why cfDNA has started to increasingly attract interest in the management of lung cancers as well. In addition to the previously mentioned EGFR and ALK inhibitors, also ROS-1 mutations and BRAF V600E mutations can be targeted.[79–82] Furthermore, the treatment of NSCLCs with certain genetic alterations is also possible with off-label drugs (e.g. trastuzumab for HER2 gene fusion positive lung cancer) or even with drugs undergoing clinical trials.[83,84] Barlesi et al conducted a nationwide NSCLC screening programme in France that included 18 679 molecular analyses of 17 664 patients.[84] They reported that at least one genetic alteration was found in 50% of the analyses. EGFR mutations were found in 11%

and ALK rearrangements in 5% of the analyses. The first-line treatment of 51% of the lung cancer patients was affected by a genetic alteration and the presence of a genetic alteration was associated with improved response in first-line treatment. The presence of a genetic alteration was also linked with a longer overall survival and a longer first-line progression-free survival. Based on their results Barlesi et al encouraged to provide access to personalized treatment.[84] The use and management of personalized treatment could potentially be improved by the use of cfDNA which could enable the easier detection and longitudinal monitoring of mutations in different phases of cancer.

Barlesi et al[84] also reported that when patients with an EGFR mutation were excluded from the final analysis, the association between a genetic alteration and a significant improvement in patients overall survival and first-line progression-free survival disappeared. This suggests that identifying EGFR mutations is currently especially important. Steendam et al compared two cfDNA mutation analysis techniques to tissue biopsy analysis in the ability of identifying EGFR primary activating mutations and p.T790M.[85] The techniques studied were droplet digital PCR (ddPCR) and New-generation sequencing (NGS). Only NSCLC patients with tissue biopsy confirmed EGFR mutations were included. T790M is an EGFR mutation that is linked with the resistance to first and second generation EGFR TKIs.[86] For detecting primary activating EGFR mutations, the concordance between NGS and tissue biopsies was 83% and between ddPCR and tissue biopsies it was 69%. However, the ddPCR technique was limited by the selected panel, which was limited to only check for exon 19 deletion and p.L858R mutation. If this was taken into consideration, the concordance was 83% as well. The cfDNA analysis methods detected a T790M mutation in three patients (8,3%) that was missed by the tissue biopsy. Nevertheless, in two of these patients the cancer did not respond to osimertinib, a third generation EGFR TKI.[85] These results suggest that a tissue biopsy may not always be necessary when considering the possibility to use a targeted treatment for NSCLC. Although, in six patients (16,7%) who had a tissue biopsy confirmed EGFR mutation, no EGFR mutations were detected in cfDNA. All of these were intrathoracic tumors.[85] This could support the assumption that the amount of cfDNA is

associated with the stage of the tumor and the use of cfDNA in localized early-stage lung cancer is more limited when compared to advanced cancer.

The detectability of ctDNA in a lung cancer seems also to be dependent on the histological subtype. In a study conducted by Abbosh et al, ctDNA was detected preoperatively in 97% of the lung SCCs and 94% of stage I SCCs.[87] For other histological subtypes the detection rate of ctDNA was 71%, adenocarcinoma only having a 19% detection rate. Abbosh et al suggested that this might be because lung SCC is more necrotic than adenocarcinoma and the amount of ctDNA in the bloodstream is associated with the necrosis of the tumor. [87,88] Moreover, the tumors in ctDNA positive lung adenocarcinoma were found to be significantly more necrotic when compared to the tumors in ctDNA negative adenocarcinoma which further supports this assumption. Other factors that predicted detection of ctDNA in NSCLC were pathologic tumor size, lymphovascular invasion, high Ki67-labeling index and cfDNA input.

The detection rate of ctDNA seems also be highly dependent on the used detection method. Contrary to Abbosh et al, a study conducted with Chaudhuri et al reported higher detection rates for adenocarcinoma.[89] They used a method they optimized for the use of detecting ctDNA in noninvasive tumors.[89,90] The study included 40 patients with a localized lung cancer (stage I-III) that were treated with a curative intent. Pretreatment ctDNA was detected in 93% of the patients. The detection rate for adenocarcinoma was 89% and for SCC 93%.[89] However, in addition to the method used, the large difference in the results between the studies is most likely at least partly caused by the patient selection. In the study from Abbosh et al 61% of the patients had a stage I tumor while only 18% of the patients in the study conducted by Chaudhuri et al had a stage I disease.

Additionally, ctDNA could possibly also be used to identify a possible minimal residual disease (MRD) and to increase the efficacy of adjuvant therapy use. Adjuvant chemotherapy has an important role in the management of NSCLCs treated with a curative intent. However, the improvement in survival at 5 years has been reported to be only 4%.[91] This could suggest that only a portion of patients benefit from adjuvant chemotherapy.[92] Since the toxicity of

chemotherapy is significant, it would be beneficial to identify this subgroup of patients.[93] In the previously mentioned study conducted by Abbosh et al, they also monitored a subgroup of 24 patients, 14 of which ended up having a NSCLC relapse. Of the relapsed patients 13 out of 14 (93%) had detectable ctDNA before or at relapse and the ctDNA was detectable on average 70 days (with a range of 10-346 days) before the relapse was confirmed by a CT-scan. Of the patients without a relapse during the median 775 days follow up time, only 1 out of 10 had detectable ctDNA. Furthermore, the molecular profiling of the ctDNA was also consistent with the resistance to adjuvant chemotherapy. In three patients, who experienced a relapse, the amount of detected single nucleotide variants (SNV) increased despite the given adjuvant chemotherapy. Contrarily, in the patient that had detectable ctDNA without clinical evidence of a relapse, the amount of SNVs was decreased. After 72h of the surgery there were 20 detectable SNVs in the ctDNA, 13 just prior the adjuvant chemotherapy and none 51 days after the completion of the chemotherapy. This patient remained relapse-free for the whole follow up period. Abbosh et al stated that ctDNA could already be feasible in identifying a residual disease and increasing the efficacy of adjuvant therapy use.[87]

Chaudhuri et al also studied the use of ctDNA in the detection of MRD in localized lung cancer.[89] Of the studied 40 patients 37 (93%) had detectable ctDNA before treatment with a curative intent. They detected ctDNA in 54% of these patients at some point after the treatment and all these patients eventually had a lung cancer recurrence. Before the recurrence the ctDNA was detectable in 72% of the patients by a median of 5,2 months before the recurrence could be confirmed by radiological imaging. They also analyzed a set MRD landmark which was prespecified as the first posttreatment blood test within 4 months after the completion of the treatment. Thirty-two patients were eligible for this analysis. At this landmark, ctDNA was detected in 53% of these patients, all of which had a recurrence within 36 months. Of the patients that had no detectable ctDNA at MRD landmark, only 1 patient (7%) experienced a recurrence within this time. Chaudhuri et al evaluated that a significant portion of the patients with detectable ctDNA MRD could potentially have benefited from posttreatment chemotherapy

or targeted therapy.[89] This would have to be studied in clinical trials but these results suggest that ctDNA has a potential role in detecting MRD and identifying patients who need adjuvant therapy.

4 Study

“Circulating tumor DNA analyses in surgically treated NSCLC” is an upcoming study which will be conducted by a research groups led by professor Marjukka Myllärniemi and docent Ilkka Ilonen in collaboration with Helsinki Biopank. The aim of the study is to research the role of ctDNA in NSCLC patients who are treated with a curative intent surgery. Preoperative blood samples will be analyzed by NGS whole exome sequencing and will be compared to the histopathological findings in the tumor tissue, TNM stage and tumor volume. Furthermore, the study aims to assess the association of ctDNA with clinical recurrence and survival and identify a possible MRD. Currently, the study is recruiting patients and aims to collect hundred patients. To meet the inclusion criteria the patients must have a histologically confirmed NSCLC that is planned to be treated with a curative intent surgery. The exclusion criteria include pregnancy, a prior metastatic solid cancer, a hematological malignancy and a known hereditary cancer syndrome. The aim is to collect four blood samples and a surgically resected tissue sample from each patient. The blood samples include a preoperative blood sample and three postoperative follow-up blood samples, which will be drawn simultaneously with clinical follow-up samples at 1, 6 and 12 months post-surgery.

4.1 Patients

Lung cancer meetings and the thoracic surgery schedule have been routinely screened for patients meeting the inclusion criteria. As of September 2020, this has yielded 31 out of 100 patients. The Covid-19 pandemic has slowed down the recruiting process because many operations have been cancelled especially in the spring of 2020. The patient data might change when collecting more patients, but the following data collected from the current patients may represent an approximation of what the final data could be. The average age of diagnosis of the patients is 67,1 years. Of the collected patients, most of the patients are female and smokers or at least have a smoking history. Of the cancers 12 (38,7%) were pathological stage IA cancers. One of the resected cancers turned out to be a metastasis of melanoma of the skin. VATS was used on nearly all patients while thoracotomy was used only on a few (3) patients. Lobectomy has

been the most common procedure. Thus far, one operation was abandoned after discovering the spread of the cancer. EGFR mutations were the most common driver mutation as it was found in the tumor tissue of 9 patients. The cfDNA is yet to be analyzed. Table 5 displays patient data in more detail.

Table 5. Data from the current patients

n=31	Amount	Percentage
General data		
Patients	31	
Age, mean	67,1	
Female	23	74,2%
Male	8	25,8%
Smoker	10	32,3%
Ex-smoker	10	32,3%
Never-smoker	9	29,0%
Passive-smoker	2	6,5%
Histology		
Adenocarcinoma	23	74,2%
Squamous cell carcinoma	1	3,2%
Carcinoid tumor	6	19,4%
Other	1	3,2%
Staging		
IA	12	38,7%
IB	5	16,1%
IIA	2	6,5%
IIB	6	19,4%
IIIA	2	6,5%
IIIB	2	6,5%
IIIC	1	3,2%
IV	1	3,2%
Surgery		
VATS	28	90,3%
Lobectomy	24	77,4%
Segmentectomy	2	6,5%
Wedge resection	1	3,2%
Thoracotomy	3	9,7%
Lobectomy	3	9,7%
Segmentectomy	0	0%
Wedge resection	0	0%
Mutations		
EGFR	9	29,0%
KRAS	6	19,4%
ALK	2	6,5%

4.2 Pilot Project

The samples of three enrolled patients were analyzed in a pilot project of the study. The histology of all three patients' NSCLC was adenocarcinoma. The aim was to test that technically the project is feasible and it is possible to extract good quality cfDNA from NSCLC liquid biopsy samples for NGS analyses. Preoperational blood samples were compared to a tissue sample. The blood samples were collected in Streck cell-free DNA BCT-tubes (Streck, USA). The tissue samples were first analyzed by HUSLAB's Laboratory of Genetics with a tumor tissue assay. The assay included all exons of PIK3CA, EGFR, KIT, KRAS, MET, NRAS and PDGFRA cancer genes and the BRAF exons 11-15. EGFR mutations were detected in all three patients, although all in different exons (19, 20 and 21). NGS whole exome sequencing was used to analyze the cfDNA blood samples and the results were compared to the mutations found in the patient's tissue sample. Compared to targeted panels NGS whole exome sequencing may allow to detect more known mutations and even discover new mutations. However, this comes with the cost of a lower sensitivity and higher financial cost.[94]

The Next-generation sequencing (NGS) method, also known as high-throughput sequencing, performs sequencing of millions of small fragments of DNA in parallel and can be used to sequence entire genomes or specific areas of interest, including a whole exome and targeted sequencing. NGS workflow contains three basic steps: library preparation, sequencing, and data analysis. The library preparation step fragments the sample into shorter 100–300 base pair length sequences. Custom adapters are added to each end of the template fragments for binding on a solid surface for library preparation. For cluster generation, PCR amplification is carried out to amplify each template. PCR process creates approximately one million copies of each template in a cluster. The most popular sequencing method is provided by Illumina. Illumina's method uses sequencing by synthesis (SBS) technology with reversible terminators where the fluorescently tagged bases, each with a different label, are incorporated and directly detected. The short sequences of nucleic acids will be then aligned to a reference genome and the data can be analyzed using bioinformatics tools or

data analysis apps.[95] When analysing the cfDNA, ctDNA can be identified by the presence of somatic mutations.

The analysis was performed by the Institute for Molecular Medicine Finland FIMM Technology Centre, University of Helsinki. The Twist Human Core Exome EF Multiplex Complete Kit (Twist Bioscience, San Francisco, CA, USA) was used to generate exome libraries from human genomic DNA from formalin-fixed paraffin-embedded (FFPE) and buffy coat samples. cfDNA libraries from plasma samples were processed using QIAseq cfDNA All-in-One Kit (Qiagen, Hilden, Germany). Libraries were sequenced on NovaSeq6000 (Illumina, San Diego, CA, USA). The samples were first sequenced with Novaseq S1 NS1-200 and analysed using Varscan program. This yielded 39 mutations in patient A, 25 in patient B and 12 in patient C. One same mutation was found in all patients. These samples were also analysed using DRAGEN Platform, which provides an accurate and ultra-rapid solution for NGS analysis and yielded 113 mutations in patient A, 62 in patient B and 230 in patient C. Eight same mutations were found in all patients. Only the somatic mutations that have passed filters selected during the Variant Call Format generation were retained. We also wanted to increase sequence depth and run second time with NovaSeq SP NSP-200 and analysed both runs with Dragen. This slightly increased the yield being 159 mutations in patient A, 92 in patient B and 342 in patient C and 14 same mutations were found in all patients. In these 14 mutations 4 genes named PLXNA4, SIPA1L1, SLC12A5 and CLVS1 have been evaluated before in ctDNA of lung cancer patient and these could be relevant targets for further analyses.[87,96] For example PLXNA4 is a receptor for semaphorins. Some semaphorins have been linked to have inhibiting effects on tumor progression while others seem to promote tumor progression.[97] Kigel et al suggested that PLXNA4 could be a possible target for anti-angiogenic and anti-tumoral drugs.[98]

Thus, from the pilot study, we can say that technically exome sequencing from this type of starting material was successful. However, we could not detect the same EGFR mutations in cfDNA samples as from tumor tissue samples using cancer panel for detection, but we may use new mutation data to design more

easily detected diagnostic panels for clinical use, especially for those patients who have non operative cancers. The results are presented in table 6.

Table 6. Results of the pilot project

Patient	**Souce material**	**exome enrichment input (ng)**	**Yield, (Gigabases)**
Patient A	cfDNA	84,26	102,0
Patient B	cfDNA	48,84	64,3
Patient C	cfDNA	15,95	19,8
Patient A	EDTA sample from the buffy coat (germ tract)	250	27,8
Patient B	EDTA sample from the buffy coat (germ tract)	250	26,9
Patient C	EDTA sample from the buffy coat (germ tract)	250	25,2
Patient A	FFPE sample	250	26,5
Patient B	FFPE sample	250	36,4
Patient C	FFPE sample	250	25,2
Patient A	cfDNA (additional reads)		96,2
Patient B	cfDNA (additional reads)		57,0
Patient C	cfDNA (additional reads)		18,6

Somatic mutations detected with whole exome sequencing in cfDNA have been detected to be consistent with that in tumor DNA, which indicated that plasma might be used for somatic mutation detection.[99] However, only two-thirds of patients shared exactly the same mutations (e.g. EGFR) in matched cfDNA and tumor DNA samples when mutations were obtained from targeted and whole exome sequencing. This indicates that cfDNA could cover most of the information of tumor DNA, but also could provide additional mutation information which could not get from tumor DNA e.g. derived from multiple metastases.[100]

5 Discussion

Despite the extensive research of NSCLC and development of better treatment options, lung cancer remains a disease with most cancer related deaths in the world and generally a poor prognosis. It is no wonder that the use of cfDNA has high hopes and expectations in the potential management of NSCLC. Arguably the biggest issues currently are the late detection of the lung cancer, the high recurrence rate after curative intent surgery and the acquired resistance to TKIs. The use of cfDNA could provide a solution to these problems in the future. In NSCLC, as in many cancers, cfDNA has multiple potential uses. However, the use of cfDNA still lacks clinical trials which is one of the reasons that there is little evidence to support the wide use of cfDNA in a clinical setting. For example, the analyzing techniques must be further developed and optimized. This is required to identify the small proportion of ctDNA in a cfDNA sample especially in early-stage cancers and MRD. Also, currently ctDNA assays seem to have a higher positive predictive value than a negative predictive value[61]. This suggests that negative results should be confirmed by a tissue sample which could be seen as an issue. However, it is already common practice in lung cancer diagnosis to use the least invasive method first and confirm with a more invasive method in case of a negative result (for example pleural fluid cytology). A similar role could be assumed by ctDNA. Nevertheless, the current studies show promising results which suggest that the use of cfDNA might be of daily use in the future in the oncologic field. Furthermore, despite the lack of clinical trials ctDNA has already gained a role in the treatment of NSCLC patients in Finland: if obtaining a sufficient tissue biopsy is not possible, a ctDNA sample is analyzed using droplet digital PCR for EGFR mutations.[18] Also, if the development of a T790M EGFR mutation is suspected, ctDNA can also be used.[18]

The introduced study “Circulating tumor DNA analyses in surgically treated NSCLC” is one of the studies that aims to research among other things the detection of potential MRD in curatively treated NSCLC. The data collected from current study participants in the introduced study has features that are expected from a study investigating NSCLC patients treated with a curative intent surgery: so far, the most common lung cancer subtype is adenocarcinoma and the

majority of the tumors are stage I or II. Interestingly there are more females enrolled in the study than male by a large margin and nearly a third of the participants are never-smokers. This could suggest that these features could be linked to a higher probability of adenocarcinoma compared to other lung cancer subtypes or otherwise a less aggressive lung cancer. However, in depth analysis of the data is yet to be performed.

The pilot project was essentially successful, however the blood sample cfDNA analysis conducted by FIMM has so far not detected the EGFR mutations that were detected in the tissue analysis. This is most likely because the amount of ctDNA in the sample is extremely low compared to the amount of cfDNA. This study includes mainly patients with early-stage lung cancers thus low ctDNA concentrations are to be expected: cfDNA is highly fragmented DNA and the total amount of ctDNA might make up as low as 0,01% of the total cfDNA in the sample, especially in early stages of the cancer. Furthermore, all three patients in the pilot project had NSCLC with a histology of adenocarcinoma which is associated with lower concentrations of ctDNA when compared to other histologies of NSCLC e.g. SCC. These extreme low concentrations make the detection of ctDNA challenging. Also, previous studies that have found EGFR mutations in ctDNA have used targeted panels rather than WGS or WES when analyzing the liquid biopsies. Unfortunately, there were no samples left from the pilot study to test whether EGFR mutations would have been found using a targeted NGS panel. Nevertheless, the NGS analysis yielded 14 mutations that all three patients in the pilot project shared and the new mutation data may be used to design a more easily detected diagnostic panel for diagnostic use.

As further studies are conducted and the cfDNA analyzing methods are further developed, the complimentary use of cfDNA could potentially turn out to be fundamental in the future management of NSCLC. The undergoing study will acquire important information on the early detection of MRD. In the future, clinical trials need to test whether this can be used as a predictive marker for oncological treatments to change patient outcomes.

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