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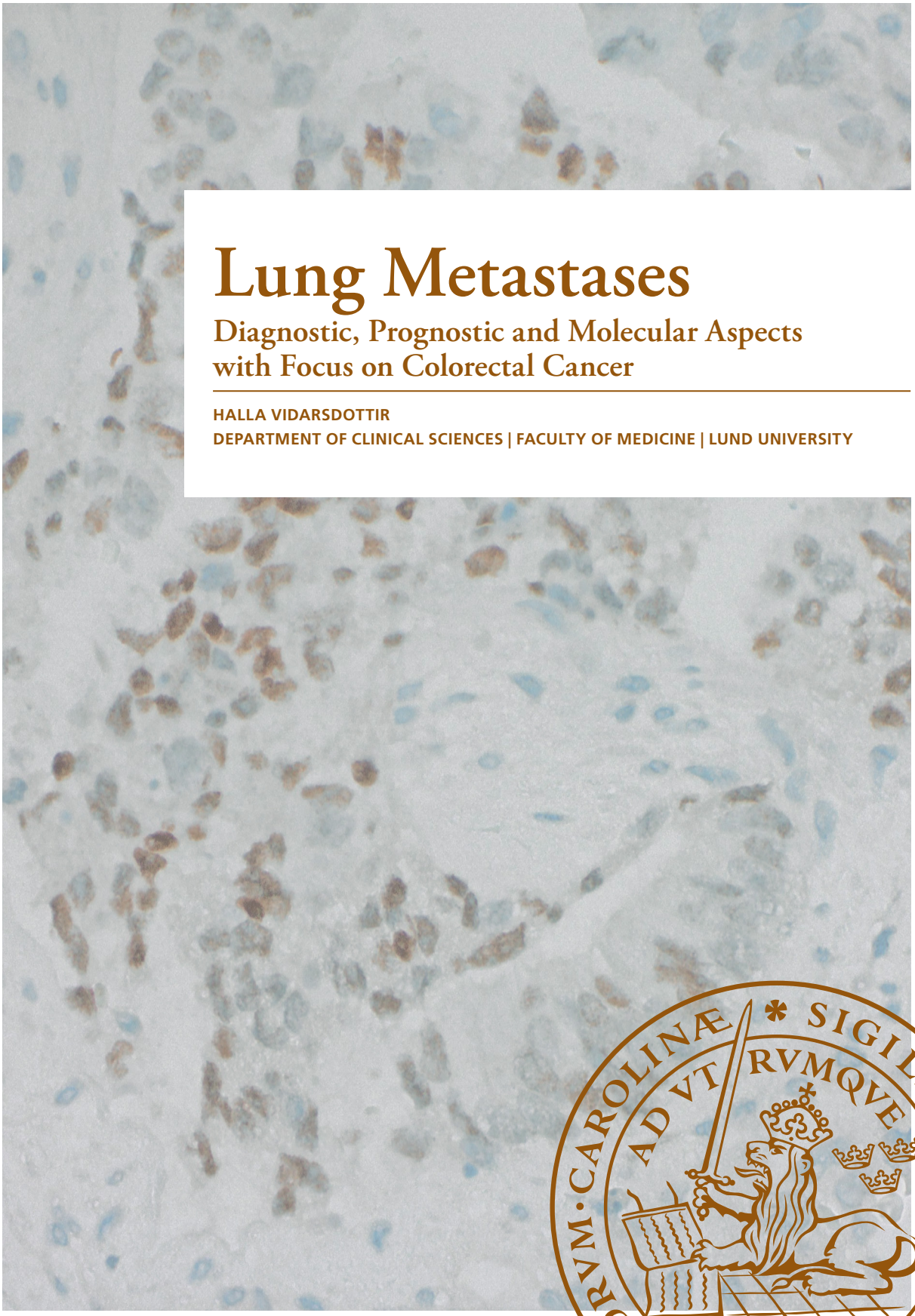

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LUND
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DOCTORAL DISSERTATION

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Abstract

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I: Lung adenocarcinoma (AC) was TTF-1 positive in 89, 93 and 93% of cases with clones 8G7G3/1, SPT24 and SP141 respectively. None of the lung squamous cell carcinoma (SqCC) was positive with clone 8G7G3/1 but 6 and 8% with clone SPT24 and SP141, respectively. Equivalent numbers for CRC lung metastases were 2, 7 and 8%.

II: Lung adenocarcinoma (AC) was TTF-1 positive in 90%, napsin A in 84%, and CK7 in 99% of cases. 68% were positive for all three markers and negative for other evaluated markers. None of the lung squamous cell carcinoma (SqCC) was expressed CK5, p40 and p63 in 94-97% of cases, while 64% were positive for all three markers, CK7+/-, and negative for other evaluated markers. CRC lung metastases were CK20+ in 83% and CDX2+ in 99% of cases, while 78% were positive for both and negative for other evaluated markers.

III: In total 216 patients with primary tumour in the rectum (57%), left colon (34%) or right colon (9%) underwent pulmonary metastasectomy. The 5-year overall survival was 56%. Age >60 years, >1 lung metastasis, size of metastasis >3 cm, disease-free interval <24 months, N2 status of the primary tumour, low RBM3 expression in the lung metastasis, and no adjuvant chemotherapy following pulmonary metastasectomy were prognostic factors for shorter overall survival.

IV: Mutations were most frequent in the *TP53, APC* and *KRAS* genes with rates of 81-85%, 70% and 41-48%, respectively in the primary tumours and corresponding lung and liver metastasis. With TST26, identical mutational profile was found in 59% of paired triplet tumours. The concordance was higher between primary tumour and lung metastasis (74%) vs. primary tumour and liver metastasis (63%). For seven (54%) of the 13 *KRAS*-mutated cases the *KRAS* mutations were concordant. With TSO500, discordant *KRAS* mutational profiles could be confirmed, sometimes with discrepancy compared to TST26. There was no significant difference in TMB between primary tumour and metastases.

Key words Lung cancer, lung metastasis, tissue microarray, TTF-1, napsin A, cytokeratin, GATA3, CDX2, PAX8, CRC, pulmonary metastasectomy, RBM3, recurrence, survival, NGS; Trusight Tumor 26, TruSight Oncology 500

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Lung Metastases

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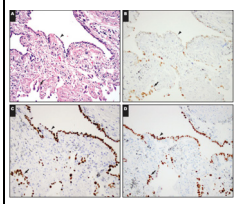
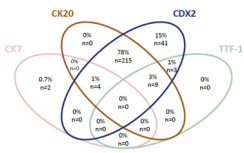
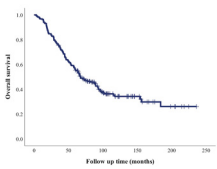
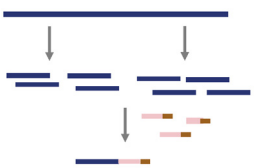
Contents

Thesis at Glance

Paper	Question	Patients & Methods	Results & Conclusions
I. 	Is there a difference in staining properties of the three TTF-1 antibody clones 8G7G3/1, SPT24 and SP141 in lung cancer and lung metastases from epithelial tumours?	TTF-1 expression was examined using the antibody clones 8G7G3/1, SPT24 and SP141 on TMAs from 665 cases of resected lung cancers and 428 lung metastases.	TTF-1 was positive in 89% 93%, 93% of lung ACs, 0%, 8%, 8% of lung SqCC and 2%, 7% and 8% of CRC lung metastases with clone 8G7G3/1, SPT24 and SP141, respectively. Clone 8G7G3/1 is more specific but less sensitive compared to clones, SPT24 and SP141.
II. 	Which are the best IHC markers to differentiate between primary lung cancers and lung metastases from epithelial tumours?	TMAs from 665 resected primary lung cancers and 425 resected lung metastases stained with TTF-1, napsin A, CK7, CK20, CDX2, CK5, p40, p63, GATA3 and PAX8	Typical IHC profile was found in 68% of lung ACs, 64% of lung SqCC and 78% of CRC lung metastases. Information on IHC markers and profiles facilitate histopathological diagnostics. Unusual immune profiles occur and may lead to incorrect diagnosis.
III. 	Which factors predict prognosis in CRC patients with surgically treated lung metastases? What is the role of RBM3 in the prognosis of CRC with surgically treated lung metastasis?	216 patients that underwent pulmonary metastasectomy at Lund University Hospital from 2000-2014. TMAs from primary tumours and lung metastases were stained with RBM3.	Median OS was 68 months, and 5-year OS was 56% after PM. Age >60 years, >1 metastasis, size of metastasis >3 cm, DFI <24 months, low RBM3 score and not receiving adjuvant chemotherapy following PM were prognostic factors for worse OS.
IV. 	What is the concordance in mutational profile between paired primary tumour and lung and liver metastases in CRC? Is TMB the same in primary tumours and paired lung and liver metastases?	27 CRC cases of paired primary tumour, lung and liver metastases. NGS analysis with TST26 gene panel. Five selected cases further analysed with TSO500 gene panel.	Based on TST26, the concordance between all three tumour samples was 59%. TMB was similar in primary tumours and metastases. There was mutational heterogeneity, also in *KRAS* mainly seen in rectal cancers, that is important from a treatment predictive perspective.

List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Vidarsdottir H**, Tran L, Nodin B, Jirström K, Planck M, Mattsson JSM, Botling J, Micke P, Jönsson P, Brunnström H. Comparison of three different TTF-1 clones in resected primary lung cancer and epithelial pulmonary metastases. *Am J Clin Pathol.* 2018;150:533–544.

II. **Vidarsdottir H,** Tran L, Nodin B, Jirström K, Planck M, Jönsson P, Mattsson JSM, Botling J, Micke P, Brunnström H. Immunohistochemical profiles in primary lung cancers and epithelial pulmonary metastases. *Hum Pathol.* 2019;84:221-230.

III. **Vidarsdottir H**[#] Siesing, C[#], Nodin B, Jönsson P, Eberhart J, Jirström K, Brunnström H. Clinical significance of RBM3 expression in surgically treated colorectal lung metastases and paired primary tumours. *J Surg Oncol.* 2021;123:1144-1156.

[#] These authors contributed equally to this paper

IV. **Vidarsdottir H**, Blackö A, Nodin B, Jirström K, Jönsson P, Rosengren F, Staaf J, Brunnström H. Mutational heterogeneity of primary colorectal carcinoma with paired lung and liver metastases investigated with targeted next generation sequencing. *Manuscript.*

Abbreviations

5-FU	5-fluorouracil
AC	adenocarcinoma
CEA	carcinoembryonic antigen
CI	confidence interval
CMS	consensus molecular subtype
CRC	colorectal cancer
CRT	classification and regression tree
CRP	c-reactive protein
CT	computed tomography
DFI	disease-free interval
DFS	disease-free survival
FFPE	formalin-fixed paraffin-embedded
FOLFIRI	5-fluorouracil, leucovorin and irinotecan
FOLFOX	5-fluorouracil, leucovorin and oxaliplatin
HR	hazard ratio
IHC	immunohistochemistry
mCRC	metastatic colorectal cancer
MSI	microsatellite instability
NGS	next-generation sequencing
NSCLC	non-small cell lung cancer
NR	not reported
OS	overall survival
PET	positron emission tomography
PM	pulmonary metastasectomy
RBM3	RNA-binding motif protein 3
RFS	recurrence-free survival
ROC	receiver operating characteristics
SCLC	small cell lung cancer
SqCC	squamous cell carcinoma
TMA	tissue microarray
TMB	tumour mutational burden
TST26	TruSight Tumor 26
TSO500	TruSight Oncology 500
TTF-1	thyroid transcription factor-1
VAF	variant allele frequency
VATS	video-assisted thoracoscopic surgery
WGS	whole genome sequencing

Abstract

In Sweden 4200 patients are diagnosed with lung cancer and 6500 patients with colorectal cancer (CRC) annually. The lungs are a common site for metastases. Immunohistochemistry (IHC) is a helpful aid in diagnostics of a pulmonary tumour. Selected patients with metastatic CRC undergo pulmonary metastasectomy and knowledge about which patients benefit from it is important. In this thesis IHC markers to distinguish between primary lung cancer and lung metastases, survival and prognostic factors of CRC patients treated with pulmonary metastasectomy and genetic profiles of paired primary CRC, lung and liver metastases are studied.

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Introduction

Historical notes

The first pulmonary metastasectomy (PM) was performed by Josef Weinlechner in 1882 for a metastasis from a chest wall sarcoma, discovered during the surgery for the primary tumour. In 1927, Divis performed the first planned, separate operation for a lung metastasis.[1] The same year Tudor Edwards performed a sublobar resection for a metastasis from sarcoma of the leg that was treated 6 years earlier, but this was not published until 1934.[2] The first publication in English literature of a planned PM was in 1930 by Torek on removal of a lung metastasis from uterine carcinoma.[3] The first PM performed in North America was by Barney and Churchill in 1939 when they removed a lung mass proving to be a metastasis from renal cell carcinoma. The patient subsequently operated the primary tumour and survived disease-free for over 20 years.[4]

In 1944, Alfred Blalock held a „recent advances" lecture to the Massachusetts Medical Society on performing right pneumonectomy for metastasis from colorectal cancer (CRC) resected 4 years earlier.[5] The first case series including 24 patients was published in 1947 by Alexander and Haight.[6] The authors also set up criteria for PM that Thomford and Clagett reformed and published in 1965 which states that: 1) The primary tumour is treated or treatable, 2) no extrapulmonary metastases (exception to this are resectable liver metastases in CRC), 3) it is possible to resect all lung metastases, 4) the patient is medically fit to tolerate the resection and 5) other curative treatment is not available.[7]

A report published in 1979 from Memorial Sloan Kettering on 35 cases where the authors recommend PM for CRC metastases confined to the lungs marked the introduction of PM for metastatic CRC (mCRC) into clinical practice.[8] In 1997 the landmark study from International Registry of Lung Metastases (IRLM) was published. It is a collection of 5206 PM cases from 18 centres. The metastases came from different primary tumours and were subdivided into 4 groups: epithelial cancers (including CRC), sarcoma, germ cell cancers and melanoma.[9] The use of PM in the management of mCRC in routine practice came about in a same way as surgery for liver metastases, as an extension of that practice and is now a recommended approach in selected cases according to clinical guidelines.[10-12]

Lung cancer

Lung cancer is the 4th most common malignancy in both men and women in Sweden. About 4200 patients are diagnosed with lung cancer each year, whereof 52% are women. Lung cancer incidence is low in Sweden from an international perspective (due to relatively low smoking frequency). The survival is poor with 5-year survival of 20% in Sweden, making it the most common aetiology for cancer-related death, with 3600 deaths each year.[13] The survival rate is higher for women compared to men both in Sweden and internationally.[14]

Smoking is the leading cause of lung cancer and alone or in combination with other factors causes at least 80% of lung cancer cases. Despite that only about 15% of smokers develop lung cancer, suggesting a genetic susceptibility. The proportion of patients that does not have a smoking history is higher among women. Other factors associated with increased risk of lung cancer are environmental factors such as asbestos, radon and metals as well as genetic factors.[15]

The most important prognostic factors in lung cancer are stage and the patient's performance status at diagnosis. Non-small cell lung cancers (NSCLC) (mainly adenocarcinoma (AC) and squamous cell carcinoma (SqCC)) are about 85% of lung cancers and small cell lung cancers (SCLC) around 15%. According to the National Lung Cancer Registry in Sweden, about 60% is AC, 18% SqCC and 12% SCLC.[13] The choice of treatment is based on stage of the disease; surgical resection with lobectomy or pneumonectomy and mediastinal lymph node dissection is the treatment for local disease (stage I, II and some stage III) as well as platinum-based adjuvant chemotherapy (for stage IB or higher). For metastatic lung cancer the treatment landscape has changed drastically with the introduction of targeted therapy with tyrosine kinase inhibitors and immune checkpoint inhibitors as a complement to chemotherapy. To decide on treatment tumours need to be evaluated for *EGFR, BRAF, ALK* and *ROS1* mutations for tyrosine kinase inhibitor and PD-L1 expression for immune checkpoint inhibitor treatment.[16]

The lungs having an extensive microvascular network and a favourable microenvironment make them the organs most often affected by metastatic spread of other cancers.[17]

A pulmonary tumour can be a primary lung cancer, a metastasis or a benign nodule. When diagnosing a pulmonary tumour, it is important to distinguish between different types of primary lung cancer and different types of lung metastases as the oncological and surgical treatment is different.

Colorectal cancer and lung metastases

Epidemiology

CRC is the third most common cancer in the world affecting 1.4 million people each year. In Sweden, about 6500 patients are diagnosed each year.[13] Metastatic spread to distant organs is considered the main reason for morbidity and mortality in CRC patients and approximately 56% of patients with CRC die from their cancer.[18] The lungs are the most common extra-abdominal site of metastasis. About 20% of CRC patients have metastatic disease at the time of diagnosis and this figure has been relatively stable over two decades.[19] About 20% subsequently develop metastatic disease.[20]

A large epidemiologic study on 49096 patients from the Swedish Cancer Registry showed that metastatic patterns differ between colon and rectal cancer with lung metastases being more frequent in rectal cancer. It was noteworthy that in rectal cancer patients with low stage primary tumour lung metastases were almost as common as liver metastases.[21]

In a population-based study from France, 19% of patients with CRC had metastases at diagnosis and of those 11% had lung metastases.[22] The proportion with synchronous lung metastases increased over time from 5.7% to 17%. Of the patients with metastases at diagnosis, the majority had both lung and liver metastases (61%), only 31% had metastases confined to the lungs (0.6% of all CRC cases in the study). Of the patients with synchronous lung metastases, 4.1% were resected for cure. The cumulative rate of developing metachronous lung metastases was 0.9% at 1 year, 4.2 % at 3 years and 5.8% at 5 years. In 54% of the patients diagnosed with metachronous lung metastasis, metastatic spread was confined to the lungs. The proportion of metachronous lung metastasis resected for cure was 14.3%. For lung location alone the proportion was 24.1% and for associated metastases it was only 3.3%. The risk of developing lung metastases was related to location of the primary tumour with rectal cancer patients having higher risk of both synchronous and metachronous lung metastases compared to patients with colon cancer.[22]

A Danish nationwide cohort study investigating occurrence of synchronous lung metastases in CRC patients as well as their outcome found a lower proportion of patients having synchronous lung metastases or 7.5%. Of these patients 37% had metastases confined to the lungs, similar to the French study. Interestingly, when looking at the group with lung metastases exclusively to the lungs the prevalence increased from 1.9% in 2001-2004 to 3.7% in 2009-2011. The same trend was seen in the French study and is most likely due to increased use of computed tomography (CT) scan instead of plain thoracic X-ray for staging. Similar to the French study 60% had both liver and lung metastases. In this study, advanced age, recent years of diagnosis, and rectal cancer were associated with higher risk of synchronous lung metastases. Proportion of patients with metastases confined to the lungs that underwent PM was low or 3.8%.[23]

In a population-based study from Iceland only 1% of all patients diagnosed with CRC in the years 1984 to 2008 underwent PM.[24] That study stretched over a long period of time and the use of PM as part of treatment of mCRC patients with lung metastases increased over the years.

In a more recent study from USA covering 28% of the US population, 20% (38,660/192,969) of CRC patients had distant metastases, and 26% (9920/38,660) of those had lung metastases.[25] Lung metastases were more common in rectal cancer as in both the French and the Danish studies. Other factors that increased the risk of lung metastasis was higher age, black race, N1 disease, having metastases in two or all three extrapulmonary metastatic sites studied (brain, bone, liver), and elevated carcinoembryonic antigen (CEA) value. Factors associated with lower risk of having lung metastasis were high grade tumours, one extrapulmonary metastatic site (brain, bone or liver) and primary tumour in the right colon.

It is interesting that these last two studies show association between increased age and synchronous lung metastases as the opposite has been reported for liver metastases.[26] Of note in the French study, age was not a risk factor for synchronous or metachronous lung metastasis.[22]

In a large study including over 38000 CRC patients diagnosed in the years 2010-2014 with single-organ metastases, 9.5 times more patients had liver only metastases (n=34694) compared to lung only metastases (n=3634) and in that study patients with lung metastases were older, more likely to be female and having the primary tumour located in the rectum compared to patients with liver metastases. Of these patients 8% of patients with lung metastases had undergone metastasectomy compared to 16% of the liver metastases patients.[27]

Pulmonary metastasectomy

As a part of standard treatment of CRC, patient follow up includes CT scans to detect asymptomatic metastases followed by surgical resection in selected patients. PM is part of a curative treatment in metastatic colorectal cancer (mCRC) and is recommended in both NCCN and NICE guidelines.[10-12]

Indications

Thomford and Clagett published in 1965 indications for PM that are still valid with some adjustments. They stressed that (1) the primary tumour should be under control, (2) no other metastatic disease elsewhere, (3) the lung metastases needs to be resectable and limited to one lung and (4) the operative risk needs to be acceptable for the patient.[7] In 2005 Kondo et al. published the new, modern indications for PM adding to the indications and updating two. Today, criteria (2) have been modified to no other extrapulmonary metastasis or if present it can be controlled by surgery or other treatment and (3) the lung metastases are thought to be completely resectable even if

they are on both sides. They added three more indications: (1) existence of effective systemic chemotherapy as a combined treatment, (2) difficulty of differential diagnosis from primary lung cancer, (3) no other effective treatment except for PM and (4) symptomatic lung metastases, e.g. pneumothorax or hemoptysis.[28]

In 2008 a survey about the practice of PM amongst the members of European Society of Thoracic Surgeons (ESTS) revealed that PM was a small part of their clinical volume, or up to 10%. Most of the responders or 99.3% performed PM for metastases from CRC. A large part (about 90%) discussed the cases in a multidisciplinary meeting. CT scan was used for the detection of metastases by all surgeons and positron emission tomography (PET) was used additionally by 44% of the surgeons. Most surgeons followed the aforementioned guidelines and considered unresectable primary tumour and incomplete metastasectomy a contraindication for PM.[29]

Operative technique

Operative technique has shifted toward minimal invasive surgery with video-assisted thoracoscopic surgery (VATS). The old recommendation of thoracotomy for possibility to palpate the lung is disappearing. A systemic review of eight studies on PM with VATS vs open thoracotomy approach noted a slightly higher odds of 1-, 3- and 5-year recurrence-free (RFS) and overall survival (OS) for patients treated with VATS but only 3-year OS was statistically significant.[30] VATS was the preferred approach for 29% of the surgeons in the ESTS survey from 2008.[29]

Wedge resection is the most common type of resection used in PM because the metastases are often peripheral in the lung or in 2/3 of the cases.[31] Additionally, it saves lung tissue compared to anatomic resections (segmentectomy or lobectomy). Moreover, a study by Vogelsang et al. showed that non-anatomic resection for PMs had better prognosis compared to anatomic resections.[32] However, recently a study found that patients with *KRAS* mutated tumours had better outcomes if they were operated with segmentectomy compared to wedge resection. They had longer time to pulmonary recurrence, a lower risk of resection margin recurrence, and improved median OS. This difference was not found in patients with wild-type *KRAS* tumours.[33] Another study (that did not look specifically at *KRAS* mutations) compared 455 wedge resections with 98 segmentectomies and found an improved 5-year OS, 5-year RFS and deceased risk for local recurrence in patients treated with segmentectomy.[34]

Systematic mediastinal lymphadenectomy or sampling is standard in the surgical management of primary lung cancer. Whether systematic lymph node dissection or sampling should be performed when performing PM is controversial. The incidence of mediastinal lymph node metastases in mCRC patients undergoing PM is reported to be between 8 and 24% and it is a known negative prognostic factor.[35, 36] In a large study on 518 mCRC patients undergoing 720 PMs, 199 (28%) did not undergo lymph node dissection, 279 had negative lymph nodes and 40 had positive nodes. Mediastinal lymph node metastasis was a negative prognostic factor and lymph node dissection did not prolong survival. The sensitivity of a PET scan to detect lymph node metastases in

this study was only 35%. The authors recommended that lymph node dissection should be performed routinely in mCRC patients as it gives prognostic information and can potentially direct further treatment.[37] A study from France on 320 patients where 140 had positive lymph nodes, did not find a statistically significant difference in median survival for patients with hilar vs. mediastinal disease.[38] The same authors found in a study of 106 patients with mediastinal involvement that the lymph node ratio was a more reliable prognostic factor than lymph node involvement.[39] A multicentre study from Spain on 522 patients found that lymph node assessment was performed in 48% of the patients and 10% of those had lymph node metastases with 20% of those having systemic nodal dissection, 35% having systemic nodal sampling and 45% having minor lymphadenectomy. Five-year disease-specific survival was 58% in those without nodal metastases, 24% with nodal metastases and 44% in those with unknown lymph node status.[40] In the ESTS survey from 2008, 55% indicated that they regularly sampled mediastinal nodes at the time of PM.[29]

Survival

Pfannschmidt et al. published a systematic review in 2010 for PM in mCRC and included 11 studies with 1307 patients operated on after 1990. The 5-year OS was 40-68%.[41] In a meta-analysis of 25 studies published between 2000 and 2011 including 2925 patients 5-year OS ranged between 27-68%.[42]

In a review of 8361 patients from 21 studies published between 2005-2015, 5-year survival rate after the first PM was between 24-82% and median survival was 35-70 months.[43] Salah et al. published a pooled analysis of 7 studies reporting on repeated PMs including 759 patients were 148 had undergone repeated PM. The 5-year OS for the whole group was 58%, for the patients undergoing one PM it was 52% vs 71% for patients undergoing second PM.[44] Looking at studies reporting on patients operated from 2000 and later the 5-year OS was 40-82% (**Table I**). For the most part, the series report good results in highly selected patients, but long-term disease-free survival (DFS) remains scarce.

The prognosis of mCRC patients treated with PM have improved over the years with better diagnostic imaging, improved oncologic treatment and surgical care. A study comparing mCRC patients treated with PM from 1985-1999 vs. 2000-2007 found that in the later period 5-year OS was 63.5% compared to 35.1% in the earlier period. The group treated during the later period were also more often treated for extrathoracic metastases and received adjuvant chemotherapy more often.[45]

Epidemiologic studies have shown that isolated lung metastases can be associated with longer survival compared to isolated liver metastases.[19, 21, 46, 47] A study on over 38.000 mCRC cases with synchronous single site metastases (lung or liver) revealed on an unadjusted analysis that median survival was longer for patients with lung metastases compared with those with liver metastases for left-sided and right-sided tumours whereas rectosigmoid and rectal cancers showed no difference. Moreover, on a multivariate analysis, patients with liver metastases had worse surival compared to patients with lung metastases.[27]

In a nationwide study from the Netherlands on 160.278 CRC patients whereof 33421 had synchronous metastases, diagnosed from 1996 to 2011 found that the proportion of patients with metastases confined to the lungs treated with PM increased from 4 to 10% during the first study period (1996-1999) compared to the last period (2008-2011). The median surival inreased from 16 to 24 months for all patients with metastases confined to the lungs comparing the same study periods.[19]

Prognostic factors

There are numerus studies on prognostic factors for resection of lung metastases in patients with mCRC and the data is often conflicting. **Table I** shows studies reporting data on patients operated on from 2000 and later as well as one large study reporting on patients operated from 1990.

In a systemic review by Pfannschmidt et al. six clinicopathological features are found as independent prognostic factors in single or few studies and either not reported or found to have no prognostic significance in the others: age, number of metastases, lymph node involvement, distribution of metastases (unilateral vs. bilateral), disease-free interval (DFI), primary tumour stage and CEA value. [41]

In a meta-analysis of 25 studies published between 2000-2011, by Gonzalez et al. including 2925 patients undergoing PM, the clinical variables associated with poor prognosis were: short DFI, multiple lung metastases, mediastinal or hilar lymph node involvement, and elevated pre-thoracotomy CEA levels.[42]

In a pooled analysis of individual data on 759 patients from seven studies three negative prognostic factors were identified: elevated CEA value, more than 2 metastases and DFI >36 months.[44]

In a best evidence article published in 2016 that looked at 19 papers (one meta-analysis, one systematic review and 17 retrospective studies) it was suggested that patients considered for PM for mCRC should be evaluated according to following factors: size and number of metastases, CEA level before resection and the response to induction chemotherapy.[48]

Reported prognostic factors of survival after PM in mCRC patients:

Age

Older age was reported as a prognostic factor in a study by Blackmon et al. and Cho et al. with age >60 and >70 years, respectively predicting poorer survival after PM[49, 50] and Onaitis et al. reported younger age (<65 years) predicting pulmonary recurrence.[51]

Gender

Female sex was a risk factor for pulmonary recurrence in the study by Onaitis et al.[51] contradictory to the study by Blackmon et al. where male sex was a risk factor for poorer survival after PM.[49]

Location and stage of the primary tumour

Rectal cancer has been reported having worse survival following PM compared to colon cancer.[52] Although not studied extensively, lymph node status (N status) of the primary tumour have been reported as a prognostic factor[53] and in one study it was a significant prognostic factor for lung metastases from rectal cancer but not colon cancer.[54]

Size and number of metastases

Multiple metastases compared to solitary metastasis have been found to be a prognostic factor by several studies (**Table I**) and also in a meta-analysis.[42] In most published series the largest part of the patients have solitary metastasis (**Table I**). Size of metastasis is less studied as a prognostic factor but has been reported.[55]

Lymph node status

Mediastinal and hilar lymph node involvement has been found to be prognostic factor with involvement leading to worse survival, also in a meta-analysis.[42]

Preoperative carcinoembryonic antigen (CEA)

An elevated CEA level is often reported as a negative prognostic factor[55-59] but there are many studies that reported it as not being a significant prognostic factor.[45, 54, 60] However, a meta-analysis on 19 studies showed that an elevated CEA was associated with an increased risk of death.[42]

Disease-free interval

Short DFI have been identified as a risk factor in several studies (see **Table I**) and was associated with shorter survival in a meta-analysis by Gonzales et al.[42] It has been pointed out that due to definition differences that direct comparison of DFI between studies is of limited value.[43]

Previously resected liver metastases

Data regarding history of resection of liver metastases is conflicting and many studies report previously resected liver metastases as not having impact on survival after PM[61-63] and a meta-analysis including seven studies confirmed that it was not a negative prognostic factor.[42] However, in 2018 a meta-analysis on individual data on 3501 patients from 17 studies concluded that a history of liver metastases resection was in fact a negative prognostic factor for survival.[64]

Biomarkers

Novel prognostic markers are being reported for mCRC patients treated with PM. A study evaluating *KRAS* and *BRAF* mutational status in the primary tumours of 180 mCRC patients treated with PM showed it was strongly correlated to survival with 5-year survival being 0%, 51.7% and 100% in patients with *BRAF* mutation, *KRAS* mutation and wild type *BRAF* and *KRAS*, respectively.[65] Overexpression of c-MET, pSTAT3 and high stromal heat-shock protein 27 analysed on resected lung metastases has been associated with worse survival.[66, 67]

Table I. Studies on pulmonary metastasectomy in CRC

Study period	N of patients	Selection of patients	Mean age (range) years	N of metastases	Chemotherapy in relation to PM	Median survival (range) months	5-year OS (%)	Prognostic factors of prolonged survival on a multivariate analysis	Post op mortality 30/90 days (%)	Reference
2000-2014	216 Colon: 93 Rectum: 123	R0	67 (37-85)	151 single, 56 multipla	Neoadjuvant: 19 Adjuvant: 99	68 (50.6-84.6)	56	Age <60, single LM, size of LM<3cm, DFI >24 months, no N2 stage of the primary tumour, adjuvant chemotherapy, high RBM3 expression in LM	0/0.46	Vidarsdottir et al. 2020 (paper III)
2002-2009	420	NR	64	256 single, 164 multiple	Neoadjuvant: 38 Adjuvant: 123	47.1	40	LM <2cm, single LM, number of LM, no lymph node invovlement	1/1	Nanji et al. (2018)[68]
2001-2016	203 Right colon:37 Left colon: 33 Rectum: 133	R0: 197 R1/R2: 6	61.7 (9-73)	133 single 70 multiple	Adjuvant: 151	NR	37	N0 of the primary tumour, no previous liver metastasectomy, adjuvant chemotherapy, single metastasis	NR	Menna et al. (2018)[69]
2004-2008 multicenter	785 Colon: 365 Rectum: 420	R0	66 (29-89)	583 single, 202 multiple	Adjuvant: 376	Not reached over the follow up of 65 months	68.1	Age <70 years, DFI>24 months, no extrathoracal metastasis treated curatively before PM, normal CEA, <3 LMs	0/0	Okumura et al. (2017)[70]
2008-2010 multicenter	522 Colon: 252 Rectum: 267	R0	NR	310 single, 212 multiple	Neoadjuvant: 111 Adjuvant: 316	54.9 (DSS)	46.1 (DSS)	DFI >12 months, normal CEA, unilateral disease, no thoracic lymph node involvment	0.38	Embun et al. (2016)[40]
2005-2010	354	R0	64.1	250 single, 104 multiple	Neoadjuvant: 27 Adjuvant: 122	NA	64.3	ASA score, <2 LM, other surgical resection than pneumonectomy	0.3	Pages et al. (2016)[71]
2001-2006 multicenter	841	R0	62.4 (27-85)	Median 1.54 (1-6)	NR	NR	63.1	N stage of primary tumour, <3 LMs, unilateral disease, DFI >24 months, normal CEA	NR	Hirosawa et al. (2015)[72]
2008-2014	190	NR	65 (36-87)	158 single, 32 multiple	NR	NR	82	N/A	0/0	Hunt et al. (2015)[73]
2003-2010	84	R0	NR	62 single, 22 multiple	NR	31.1 (1.5-88)	41.3 (DSS)	Single LM, metachronous LM, not detected during adjuvant chemotherapy for primary tumour	0/0	Cho et al. (2103)[74]
1990-2008 multicenter	1030	R0: 979 R1, R2: 51	64 (58-71)	597 single, 433 multiple	Neoadjuvant or adjuvant: 241	69.5	53.5	Tumour size, normal CEA, no lymph node involvement, R0	NR	Iida et al. (2013)[55]
2000-2010	229 Colon: 130 Rectum: 85 Both: 14	R0: 196 R1/R2: 33	60 (24-82)	Median: 2 (1-16)	Neoadjuvant: 114	70.1	55.4	Age <60, female gender, <3 LMs	0/NR	Blackmon et al. (2012)[49]
2001-2007	125 Colon: 35 Rectum: 90	R0	60 (32-80)	77 single, 48 multiple	Adjuvant chemotherapy: 41	37	48	No pulmonary recurrencec <6 months, no extrapulmonary metastasectomy, no lymph node involvement, normal CEA level	NR	Hwang et al. (2010)[56]

LM: Lung metastasis, DFI: disease-free interval; DSS; disease-specific survival; CEA: carcinoembryonic antigen; PM: pulmonary metastasectomy; RBM3: RNA-binding motif protein 3; NR: not reported.

Chemotherapy and pulmonary metastasectomy

As of now there is no consensus regarding the use of chemotherapy, neoadjuvant or adjuvant, in connection to PM in mCRC patients. International guidelines recommend the same perioperative oncologic treatment as for liver metastases and the recommendations are based on research on liver metastases. Various proportion of patients have received chemotherapy in published studies, from 7.6-55% and 27-80% for neoadjuvant and adjuvant chemotherapy, respectively.[50, 71, 75-77] A recent systematic review and meta-analysis including 18 studies with 3885 patients found that adjuvant chemotherapy did not provide survival benefit for patients undergoing PM for mCRC although in most of the studies patients were treated with 5-fluorouracil (5-FU) and not the more effective oxaliplatin based treatment. In the review neither neoadjuvant or adjuvant treatment had effect on RFS or OS.[78] In fact, a study from Japan comparing survival between different study periods showed that OS for patients treated with adjuvant chemotherapy was significantly better for patients treated between 2005-2008 vs 2000-2004 and 1990 to 1999 with 5-year OS 70%, 47% and 32%, respectively. There was a difference in chemotherapy regimen between periods with FOLFOX or FOLFIRI with or without bevacizumab mainly given to patients in the latest period vs. 5-FU and leucovorin and 5-FU monotherapy during the other the other periods. Moreover, there was no significant difference in survival between study periods in patients not receiving adjuvant chemotherapy.[79]

A study by Park et al. on 221 patients, whereof 176 received adjuvant chemotherapy, showed a DFS benefit in low risk patients, but patients treated with adjuvant chemotherapy had no OS benefit compared to patients treated with surgery alone.[76] In a study from Canada exploratory analysis suggested a survival benefit among chemo-naïve patients.[75] One large single centre study with 615 patients (75% treated with adjuvant chemotherapy) showed OS benefit of adjuvant chemotherapy but not neoadjuvant therapy. No information was given on chemotherapy agents used.[50]

The use of neoadjuvant and adjuvant chemotherapy is more standardized in the treatment of liver metastases from CRC and there is evidence for better DFS in patients treated with adjuvant chemotherapy vs. surgery alone.[80]

The evidence for the benefit of pulmonary metastasectomy

The evidence for benefit of PM in mCRC is based on single arm follow up studies of highly selected patients and its practice has been criticised. In this criticism Tom Treasure has been the strongest advocate. In 2019 the anticipated, prospective, randomized controlled trial of his, the PulMiCC trial, was stopped due to poor and worsening recruitment with only 65 randomized patients between December 2010 and December 2016. The 5-year estimated survival of the patients undergoing PM in this trial was 38% vs 29% in matched control patients that did not undergo operation. The study was underpowered, and this difference did not reach statistical significance.[81] The problem with conducting a randomized study today is that even the advocates of the

need of such trial, as the PulMiCC trial researchers accept that there is a group of patients where the argument for PM is so convincing that they can´t be reasonably randomised. Patients who are young and fit with solitary lung metastasis would be excluded and then you are left with the problem of randomising a borderline group of patients and if such a study would show no benefit of PM it would not say anything about the effect of PM in patients excluded from randomisation in the study.

A retrospective study published in 2014 matched patients treated with chemotherapy and PM against patients treated with chemotherapy without PM. The groups were matched for age, gender, stage and location of the primary tumour (colon vs rectum), but there was a difference between the groups in number of metastases (more patients with two metastases in the chemotherapy control group and more patients with a single metastasis in the PM group) and the median CEA value was higher in the chemotherapy control group (median 2.3 and 16 in the PM and chemotherapy group, respectively). This analysis showed a significantly increased survival for patients in the PM group. The median survival was 21.8 and 18.9 months for different chemotherapy regimens vs. 44.5 months for operated patients. There were 43 patients in each group and the study period was long, from 1980 to 2006. The authors concluded that PM was of value in patients with mCRC.[54]

It is possible that the survival benefit seen in the follow-up studies published in the literature as well as the meta-analyses on published series is due to selection of patients with favourable biology and early detection. More and more metastasectomies are being performed and they have been made possible by better oncological therapies, that might be the reason for the benefit, raising the possibility of reverse causation meaning that longer survival gives opportunities for more treatments rather than more treatments necessarily being the cause of longer survival.[82]

Resection of lung metastases in CRC is today a well-accepted treatment in selected patients despite the lack of randomized controlled trials and is the recommended treatment in international guidelines.[10-12] It can be performed safely with low morbidity and mortality. The reported complication rates are 1.7-15.7% and operative mortality 0-1.3%.[24, 34, 83] Herein, it remains widely practiced and it is likely to continue to be.

Genetics & Colorectal Cancer

CRC is a heterogenous disease at the molecular level. CRC was one of the first solid tumours to be molecularly characterised and a model describing accumulation of genetic and epigenetic events leading to adenoma and carcinoma formation (adenoma-carcinoma sequence) was published in 1990 by Fearon and Vogelstein. The model gives understanding of the role of driver mutations in tumour suppressor genes (e.g., *APC*, *TP53* and *SMAD4*) and (proto)oncogenes (e.g., *KRAS, PIK3CA*) that confer growth advantages and give rise to CRC progression.[84, 85] Since the original description information on molecular pathogenesis of CRC has expanded and this traditional adenoma-carcinoma sequence is thought to be behind only 50-60% of CRCs. Other

CRCs are thought to develop through other routes e.g., the serrated pathway (serrated adenomas with frequent *BRAF* mutations) and colitis-associated CRC with *TP53* mutations.[86]

Three major molecular pathways involved in origin and progression of CRC have been described: the chromosomal instability (CIN), the microsatellite instability (MSI) and the CpG island methylator phenotype (CIMP) pathways (**Table II**).[87]

Table II. Major molecular pathways in colorectal cancer

Molecular pathway	Characteristics	Genes involved /specific pathways
CIN	Alterations in number and structure of chromosomes. Combination of oncogene activation and tumour suppressor gene inactivation. The classic carcinoma-adenoma sequence.	*APC, TP53, PTEN, KRAS, PIK3CA* Wnt signalling pathway, MAPK pathway
MSI	Generalised instability of short tandemly repeated DNA sequences known as microsatellites. Result of a mutation in one of the MMR genes (hereditary MSI tumours) or silencing of the MLH1 promoter by hypermethylation (sporadic MSI tumours),	MMR genes: *MLH1, MSH2, MSH6, PMS2*
CIMP	Widespread hypermethylation of promoter CpG island loci. Silencing of MLH1 gene through hypermethylation (sporadic MSI tumours).	*MLH1, BRAF*

CIN: chromosomal instabilty, MSI: microsatellite instability, CIMP:CpG island methylator phenotype

NGS studies of the CRC genome have shown that the number of mutations is very high, each tumour harbouring around 75 mutations and individual CRCs contain around 15 predicted driver mutations. The heterogeneity between cancers is remarkable with few mutations being the same in two given primary CRCs.[86] A large study with whole genome sequencing (WGS) on 429 metastases from CRC found that compared to primary CRC the metastases showed significant enrichment in 4 out of 23 driver genes (*TP53, ZFP36L2, KRAS* and *APC*). Of identified driver genes only *PIK3CA* mutations were decreased in the metastases.[88]

About 70% of CRC cases are sporadic and due to somatic mutations and 25% are familial CRC, were patients have predisposition to develop CRC caused by single-nucleotide polymorphism and/or germline minor variant in oncogene or tumour suppressor gene. About 5% have hereditary diseases caused by inactivating mutations in oncogene or tumour suppressor gene. The hereditary syndromes involved in CRC and the genes involved are shown in **Table III**.

Table III. Hereditary colorectal cancer syndromes

Syndrome	Gene
Hereditary non-polyposis colorectal cancer (HNNPCC) (Lynch syndrome)	*MLH1, MSH2, MSH6, MLH3, MSH3, PMS2*
Familial adenomatous polyposis (FAP)	*APC*
MUTYH-associated poplyposis	*MUTYH*
Peutz-Jeghers syndrome	*STK11*
Juvenile polyposis syndrome	*SMAD4, BMPRIA*
PTEN hamartoma tumours syndrome	*PTEN*
Polymerase proofreading-associated polyposis (PPAP)	*POLE, POLD1*

The Cancer Genome Atlas Network study on 276 CRCs showed that activation of the Wnt signalling pathway and inactivation of TGF-β signalling pathway are altered in nearly all CRCs. They identified 32 recurrently mutated genes and after removal of non-expressed genes there were 15 and 17 genes in hypermutated and non-hypermutated tumours, respectively. The only genes that were mutated in both types of tumours were *APC* and *TCF7L2*. When hypermutated cancers were excluded, colon and rectum cancers had similar patterns of genomic alteration. Twenty-four genes were significantly mutated. The expected genes, *APC, TP53, SMAD4, PIK3CA* and *KRAS* and additionally *ARID1A, SOX9* and *FAM123B/WTX* were frequently mutated.[85]

The "big bang" model of human CRC postulates that most driver events in CRC including *APC, KRAS, TP53* mutations and most subclonal mutations occur before or early after the transition to carcinoma. According to this model public mutations already present in the first transformed tumour cell will persist and be found in all tumour cells. Private mutations that arise early will become pervasive in the final tumour while remaining non-dominant and as a result create subclones. Mutations that occur late are only present in small regions of the tumour. Thus according to this model the timing of a mutation rather than clonal selection determines the pervasiveness.[89]

A new classification system called the consensus molecular subtypes in CRC was published in 2015. According to this system CRC is divided into four groups based on gene expressed molecular characteristics (**Table IV**).[90] This molecular subtyping of CRC was formed by international consortium of six groups previously reporting gene-expression based CRC classifications and represent a major step forward in CRC management. The first group CMS1 is the hypermutated group with microsatellite instability (MSI) comprising about 14% of all CRCs while the large chromosomal instability group (CIN) comprising 85% of CRCs is divided into three groups, CMS2-4. About 13% of CRCs have mixed features that possibly represent intra-tumoral heterogeneity or a transition phenotype, typically with characteristics of multiple CMS. No genetic aberration is limited to a subtype although *BRAF* mutations are frequent in CMS1 and there is an overrepresentation of *KRAS* mutations in CMS3. Loree et al. showed that prevalence of CMS1 rises from the cecum to the ascending colon and hepatic flexure before falling throughout the rest of the colon. CMS2 shows an increase moving distally from the cecum, with a peak in the sigmoid and rectosigmoid regions. CMS3 shows a gradual decrease moving distally, while CMS4 stays relatively stable a part from an increased prevalence in the descending colon.[91] CMS4 tumours have

higher rate of colitis-related CRC.[92] Notably, in the previously mentioned study with WGS on CRC metastases, CMS classification was possible for 91 of the cases and of those no tumour classified as CMS3.[88] A study found that CMS might serve as a predictive factor for the efficacy of chemotherapy in mCRC with irinotecan being superior to oxaliplatin in CMS4. CMS1 showed poor response with anti-EGFR therapy and CMS2 particularly good response compared to the other subtypes.[93]

Table IV. Consensus molecular subtypes in colorectal cancer

CMS type	% of CRC cases	Molecular characteristics	Genetic drivers	Associated precursors	Gene-expression signature	Sideness	Prognosis
CMS1 (MSI immune)	14	Hypermutated, microsatellite unstable, strong immune activation	*BRAF* mutations	Serrated	Immune infiltration and activation	More common in right-sided tumours	Worse after relapse
CMS2 (Canonical)	37	SCNA high CIN phenotyoe	*APC*	Tubular	Epithelial differentation, Wnt and MYC signaling activation	More common in left-sided tumours	Better surival after relapse compared to the other groups
CMS3 (Metabolic)	13	Mixed MSI status, SCNA low, CIMP low	*KRAS* mutations	Unkown	Metabolic dysregulation		
CMS4 (Mesenchymal)	23	SCNA high	Unknown	Serrated	Mesenchymal transition, complement activation, immunosuppression Stromal infiltration, TGFβ activation, angiogenesis		Worse relapse free and overall survival

CIN: chromosomal instability pathway CIMP: CpG island methylator phenotype, SCNA: Somatic copy number alterations, TGF: transforming growth factor

Specific mutations

In CRC two oncogenes have been widely studied, *KRAS* and *BRAF* in relation to resistance to anti EGFR therapies.

KRAS mutations are found in about 40% of CRC, typically in codon 12 or 13. They are of clinical importance since patients with *KRAS* mutated tumours do not benefit from EGFR inhibitor therapy with cetuximab and panitumumab. *KRAS* mutations are more frequent in CRCs with lung metastases.[94-96] Furthermore, they have been connected to more diffuse metastatic pattern and a high risk of lung recurrence in mCRC patients treated with PM.[97] One study has shown difference in prognosis after PM based on the type of *KRAS* mutation, with exon 2 codon 13 mutation having better outcome following PM compared to codon 12 mutations.[98] In a study on mCRC patients with single organ metastases *KRAS* mutation was associated with decreased OS. However when looking at patients with metastases confined to the lungs *KRAS* mutation was not associated with worse survival. On the other hand in patients with metastases confined to the liver *KRAS* mutation was associated with worse survival in patients with left colon and rectal cancers but not right sided tumours.[27]

BRAF can be found in about 5-15% of mCRC and is also a contraindication for *EGFR* inhibitor therapy. The typical mutation, *BRAF* V600 is 95% of the mutations observed. *BRAF* mutated tumours have morphological, clinical and therapeutic characteristics that differ from wild type *BRAF* tumours. *BRAF* mutations are usually mutually exclusive with *KRAS* mutations but rare cases harbouring both *KRAS* and *BRAF* mutations have been reported, 0.3% (8/2530) in three randomised trials on mCRC.[99] CRC with *BRAF* V600 mutation is associated with right sided tumour, patients older than 70 years, female gender, and mucinous tumours with peritoneal and nodal metastases. Lung metastases are less frequent.[47] *BRAF* mutations are associated with MSI and present in 40-60% of sporadic MSI CRC tumours but not described in Lynch syndrome. Patients with *BRAF* mutations have in general worse prognosis compared to patients with wild type *BRAF* with median OS of 12 compared to 30 months, respectively.[99]

Diagnosis

The lungs are a common site of metastases and differentiation between a primary lung cancer and metastasis is highly important when planning treatment. When differentiating between primary lung cancer and metastasis, diagnostic pathology is of essence. IHC is an essential aid to morphology in the diagnosis of a pulmonary tumour in combination with patient's former cancer history, age, gender, risk factors and radiology.

Immunohistochemistry (IHC)

IHC is a technique used to detect specific antigens (typically proteins) in tissues or cells based on antigen-antibody recognition. It uses the specificity provided by the binding of an antibody with its antigen. IHC has a history dating back more than 70 years. However it was not until the 1990s it became generally used in diagnostic pathology.[100] The role of IHC in diagnostic pathology has expanded and it is used in about 11-38% of cases in the diagnosis of carcinoma.[101]

The IHC process involves the following key steps shown in **Figure 1.** Unmasking is needed because formalin-fixation and paraffin-embedding might have altered the antigens. The indirect method or sandwich procedure is more commonly used. This method has few advantages: versatility is increased, primary antibody can be used at a higher working dilution and the secondary antibody is readily prepared with high specificity and affinity.[102]

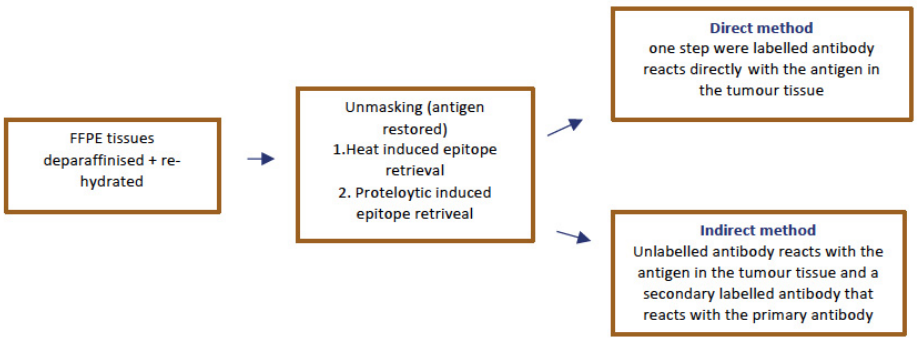


Figure 1. The process of immunohistochemistry
FFPE: formalin-fixed paraffin embedded

Pitfalls

Many factors can affect the outcome of IHC stainings. They can be divided into:

- Pre-analytical factors, i.e., factors relating to the tissue: pre-fixation conditions such as freezing, time to fixation, the fixation medium used, fixation time, processing (dehydration etc.) and sectioning/drying of slides etc.
- Analytical factors, i.e., factors relating to the staining: epitope retrieval, blocking, choice of antibody clone, other reagents, time and temperature for primary and secondary antibody, detection system, platform and double staining.
- Post-analytical factors, i.e., factors relating to the evaluation: choice of cut-off value and evaluation of the correct cells.
- Factors that can lead to weak or absent immunoreactivity include inadequate fixation, incomplete dehydration and prolonged heating. Background staining can be caused by too thick sections of tissue, delayed fixation and necrotic tissue.

Tissue microarray (TMA)

IHC analysis on whole tumour slides works well in the clinical situation but when looking at great number of tumours and markers it costs both time, tissue and money. An alternative, well established method is the TMA technique, first described by Kononen et al.[103] In this method small cores (in our studies 1 mm) are taken from the formalin-fixed paraffin-embedded (FFPE) samples of interest (donor blocks) and placed in a recipient block, thus placing cores from multiple tumours in a single block. Sections from the recipient block can then be sectioned and prepared as any other tumour block (see **Figure 2**). In addition to the clear advantage of TMAs with respect to the amount of tissue used and thus preservation of valuable tissue due to the relatively

small amount of tissue required for construction, TMAs have advantages in several other key areas including reproducibility, analysis time, cost and applicability. As TMAs contain small cores representing all samples on a single slide, assay conditions are uniform across all samples, leading to greater reproducibility of results and reduced assay analysis time than individual slide analysis of each sample, and reagent costs are kept at a minimum since only one (or few) slides need to be analysed. Additionally, tissue analysis methods that can be performed on whole tissue sections can be applied to TMAs, including IHC. Furthermore, recent advances have enabled efficient extraction of DNA and/or RNA from TMA cores, enabling TMA technology to be coupled with advanced molecular testing.[104] In the diagnostics of a pulmonary tumour, the tissue is often a small biopsy and TMAs can in that sense imitate the clinical situation. However, it must also be recognized that TMAs may miss or underestimate heterogeneous protein expression, and while biopsies are practically always well fixed, TMA cores may be taken from poorly fixed areas especially in large tumours, which may affect the validity of TMA-based data.

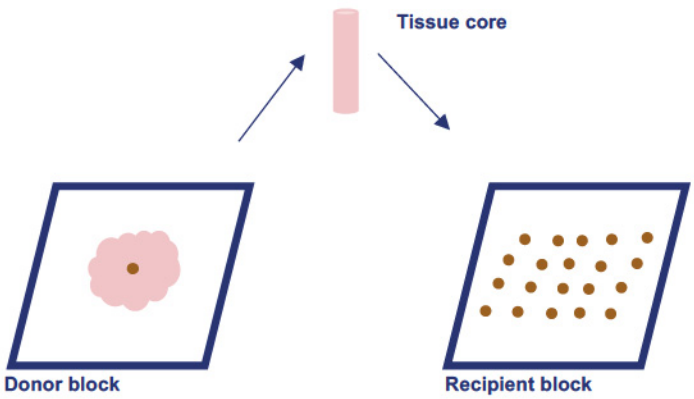


Figure 2. Tissue microarray preparation

Immunohistochemical markers

IHC markers are used alone or more commonly in panels to confirm or reject a diagnosis. Here follows a short description of the ten IHC markers that were used in studies I and II.

Thyroid transcription factor-1 (TTF-1)

TTF-1 is a 38 kd homeodomain containing DNA-binding protein originally identified in follicular cells of the thyroid and subsequently in pneumocytes. The gene is located on chromosome 14q13. This marker is recommended in the diagnosis of primary lung AC and it is one of the most reliable method to distinguish primary lung AC from both

primary lung SqCC and metastatic AC. It is also highly specific for thyroid ACs and high-grade neuroendocrine carcinomas.[105, 106]

Several different clones are available, e.g., 8G7G3/1, SPT24 and SP141. The different clones are directed against different epitopes leading to different IHC staining patterns. Comparison between clone SPT24 and 8G7G3/1 has shown SPT24 to be more often positive in lung SqCC and sometimes in lung metastases[105, 107-109] while clone 8G7G3/1 has weaker staining intensity and was less frequently positive in lung metastases.[107, 109] However, one study has shown lung metastases positive with both clones in about same extent.[110] NordiQC, an organisation contracted for external technical quality assurance of IHC staining by more than 200 different pathology departments (also in Sweden) recommends a more sensitive marker e.g. clone SPT24 in the diagnostics of a pulmonary tumour.[111] Due to this many pathology departments in Sweden use the clone SPT24 instead of clone 8G7G3/1 that is recommended in the WHO guidelines for diagnostics of lung cancer.[112, 113]

TTF-1 expression in primary tumours outside of lungs have been studied quite extensively but few studies have focused on lung metastases which is more applicable to the clinical situation of lung pathology. Extra-pulmonary tumours that to a varying extent have shown TTF-1 positivity are e.g., colorectal,[107, 109, 110, 114, 115] gastric,[105] cervical, endometrial and ovarian,[116-119] breast,[120, 121] and prostatic[105, 122] ACs as well as primary brain tumours,[123-125] salivary gland tumours,[105] urothelial[105], renal cell[115] and cholangiocarcinomas.[126] Some lung metastases from colorectal,[107, 110, 114, 115] renal, prostatic, ovarian, endometrial and salivary gland carcinoma[115] have been reported as TTF-1 positive.

Napsin A

Napsin A is a novel aspartic proteinase of the pepsin family involved in the maturation of surfactant protein B. It is found mainly in lung and kidney. Together with TTF-1, napsin A serves as a diagnostic marker for lung AC and differentiates it from lung SqCC.[127] Clear cell and renal cell carcinomas [128] as well as clear cell ovarian and endometrial carcinomas[129, 130] express napsin A as well. Some studies have shown that napsin A is both more sensitive and more specific than TTF-1 for diagnosing lung AC.[127, 131-133] Renal cell carcinoma metastases have also been shown to retain napsin A positivity.[128]

CK5

CK5 is a basal cytokeratin and a marker of squamous differentiation. It can be positive in mesotheliomas, basal-like breast carcinomas, thymomas and some urothelial carcinomas and salivary gland tumours. It is used to distinguish mesothelioma from lung AC.[134] It stains basal cells of the prostate and basal/myoepithelial cells of the breast and may thus be used to rule out invasion in these cancers.

p63

p63 is a nuclear marker that is a member of the p53 gene family but does not appear to be a tumour suppressor gene. It is said to determine squamous differentiation (p63+) as part of panel but is today seldom used in lung pathology due to limited specificity.

It may be used to differentiate renal collecting duct carcinoma (p63-, PAX8+) from upper tract urothelial carcinoma (p63+,PAX8-). [135] Other use of this marker is to rule out invasion in breast tumours and salivary gland tumours by determining presence of myoepithelial cells.

p40

p40 is one of ten p63 isoforms, and a nuclear marker of squamous cell differentiation. It is used to differentiate between primary lung AC and a primary lung SqCC. It is more specific for lung SqCC compared to p63 with p40 staining 1% of lung AC compared to 31% for p63.[136]

CK7

CK7 is an intermediate filament protein (54 kDa) that recognizes the simple epithelium found in most glandular and transitional epithelium, but not in stratified squamous epithelium. CK7 is a basic cytokeratin and is generally expressed in e.g., ovary, lung, breast and upper gastrointestinal ACs, but not CRC.

CK20

CK20 is an epithelial marker with restricted expression compared to CK7. Colorectal and urothelial carcinomas are typically positive. It can be less sensitive in poorly differentiated colon carcinoma. CK20 is often used together with CK7 to distinguish ovarian, lung, and breast carcinomas (CK7+, CK20-) from colon carcinomas (CK7-, CK20+) and renal, prostatic carcinomas, and hepatocellular carcinoma (CK7-, CK20-).[137-139]

CDX2

CDX2 is a homeobox gene that encodes an intestine-specific transcription factor. The CDX2 protein is expressed in primary and metastatic CRC. It is a useful marker for establishing gastrointestinal origin since most ACs of the colon, small intestines, stomach and oesophagus are CDX2 positive. CDX2 have been shown to be more sensitive but less specific compared to CK20 in diagnosing CRC, at least in some studies.[140, 141] CDX2 has been shown to be useful in differentiating between mucinous lung AC and metastatic mucinous CRC.[142] Of note, a rare form of lung AC with enteric differentiation is often CDX2 positive.[143]

GATA3

GATA3 is one of six members of the GATA family of transcription factors. It is a nuclear marker expressed in many epithelial tumours including most breast and urothelial carcinomas and as such used to diagnose those tumours. An increasing number of other tumours have though been found to express GATA3 rather frequently, including epithelial skin tumours, chromophobe renal cell carcinoma and mesothelioma. Some lung cancers (both ACs and SqCC), ductal pancreatic and salivary gland ACs may also be GATA3 positive.[144,145] About 70% of triple negative breast cancers are GATA3 positive.[146]

PAX8

PAX8 is a transcription factor located on chromosome 2p13 and critical for the development of eye, urinary, thyroid and reproductive organs. PAX8 is expressed in carcinomas arising in endometrium, endocervix, ovary, thyroid, kidney, and urothelium but not in primary lung AC. This suggest that PAX8 has potential value for differential diagnosis of primary lung carcinoma from lung metastases and may be helpful in determining primary site. All ACs of the breast, prostate, stomach, colon, bladder, salivary gland, bile duct and ampulla, hepatocellular carcinoma, adrenal cortical tumours, acinar cell carcinomas of the pancreas, and all types of lung carcinomas that have been investigated have been consistently PAX8 negative.[147]

The IHC markers used in paper I and II and their main use in clinical practice and how they can be used in panels to help diagnose tumour´s origin is shown in **Table V**.

Table V. IHC markers and their main use

IHC markers	IHC staining pattern	Main use
TTF-1, napsin A	Nuclear, cytoplasmic	Markers of pulmonary AC, also thyroid and neuroendocrine (TTF-1+), RCC, clear cell ovarian/ endometrial cancer (napsin A+)
CK5, p40, p63	Cytoplasmic, nuclear, nuclear	Markers of squamous differentiation
CK7, CK20	Cytoplasmic	Distinguish ovarian, pulmonary and breast carcinomas (CK7+, CK20 -) from colon (CK7-, CK20+), urothelial (CK7+, CK20+) renal and prostatic carcinomas (CK7- ,CK20-)
CDX2	Nuclear	Marker of gastrointestinal origin
GATA3	Nuclear	Marker for breast and urothelial carcinoma
PAX8	Nuclear	Marker of renal, ovarian and endometrial cancer (also thyroid and thymic tumours)

RNA binding-motif protein 3 (RBM3)

The RNA-binding motif protein 3 (RBM3) was first identified in a human fetal brain tissue cDNA library.[148] RBM3 binds to RNA and DNA and facilitates protein synthesis in response to stress and is transcriptionally upregulated in response to hypoxia, ischemia and cold.[149, 150] RBM3 has anti-apoptotic, cell proliferation enhancement, and a proto-oncogene function. RBM3 has emerged as a prognostic biomarker in several types of solid tumours.[151-156] High RBM3 expression has been associated with improved survival in CRC.[157, 158] Studies on mCRC, as well as ovarian, testicular, and pancreatic cancer, have also revealed a potential link between RBM3 and improved response to platinum-based chemotherapy.[151, 156, 158, 159] A study of 1800 CRC cases found loss of RBM3 expression to be associated with advanced tumour stage and right-sided tumours.[160] Strong RBM3 expression was seen in left-sided and rectal tumours, in 84% and 90%, respectively. Interestingly, this study showed a significant prognostic effect of RBM3 in colon cancer but not in rectal cancer[160] and this difference between colon and rectal cancer was also noted in another study.[157] However there is a contradicting study on 455 mCRC cases (both from colon and rectum) were RBM3 expression was

a prognostic factor in both colon and rectal tumours.[158] The expression of RBM3 has been shown to be reduced in metastatic compared to primary melanoma[153] and to be higher in metastatic compared to primary pancreato-biliary periampullary cancers.[159]

Next-generation sequencing (NGS)

Cancer is driven by genetic mutations. Next-generation sequencing (NGS) is a high-throughput DNA sequencing methodology that has been rapidly evolving since it first came on the market in 2004. It has become an affordable and powerful tool to assess complete mutational profiles of cancer patients. It is the basis for personalised medicine in cancer allowing tumours to be genotyped and specific treatments directed against specific gene mutations e.g. *EGFR* tyrosine kinase inhibitors in NSCLC and *BRAF* inhibitor in melanoma can be used. One study showed that with sequencing the outcome of one in four patients with advanced cancer can be approved[161] and another WGS study on 429 metastases from CRC found that for 55% of the patients one or more targeted treatments were potentially available based on the molecular profile of their cancer.[88]

In paper IV we used the Illumina TruSight Tumor 26 (TST26) and TruSight Oncology 500 (TSO500) panels to study the concordance in mutational status between primary CRC and paired liver and lung metastases as well as spatial and temporal heterogeneity and tumour mutational burden (TMB) within and between primary tumours and metastases.

There are two main NGS platforms: Ion-Torrent and Illumina. Ion-Torrent uses pH (voltage) change on nucleotide binding and amplifies with bead and emulsion while Illumina uses fluorescence to detect nucleotides and amplifies on a flow cell. The base pair read length is a little longer for Ion Torrent or up to 400 bp while being 300 bp for Illumina. Ion Torrent has a shorter read time compared to Illumina but has homopolymer error while Illumina has errors in GC rich regions.[162]

Here the focus is on the Illumina platform as it is used in paper IV.

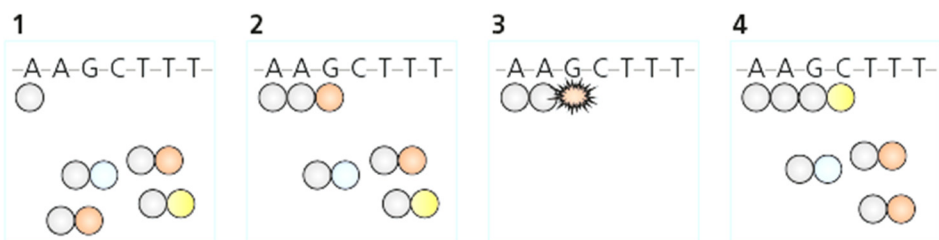


Figure 3. Illumina sequencing by synthesis. Adapted from Comprehensive genomic profiling, Karger Publishers Ltd. 2020.[163]

(1) Nucleotides with fluorescent tags compete for the next space on a DNA strand. (2) A complementary tagged nucleotide is incorporated, blocking further binding. (3) Washing removes the unbound tagged nucleotides, and the signal from a fluorocent emission is captured. (4) The fluorescent tag and blocker are washed away,allowing the process to be repeated in the next cycle. This process happens simultaneously for all DNA strands in a cluster and all clusters on the flow cell.

Illumina sequencing is based on a technique known as "bridge amplification". DNA molecules with adapters ligated on each end are used as substrates for repeated amplification synthesis reactions on a surface that contains oligonucleotide sequences complementary to a ligated adapter. The oligonucleotides on the slide are spaced such that the DNA, which is then subjected to repeated rounds of amplification, creates clonal "clusters" consisting of about 1000 copies of each oligonucleotide fragment. Each glass slide can support millions of parallel cluster reactions. During the synthesis reactions, modified nucleotides, corresponding to each of the four bases, each with a different fluorescent label, are incorporated and then detected. The nucleotides also act as terminators of synthesis for each reaction, which are unblocked after detection for the next round of synthesis. The reactions are repeated for 300 or more rounds. The use of fluorescent detection increases the speed of detection due to direct imaging, in contrast to camera-based imaging.

NGS has pre-analytic, analytic and post-analytic limiting factors:

- *Pre-analytic factors:* The ratio of tumour cells to non-tumour cells must be above detection limit. The best quality of the DNA is reached with minimum of cold ischemia. When using FFPE tissue the formalin fixation causes cross-linking and fragmentates nucleic acids leading to low-quality and low molecular weight DNA. When using FFPE DNA as in our fourth study, AT/GC drop out, PCR errors and deamination artifacts are more likely.
- *Analytic errors* can occur due to numerous factors: wrong template, inaccurate dilution of the libraries and batch variations for reagents.
- *Post-analytic factors*: The data volumes acquired by NGS are substantial. Data needs to be optimized and meaningful variations differentiated from non-meaningful variations. There are limitations in the knowledge on how to interpret novel or rare mutations. Also, the information needs to be put in clinical context and integrated into the medical care of patients. Tumour heterogeneity can be a problem and mutations can mean different things in different tumour types.[163]

Aims of the thesis

Overall aim

To investigate lung metastases, firstly different IHC markers to improve the histopathological diagnostics of them, secondly, find prognostic factors for mCRC patients treated with PM and thirdly to look at the genetic heterogeneity of CRC with lung metastases.

Paper I
To compare the staining properties of three available TTF-1 clones, 8G7G3/1, SPT24 and SP141 in large cohorts of primary lung cancer and lung metastases from epithelial tumours. This is the first study to compare all these three clones of TTF-1 in primary lung cancer and epithelial lung metastases.

Paper II
To evaluate expression of ten commonly used IHC markers, TTF-1, napsin A, CK5, p40, p63, CK7, CK20, CDX2, GATA3 and PAX8 in primary lung cancers and lung metastases to investigate their usefulness in the differential diagnostics of lung tumours.

Paper III
To examine prognostic factors including the expression of RBM3 in lung metastases and paired primary tumours from a well-defined, retrospective cohort of mCRC patients treated with PM and to further assess the utility of RBM3 as a biomarker for better selection of patients who will benefit from surgical resection and chemotherapy.

Paper IV
To describe the mutational profiles and spatial and temporal genetic heterogeneity of primary CRC tumours and matched lung and liver metastases.

Patients & methods

Study population

Primary lung cancer cohort

Papers I and II in this thesis included 665 resected primary lung cancers from 657 individuals (eight cases with two synchronous primary lung cancers each) originally included in three independent unselective cohorts (see **Figure 4**). Types of primary lung tumours in the combined cohort were the following: 415 ACs, 193 SqCC, 12 large cell carcinomas, eight adenosquamous carcinomas, six sarcomatoid carcinomas, 21 large cell neuroendocrine carcinomas (six of which had AC component), three SCLC and seven carcinoid tumours. The AC and SqCC components of combined cases were evaluated separately and grouped with AC and SqCC.

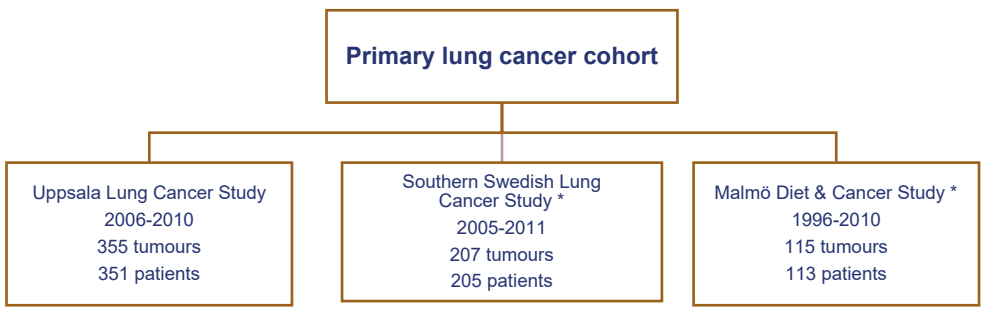


Figure 4. The primary lung cancer cohort

*12 cases were included in both of these cohorts and each of these cases was only included once in the present study.

Lung metastases cohort

The lung metastases cohort is a retrospective, consecutive study from the Skåne University Hospital in Lund, where epithelial malignant tumours consistent with metastases were included. The cohort included 440 resected lung metastases from 351 patients. Sixty cases had two and 12 cases had three resected metastases to the lungs originating from the same tumour. There were 12 and 15 metastases with no tumour tissue on the TMA slides in papers I and II and therefore 428 and 425 lung metastases

included in the studies, respectively. The site of origin for the lung metastases can be seen in **Table VI.**

Table VI. Types of tumours in the lung metastases cohort

Site of origin	N of patients	N of lung metastases (%)
CRC	221	280 (65)
Renal cell carcinoma	34	42 (10)
Breast carcinoma	23	27 (6.3)
Other GI cancers	17	19 (4.4)
Oesophagus	3	3 (0.7)
Liver	2	4 (0.9)
Gallblader	1	1 (0.2)
Pancreas	5	5 (1.2)
Small bowel	2	2 (0.5)
Appendix	4	4 (0.9)
Gynecologic cancers	15	20 (4.7))
Cervix	6	9 (2.1)
Ovarium	2	2 (0.5)
Uterus	5	6 (1.4)
Vulva	2	3 (0.7)
Urothelial cancers	8	8 (1.9)
Bladder	7	7 1.6)
Renal pelvis	1	1 (0.2)
Prostatic cancer	10	11 (2.6)
Head & neck cancers	5	9 (2.1)
Salivary gland	2	4 (0.9)
Tonsil	2	4 (0.9)
Mouth	1	1 (0.2)
Thymoma	4	5 (1.2)
Skin cancer	4	4 (0.9)
Thyroid cancer	3	3 (0.7)
Total:	**344**	**428**

The NGS study population

For the study in paper IV, 27 patients were selected from the lung metastases cohort. The selected patients had been surgically treated for CRC with both lung and liver metastases and had available tumour tissue from all three sites suitable for NGS.

Definitions

- Synchronous metastasis was defined as a metastasis diagnosed at the time of or within six months from the diagnosis of the primary tumour.
- DFI was defined as the interval between curative resection of the primary tumour and surgery of the first lung metastasis. For patients with a history of liver resection prior to PM, the DFI was defined as the period between surgery of the liver metastasis and PM.
- Time to recurrence was calculated from the date of PM to the date of the first documented recurrence.
- The follow-up time was measured from the first PM when more than one was performed.
- Left sided colon cancer was defined as splenic flexure and distal colon to the rectum.
- Prolonged air leakage after PM was defined as a need for a chest tube drainage for five or more days.

TMA construction

The TMAs were constructed with two cores, 1 mm in diameter, for the Uppsala Lung Cancer Study and Malmö Diet Cancer Study and lung metastases cohorts, whereas three cores were used from each tumour for the Southern Swedish Lung Cancer Study cohort. For the lung metastases cohort cores were taken from each metastasis if several lung metastases had been surgically treated. Cores where also taken from the primary tumour when available (70% of the cases). Reasons for not including the primary tumour were nonsurgical treatment (e.g., radiation therapy of prostatic cancer, transurethral resection of bladder cancer, or combined chemotherapy and radiation therapy of advanced disease), neoadjuvant treatment with limited viable tumour in the surgical specimen (e.g., rectal cancer), primary tumour surgically treated outside the Region Skåne county, and tumour blocks missing due to inclusion in other studies or for unknown reason. For CRC cases, cores were also taken from surgically treated liver metastases in cases with available primary tumour.

Immunohistochemical evaluation

Paper I & II

The 4-µm-thick tissue sections from the TMAs were automatically pre-treated and stained on a Ventana BenchMark Ultra (Ventana Medical Systems, Tucson, AZ) with the IHC markers, TTF1, three different clones in paper I, and nine other IHC markers: napsin A, CK5, CK7, p40, p63, CK20, CDX2, GATA3, and PAX8 in paper II. Detailed information of the antibodies, pre-treatment, and control tissue is found in **Table VII** and representative IHC images are shown in **Figure 5.**

The fraction of IHC-positive viable tumour cells was divided into five categories: less than 1%, 1% to 9%, 10% to 24%, 25% to 49%, and 50% or more.

Special care was taken to not interpret e.g., trapped alveolar or bronchiolar epithelium as positive tumour cells. Cytoplasmic staining for napsin A, CK5, CK7, and CK20 and nuclear staining for TTF-1, CDX2, p40, p63, GATA3, and PAX8 were considered positive.

Table VII. Details on IHC markers used in papers I and II

Antibody	Clone	Vendor	Staining pattern	Positive control	Negative control	Internal positive control [a]
TTF-1	8G7G3/1	Ventana Medical Systems (Tuscon, AZ)	Nuclear	Thyroid	Tonsil, kidney	Type II pneumocytes, terminal bronchioles
TTF-1	SP141	Ventana	Nuclear	Thyroid	Tonsil, kidney	Type II pneumocytes, terminal bronchioles
TTF-1	SPT24*	Leica Biosystems (Nussloch, Germany)	Nuclear	Thyroid	Tonsil, kidney	Type II pneumocytes, terminal bronchioles
CK7	SP52	Ventana	Cytoplasmic	Liver (bile ducts)	Liver (hepatocytes)	Type II pneumocytes
CK20	SP33	Ventana	Cytoplasmic	Appendix	Tonsil, liver	Gastrointestinal ACs
CDX2	EPR2764Y	Ventana	Nuclear	Pancreas (ducts), small intestine [b]	Tonsil [b]	Gastrointestinal ACs
CK5	XM26	Leica	Cytoplasmic	Tonsil (epithelium)	Liver, appendix	Basal cells
p40	BC28	Histolab/Biogcare Medical (Concord, CA)	Nuclear	Tonsil (epithelium)	Thyroid, kidney	Basal cells
p63	4A4	Ventana	Nuclear	Tonisl (epithelium)	Thyroid, kidney	Basal cells
Napsin A	IP64	Leica	Cytoplasmic	Kidney (proximal tubules)	Tonsil, thyroid	Type II pneumocytes, alveolar macrophages
GATA3	L50-823	Cell Marque (Rocklin, CA)	Nuclear	Kidney (collecting ducts), tonsil (T lymphcytes)	Tonsil (B lymphocytes), thyroid	T lymphocytes
PAX8	MRQ-50	Cell Marque	Nuclear	Kidney, thyroid	Muscle	Lymphocytes

*For the primary lung cancers staining was performed on a Dako Autostainer. a Internal control here denotes cell types that were present on all slides but not for all individual cases. b for about half of the slides, either tonsil was missing as negative control or appendix was used as positive control

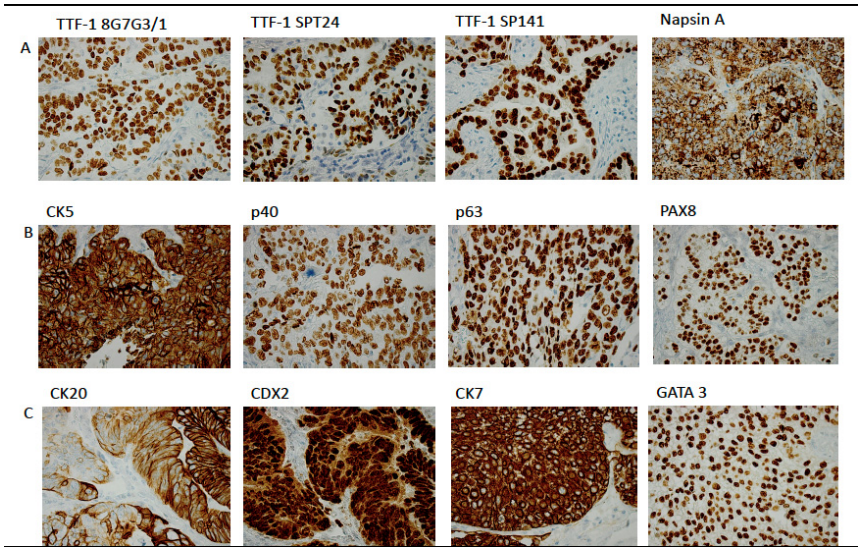


Figure 5. Representative IHC images of the IHC markers used in paper I and II.
A. Lung adenocarcinoma. B. Lung squamous cell cancer except PAX8 a lung metastasis from renal cell cancer . C. CK20 and CDX2 a lung metastasis from CRC and CK7 and GATA3 a lung metastasis from urothelial cancer.

Paper III

4 µm thick TMA sections were automatically pre-treated with the PT-link system (Agilent Technologies, Santa Clara, CA) and stained on an Autostainer Plus (Agilent Technologies) with a monoclonal antibody; RBM3 clone AMAb90655 (Atlas Antibodies, Bromma, Sweden) diluted 1:750. Representative IHC images are shown in **Figure 6**.

The fraction of tumour cells with positive nuclear RBM3 expression was denoted as 0 (<1%), 1 (1-24%), 2 (25-49%), 3 (50-74%) or 4 (≥75%), and the intensity of the staining as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). A nuclear score (0-12) was then constructed by multiplying the intensity and fraction of stained tumour cells.

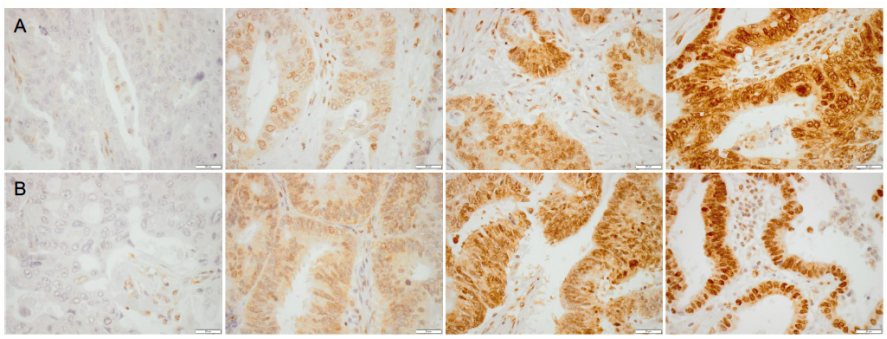


Figure 6. IHC images of RBM3 staining. From left to right (negative, weak, moderate, strong). A. CRC primary tumour. B. CRC lung metastasis.

NGS analysis

In paper IV DNA from tissue cores from 27 cases of primary CRC with matched resected liver and lung metastases (thereby three tumours from each case) were sequenced with Illumina TST26 gene panel. One tissue core, 1 mm i diameter was taken from FFPE tissue from the surgical specimens. Only one lung metastasis and one liver metastasis were sampled even if a patient had been surgically treated for multiple metastases. Five of these cases were selected to be analysed with Illumina TSO500 gene panel. The selection was based on the mutational profile of the cases with focus on cases with discordant mutations between tumour sites. For this analysis several different areas from the primary tumour as well as all available metastases were included, and macro-dissected whole tissue sections were used instead of tissue cores. Normal tissue from the same surgical specimen were also included.

Next generation sequencing

DNA was extracted from the tumour tissue samples using the GeneRead DNA FFPE Kit (Qiagen, Hilden, Germany). More than 10% viable tumour cells were required from all sampled areas, and delta Ct values of <8 were required for all samples after DNA extraction. Libraries were prepared using the TST26 or TSO500 gene panels (Illumina, San Diego, CA), respectively, and NGS was carried out on a MiSeq (TST26) or NextSeq (TSO500) instrument (Illumina) in accordance with the manufacturer's instructions. Variant calling and annotations were performed using the standard reporter software/analysis pipeline (Illumina), for TST26 in accordance with previous routine in the clinical setting.[164] Additional filtration was possible for mutations detected with TSO500 if also detected in normal tissue.

TruSight Tumor 26 (TST26)

A platform of 26 genes (**Table VIII**) selected as relevant from Collage of American Pathologists (CAP) and The National Comprehensive Cancer Network (NCCN) guidelines and late-stage pharmaceutical trials to provide a view of somatic variation in colon, lung, melanoma, gastric and ovarian tumours. This panel has specifically been optimized for use on FFPE tissue.

TableVIII. Genes in the TST26 panel

AKT 1	*EGFR*	*GNAS*	*NRAS*	*STK11*
ALK	*ERBB2*	*KIT*	*PDGFRA*	*TB53*
APC	*FBXW7*	*KRAS*	*PIK3CA*	
BRAF	*FGFR2*	*MAP2K1*	*PTEN*	
CDH1	*FOXL2*	*MET*	*SMAD4*	
CTNNB1	*GNAQ*	*MSH6*	*SRC*	

TruSight Oncology 500 (TSO500)

A pan-cancer platform that analysis 523 cancer relevant genes (**Table IX**) and identifies known and emerging biomarkers. It identifies somatic variants, including small variants, gene fusions and splice variants. It can also measure TMB and MSI. The platform covers a large number of genes as well as 1.94 megabases of the genome to measure TMB.[163] It has been shown to measure TMB with great accuracy comparable to WGS.[165]

Table IX. The genes in the TSO500 panel

ABL1	*BRD4*	*CUX1*	*FAM175A*	*GATA6*	*IGF1*	*MAP3K13*	*NOTCH4*	***POLE***	***RPTOR***	***TAF1***
ABL2	***BRIP1***	*CXCR4*	*FAM46C*	*GEN1*	*IGF1R*	*MAP3K14*	*NPM1*	*PPARG*	***RUNX1***	***TBX3***
ACVR1	*BTG1*	***CYLD***	***FANCA***	*GID4*	***IGF2***	*MAP3K4*	***NRAS***	*PPM1D*	*RUNX1T1*	*TCEB1*
ACVR1B	*BTK*	*DAXX*	***FANCC***	*GLI1*	*IKBKE*	***MAPK1***	***NRG1***	***PPP2R1A***	*RYBP*	*TCF3*
AKT1	***C11orf30***	*DCUN1D1*	***FANCD2***	***GNA11***	*IKZF1*	*MAPK3*	***NSD1***	***PPP2R2A***	*SDHA*	***TCF7L2***
AKT2	*CALR*	*DDR2*	***FANCE***	*GNA13*	*IL10*	*MAX*	***NTRK1***	*PPP6C*	*SDHAF2*	*TERC*
AKT3	*CARD11*	*DDX41*	***FANCF***	***GNAQ***	*IL7R*	*MCL1*	***NTRK2***	*PRDM1*	*SDHB*	*TERT*
ALK	***CASP8***	*DHX15*	***FANCG***	*GNAS*	*INHA*	*MDC1*	***NTRK3***	*PREX2*	*SDHC*	*TET1*
ALOX12B	*CBFB*	***DICER1***	***FANCI***	*GPR124*	*INHBA*	***MDM2***	*NUP3*	*PRKAR1A*	*SDHD*	***TET2***
ANKRD11	*CBL*	*DIS3*	*FANCL*	*GPS2*	*INPP4A*	***MDM4***	*NUTM1*	*PRKCI*	*SETBP1*	*TFE3*
ANKRD26	***CCND1***	*DNAJB1*	*FAS*	*GREM1*	*INPP4B*	***MED12***	*PAK1*	*PRKDC*	***SETD2***	*TFRC*
APC	***CCND2***	*DNMT1*	*FAT1*	*GRIN2A*	*INSR*	*MEF2B*	*PAK3*	*PRSS8*	*SF3B1*	*TGFBR1*
AR	***CCND3***	***DNMT3A***	***FBXW7***	*GRM3*	*IRF2*	*MEN1*	*PAK7*	***PTCH1***	*SH2B3*	***TGFBR2***
ARAF	***CCNE1***	*DNMT3B*	*FGF1*	*GSK3B*	*IRF4*	***MET***	***PALB2***	***PTEN***	*SH2D1A*	*TMEM127*
ARFRP1	*CD274*	*DOT1L*	*FGF10*	*HRF3A*	*IRS1*	*MGA*	*PARK2*	***PTPN11***	*SHQ1*	*TMPRSS2*
ARID1A	*CD276*	*E2F3*	*FGF14*	*H3F3B*	*IRS2*	*MITF*	***PARP1***	*PTPRD*	*SLIT2*	*TNFAIP3*
ARID1B	*CD74*	*EED*	*FGF19*	*H3F3C*	*JAK1*	***MLH1***	*PAX3*	*PTPRS*	*SLX4*	*TNFRSF14*
ARID2	*CD79A*	*EGFL7*	*FGF2*	***HGF***	*JAK2*	*MLL*	*PAX5*	*PTPRT*	***SMAD2***	*TOP1*
ARID5B	*CD79B*	***EGFR***	*FGF23*	*HIST1H1C*	*JAK3*	*MLLT3*	*PAX7*	*QKI*	*SMAD3*	*TOP2A*
ASXL1	*CDC73*	*EIF1AX*	*FGF3*	*HIST1H2BD*	*JUN*	*MPL*	*PAX8*	*RAB35*	***SMAD4***	***TP53***
ASXL2	*CDH1*	*EIF4A2*	*FGF4*	*HIST1H3A*	*KAT6A*	***MRE11A***	*PBRM1*	*RAC1*	***SMARCA4***	*TP63*
ATM	***CDK12***	*EIF4E*	*FGF5*	*HIST1H3B*	*KDM5A*	***MSH2***	*PDCD1*	*RAD21*	***SMARCB1***	*TRAF2*
ATR	***CDK4***	*EML4*	*FGF6*	*HIST1H3C*	*KDM5C*	***MSH3***	*PDCD1LG2*	***RAD50***	*SMARCD1*	*TRAF7*
ATRX	***CDK6***	*EP300*	*FGF7*	*HIST1H3D*	***KDM6A***	***MSH6***	***PDGFRA***	***RAD51***	*SMC1A*	***TSC1***
AURKA	*CDK8*	***EPCAM***	*FGF8*	*HIST1H3E*	***KDR***	*MST1*	***PDGFRB***	***RAD51B***	*SMC3*	***TSC2***
AURKB	***CDKN1A***	*EPHA3*	*FGF9*	*HIST1H3F*	***KEAP1***	***MST1R***	*PDK1*	***RAD51C***	*SMO*	*TSHR*
AXIN	***CDKN1B***	*EPHA5*	***FGFR1***	*HIST1H3G*	*KEL*	***MTOR***	*PDPK1*	***RAD51D***	*SNCAIP*	*U2AF1*
AXIN2	***CDKN2A***	*EPHA7*	***FGFR2***	*HIST1H3H*	*KIF5B*	***MUTYH***	*PGR*	*RAD52*	*SOCS1*	*VEGFA*
AXL	*CDKN2B*	*EPHB1*	***FGFR3***	*HIST1H3I*	***KIT***	*MYB*	*PHF6*	*RAD54L*	*SOX10*	***VHL***
B2M	*CDKN2C*	***ERBB2***	*FGFR4*	*HIST1H3J*	*KLF4*	***MYC***	*PHOX2B*	***RAF1***	*SOX17*	*VTCN1*

BAP1	*CEBPA*	***ERBB3***	*FH*	*HIST2H3A*	*KLHL6*	*MYCL1*	***PIK3C2B***	*RANBP2*	*SOX2*	*WISP3*
BARD1	*CENPA*	*ERBB4*	*FLCN*	*HIST2H3C*	*KMT2B*	*MYCN*	*PIK3C2G*	*RARA*	***SOX9***	*WT1*
BBC3	*CDH2*	*ERCC1*	*FLI1*	*HIST2H3D*	*KMT2C*	*MYD88*	*PIK3C3*	*RASA1*	*SPEN*	*XIAP*
BCL10	***CDH4***	***ERCC2***	***FLT1***	*HIST3H3*	***KMT2D***	*MYOD1*	***PIK3CA***	***RB1***	*SPOP*	***XPO1***
BCL2	***CHEK1***	***ERCC3***	***FLT3***	*HLA-A*	***KRAS***	*NAB2*	***PIK3CB***	*RBM10*	*SPTA1*	*XRCC2*
BCL2L1	***CHEK2***	***ERCC4***	***FLT4***	*HLA-B*	*LAMP1*	***NBN***	*PIK3CD*	*RECQL4*	***SRC***	*YAP1*
BCL2L11	*CIC*	***ERCC5***	*FOXA1*	*HLA-C*	*LATS1*	*NCOA3*	*PIK3CG*	*REL*	*SRSF2*	*YES1*
BCL2L2	*CREBBP*	*ERG*	*FOXL2*	*HNF1A*	*LATS2*	*NCOR1*	***PIK3R1***	***RET***	*STAG1*	*ZBTB2*
BCL6	***CRKL***	*ERRFI1*	*FOXO1*	*HNRNPK*	*LMO1*	*NEGR1*	***PIK3R2***	*RFWD2*	*STAG2*	*ZBTB7A*
BCOR	*CRLF2*	*ESR1*	*FOXP1*	*HOXB13*	*LRP1B*	***NF1***	*PIK3R3*	*RHEB*	*STAT3*	*ZFHX3*
BCORL1	***CSF1R***	*ETS1*	*FRS2*	***HRAS***	*LYN*	***NF2***	*PIM1*	*RHOA*	*STAT4*	*ZNF217*
BCR	*CSF3R*	*ETV1*	*FUBP1*	*HSD3B1*	*LZTR1*	*NFE2L2*	*PLCG2*	***RICTOR***	*STAT5A*	*ZNF703*
BIRC3	*CSNK1A1*	*ETV4*	*FYN*	*HSP90AA1*	***MAGI2***	*NFKBIA*	*PLK2*	*RIT1*	*STAT5B*	*ZRSR2*
BLM	*CTCF*	*ETV5*	*GABRA6*	*ICOSLG*	*MALT1*	*NKX2-1*	*PMAIP1*	***RNF43***	***STK11***	
BMPR1A	*CTLA4*	*ETV6*	*GATA1*	*ID3*	***MAP2K1***	*NKX3-1*	***PMS1***	***ROS1***	*STK40*	
BRAF	*CTNNA1*	*EWSR1*	*GATA2*	***IDH1***	***MAP2K2***	***NOTCH1***	***PMS2***	*RPS6KA4*	*SUFU*	
BRCA1	***CTNNB1***	***EZH2***	***GATA3***	*IDH2*	***MAP2K4***	***NOTCH2***	*PNRC1*	*RPS6KB1*	*SUZ12*	
BRCA2	*CUL3*	***FAM123B***	*GATA4*	*IFNGR1*	***MAP3K1***	*NOTCH3*	***POLD1***	*RPS6KB2*	*SYK*	

Bold: Genes that have been found mutated in CRC. Based on data from the The Cancer Genome Atlas Network and genes listed as significant genes in CRC at www.mycancergenome.org

Statistical methods

Paper I

The frequency of TTF-1 positive cases was compared between the three clones using a paired non-parametric test, Wilcoxon's test. A p-value of <0.05 was considered statistically significant. Receiver operating characteristics (ROC) analysis was used to identify the best cut-off for the TTF-1 clones to separate non-squamous lung cancers and lung AC, respectively, from other tumours. All analyses were performed with MedCalc Statistical Software version 14.12.0 (MedCalc Software, Ostend, Belgium).

Paper II

Descriptive statistics.

Paper III

The IBM SPPS Statistics version 26 (SPSS, Inc., Chicago, IL) software was used for all statistical calculations. The Kaplan-Meier method and log-rank test were used for survival analysis and Cox regression proportional hazard models were used for estimation of hazard ratios (HR) for death and recurrence. Graphic presentation of the cumulative hazard function for each variable was checked to see if the assumption of hazard proportionality was supported. Survival was assessed from the time of PM to the time of death or last follow-up. Wilcoxon signed-rank test was used for comparison of RMB3 expression in primary tumours and lung metastases. Classification and regression tree (CRT) analysis was applied to estimate the optimal prognostic cut-off for RBM3 expression. Chi-square test was used to evaluate associations of RBM3 expression in primary tumours and lung metastases, respectively, with established clinicopathological characteristics. All tests were two-sided and $p<0.05$ was considered statistically significant.

Paper IV

Fischer's exact test was used to compare mutation and concordance levels between tumour sites. Wilcoxon signed rank test was used to compare TMB between the primary tumours and the metastases. A p-value <0.05 was considered significant.

Ethics

The studies were conducted in adherence to the Declaration of Helsinki and approved by the regional ethical review boards in Uppsala, Dnr 2012/532 and Lund, Dnr 2004/762 and 2008/702, and Dnr 2007/445, 2008/35 and 2014/748, respectively.

Results

Paper I

Expression of TTF-1 in primary lung cancers

In the lung cancer cohorts there were 665 primary lung cancers from 657 patients, 54% women, with a median age of 68 years (range, 43-84 years) at the time of surgery.

If 1% was used as a cut-off value for positive TTF-1 staining for all three clones, then clone SPT24 and SP141 stained more cases of all histological subtypes except SCLC and sarcomatoid carcinomas (both of which we had few cases in our cohort) compared to clone 8G7G3/1.

Table X. Results of IHC staining with three different TTF-1 clones in primary lung cancers

	TTF-1 clone / cut of value for positive staining					
Tumour type	**8G7G31**		**SPT24**		**SP141***	
	≥1 %	≥10 %	≥1%	≥10%	≥1%	≥10%
AC (n=429)	380 (89%)	361 (84%)	397 (93%)	386 (90%)	388 (93%)	383 (91%)
SqCC (n=201)	0 (0%)	0 (0%)	12 (6%)	6 (3%)	16 (8%)	8 (4%)
Other[a]	23 (7%)	18 (37%)	30 (61%)	28 (57%)	30 (61%)	30 (61%)

[a] 21 SCLC, 21 LCNEC, 7 carcinoid, 12 large cell carcinomas, 6 sarcomatoid carcinomas.
*missing information in 10 AC and 4 SqCC cases.

Most lung ACs were positive with TTF-1 irrespective of what clone was used 89%, 93%, and 93%, were positive with TTF-1 clones 8G7G3/1, SPT24, and SP141, respectively. There was a difference in the staining of lung SqCC between clones. None was positive with clone 8G7G3/1 while SPT24 and SP141 were positive in 6% and 8% of the SqCC cases, respectively (**Table X**).

To separate lung AC from non-adenocarcinoma in lung and lung metastases a ROC analysis identified 1% positive cells as the best cut-off for the 8G7G3/1 clone and 10% for the SPT24 clone and 50% for the SP141 clone (**Table XI**).

When separating non-squamous lung cancer from cases with lung SqCC and lung metastases 1% was the best cut off for the SPT24 and 8G7G3/1 clones and 10% for the SP141 clone.

Table XI. Cut-off values for seperating primary lung AC from lung metastases

Clone	The best cut-off value (% positive cells)	Sensitivity (%)	Specificity (%)
8G7G3/1	1%	88	96
SPT24	10%	90	93
SP141	50%	89	94

Expression in lung metastases

The lung metastases cohort included metastases from 344 patients, 52% men, with a median age of 66 years (37-87 years) at the time of PM. Seventy-two of the patients were operated for more than one metastasis giving 428 evaluable metastases in the cohort. CRC metastases were the most common (66%) followed by renal cell carcinoma and breast cancer metastases. The types and number of cases can be seen in **Table VI** under the Patients & methods section.

Thirty (7%) of the cases in the lung metastases cohort were positive for TTF-1 with any of the clones and in eight cases with all three clones. All lung metastases that were positive with the 8G7G3/1 clone were positive with all 3 clones. Fifteen metastases were positive in both SPT24 and SP141 and three with SPT24 alone and four cases with SP141 alone. TTF-1–positive lung metastases were from colorectal, thyroid, urothelial, pancreatic, small bowel, and cervix carcinomas. All included lung metastases from the gastrointestinal tract other than CRC (n=19) were negative with TTF-1 clone 8G7G3/1. One metastasis of small bowel AC was positive with clones SPT24 and SP141 in less than 10% of the tumour cells. The primary tumour was TTF-1 negative. One metastasis of pancreatic AC was positive with the SPT24 clone in less than 10% of the tumour cells but negative with clones SP141 and 8G7G3/1.

Significantly more metastases are positive with clones SPT24 and SP141 compared to 8G7G3/1 when the cut off value is 1%. The difference is still significant with cut off value at 10% but at 25% there is no statistically significant difference between the threeTTF-1 clones (**Table XII**).

Table XII. Comparison of different cut-off values for TTF-1 clones 8G7G3/1, SPT24 and SP141

Cut-off for SPT24, SP141	N of positive cases 8G7G3/1 (cut -off 1%)	N of positive cases SPT24	N of positive cases SP141	p-value*
1%	8	26	27	<0.0001
10%	8	15	16	<0.005
25%	8	13	14	0.06

*Wilcoxon's test

Colorectal carcinoma

280 lung metastases from CRC were evaluated and were more often positive with clones SPT24 and SP141 compared to clone 8G7G3/1 with 2% (n=5), 7% (n=19) and 8% (n=21) positive with clone 8G7G3/1, SPT24 and SP141, respectively.

Five cases were positive with all three clones, while 13 cases were positive with clones SPT24 and SP141. One case was positive with clone SPT24 only, while three cases were positive with clone SP141 and negative with the other clones. There were no TTF-1 positive metastases from the 23 right-sided CRCs. Primary tumours from 166 of these patients were evaluated. Three (2%), seven (4%) and seven (4%) were TTF-1 positive with clones 8G7G3/1, SPT24 and SP141, respectively. All 8G7G3/1 positive case were positive with the other clones.

57 liver metastases from 48 CRC patients were evaluated and seven (12%) from six patients were TTF-1 positive. Five of the liver metastases were positive with all three clones and two with SPT24 and SP141 clones.

One interesting case of rectal AC was positive for TTF-1 with all three clones in >25% of the tumour cells of the primary tumour whereas the lung and liver metastases were TTF-1 negative with all three clones. Whole tumour sections of the metastases were also negative.

TTF-1 expression in the largest primary lung cancer and lung metastases groups is shown in **Table XIII**. Cut-off value for positive staining >1% of tumour cells and IHC figures from TTF-1 positive lung metastasis and lung SqCC are shown in **Figure** 7.

Table XIII. TTF-1 expression in primary lung cancer and the largest lung metastases groups

Primary lung cancer	**N of cases**	**8G7G3/1 n (%)**	**SPT24 n (%)**	**SP141 n (%)**
Adenocarcinoma	429	380 (89)	397 (93)	388 (93)
Squamous cell carcinoma	201	0	12 (6)	16 (8)
Lung metastases	N of cases	8G7G3/1 n (%)	SPT24 n (%)	SP141 n (%)
All	428	8 (2)	26 (6)	27 (6)
CRC	280	5 (2)	19 (7)	21 (8)
Renal cell carcinoma	42	0	0	0
Breast cancer	27	0	0	0

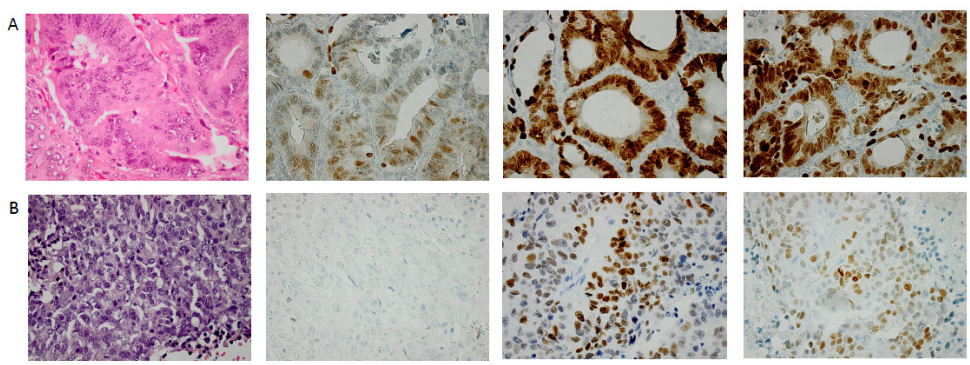


Figure 7. IHC staining with three different TTF-1 antibody clones in A) CRC lung metastasis and B) Primary lung SqCC. The stainings are H&E, TTF-1 clone 8G7G3/1, SPT24 and SP141 from left to right.

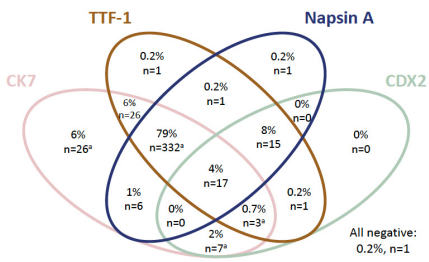
We compared the TTF-1 expression with clone 8G7G3/1 in the TMAs to whole tumour slides in 123 of the lung metastases (60% CRC origin). Concordance was 97%, in 118 of the cases both were negative and in one case both were positive. Moreover, we compared TTF-1 expression with clone SPT24 in 18 TTF-1 positive lung metastases (83% CRC origin). All cases were positive on both whole tumour slide and TMA, with 61% having the same score.

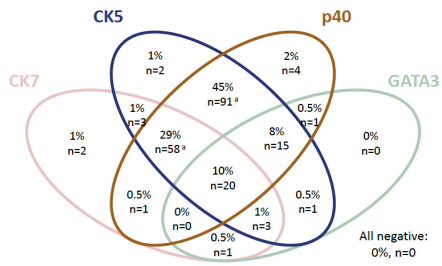
Paper II

IHC panels

Lung AC was characteristically positive for CK7, TTF-1 (here clone SPT24) and napsin A (**Figure 8**). However, only 68% of the cases expressed all these three and no other of the evaluated markers. One invasive mucinous type of AC was positive for both CK20, CDX2 and CK7 and at the same time negative for TTF-1 and napsin A. 83% of lung AC expressed both TTF-1 and napsin A and 92% at least one of the two markers. If using ≥1% positive tumour cells (instead of ≥10%) as cut-off for a positive staining the numbers were 86% and 94%, respectively. There were no other cases than lung AC in the study material with co-occurrence of TTF-1 and napsin A (also true if ≥1% positive tumour cells was used as cut-off for a positive staining).

The typical markers for lung SqCC were CK5, p40 and p63, with CK7 either positive or negative and negative for all other evaluated markers (**Figure 8**). This was true for 64% of the lung SqCC cases evaluated. All 13 cases of SqCC that were CDX2 positive were CK20 negative. IHC figures of lung AC and lung SqCC with untypical stainings are shown in **Figure 9**.





Tumour type	n	CK7	CK20	CDX2	CK5	p40	p63	TTF-1	Napsin A	GATA3	PAX8
Adenocarcinoma	431	99/99	4/2	11/7	0.5/0.5	2/0.2	26/10	93/90	88/84	4/2	0.5/0
Squamous cell carcinoma	202	46/44	2/1	13/7	97/96	97/94	98/97	6/3	2/0.5	30/20	2/2

Figure 8. Immunohistochemical profiles with ≥10% positive tumour cells defining a positive staining in the pictures. Table: Frequency (%) of positive primary lung cancers for different IHC stains presented as ≥1% or ≥10% positive tumour cells.

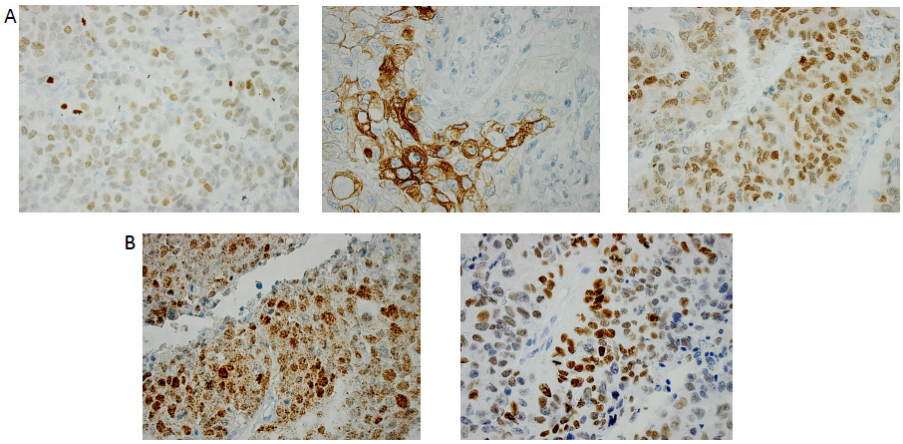
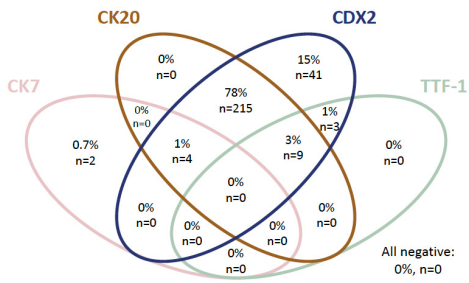


Figure 9. Untypical immunohistochemical stainings. A. Primary lung AC positive for GATA3, CK20 and CDX2. B. Primary lung SqCC positive for PAX8 and TTF-1 (clone SPT24.)

Lung metastases from colorectal cancer

Lung metastases from CRC (all AC whereof 18 mucinous) were CK20 positive in 83% of the cases and CDX2 positive in 99% and 78% were positive for both CK20 and CDX2 with all other markers negative. All lung metastases from mucinous AC were CK20 and CDX2 positive and one was CK7 positive. All other tested markers were negative. Rare cases expressed CK7, p63, and PAX8 and 4% were TTF-1 positive. A larger proportion of rectal cancers had deviant immune profile compared to colon tumours (overall 26% vs. 16%). Of 46 CK20 negative lung metastases from CRC, 30 had their primary tumour in the rectum. Additionally, five of six CK7 positive metastases were from rectal cancers. Two lung metastases from the same rectal cancer were CK7+/CK20-/CDX2-/PAX8+. IHC profile for CRC lung metastases can be seen in **Figure 10.**



Tumour origin	N	CK7	CK20	CDX2	CK5	p40	p63	TTF-1	Napsin A	GATA3	PAX8
Colorectal carcinoma	227	3/2	91/83	99/99	1/0	0/0	0.7/0.4	7/4	0/0	0/0	0.7/0.7

Figure 10. Immunohistochemical profiles for lung metastases from CRC with ≥10% positive tumour cells defining a positive staining. Table: Frequency (%) of positive CRC lung metastases for different immunohistochemical stains presented as ≥1% or ≥10% positive tumour cells.

Other lung metastases

Renal cell carcinoma metastases were positive for PAX8 in 74% and for CK7 and napsin A in 7% of the cases. PAX8 was positive with napsin A positive or negative and all other evaluated markers negative (the typical profile) in 71% of the cases. One of the renal cell carcinoma lung metastases was positive for both CDX2 and CK7 with all other evaluated markers negative i.e., the same immune profile as many upper gastrointestinal tumours. Lung metastases from breast cancer were positive for CK7 in 78% and CK5 in 15% of the cases. Most lung metastases from breast cancer (93%) and all metastases from urothelial carcinoma were positive for GATA3.

By using >50% positive tumour cells as a cut off for a positive GATA3 staining, 85% of lung breast cancer metastases and 100% of urothelial metastases were positive while only six other lung metastases and 26 primary lung cancers were positive (**Table XIV**).

Table XIV. GATA3 expression in primary lung cancer and lung metastases from breast and urothelial carcinoma at different cut-off values

N of GATA3 + cases (%)	Cut off >1%	Cut off >10%	Cut off >25%	Cut off >50%
Breast carcinoma LM	25 (93%)	25 (93%)	24 (89%)	23 (85%)
Urothelial carcinoma LM	8 (100%)	8 (100%)	8 (100%)	8 (100%)
Lung SqCC	62 (31%)	41 (20%)	34 (17%)	19 (9%)
Lung AC	18 (4%)	9 (2%)	6 (1)	6 (1%))

LM: lung metastasis

Frequency of the IHC markers in different types of lung metastases other than CRC where we had eight or more included cases in our study is shown in **Table XV** (more information on other tumour types where we had fewer cases in Table III in paper II).

Table XV. Frequency (%) of positive epithelial lung metastases for different immunohistochemical stains presented as ≥1% or ≥10% positive tumour cells

Tumour origin	N	CK7	CK20	CDX2	CK5	p40	p63	TTF-1	Napsin A	GATA3	PAX8
Renal cell carcinoma[a]	42	10/7	0/0	2/2	0/0	0/0	0/0	0/0	10/7	2/2	86/74
Breast carcinoma[b]	27	78/78	0/0	0/0	19/15	7/4	19/7	0/0	0/0	93/93	4/0
Gynecological carcinomas[c]	17	71/71	6/6	53/41	6/6	0/0	6/6	12/0	0/0	29/24	71/71
Prostatic carcinoma	11	0/0	9/0	55/36	0/0	0/0	0/0	0/0	0/0	0/0	9/0
SqCC[d]	11	18/18	0/0	60/50	100/100	100/100	100/100	0/0	0/0	36/36	18/0
Urothelial carcinoma	8	100/100	50/50	25/13	38/0	100/100	100/100	13/13	0/0	100/100	0/0

a. Thirty-three clear cell, 4 papillary, and 5 other/intermediate ACs
b. Twenty-six ACs of ductal/ no special type (whereof 1 mixed mucinous) and 1 malignant adenomyoepithelioma
c. Six from uterus (5 endometrioid ACs, 1 carcinosarcoma), 8 from cervix (6 ACs and 2 AC component of adenosquamous carcinomas), 2 from ovarium (1 clear cell and 1 mucinous AC), and 1 AC from vulva.
d. Four from tonsil, 3 from anus, 2 from esophagus, 1 from uterine cervix, and 1 from oral cavity.

Paper III

This study included 216 patients (130 males, 86 females) with a median age of 67 years (range, 37-85). The primary tumour was located in the rectum in 57% (n=123), left colon in 34% (n=74) and right colon in 9% (n=19). Fifty-four patients (25%) had synchronous metastasis. Most patients (70%) were treated for a solitary lung metastasis or 70%. Twenty-one patient had bilateral disease, 15 were treated with two separate surgeries and six patients through a sternotomy. The number of PMs increased during the study period from 32 PMs during the first five years of the study to 113 during the last five years (**Figure 11**).

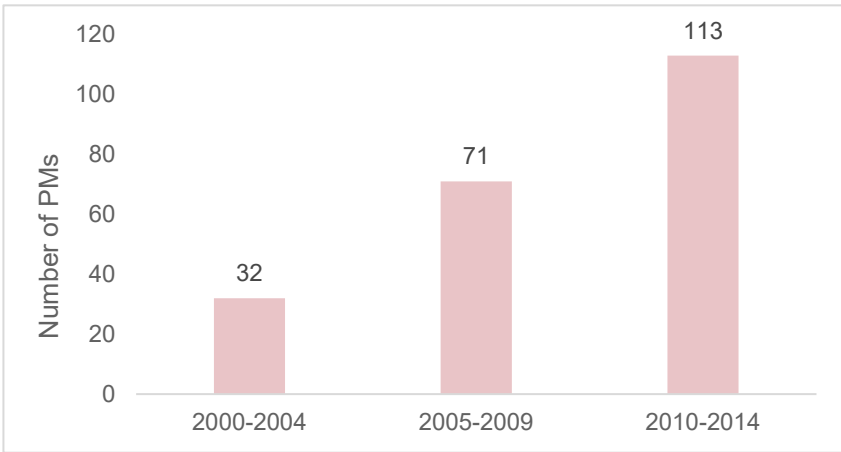


Figure 11. The number of PMs performed during five year periods of the study.

Thoracotomy was the most common surgical approach (63%) but the use of video-assisted thoracoscopic surgery (VATS) increased during the study period, from 15% of PMs during the first five years of the study to 47% during the last five years. The most common type of resection was wedge resection (78%) followed by lobectomy (16%) (**Figure 12**).

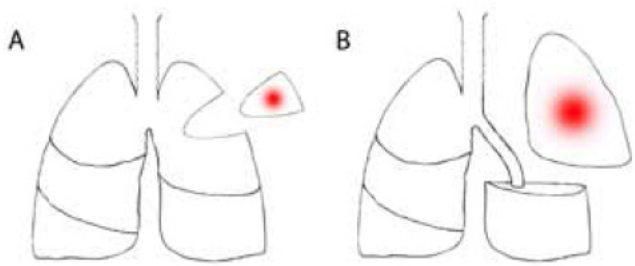


Figure 12. The most common types of resections for lung metastases. A. Wedge resection. B. Lobectomy.

Seventy of the patients were also surgically treated for liver metastases, 58 before and 12 after the PM. Eleven patients underwent two liver operations for recurrent metastases. Most patients or 159 underwent a single PM while 42 and 15 patients were treated with PM two or three times, respectively due to recurrent lung metastases.

Lymph node sampling was performed in 30% of all patients. The six patients with positive hilar/mediastinal lymph nodes at PM showed significantly worse survival compared to 59 patients with histopathologically confirmed negative lymph nodes, with median survival 18 months vs. 66 months (log rank test, p=0.0001). Of these six patients two had a mediastinoscopy with negative nodes before the PM. The proportion of PM patients where lymph node sampling was performed decreased during the study period, from 44% of PMs during the first 5 years to 35% next five years and then 23% during the last 5 years of the study. The difference was statistically significant (p=0.013 and p= 0.0001, respectively).

In 129 cases we had information on the size of the surgical margin ranging from 0.5 mm to 70 mm with a median of 6 mm. The size of the surgical margin was not a prognostic factor for OS or RFS (p=0.82 and p=0.63, respectively). When the patients with available data was divided into two groups based on the ratio between size of the surgical margin and size of the metastases (<0.5 vs ≥0.5) there was no difference in OS or RFS (log-rank test, p= 0.89 and p=0.52, respectively).

In total, 53 patients (25%) had complications after lung surgery most of them minor complications. The most common complication was persistent air leakage in 20 of the patients (defined as need for chest tube drainage >5 days). Other complications were atrial fibrillation (n=5), post-operative bleeding (n=4), pneumonia (n=3), paralysis of the phrenic nerve (n=2), empyema (n=1), and pulmonary embolism (n=1). Of the four patients with postoperative bleeding, three were re-operated and one received blood transfusion. The 30- and 90-days mortality after PM was 0 and 0.46%, respectively, with one patient dying 90 days postoperatively from cardiac infarction and sepsis while receiving adjuvant chemotherapy.

Oncologic therapy

Primary tumour

A total of 29 (14.5%) of the patients received neoadjuvant chemotherapy prior to surgery of the primary tumour. Twenty-five of those had this in form of chemoradiotherapy for rectal cancer (with the function of the chemotherapy being to enhance the effect of radiotherapy and not as a systematic treatment). Adjuvant chemotherapy was given to 77 of the patients and 15 of those received both neoadjuvant and adjuvant treatment. The most common regimen for adjuvant therapy was FOLFOX (5-fluorouracil and oxaliplatin), followed by monotherapy with fluoropyrimidine. One patient received FOLFIRI which is not recommended in the adjuvant setting.

Lung metastases

Twenty-three patients (11%) received neoadjuvant chemotherapy before PM. Half of the patients (n=99) received adjuvant therapy. Nine patients received both. The most common chemotherapy regimen was as for the primary tumours, FOLFOX followed by monotherapy with fluoropyrimidine. Median OS was significantly shorter for patients that received neoadjuvant chemotherapy before PM compared to patients that did not (42 vs 78 months, p=0.002). On the other hand, patients receiving adjuvant chemotherapy after PM had better OS (92 vs. 57 months, p=0.004).

Survival

Median follow-up time was 65 months (range, 3-236). The 3- and 5-year OS was 75% and 56%, respectively (**Figure 13A**). Median OS was 68 months (95% CI: 50.6-84.6 months). Disease-specific 3- and 5-year survival was 77% and 61%, respectively and median disease-specific survival was 72 months (95% CI: 58.8-85.8 months). One hundred and thirty-seven patients had disease recurrence (63%). The recurrence site was known for 131 (96%) of these patients. The most frequent site was the lungs (n=112), followed by the liver (n=44). Other sites were, local recurrence, peritoneum, abdominal/mediastinal/inguinal lymph nodes, abdominal wall, adrenal glands and retroperitoneum. Recurrence free survival (RFS) was 51 and 46% at 3 and 5 years, respectively and the median time to recurrence was 36 months. (**Figure 13B**).

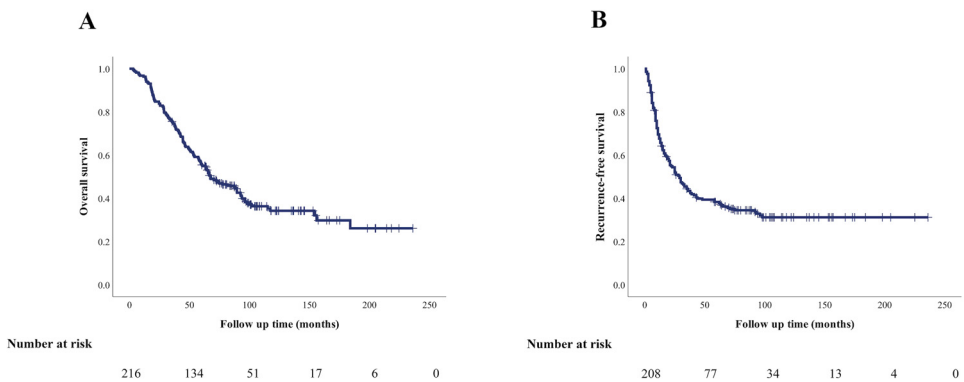


Figure 13. Kaplan-Meier graph showing A) overall survival and B) Recurrence free surival after surgical treatment of lung metastases from colorectal cancer.

RMB3 and other prognostic factors

RBM3 expression was evaluated in 76% of primary tumours and in 98% of patients in at least one of the lung metastases. For 161 patients the RBM3 expression was evaluated in one, in 42 patients in two and in 8 patients in three lung metastases. The distribution

of the RBM3 score for both primary tumours and lung metastases are shown in **Figure 14.**

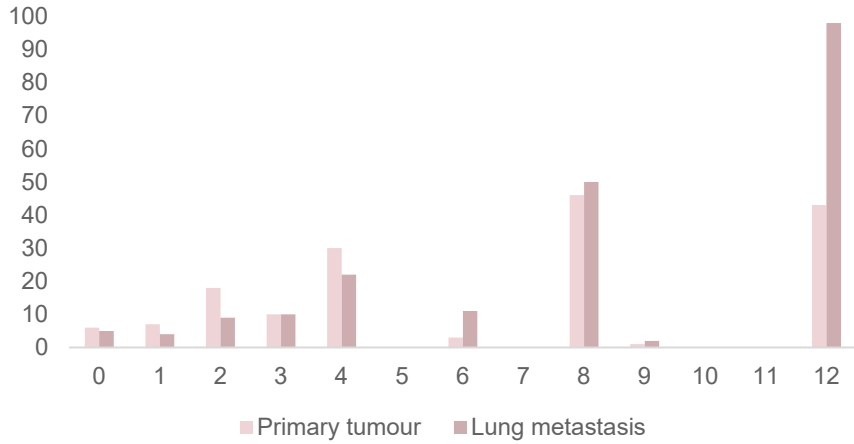


Figure 14. Distribution of the RBM3 score in primary tumours and lung metastases.

CRT analysis determined an optimal cut off value for RBM3 score for both primary tumours and lung metastases to be 6. The RBM3 expression was therefore dichotomized into low (≤6) and high (>6) for further analysis. The RBM3 expression was high in 90 and low in 74 primary tumours. The lung metastases had high RBM3 score in 150 cases and low in 61.

Lung metastasis size > 3 cm and high c-reactive protein (CRP) level before PM were associated with low RBM3 expression in the lung metastasis. For details on associations between RBM3 score in primary tumours and lung metastases with clinicopathological factors see Supplementary Table 1 in paper III.

RBM3 expression was significantly higher for lung metastases compared to primary tumours ($p<0.001$), also in patients with metachronous disease and patients that had not received neoadjuvant treatment ($p<0.001$ for both groups). No difference was found for patients receiving neoadjuvant treatment or with synchronous disease (**Figure 15**).

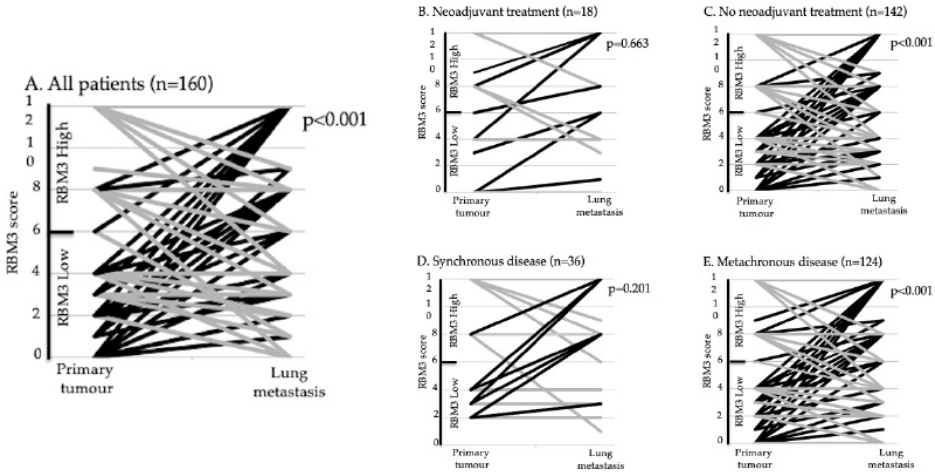
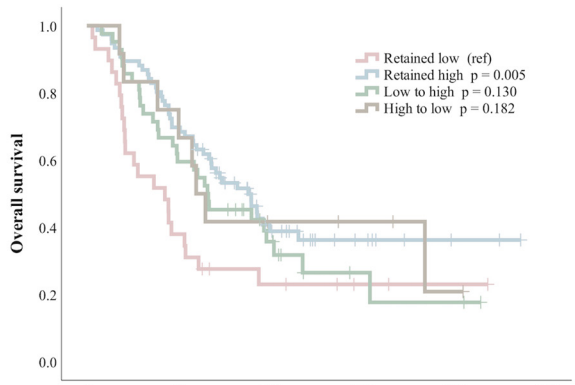


Figure 15. Slope graphs showing the differences in RBM3 expression between primary tumours and lung metastases. A) All patients B) Patients receiving neoadjuvant treatment before PM C) Patients not receiving neoadjuvant treatment before PM D) Patients with synchronous disease E) Patients with metachronous disease. RBM3: RNA-binding motif protein 3

Patients with low RBM3 expression in both primary tumour and lung metastasis had the shortest OS and patients with high RBM3 expression in both the primary tumour and lung metastasis had the longest OS (p=0.005) (**Figure 16**).



Number at risk						
Retained low	29	11	5	2	1	0
Retained high	76	52	20	7	3	0
Low to high	42	25	11	3	2	0
High to low	14	10	4	3	1	0

Figure 16. Kaplan-Meier graphs showing differences in overall survival after surgical treatment of lung metastases from colorectal cancer in strata according to RBM3 expression in the primary tumour and lung metastasis.

High RBM3 expression in the lung metastasis was associated with both prolonged OS (p=0.002) and RFS (p=0.013) after PM (**Figure 17**). There was a non-significant trend of high RBM3 score in the primary tumour and prolonged OS (p =0.104) and RFS (p=0.050) (see Figure 2 in paper III).

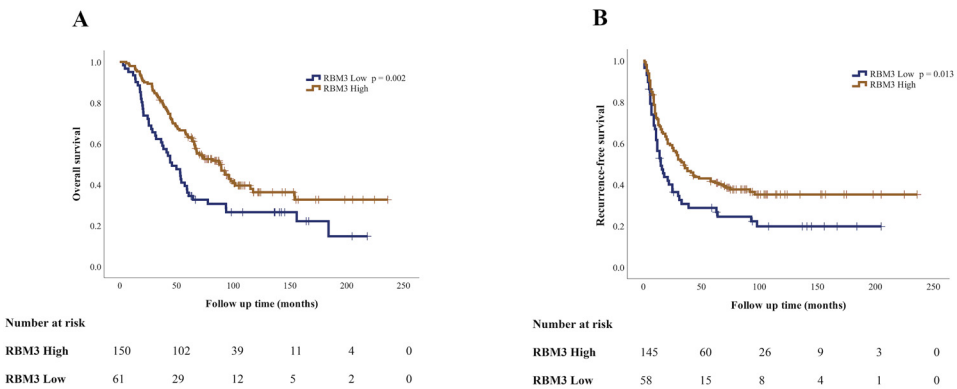


Figure 17. Kaplan-Meier graphs showing difference in overall (A) and recurrence-free (B) survival after surgical treatment of lung metastases from colorectal cancer for patients with high (bronze line) vs. low (blue line) RBM3 in the lung metastases.

Univariable analysis of HRs for death and recurrence showed age >60 years, larger number and size of lung metastases, open vs thoracoscopic surgical approach, elevated CEA and CRP values before PM, N2 stage of the primary tumour, receiving neoadjuvant chemotherapy, not receiving adjuvant chemotherapy and low RBM3 score in the lung metastasis to be significant prognostic factors for shorter OS. For shorter RFS larger number of metastases, DFI ≤ 24 months, elevated CEA value, receiving neoadjuvant chemotherapy and low RBM3 score in lung metastasis were significant prognostic factors in univariable analysis.

In multivariable analysis age >60 years, >1 metastasis, size of metastasis >3 cm, DFI ≤24 months, N2 stage of the primary tumour, not receiving adjuvant chemotherapy and low RBM3 score in the lung metastasis remained significant prognostic factors for shorter OS. Age >60 years, >1 metastasis, DFI ≤24 months, elevated CEA value, and low RBM3 expression in the lung metastasis were significant prognostic factors for shorter RFS (**Table XVI**).

Table XVI. Multivariable hazard ratios for death and recurrence

	Overall survival		Recurrence-free survival	
Factor analysed	**HR, 95% CI**	**P-value**	**HR, 95% CI**	**P-value**
Age >60 years vs. ≤60 years	**2.48 (1.41-4.38)**	**0.002**	**1.90 (1.14-3.17)**	**0.014**
VATS vs. thoracotomy	0.99 (0.61-1.60)	0.97	1.45 (0.92-2.27)	0.11
>1 metastasis	**1.75 (1.10-2.79)**	**0.019**	**1.67 (1.06-2.62)**	**0.027**
Size of metastasis >3 vs. ≤3 cm	**2.08 (1.09-3.97)**	**0.026**	1.34 (0.69-2.60)	0.39
DFI >24 vs. ≤ 24 months	**0.50 (0.32-0.79)**	**0.003**	**0.49 (0.32-0.75)**	**0.001**
N2 vs N0 and N1	**1.74 (1.07-2.82)**	**0.026**	1.57 (0.99-2.49)	0.056
CEA >5 µg /L before PM*	1.76 (0.91-3.39)	0.09	**1.96 (1.05-3.63)**	**0.033**
Neoadjuvant vs. no neoadjuvant chemotherapy before PM	1.28 (0.63-2.60)	0.49	1.71 (0.88-3.30)	0.11
Adjuvant vs. no adjuvant chemotherapy after PM	**0.53 (0.34-0.82)**	**0.004**	0.68 (0.45-1.01)	0.058
High vs. low RBM3 score in the LM	**0.43 (0.27-0.68)**	**0.0001**	**0.50 (0.31-0.78)**	**0.003**

The analyses include 169 patients. Only factors that were significantly associated with RFS or OS in the univariable analysis were included in the multivariable analysis.
CI, confidence interval; DFI, disease-free interval (after surgery of primary tumour); HR, Hazard ratio; LM: lung metastasis; PM, pulmonary metastasectomy; VATS, video-assisted thoracoscopic surgery
*Missing cases included as an own category

RBM3 and oncologic treatment

Patients with high RMB3 expression in the lung metastases had a longer OS if they were treated with oxaliplatin at any time during their disease as compared to patients that had not received oxaliplatin treatment. OS did not differ between oxaliplatin treated and untreated patients that had low RBM3 expression in their metastasis (**Figure 18**).

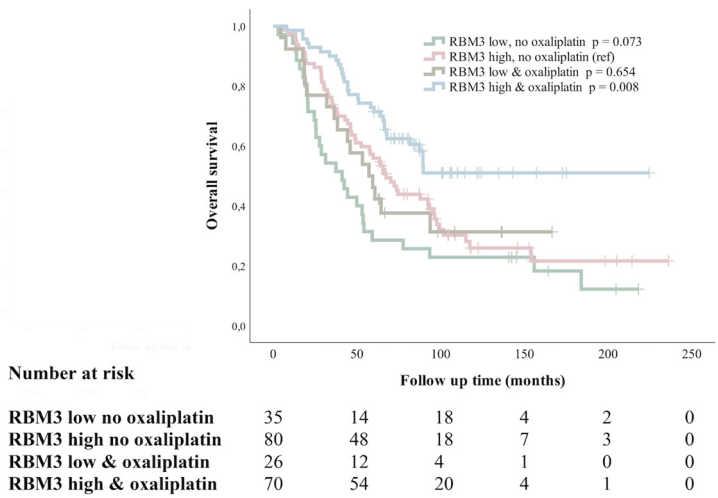


Figure 18. Kaplan-Meier graphs showing differences in overall survival after PM in strata according to RBM3 expression in the lung metastases and treatment with oxaliplatin at any time during the course of disease.

Paper IV

In total 27 patients (17 males, 10 females) with a median age of 69 years, surgically treated for CRC and both lung and liver metastases were included in the study. The location of the primary tumour was rectum in 15, left colon in 10 and right colon in two patients. Thirteen of the metastases were synchronous (10 liver and three lung metastases) and 41 were metachronous (17 liver and 24 lung metastases). For 12 out of 15 patients with rectal cancer, neoadjuvant radiotherapy was given preoperatively, whereof three in combination with chemotherapy (as a radiosensitiser). One other patient received neoadjuvant chemotherapy without radiotherapy. One patient with colon cancer received neoadjuvant chemotherapy. All but two of the 27 patients were treated with chemotherapy at some point prior to resection of liver metastases and all but three patients were treated with chemotherapy at some point prior to PM (**Table XVII**).

Table XVII. Patients characteristics and oncologic treatment

Case	Gender	Age (years)	Synchronous LM **	Synchronous PM **	Location of primary tumour	Order of operations	Time P/LM (months)	Time P/PM (months)	Radiotherapy rectal cancer	Administration of chemotherapy	Chemotherapeutic agent
1*	M	76	Yes	No	Rectum	Primary, liver, lung	9	32	CXR	Before primary, LM and PM	5-FU
2	M	69	Yes	No	Left colon	Primary, liver, lung	4	19	N/A	Before both LM and PM	FOLFOX
3	M	69	Yes	Yes	Left colon	Primary, liver, lung	8	11	N/A	Before both LM and PM	FOLFOX
4	F	57	Yes	No	Left colon	Primary, liver, lung	4	16	N/A	Before both LM and PM	FOLFOX
5	M	72	No	No	Left colon	Primary, lung, liver	85	71	N/A	Before both LM and PM	FOLFOX
6	F	65	No	No	Rectum	Primary, lung, liver	37	16	25 Gy	Before LM	FOLFOX
7*	M	70	No	No	Rectum	Primary, liver, lung	25	27	No	Before both LM and PM	FOLFOX, bevacizumab
8	M	69	Yes	No	Right colon	Primary, liver, lung	19	21	N/A	Before both LM and PM	FOLFOX, (FOLFIRI, bevacizumab before PM)
9*	F	64	No	No	Rectum	Primary, liver, lung	20	22	25 Gy	Before both LM and PM	FOLFIRI (before PM)
10	F	66	Yes	No	Left colon	Liver, primary, lung	0	18	N/A	Before primary, LM and PM	FOLFOX
11	F	65	No	No	Left colon	Primary, liver, lung	6	19	N/A	Before both LM and PM	FOLFOX, bevacizumab
12	M	65	No	No	Rectum	Primary, liver, lung	18	42	25 Gy	Before both LM and PM	FOLFOX
13	F	79	Yes	No	Rectum	Liver + primary, lung	0	29	No	No	
14*	M	70	No	No	Rectum	Primary, liver, lung	12	27	No	Before PM	FOLFOX
15	M	62	No	Yes	Rectum	Primary, liver, lung	9	15	25 Gy	Before both LM and PM	FOLFOX

16	M	57	No	No	Rectum	Primary, liver, lung	23	37	CXR	Before primary, LM and PM	FOLFOX
17	M	69	Yes	No	Rectum	Primary, liver, lung	26	87	25 Gy	Before both LM and PM	FOLFIRI, cetuximab, bevacizumab (before LM), FOLFOX (before PM)
18	M	58	No	No	Left colon	Primary, lung, liver	84	78	N/A	Before both LM and PM	FOLFOX
19	M	46	No	Yes	Rectum	Lung, primary, liver	18	0	25 Gy	Before LM	FOLFIRI
20	F	61	No	No	Rectum	Primary, liver, lung	10	28	25 Gy	Before both LM and PM	FOLFOX
21	F	60	Yes	No	Rectum	Primary, liver, lung	5	19	25 Gy	Before both LM and PM	FOLFIRI, bevacizumab
22	M	75	Yes	No	Rectum	Primary, liver, lung	3	25	25 Gy	Before both LM and PM	FOLFOX
23	F	76	No	No	Right colon	Primary, liver, lung	27	41	N/A	Before both LM and PM	5-FU
24	F	67	No	No	Left colon	Primary, liver, lung	17	23	N/A	Before both LM and PM	FOLFIRI + bevacizumab (before LM), FOLFOX (before PM)
25*	M	70	No	No	Rectum	Primary, liver, lung	12	27	CXR	Before primary, LM and PM	5-FU (with radiotherapy)
26	M	83	No	No	Left colon	Primary, liver, lung	15	17	N/A	Before both LM and PM	5-FU
27	M	73	No	No	Left colon	Primary, liver, lung	14	38	N/A	Before both LM and PM	FOLFOX

5-FU, 5-fluorouracil; CXR, chemoradiotherapy; FOLFIRI, 5-FU and irinotecan; FOLFOX, 5-FU and oxaliplatin; LM, operation for liver metastasis; N/A, not applicable; PM, pulmonary metastasectomy; Time P/LM, time between primary and LM surgery; Time P/PM, time between primary and PM surgery
* Case also sequenced with the TSO500 panel; ** No = metachronous

TST26

Frequencies of all mutations (pathogenic/presumed pathogenic and of unknown significance) detected by the TST26 panel can be found in **Figure 19.**

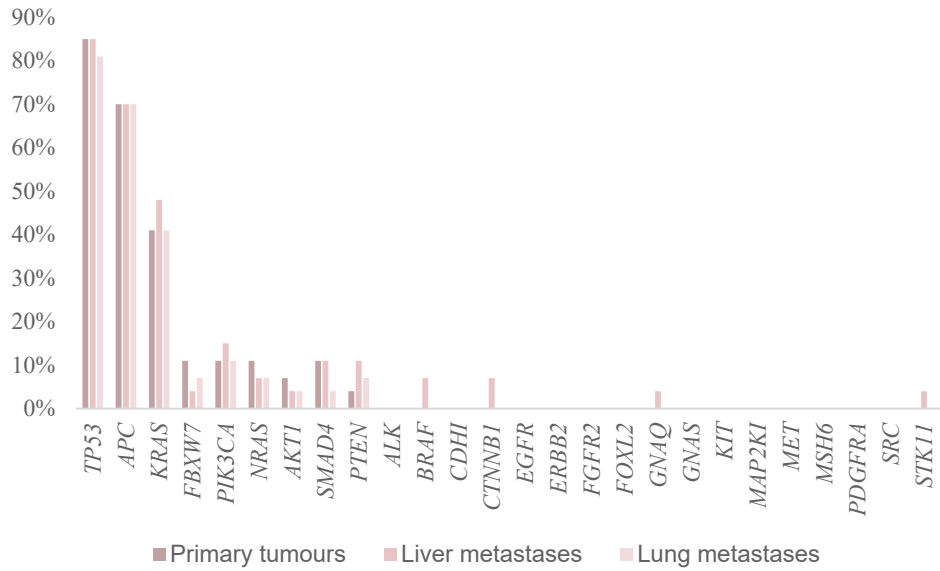


Figure 19. Frequency of mutations from TST26 in 27 cases of primary CRC with matched lung and liver metastases.

As seen mutations were most frequent in *TP53, APC* and *KRAS* genes with rates of 85%, 85% and 81% (*TP53*), 70% (*APC*) and 41%, 48% and 41% (*KRAS)* in primary tumours and corresponding liver and lung metastasis, respectively. Global concordance for these three most frequently mutated genes can be seen in **Figure 20.**

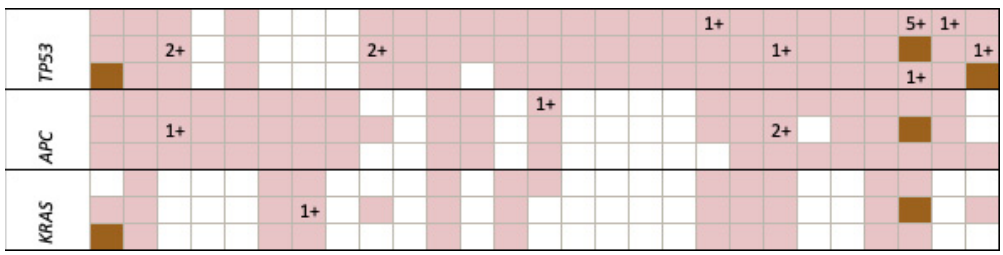


Figure 20. All mutations for the three most frequently mutated genes in 27 CRC cases with match liver and lung metastases.The top row is primary tumour, the middle liver metatasis and the bottom row is lung metastases. No colour: no mutation, same colour= same mutation, different colour = different mutation. 1+ one extra mutation, 2+ two extra mutations, 5+ five extra mutations.

78% of the cases had pathogenic/presumed pathogenic mutations (**Figure 21**). The number of pathogenic/presumed pathogenic mutations was highest in liver metastases (n=41), followed by primary tumours (n=32) and lung metastases (n=25). *APC* and *KRAS* mutations were more frequent in liver metastases compared to primary tumours and lung metastases but the difference was not statistically significant with pairwise comparison. Mutations in the *TP53* gene were most frequent in the primary tumours followed by liver and lung metastases (n=15/14/11) but the difference was not significant.

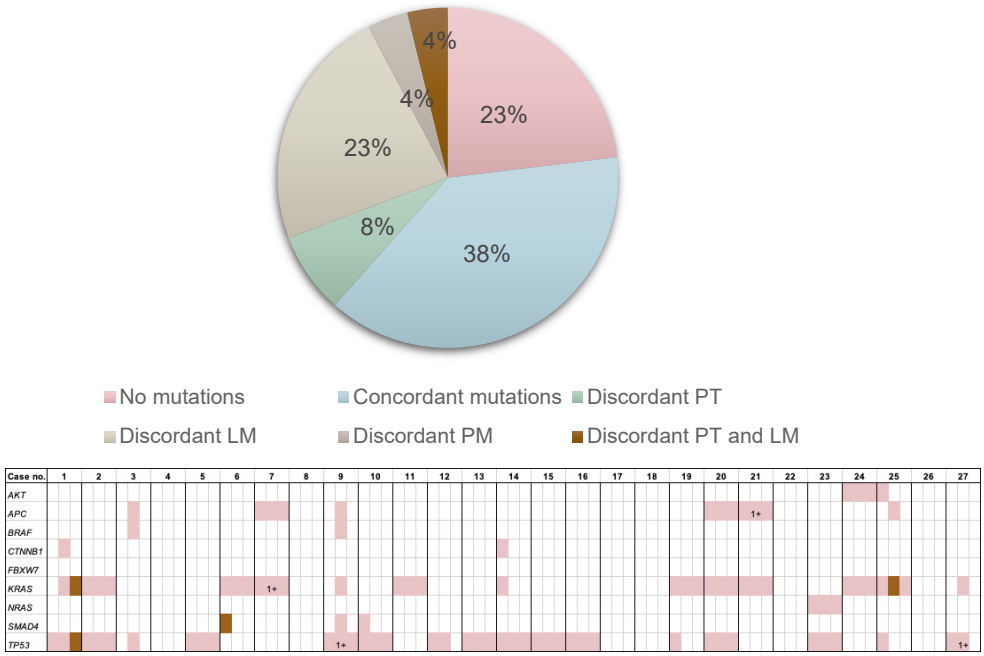


Figure 21. Pathogenic/presumed pathogenic mutations from the TST26 panel in primary tumour (first column), liver metastasis (second column) and lung metastasis (third column) from 27 cases of CRC.

No colour = no mutation (or only of unknown significance, data not shown); same colour = same mutation; different colour = different mutation. 1+ = same mutation plus extra mutation. PT: primary tumour, LM: liver metastasis, PM: lung metastasis.

With the TST26 panel 16 out of 27 cases (59%) showed identical pathogenic/presumed pathogenic mutation calls (10 with mutations and six without any mutations identified) (**Figure 21**). Concordance between primary tumours and lung metastases was 74% (20/27), between primary tumour and liver metastases 63% (17/27), and between liver and lung metastases 70% (19/27). The difference in concordance was not statistically significant.

The *APC, BRAF, CTNNB1, KRAS, SMAD4,* and *TP53* genes had novel presumed pathogenic/pathogenic mutations in the metastases, with novel mutations being most frequent in liver metastases (7/27). The primary tumour had mutations that were not

found in the metastases in three cases. *BRAF* mutations were seen in a liver metastasis in two different patients. Both were *BRAF* V600 mutations.

KRAS mutation was found in 13 cases, and of those 7 cases (54%) had the same *KRAS* mutation (and no additional mutation) in the primary tumour and both metastases (**Figure 21**). Five cases had mutations in codon 12 and two in codon 13. Of the six cases with discordant *KRAS* mutational profiles (including 4 cases with a novel *KRAS* mutation in the liver metastasis) five were rectal cancers.

TSO500

The five cases sequenced with TSO500 were all rectal cancers, (cases no. 1, 7, 9, 14 and 25 in **Table XVII** above). A total of 35 genes were mutated in any of the samples, whereof 12 were known to be significant genes in CRC. Pathogenic/presumed pathogenic mutations were found in 25 of mutated genes. Pathogenic/presumed pathogenic mutations for the different tumour sites and areas can be found in **Figure 22**. None of the five cases had identical mutational profile in all tested tumours. The *APC* gene was mutated in all sites in 3 out of 4 cases with mutations. Mutations found in only one site had often a variant allele frequency (VAF) of ≤3%, i.e., *ARID1A*, *CDK4*, *FGFR4*, *FOXP1*, *GNAQ*, *PTPRT*, and *SDHA*, but for some in the range 5-31%, i.e., *FBXW7*, *MAP3K1*, *MDC1*, *NFE2L2*, and *QKI*. Correspondingly, VAF ≤5% was uncommon but occurred for mutations that were concordant between sites (i.e., *ABL1*, *ALK*, *ERBB3*, *ETV1*, *PIK3CA*, *PTCH1*, *TP53*).

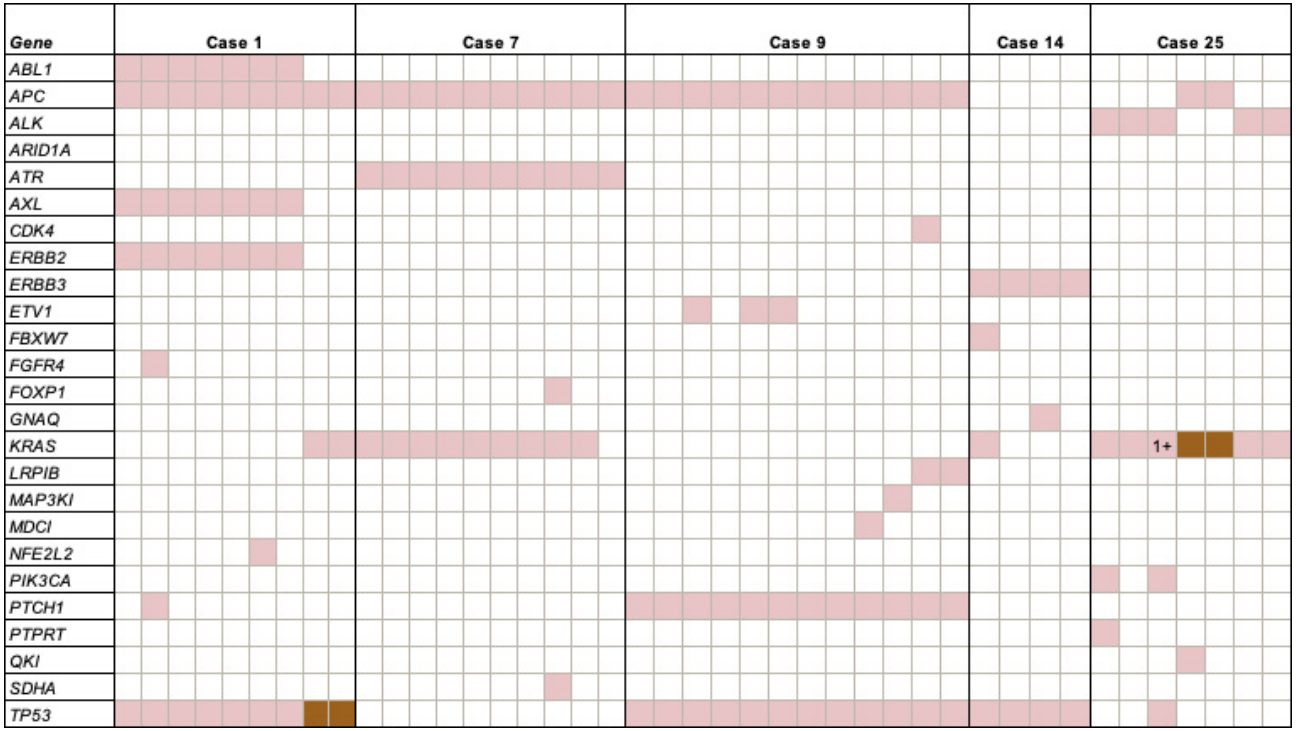


Figure 22. Pathogenic/presumed pathogenic mutations from the TSO500 panel in five cases of CRC with multiple areas and metastases investigated.
No colour = no mutation (or only of unknown significance, data not shown); same colour = same mutation; different colour = different mutation. 1+ = same mutation plus extra mutation.
Case 1: Primary tumour, primary tumour, lymph node metastasis, Liver metastasis 1, livermetastasis 1, liver metastasis 2, liver metastasis 2, Lung metastasis 1, lung metastasis 1
Case 7: Primary tumour, primary tumour, primary tumour, liver metastasis 1, liver metastasis 1, liver metastasis 2, liver metastasis 2, lung metastasis 1, lung metastasis 1, lung metastasis 2
Case 9: Primary tumour, primary tumour, primary tumour, lymph node metastasis, liver metastasis 1, liver metastasis 1, lung metastasis 1, lung metastasis 2, lung metastasis 3, lung metastasis 4, lung metastasis 5, lung metastasis 5
Case 14: Primary tumour, lymph node metastasis, liver metastasis, lung metastasis
Case 25: Primary tumour, primary tumour, primary tumour, liver metastasis 1, liver metastasis 1, liver metastasis 2, lung metastasis

Case by case description

In case 1 the lung metastasis had a distinct different mutational profile with less mutations than the primary tumour, lymph node metastasis and one of the liver metastases. Moreover, the lung metastasis had gained a pathogenic mutation in *KRAS.* All tumours had a mutation in the *TP53* gene, but the lung metastasis had a different one. All the tumours had the same *APC* and *LRP1B* mutations.

In case 7 the primary tumour, liver metastases and one of two lung metastases had the same mutational profile while one of the lung metastases had two novel mutations.

In case 9 all tumours had the same *APC, PTCHI* and *TP53* mutations. One out of five lung metastases had gained *LRP1B* mutation, another had a *MAP3K1* mutation, and one had gained a *MDC1* mutation. All five lung metastases had gained *PIKA3CA* mutation of unknown significance. In one out of three cores from the primary tumour *ETV1* mutation was found that was also in the liver metastasis.

In case 14 all the tumours had the same *APC, ERBB3, TP53* mutations. The primary tumour areas had mutations in *APC, FBXW7* and *KRAS* not found in lymph node, liver or lung metastasis.

In case 25 one of two liver metastasis was different from the other tumours in that the *ALK* mutation was missing, and it had different *APC, KRAS* and *TP53* mutations compared to the other tumours from the case.

KRAS mutations were found in four out of five cases with TSO500. In cases 1,14 and 25 all of the mutations were in codon 12 and in case 7 all the tumours had a concordant mutation in codon 146.

Of all 59 different mutations (both pathogenic and of unknown significance) the consequence was in 71% of cases missense mutations followed by nonsense mutations and frameshift deletions (**Figure 23**). All *KRAS* mutations were missense mutations while one *LRP1B* mutation found in all the tumours in case 1 was a splice site mutation.

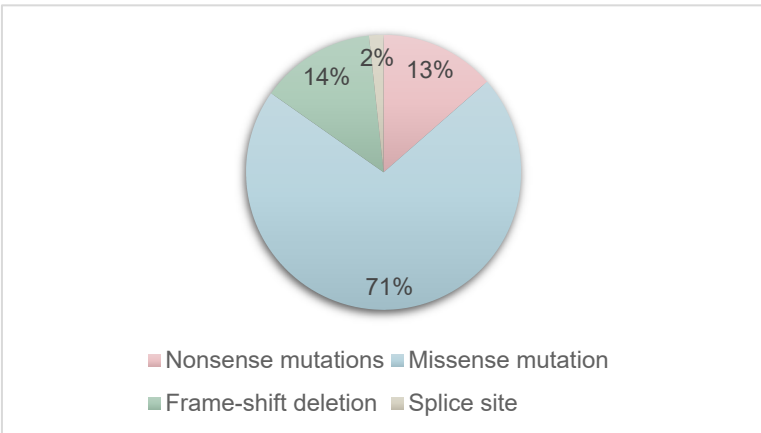


Figure 23. Conseqence of mutations found with TSO500

TMB (mean values for all primary tumours areas and for each metastasis for each case) was slightly higher in two and slightly lower in three metastases compared to the primary tumour. There was no significant difference in TMB between primary tumours and metastases (p=0.93, Wilcoxon signed rank test) (**Figure 24**). None of the cases were MSI high. Information on MSI and TMB can be found in **Table XVIII**.

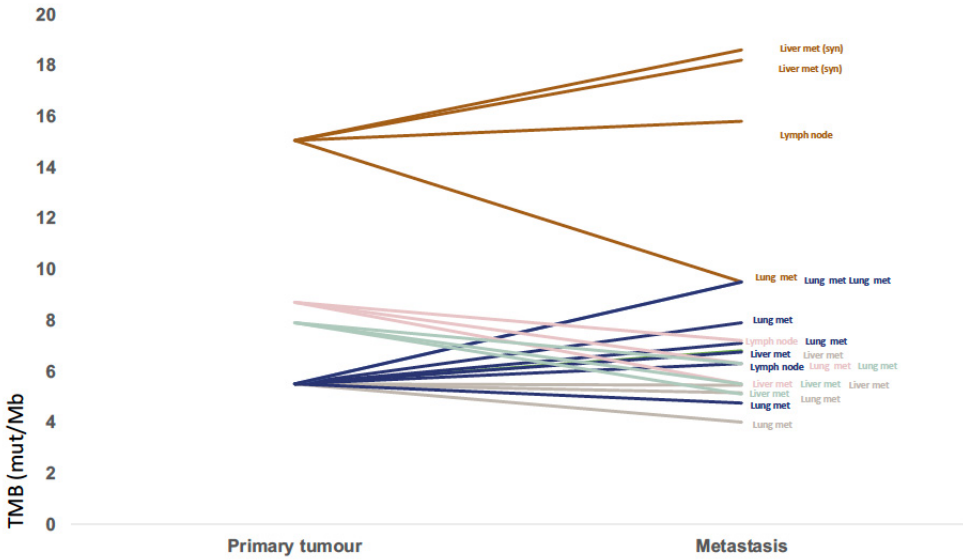


Figure 24. Tumour mutational burden (TMB) based on TSO500 in primary tumour (mean value when different areas that were assessed) and matching liver, lung, and lymph node metastases for five CRC cases.
Syn: synchronous

Table XVIII. Information on MSI and TMB for the five cases sequenced with the TSO500 gene panel

Case	Site	Months after first surgery	Delta Ct	Total TMB (mut/Mb)	Unstable MSI sites (%)
1	Primary tumour area A	0	1,35	11,1	0,99
	Primary tumour area B	0	1,53	19	1,01
	Lymph node metastasis	0	1,25	15,8	0,96
	Liver metastasis 1 area A (syn)	9	0,77	17,4	2,04
	Liver metastasis 1 area B (syn)	9	0,48	19	0
	Liver metastasis 2 area A (syn)	9	1,24	19	2,97
	Liver metastasis 2 area B (syn)	9	1,33	18,2	1,96
	Lung metastasis area A	32	0,92	9,5	0
	Lung metastasis area B	32	0,65	9,5	0,9
	Normal colon	0	1,02	1,6	1,89
	Normal liver	9	1,41	1,6	0
7	Primary tumour area A	0	-0,26	5,5	4,59
	Primary tumour area B	0	-0,42	3,2	4,59
	Primary tumour area C	0	-0,42	6,3	2,75
	Liver metastasis 1 area A	25	3,23	4,5	0
	Liver metastasis 1 area B	25	1,92	6,4	4,05
	Liver metastasis 2 area A	25	1,71	5,6	3,41
	Liver metastasis 2 area B	25	1,82	8	3,95
	Lung metastasis 1 area A	27	0,06	5,5	3,42
	Lung metastasis 1 area B	27	1,31	4,8	4,05
	Lung metastasis 2	27	0,04	4	2,56
	Normal liver	25	2,90	0	25
	Normal lung	27	0,23	2,4	2,63
9	Primary tumour area A	0	-0,53	5,5	4,5
	Primary tumour area B	0	-0,56	4	5,04
	Primary tumour area C	0	-0,39	7,9	5
	Lymph node metastasis	0	-0,66	6,3	4,24
	Liver metastasis area A	20	-0,10	6,3	5,04
	Liver metastasis area B	20	1,12	7,2	5,22
	Lung metastasis 1	22	2,11	7,9	5,77
	Lung metastasis 2	22	1,65	7,1	6,03
	Lung metastasis 3	22	1,50	9,5	3,48
	Lung metastasis 4 area B	33	1,34	9,5	5,36
	Lung metastasis 5 area A	33	1,38	10,3	5,31
	Lung metastasis 5 area B	33	1,19	8,7	4,46
	Normal lung	33	1,25	0	2,5
14	Primary tumour	0	0,07	8,7	1,72
	Lymph node metastasis	0	0,19	7,2	1,33
	Liver metastasis	12	-0,34	5,5	0,84
	Lung metastasis	27	-0,36	6,3	0
	Normal lung	27	0,10	0	1,65
25	Primary tumour area A	0	-0,59	3,1	0,85
	Primary tumour area B	0	-0,43	7,9	0,85
	Primary tumour area C	0	1,49	9,6	1,75
	Liver metastasis 1 area A	12	-0,41	4,7	1,8
	Liver metastasis 1 area B	12	-0,44	5,5	2,63
	Liver metastasis 2	12	-0,66	5,5	1,79
	Lung metastasis	27	NA	6,3	3,31
	Normal liver	12	0,25	2,4	0,83

TMB: tumour mutational burden, MSI: microsatellite instability

A comparison between TST26 and TSO500

A comparison between pathogenic/presumed pathogenic mutations in *APC, KRAS* and *TP53* genes with the two panels, TST26 and TSO500 revealed that perfect concordance between these panels was limited to *TP53* for case 1, *APC* for case 7 and all genes for case 14 (with comparison only possible for the primary tumour) (**Table XIX**).

Table XIX. Comparison of detected pathogenic/presumed pathogenic *APC*, *KRAS*, and *TP53* mutations between the TST26 and TSO500 panels where the same tissue block was used for both analyses.

Case	Block	Panel	*APC*	*KRAS*	*TP53*
1	Primary tumour	TST26			
1	Primary tumour	TSO500			
1	Liver metastasis	TST26			
1	Liver metastasis	TSO500			
1	Lung metastasis	TST26			
1	Lung metastasis	TSO500			
7	Primary tumour	TST26			
7	Primary tumour	TSO500			
7	Liver metastasis	TST26		+1	
7	Liver metastasis	TSO500			
7	Lung metastasis	TST26			
7	Lung metastasis	TSO500			
9	Primary tumour	TST26			
9	Primary tumour	TSO500			
9	Liver metastasis	TST26			+1
9	Liver metastasis	TSO500			
9	Lung metastasis	TST26			
9	Lung metastasis	TSO500			
14	Primary tumour	TST26			
14	Primary tumour	TSO500			
25	Primary tumour	TST26			
25	Primary tumour	TSO500		+1	
25	Liver metastasis	TST26			
25	Liver metastasis	TSO500	+1		
25	Lung metastasis	TST26			
25	Lung metastasis	TSO500			

No color = no mutation or only mutation of unknown significance (data not shown); same colour = same mutation; different colour = different mutation; +1 = same mutation plus extra mutation.

General discussion

The correct diagnosis of a pulmonary tumour is important from a treatment perspective. The lungs are the most common metastatic site due to extensive microcirculation and primary lung cancer is the 4th most common cancer in both men and women in Sweden. CRC is the 3rd most common cancer in Sweden and the lungs are the second most common site for metastases from CRC. Advances in CRC treatment in recent years with better surgical and oncological treatment has increased survival. Despite that still 30% of CRC patients suffer from distant metastases which is the leading cause of death for this disease. There has been an increase in surgical treatment of lung metastases in recent years and CRC is the most common indication for PM. In this thesis the focus in the first two papers was evaluation of IHC markers to help in the diagnostics of a pulmonary tumour. In the third paper we investigated the outcome for PM for CRC lung metastases and found a possible new prognostic marker, RBM3, for these patients. The last study concerned the rapidly evolving field of molecular profiling of CRC, with focus on heterogeneity between primary CRC with paired lung and liver metastases.

In the first part of this thesis ten different IHC markers all in clinical use were evaluated. When looking at individual IHC markers untypical staining pattern is infrequent but more common if looking at IHC profiles including more than one IHC marker.

In paper I we looked at three different clones of TTF-1, a well-established marker for primary lung AC. The 8G7G3/1 clone was the first TTF-1 clone to become available and is the most widely used. The WHO group recommends that clone in the diagnostics of lung cancer.[112, 113] As evident, in Ordonez et al. review from 2012, published TTF-1 studies that used the 8G7G3/1 clone greatly outnumbered those that used the SPT24 clone.[106] The third clone SP141 came on the market in 2013 and has not been used in clinical work in Skåne whereas clones, 8G7G3/1 and SPT24 are used in Lund and Malmoe, respectively. An assessment performed by Nordic Quality Control found the SP141 clone to be as SPT24 more sensitive than the 8G7G3/1 clone and they recommend these clones[111] in contrast to the WHO guidelines. Due to this many pathology departments in Sweden use the SPT24 clone. We found the TTF-1 clone 8G7G3/1 to be more specific but less sensitive in diagnosing lung AC compared to clones, SPT24 and SP141. Our study was the first to compare the well-known clones 8G7G3 and SPT24 with the new clone SP141 in primary lung cancers as well as lung metastases of different types. A previous study of the three clones for primary CRC and corresponding mixed metastases was available.[114]

Lung AC was most often positive of all tested tumours and more often so with clones SPT24 and SP141 compared to clone 8G7G3/1 but at the cost of staining more lung SqCC and lung metastases. No lung SqCC tumour was positive with the 8G7G3/1 clone but 6% and 8% were positive with clones, SPT24 and SP141, respectively. By using a different cut-off value than that of 1% positive tumour cells recommended by WHO guidelines[112], the risk of positive SqCC could be reduced. We found these cut-off values being 10 and 50% positive tumour cells for SPT24 and SP141, respectively. In a previous study by Ye et al. only 82.5% of the primary lung AC cases were positive with clone, 8G7G3/1 (99/120) compared to 88% in our study. They used 5% positive tumour cells as a cut-off value instead of 1% in our study, which could contribute to fewer positive cases.[133] Another study on both clones, 8G7G3/1 and SPT24 showed much lower frequency with 65% and 84% TTF-1 positive lung ACs with clone 8G7G3/1 and SPT24, respectively.[107] In the review from 2012 76.7% (2004/2614) were positive with clone 8G7G3/1 and 81.3% (471/579) with clone SPT24 compared to 88% and 92.5% in our study for clones 8G7G3/1 and SPT24, respectively.[106] A probable contribution to the difference in staining frequency is different staining dilutions. Another contributing factor may be AC subtypes in the cohorts, as it is known that mucinous AC are less frequently TTF-1 positive compared to non-mucinous.[106]

In a review of 47 studies on TTF-1 expression in lung SqCC, 23 of them reported 3-38% TTF-1 positive cases and 24 studies reported all cases negative. Of reported cases investigated with clone 8G7G3/1 and SPT24 clone, 4% (2/1057) and 16.2% (23/142) were TTF-1 positive, respectively. One of the most likely reasons for the large differences is that entrapped type II pneumocytes (that are TTF-1 positive) have been interpreted as cancer cells.[106] We took great care not to do that in our study. Furthermore, all cases of SqCC outside of lungs, although few included in our study, were TTF-1 negative.

In our study 2% of the primary CRC were TTF-1 positive with clone 8G7G3/1 and 4% with clones SPT24 and SP141. This is comparable to a study on 104 primary CRC (published after our study) comparing TTF-1 clones 8G7G3/1, SPT24 and SP141 that found 5.7% to be positive with SPT24 and SP141 while 2% were positive with 8G7G3/1.[166] Bae et al. published a large study on 1319 CRC primary tumours and found 5% positive with SPT24 and SP141 while no case was positive with clone 8G7G3/1.[114] Compérat et al. had similar results with clone SPT24 where 5% of primary CRC tumours were TTF-1 positive but none of the 90 primary CRC tumours were positive with clone 8G7G3/1.[107] Similar results with clone 8G7G3/1 being negative in all CRC cases was reported from a large study including 1300 cases by Turner et al.[127] Dettmar et al. examined 555 primary CRC cases with clones 8G7G3/1 and SPT24 and found 3.2% and 4.3 % of primary CRC to be TTF-1 positive with clones 8G7G3/1 and SPT24, respectively.[167] Although most studies are in line with ours that clone SPT24 is more often positive compared to clone 8G7G3/1 Matoso et al. found the clones 8G7G3/1 and SPT24 to be positive to the same extent in non-pulmonary tumours, in their study 2.5% of primary CRC were positive with both clones.[105]

Right sided colon cancers, both primary tumours and lung metastases were TTF-1 negative with all three clones in our study. Bae et al. found TTF-1 expression to be associated with distal location of the primary tumour [114] and in the study by Dettmar et al. only one of the 24 TTF-1 positive cases was a right- sided colon cancer.[167]

In our study, 7% of the lung metastases cohort were TTF-1 positive with any of the clones which was lower than previously reported by Ye et al were 13.6% of 103 lung metastases of mixed origin were positive with TTF-1, clone 8G7G3/1. In that study larger tissue cores, 5 mm compared to 1 mm in our study were used for the TMAs which could explain more cases being positive.[115]

Of the CRC lung metastases 2%, 7% and 8% were TTF-1 positive with clones, 8G7G3/1, SPT24 and SP141, respectively in our study. Compérat et al. reported 10% of CRC lung metastases in their study TTF-1 positive with clone SPT24 but 0% with clone 8G7G3/1.[107]

Both Bae and Comperat suggested two possible explanations for this low level TTF-1 expression in CRC and the clone-type difference. The first one that CRCs have a low level of TTF-1 expression and it is known that clone SPT24 and SP141 have higher affinity for TTF-1 compared to clone 8G7G3/1. The second hypothesis was that the aberrant TTF-1 expression is caused by cross-reactivity with other proteins.[107, 114] The first hypothesis has been confirmed in a study published in 2020 were the TTF-1 expression in CRC was confirmed by mRNA expression.[166]

During the covered years of the first study, we identified three cases that were diagnosed as lung metastases from CRC on resected specimens when they were in fact primary lung cancers. We made the correct diagnoses after comparison with the primary CRC tumour including comprehensive IHC panels. In these cases routine staining with TTF-1 would have been beneficial in the clinical setting (for all three cases, the colorectal and the primary lung cancers had different expression of napsin A, CDX2, CK7 and CK20 as well).

Contrary to recommendations in the current WHO guidelines for lung tumours[112] we think that routine staining with TTF-1 should be considered also on biopsy/cytology from AC with obvious glands or mucin inclusions encountered in the lung since a positive test strengthens the diagnosis of a pulmonary origin. It is important to consider that CRC lung metastases can express TTF-1, also with clone 8G7G3/1 and not all lung ACs are TTF-1 positive.

In paper II we looked at nine other IHC markers in the same cohorts of primary lung cancers and lung metastases and included the results of TTF-1 clone SPT24 in the analysis. We systematically assessed the diagnostic value of these markers. In the following discussion positive staining refers to ≥10% positive tumour cells.

A deviant IHC profile was quite frequent although an untypical expression of individual markers for each histopathological diagnosis were uncommon.

Typical IHC profiles were seen in 68% of lung AC (TTF-1+/napsin A+/CK7+), 64% of lung SqCC (CK5+/p40+/p63+/CK7+/-), and 78% of CRC lung metastases (CK20+/CDX2+) with all other tested markers negative.

Most breast cancers and urothelial cancers were GATA3 positive and in only few cases of lung metastases from renal cell, gynaecological, squamous cell, adenocystic and thyroid carcinoma. This is in line with the study by Miettinen et al.[145] For the primary lung cancers, we had a lower number of GATA3 positive lung AC in our study or 3.7% compared to 8%. On the other hand, our study showed a higher number of GATA3 positive lung SqCC or 20% (or 30% if cut-off value was 1%) compared to 12% in the study by Miettinen et. al.[145] It is noteworthy that the GATA3 positivity in our lung cancer cases was typically rather weak. In another study by Laurent et al. 2.1% of TTF-1 negative primary lung AC and 0% of TTF-1 positive lung AC were GATA3 positive[168] and in a study on 25 lung SqCC none was positive for GATA3.[169] In a study by Hattori et al. that used a score made up from intensity and fraction of stained cells 72.7% of 33 lung metastases from breast carcinoma were GATA3 positive and only one out of 156 lung SqCC and none of 170 lung AC cases were GATA3 positive.[170] The same was true for a large study by Liu et al. where GATA3 was almost 100% specific for breast and urothelial carcinoma with only two endometrial cancers staining positive and all other tested tumours, including 49 lung SqCC and 61 lung AC were negative.[144] A possible explanation for the difference compared to our results may be that our staining protocol was rather strong and over-staining must be considered.

Some untypical patterns may not cause much concern e.g., CDX2 expression in a TFF-1+/napsin A+ primary lung AC or in a CK5+/p40+ primary lung SqCC. Other deviations from normal IHC profile are more problematic e.g., CK20+ primary lung SqCC, CK7-/TTF-1 lung AC, and CK7+ CRC. This means that lung SqCC can have the same IHC profile as urothelial carcinoma and basal-like breast cancer.

CK7 was positive in 99% of primary lung AC in line with previous studies[171, 172] Interestingly, 44% of the primary lung SqCC expressed CK7. Previous studies have reported frequency of 13-28%.[171-177] In the largest study with 456 lung SqCC cases 21% were CK7 positive.[171] One large study with 225 cases had a similar frequency to our study with 37% of the cases CK7 positive[178] and a small study of 30 SqCC cases reported half of them as CK7 positive.[179] The reason for the high frequency of CK7 positive lung SqCC cases in our study could be a strong staining, the cut off value chosen and the fact that we included even weak staining as positive. It could also have a possible biological explanation, that in countries where smoking is more common there might be more cases of typical CK7 negative lung SqCC cases compared to Sweden.

Previous studies on primary and metastatic CRC and other gastrointestinal cancers have found CDX2 to be less specific but more sensitive compared to CK20 for gastrointestinal origin[140, 180] and our results confirm that. In some studies all primary lung AC cases have been negative for CDX2[140, 141, 181] but in our primary lung cancer cohort, 7% of all lung ACs had positive CDX2 expression. One large study has reported higher frequency of CDX2 expression or 14%[182] and yet other studies 1-3%.[183-187]

CDX2 expression is seen in selected non-gastrointestinal AC e.g., mucinous ovarian carcinomas and AC of the urinary bladder.[180] In our study 4 out of 8 urothelial carcinomas and 9 out of 17 gynaecological carcinomas showed CDX2 expression. One lung metastasis from prostatic carcinoma was CDX2 positive. This is a rare but previously reported.[188]

During study II we found one case erroneously diagnosed as primary lung SqCC in the clinical setting. Comparison to patient's previously diagnosed urothelial carcinoma and further IHC markers such as uroplakin II were performed and proved it to be a lung metastasis from patient's previous urothelial cancer. We also found one case diagnosed as lung metastasis from endometrial cancer in the clinical setting that proved to be a primary lung cancer, when compared to the primary tumour, they had distinctly different IHC profiles including differently expressed napsin A, TTF-1 and PAX8.

In the second part of this thesis the focus was on lung metastases from CRC. CRC poses the tumour type that is most often treated with PM. It is uncontroversial that PM can be performed with low morbidity and mortality, but it is also true that it is not of benefit for all mCRC patients. The ESMO guidelines for metastatic CRC, recommend R0 resection of lung metastases in analogy with resection of liver metastases although PM is less well studied.[189, 190] PM is also the recommended approach of resectable lung metastases in the NCCN and NICE guidelines.[10-12]

The evidence for the effect of PM lies on case series and retrospective studies where selection bias poses a great problem. There are no randomised, controlled trials and the only serious attempt was the PulMiCC trial that randomised patients between December 2010 and December 2016 but was then discontinued due to poor and worsening recruitment. Due to few randomised patients (n=65), the study was underpowered and the survival difference between the group undergoing PM and the control group 38% vs 29%, respectively was not statistically significant.[81] Other comparative studies are scarce. One small now outdated Swedish study published in 1970 included 70 patients treated with PM, with a mixture of different primary tumours, most common was renal cell cancer (n=30) and only 8 gastrointestinal cancers. The survival of these 70 patients was compared to a historical control group of 12 patients not treated with PM and the authors found no difference in 5-year survival (31% vs 25%, respectively).[191] This study is merely of historical interest and not applicable to modern cancer treatment or mCRC patients.

In 2014 a Danish register study was published on synchronous lung metastases from CRC. Of 26200 patients diagnosed with CRC, 1970 (7.5%) had synchronous lung metastases and of those 736 (37%) had metastases confined to the lungs. Only 3.8% (n=28) of those underwent PM but these patients had superior survival compared to the patients not treated with PM (but with metastases confined to the lungs), median OS 1470 days vs. 361 days, respectively. This study as others could not conclude whether the survival benefit was due to the PM per se or reflected the patient selection.[23] Another study published the same year compared mCRC patients that were either treated with PM and chemotherapy or chemotherapy only. The survival in the PM group was better (median survival 44.5 vs. 21.8/18.9 months for different

chemotherapy regimens) but in that study the PM group had a lower CEA value and more patients had single metastasis so selection bias cannot be excluded.[54]

There are numerous cases series reporting prognostic factors and survival after PM from CRC. The studies are often small including less than 100 patients and with a long study period. Three systematic reviews and one meta-analysis have been published from these series,[41-43, 192] many of them including the same studies. Several prognostic factors have been identified and some predictive models presented. In paper III the results of PM on CRC patients performed at Skåne University Hospital, Lund are presented, and traditional prognostic factors analysed as well as RBM3 expression, a protein that has in recent years emerged as a prognostic biomarker in several solid tumours including CRC.[151-156] We found high RBM3 expression in the lung metastasis to predict prolonged OS and RFS after PM. Moreover, in a multivariate analysis age >60 years, >1 metastasis, size of metastasis >3 cm, N2 status of the primary tumour, and DFI <24 months were prognostic factors for shorter OS. Age >60 years, >1 metastasis and elevated CEA value were prognostic factors for shorter RFS.

The 5-year OS after PM have been reported ranging from 40% to 66%[41-43] and the results in paper III were in line with that or 56%.

Number of PMs performed per year increased during the study period from being four to 10 during the first half of the study period to 16-29 per year during the second half of the study period. This is in line with a study from the Netherlands on PMs from 2012 to 2017.[193]

Interestingly, the number of patients with rectal cancer compared to colon cancer was higher in our cohort in paper III. This is in line with other studies showing lung metastases being more common in rectal vs colon cancer.[22, 23, 194] This has a few possible explanations, one being the anatomical difference. A study has shown that the liver filters circulating viable tumour cells from the colon.[195] Rectal cancer on the other hand drains tumour cells directly to the lungs via vv. hemorroidalis inferior bypassing the liver.[196, 197] This could also have a biological explanation as tumours in the left colon are also more prone to metastasize to the lungs compared to right sided colon tumours and it is well-known that tumours right vs left colon are embryonically and biologically different. *KRAS* mutations have been linked to lung metastases in CRC [33, 65, 94, 97] and *BRAF* mutations (more common in right-sided colon carcinomas) are linked to less frequent lung metastases.[47]

Few studies to date have investigated the prognostic or predictive value of investigative biomarkers after PM in mCRC, and our study in paper III is the first study to examine the role of RBM3 in this context. Other biomarkers that have been studied are neutrophil to lymphocyte ratio (NLR) found to be a prognostic factor for OS and pulmonary recurrence in mCRC patients treated with PM.[198] The same authors also published a study demonstrating that *KRAS* and *BRAF* mutations were prognostic factors for mCRC patients undergoing PM.[65] In the VICTOR study patients with *KRAS* mutations in the primary tumour had higher risk of lung relapse.[95] In a large study from Australia on molecular markers in mCRC, metastases limited to the lungs were more likely in patients with *KRAS* mutations. No association was found with other

metastatic sites. *BRAF* mutations are more common in right-sided tumours and are associated with peritoneal carcinomatosis and lower incidence of liver or lung limited metastases.[47] Overexpression of c-MET, pSTAT3[67] and high stromal heat-shock protein 27[66] analysed on resected lung metastases have been associated with an impaired survival in mCRC patients undergoing PM. A reduced expression of E-cadherin, aerogenous spread with floating cancer cell clusters and vascular invasion were found to be negative prognostic factors after PM in a study on 86 lung metastases by Shiano et al. In the same study insulin-like growth factor-1 receptor (IGF1-R), Eß-catenin, and p53 were not found to be significant.[199] FOS-B, VEGDF-D and MAGE-A have been studied but were not found to be significant prognostic factors.[200]

Possible reasons for shorter DFI being a risk factor for death after PM might be that earlier metastatic spread means a more biologically aggressive disease. Interestingly this does not translate to synchronous metastases being a risk factor in our study.

CEA value before PM was a significant prognostic factor for both OS and RFS in univariate analysis but only for RFS in a multivariate analysis in our study. This is in contrast to several previous studies including two large studies with 1030 and 1112 patients, respectively.[44, 55] However, our finding of elevated CEA value being a negative prognostic factor for RFS was in line with systematic review and meta-analysis of studies on PM in CRC patients published after 2001 including 2925 patients, where elevated CEA nearly doubled the likelihood of early recurrence.[42]

Although a small number of patients underwent lymph node dissection in our study the finding of positive lymph nodes effected survival negatively. This is in line with other studies.[35, 37, 38, 201] Interestingly some studies have not found survival difference in patients with hilar lymph node disease versus mediastinal lymph node disease[37, 38, 201] although at least one study has.[63] It may be of benefit to evaluate all patients subject to PM with a PET-CT to identify patients with risk for lymph node metastases although given the low sensitivity of 35% reported by Hamajii et al.[37] it does not seem to be an appropriate screening tool for mediastinal lymph nodes before PM. Another method could be endobrochial ultrasound-guided sampling (EBUS) as is part of the treatment of primary lung cancer. Due to negative effect on prognosis it has been suggested that positive mediastinal lymph nodes should be a contraindication to PM in CRC patients.[35, 37] Not all authors agree with this and Renaud et al. that published the results for 320 mCRC patients undergoing PM whereof 140 had positive lymph nodes (91 hilar, 49 mediastinal) found lymph node positive patients having median survival of 47 and 37 months for hilar and mediastinal disease, respectively. They concluded that patients should not be excluded from surgery despite positive lymph nodes. [38]

Nelson et al. studied risk of local recurrence after wedge resection of CRC lung metastases in 679 wedge resections from 355 patients. Margin size and tumour size were risk factors for local recurrence. Moreover a pathologic margin length of at least half the tumour size was estimated to lead to a local recurrence rate <11%.[202] We did not find size of margin or the ratio between surgical margin and size of the metastasis being associated with OS or RFS in our study (information on margin size was not available for all cases in our study).

In paper III, 11% and 50% of the patients were treated with neoadjuvant and adjuvant chemotherapy, respectively. Neoadjuvant chemotherapy was associated with shorter OS and RFS on an unadjusted analysis and adjuvant chemotherapy was an independent prognostic factor for longer OS. A single institution study including 615 patients found as our study adjuvant chemotherapy and not neoadjuvant chemotherapy being a significant prognostic factor for OS.[50] A similar portion of patients received adjuvant chemotherapy in a large, multicentre study on 785 patients whereof 376 (48%) received adjuvant chemotherapy but the authors did not find any significant survival benefit of adjuvant therapy on either DFS or OS. That study also looked at the effect of adjuvant chemotherapy based on prognostic factors and did not find survival benefit in any of the risk groups although there was a trend toward increased OS in the high-risk group.[70] Possible explanation for this study not showing survival benefit of adjuvant chemotherapy (in contrast to our study) is that 68% were treated with 5-FU alone without addition of oxaliplatin which is a much higher proportion than in our study (32%). Oxaliplatin containing adjuvant chemotherapy has shown additional survival benefit compared to 5-FU alone after resection of primary CRC.[203, 204] A study on neoadjuvant chemotherapy before PM showed a potential role for oxaliplatin-based regimen and a worse OS for patients treated with irinotecan based chemotherapy.[77] A systematic review and a meta-analysis of 18 studies including 3885 patients found no significant difference in OS or DFS and the authors concluded that adjuvant chemotherapy was not suggested for CRC patients treated with PM.[78]

RBM3 is an RNA and DNA binding protein that is induced in response to various types of stress e.g. hypothermia and hypoxia. It has been introduced in the last decade as a prognostic biomarker. In paper III we found high RBM3 expression in the lung metastasis to be a significant prognostic factor for both RFS and OS in CRC patients undergoing PM. Moreover, patients with high RBM3 expression treated with oxaliplatin did considerably better than patients not receiving oxaliplatin. This difference was not found in patients with low RBM3 expression, indicating a predictive value of RBM3 for oxaliplatin treatment. The association of RBM3 with platinum-based chemotherapy has been described for ovarian, testicular and pancreas cancer as well as mCRC.[151, 156, 158, 159] The study on ovarian cancer showed that expression of RBM3, both at the mRNA and protein levels was a positive prognostic marker. It also showed that decreased RBM3 expression conferred to reduced sensitivity to cisplatin in ovarian cancer cells.[151]

Oxaliplatin is a platinum-based chemotherapy drug classified as alkylating agent. It was developed when finding an alternative to cisplatin that does not have an effect in CRC. Oxaliplatin has cytotoxic effect like other platinum-based compounds through DNA damage.[205] It also exhibits synergism with 5-FU probably via down regulation of thymidylate synthase.[206] The breakthrough of oxaliplatin in combination with 5-FU came with the study by de Gramont in 2000.[207] With the side effects of oxaliplatin treatment, such as neuropathy, knowledge on markers helping to choose patients benefitting from the treatment are important.

Notably in two previous studies on RBM3 expression the prognostic value found has been restricted to colon cancer and no difference in survival of patients with rectal

cancers that made up 57% of the cohort in our study.[157, 160] The study by Siesing et al. showed however prognostic effect of RBM3 expression in mCRC for both colon and rectal tumours.[158]

Worth mentioning in paper III, the RBM3 expression in the primary tumours was not a prognostic factor, although it was borderline significant for RFS. In our study, the RBM3 expression was significantly lower in the primary tumours compared with lung metastases, especially in patients that had not received neoadjuvant chemotherapy and in patients with metachronous metastases. In a study on 1800 CRCs Melling et al. found that loss of RBM3 expression was associated with right sided tumour location, poor prognosis and more advanced tumour stage.[160] Another study on 455 cases of mCRC showed that RBM3 expression was significantly higher in patients undergoing surgery for metastases.[158] In paper III only 9% of the patients had right-sided colon cancer, but RBM3 expression did not differ according to tumour location in our study. Our finding of an elevated RBM3 expression in the lung metastases compared to primary tumours is of interest, particularly as it was demonstrated to provide prognostic information. The difference in RBM3 expression between synchronous and metachronous disease with higher RBM3 expression in metachronous lung metastases and not synchronous is also of note. Hypothetically, RBM3 could have a role in processes that drive metastatic formation over time and nonetheless signify less aggressive disease. Only 11% of the patients had received neoadjuvant chemotherapy before PM in our study but in those patients RBM3 did not differ between primary tumour and lung metastasis. As mentioned earlier patients with high RBM3 expression treated with oxaliplatin had prolonged survival. Speculatively, chemotherapy might have selective effect on microscopic metastases with high RBM3 expression.

There are not many studies reporting on complications after PM. In our study 25% of the patients had complications after PM, most often persistent air-leakage. The same was found in my study from Iceland.[24] In a nationwide study on PMs from the Netherlands where 52% of the cases were CRC lung metastases 3.6% had complicated postoperative course defined as complication leading to prolonged hospital stay, re-intervention or mortality.[193] The reported 30 and 90 days postoperative mortality is 0-2.4%[41] that is in line with our study where 30 days mortality was 0% and 90 days mortality 0.46%. A study that measured lung function after PM found not surprisingly that spirometric changes were affected by the volume of the resected lung parenchyma. Functional loss after three or more non-anatomical resections was comparable to that recorded after lobectomy. Three months after PM none of the functional changes remained.[208]

In paper IV (still in manuscript) paired primary tumours, liver and lung metastases from CRC patients were analysed with targeted NGS.

Vignot et al. studied 12 pairs of primary CRC and liver metastasis and one pair of primary tumour and peritoneal metastasis and had 90% concordance when looking at 12 known, recurrent mutations but when looking at global concordance rate it was 78%.[209] This is higher concordance than in paper IV where the concordance of pathogenic/presumed pathogenic mutations for all three tumours (primary, liver and

lung metastases) was 59% and concordance between primary tumours and liver metastases was 63%. In both the study by Vignot et al. and our study almost all of the patients had received oncologic treatment before the surgery of the metastasis. In our material 33% (9/27) of the liver metastases were synchronous where as 46% (6/13) were synchronous in the study by Vignot et al. Two of the patients (15%) in their study had received neoadjuvant treatment before surgery for the primary tumour compared to 44% (12/27) in our study. More than half of our patients had their primary tumour in the rectum compared to one case Vignot's study.

Schweiger at al. sequenced 24 primary tumours and 47 matched lung metastases in CRC patients and found the concordance between primary tumour and lung metastasis to be 83.5%, which is higher compared to our study (74%). They also found that the frequency of mutated genes was comparable between patients irrespective if they had received chemotherapy prior to PM or not.[17] As most of the patients in our study had received chemotherapy, we could not access its effect.

In our study only 54% of *KRAS* mutations were concordant between all three included sites, but a better concordance was seen between primary tumour and lung metastasis (93%) than between primary tumour and liver metastasis (78%). In contrast, Kim et al. showed a significantly higher concordance in *KRAS* mutational status between primary tumour and liver metastasis compared to primary tumour and lung metastasis (89.4% vs. 67.6%).[94] *KRAS* mutations in codon 12 are most common and it was in line with our study. One of the concordant *KRAS* mutations found in case 7 was in codon 146 (C437C>T:pAla146Val), a rare mutation found in only 0.9% of 1267 cases in the study by Imamura et al.[210] *KRAS* mutations are more often found in lung metastases compared to liver metastases and a role of *KRAS* mutations in the propensity of CRC to metastasize to the lung has been suggested.[96] However, *KRAS* mutations have been linked not only to early pulmonary recurrence but also to a more diffuse pattern of metastases.[97] This may lead to lower frequency of *KRAS* mutations in lung metastases if investigated in surgically treated cases (like our study) since patients with disseminated disease are not candidates for PM. Subset of discordant *KRAS* status have been seen in some studies as ours but more studies have shown high concordance between primary tumours and metastases. A meta-analysis published in 2012 including 19 studies on concordance between primary tumour and metastases concluded that *KRAS* was highly concordant in primary and distant metastatic tumours. However, there was a discordance between primary tumour and lymph node metastases. The concordance rate between primary tumours and distant metastases in the studies were 76.5%-100% and between primary tumours and lymph node metastases 67.9-100%. In the meta-analysis the concordant rate was 94.1% (95% CI: 88.3%–95.0%) and 81.3% (95% CI: 69.6%–97.4%) for primary tumour and distant metastases vs primary tumour and lymph node metastases, respectively.[211] A later study including 343 lymph node metastases confirmed this finding that *KRAS* concordance was lower for lymph node metastases than distant metastases.[212] A meta-analysis published in 2019 included 50 studies on concordance in *KRAS* between primary tumours and metastases and found the discordance rate to be 8% (95% CI: 5-10%). In this meta-analysis there were 10 included studies where *KRAS* status was evaluated with NGS.[213]

A possible explanation for *KRAS* discordance could be intra-tumour heterogeneity. In a study from 2011 on 43 patients with primary CRC and 113 metastatic tumours the frequency of *KRAS* mutations in the primary tumour was 34.9% and the concordance rate was high between primary tumours and metastases. The authors microdissected the five cases where primary tumour and metastases had different *KRAS* status. In all of these cases the primary tumour had heterogenous mutational pattern with a mixture of different *KRAS* statuses, mutant type and wild type. Ten concordant cases were microdissected as well and in all those cases the *KRAS* status was homogenous. The authors suggested that different areas of the primary tumour should be examined for *KRAS* status to correctly predict the *KRAS* status in the metastatic lesions.[214] This may be especially important for the first part of our study IV since small tissue cores were used. Another possible explanation for the high discordance in *KRAS* status in our study is that all our cases come from patients with limited metastatic disease. An interesting study on genomic profiling of 158 mCRC patients with matched primary tumours and metastases suggested that concordant *KRAS* status was associated with more disseminated metastases[215] and patients with disseminated metastatic disease are not candidates for PM.

According to ESMO guidelines other metastatic sites such as lymph node or lung metastases may be used only if primary tumour or liver metastases samples are not available for biomarker testing.[189]

Zou et al. investigated eight different genes *(EGFR, KRAS, NRAS, PIK3CA, ERBB2, BRAF, KIT,* and *PDGFRA*) in primary CRC tumours and matched lung or liver metastases.[216] Gene mutations were significantly more prevalent in lung metastases compared to liver metastases, 87% vs. 44% in contrast to our study were pathogenic/presumed pathogenic mutations were more common in liver metastases compared to lung metastases (n=41 vs n=32). Moreover, *KRAS* mutations were significantly more common in lung compared to liver metastases (57% vs. 22%), in contrast to our study where 41% of the lung metastases and 48% of the liver metastases harboured *KRAS* mutations. An important difference between the studies was that we only included cases with both lung and liver metastases while Zou et al. included cases with either.

We found no significant difference in TMB between the primary tumour and the matched lung and liver metastases (although the comparison was limited due to sample size). This is in line with a study by Stein et al. that compared TMB in primary CRC and unmatched peritoneal metastases from CRC and found no difference.[217]

It is noteworthy that there was a difference in detected mutations between the TST26 and TSO500 panels for common genes in our study. Our material showed good DNA quality and quantity, why these factors probably do not contribute. Giardina et al. showed robust data for the TST26 assay using a broad range of input DNA (10-200 ng).[218, 219] A study on different cancer types by Prieto-Potin et al. demonstrated that the TST26 panel is highly accurate but the sequencing was only successful in two-thirds of the patients, while the remaining third failed due to unsuccessful quality-control filtering. Only 14% of patients received targeted treatment based on the variant

determined by the panel but for the CRC (29% of tested tumours) 24 of 45 clinically relevant mutations affected treatment decision.[220]

Other causes of discrepancy between the panels may be related to gene coverage and difference in sampled areas in the tissue blocks, which may lead to sampling of subclonal populations but also variation in tumour cell content e.g., Pestinger et al. have shown a good correlation (and reproducibility) between mutations, TMB, and copy number variations when predicted by TSO500 and WGS.[165]

Limitations of the studies in this thesis

The use of TMAs instead of whole tumour slides can lead to missing of a focal positivity for IHC markers. It can however be argued that TMAs are more resembling biopsies that are often the diagnostic material for a pulmonary tumour in the clinical work (but with risk of poor fixation which is not seen in biopsies). In paper I comparison between TMAs with whole tumour sections of lung metastases for selected relevant cases were made and in 118 out 123 cases both were negative. TMA sections are in part ideal for method comparisons since the same areas from a large number of cases can be evaluated. The TMA method has also been shown to be reliable tool to demonstrate links between clinical endpoints and molecular characteristics.[221]

In the cohorts used in studies I and II there were a very few cases of some types of primary lung cancer (e.g. SCLC) as well as most types of lung metastases and especially the lung metastases cohort does not represent all patients with lung metastases as they were a selected group of patients with surgically treated metastases. This naturally limits a general applicability of the findings.

The retrospective and non-randomised design of the study in paper III and the fact that the data for some of the cases was incomplete, are limiting factors for that study.

The main limitation in study IV is the small number of included cases. All the included cases were patients with surgically resected liver and lung metastases creating a selection bias possibly to patients with more auspicious biology compared to patients with more disseminated disease. There was also a variation of tumour cell content between samples studied. Furthermore, normal tissue was not included in TST26 analysis which could have aided the filtering and interpretation process of found alterations.

Conclusions

TTF-1 expression differs between clones. TTF-1 clone 8G7G3/1 is more specific and less sensitive for primary lung adenocarcinoma compared with clones SPT24 and SP141.

A significant number of colorectal carcinomas are TTF-1 positive, more so with clones SPT24 and SP141 which should be considered when distinguishing between primary lung cancer and lung metastasis from CRC.

IHC markers alone or in combination aid in the diagnosis of a pulmonary tumour, but non-typical IHC profiles are fairly common. Profiles that deviate from normal IHC expression occur and may lead to incorrect diagnosis.

Lung adenocarcinomas that are TTF-1 and napsin A negative have the same IHC profile as several other tumour forms and in exceptional cases the same as CRC.

PM is a well-accepted treatment strategy for CRC metastases. Prognostic factors should be taken into account when deciding on treatment for these patients, and adjuvant chemotherapy might be a good option for patients undergoing PM but needs to be investigated further.

RBM3 expression in lung metastases is indicated to be a prognostic factor in CRC patients undergoing PM.

Primary CRC and metastases to liver and lung have the same mutational profile in slightly more than half of the cases. The concordance between primary tumour and liver or (especially) lung metastases is higher. The mutational heterogeneity, for *KRAS* mainly seen in rectal cancers, is important from a treatment predictive perspective.

Future perspectives

Future studies on diagnostics of a pulmonary tumour with more cases of lung metastases from tumours other than CRC for evaluation of the IHC markers are needed. Given the expression of e.g., CDX2 in lung AC, other markers of gastrointestinal origin should be investigated for comparison.

The potential risk and actual occurrence of misdiagnoses in the clinical setting due to deviant IHC expression or profiles should be investigated in real-world samples.

It would be valuable with a randomised study comparing PM and modern chemotherapy where all know parameters are taken into account when randomising patients with lung metastases from CRC. This would indeed be challenging, and we are probably beyond this point. It might though be possible to randomise patients that are not usually subject for PM today e.g. patients with multiple synchronous metastases (4 or more).

A randomised study on perioperative chemotherapy in relation to PM would be of value.

Also, a study on PM for CRC in Sweden would be of interest. Are there regional differences in the treatment of lung metastases from CRC? If there are differences there might be of value to assess the outcome of all CRC patients with lung metastases diagnosed in Sweden during a certain time period, comparing different treatments. This could be done as a register study with data from the Swedish Colorectal Cancer Registry.

For further data on a potential value in the clinical setting, more studies confirming the prognostic role of RBM3 expression in the context of lung metastases in CRC are needed. It would also be of interest to examine this marker in a group of CRC patients with lung metastases not treated with PM.

NGS has significantly contributed to personalised medicine and with increasingly higher sensitivity, broader panels, lower cost and faster kits this will most probably become standard in all cancer diagnostics and lead to treatment decisions based on specific properties of the tumour. The role of mutational heterogeneity for treatment selection, response and tumour progression in clinical practice is one area that should be further investigated as precision medicine is the way forward in cancer treatment.

Populärvetenskaplig sammanfattning

Lungorna är vanlig plats för dottertumörer från cancer (så kallade lungmetastaser). Att skilja mellan primär cancer i lungan och lungmetastaser av olika typer är viktigt för att välja rätt behandling. För rätt diagnos krävs så kallad histopatologisk undersökning där cellerna undersöks i mikroskop. För att skilja mellan olika typer av cancerceller används ofta speciella analyser, framför allt immunhistokemiska färgningar.

Varje år drabbas över 4500 personer av tjocktarmscancer och 2000 av ändtarmscancer i Sverige, vilket gör kolorektalcancer (som de tillsammans kallas) till den tredje vanligaste cancerformen. Var nionde patient i Sverige med nydiagnostiserad cancer har en kolorektalcancer. Lungan är andra vanligaste platsen för metastaser från kolorektalcancer och runt 10–20% av patienterna som diagnostiseras med kolorektalcancer har lungmetastaser vid diagnostillfället, medan risken för att utveckla lungmetastaser inom fem år är 6%. Kirurgi används som behandling av lungmetastaser från kolorektalcancer i utvalda fall och ger då 5-årsöverlevnad mellan 40–70%. I många fall är kirurgi tyvärr inte möjlig för att metastaserna är för utbredda. Att hitta vilka patienter som kan ha nytta av kirurgi vid lungmetastaser från kolorektalcancer är av intresse.

I delarbete I tittade vi på tre olika typer av immunhistokemisk färgning för markören TTF-1. Vi undersökte denna färgning i 665 fall av primär lungcancer och 428 fall av olika typer av lungmetastaser. Denna färgning används i kliniken för att skilja mellan lungcancer av körteltyp (positiv för TTF-1), lungcancer av skivepiteltyp (negativ för TTF-1) och lungmetastaser (negativ för TTF-1). Vi ville jämföra dessa tre olika typer av TTF-1-färgningar för att se vilken som var bäst (om någon) på att skilja mellan lungcancer och lungmetastaser. Det fanns skillnader, där en av färgningarna var mer specifik jämfört med de andra två men samtidigt mindre känslig. Det fanns en del lungmetastaser som var positiva och det måste man ha i åtanke vid diagnostik av oklar tumör i lungan.

I delarbete II använde vi oss av fallen från delarbete I, dvs. 665 fall av primär lungcancer och 425 fall av lungmetastaser men tittade på flera immunhistokemiska färgningar utöver TTF-1: CK5, p40 och p63 som färgar skivepiteltumörer, CK7, CK20 som används för att skilja till exempel cancer i äggstockar, lungor och bröst från tarmcancer, CDX2 som färgar tumörer i mag-tarmkanalen, napsin A som färgar lung- och njurcancer, GATA3 som är markör för bröst- och urinvägscancer och PAX8 (som är markör för njur-, äggstocks- och livmodercancer). Vi fann generellt stöd för vad som rapporterats i litteraturen hur de olika immunhistokemiska färgningarna typiskt utrycks

i olika tumörer, vilket används för att underlätta att komma till rätt diagnos men att det också finns ovanliga immunprofiler som kan leda till fel diagnos.

I delarbete III tittade vi på lungmetastaser enbart från kolorektalcancer. Vi skapade en databas omfattande 216 patienter som behandlats med kirurgi för lungmetastas från kolorektalcancer på thoraxkliniken i Lund från 2000 till 2014. Vi tittade på vilka faktorer som kunde påverka överlevnad och tid till återfall efter operation för lungmetastaser från kolorektalcancer. Vi analyserade också tumörvävnad för RBM3-protein för att se om det kunde ge information om överlevnad och tid till återfall. Vi fann att ålder <60 år, endast en metastas, mindre storlek av metastasen, längre tid än två år mellan diagnos av primärtumören och metastasen, att få cellgiftbehandling efter lungkirurgi samt högt uttryck av RBM3 i lungmetastasen ledde till bättre överlevnad och ålder <60 år, endast en metastas, längre tid än två år mellan diagnos av primärtumören och metastasen, normal nivå i blodet av en tumörmarkör som kallas CEA samt högt uttryck av RBM3 i lungmetastasen ledde till längre tid till återfall. Detta skulle kunna bidra till att förutsäga prognos och bättre identifiering av patienter som har nytta av kirurgisk behandling av lungmetastaser.

I delarbete IV undersöktes mutationer i 27 tripletter (varje triplett från en individ) av primärtumör (i tarmen), lungmetastas och levermetastas. Vi använde en metod som kallas „next-generation sequencing“ för att titta på i första steget 26 gener. Vi valde ut fem fall i det andra steget för att titta på 523 gener i flera prov från primärtumören samt flera metastaser. Vi ville se om mutationer som uppstått i tumörcellerna var desamma eller skiljde sig mellan primärtumören och de olika metastaserna. Vi fann att mutationer var vanligast i *TP53*, *APC* och *KRAS* generna. Samma mutationsprofil fanns i alla tre tumörer (från samma patient) i drygt hälften av patienterna (59%). Det var högre överensstämmelse mellan primärtumör och lungmetastas (74%) jämför med primär tumör och levermetastas (63%). Fynden kan bidra till förståelse för hur tarmcancer förändras under sjukdomsförloppet och kan även visa på skillnader mellan primärtumör och metastaser som är viktiga för val av behandling.

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References

[1] Downey RJ: Surgery for colorectal and sarcomatous pulmonary metastases: history, current management, and future directions. Thorac Surg Clin 2006, 16:133-7, v-vi.

[2] Edwards A: Malignant disease of the lung. J Thorac Surg 1934, 4:107-24.

[3] Martini N, McCormack PM: Evolution of the surgical management of pulmonary metastases. Chest Surg Clin N Am 1998, 8:13-27.

[4] Barney JD, Churchill, E.J: Adenocarcinoma of the Kidney with Metastasis to the Lung: Cured by Nephrectomy and Lobectomy. The Journal of Urology 1939, 42:8.

[5] Blalock A: Recent advances in surgery. New England Journal of Medicine 1944, 231:261-7.

[6] Alexander J, Haight C: Pulmonary resection for solitary metastatic sarcomas and carcinomas. Surg Gynecol Obstet 1947, 85:129-46.

[7] Thomford NR, Woolner LB, Clagett OT: The Surgical Treatment of Metastatic Tumors in the Lungs. J Thorac Cardiovasc Surg 1965, 49:357-63.

[8] McCormack PM, Attiyeh FF: Resected pulmonary metastases from colorectal cancer. Dis Colon Rectum 1979, 22:553-6.

[9] Pastorino U, Buyse M, Friedel G, Ginsberg RJ, Girard P, Goldstraw P, Johnston M, McCormack P, Pass H, Putnam JB, Jr., International Registry of Lung M: Long-term results of lung metastasectomy: prognostic analyses based on 5206 cases. J Thorac Cardiovasc Surg 1997, 113:37-49.

[10] National Institute for Health and Clinical Excellence. Colorectal cancer NICE guidelines. [Internet]. 2020. Available from: URL:https://www.nice.org.uk/guidance/ng151/resources/colorectal-cancer-pdf-66141835244485

[11] National Comprehensive Cancer Network NCCN Clinical Practice Guidelines in Oncology: colon cancer. [Internet]. 2021. Available from: https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

[12] National Comprehensive Cancer Network NCCN Clinical Practice Guidelines in Oncology: rectal cancer. [Internet]. 2020. Available from: https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf

[13] Statistik för cancer Sweden. Socialstyrelsen.

[14] Cancer Statistics Review – SEER Statistics: National Cancer Institute (NIH); [cited 2020 9 November].

[15] Nasim F, Sabath BF, Eapen GA: Lung Cancer. Med Clin North Am 2019, 103:463-73.

[16] Duma N, Santana-Davila R, Molina JR: Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. Mayo Clin Proc 2019, 94:1623-40.

[17] Schweiger T, Liebmann-Reindl S, Glueck O, Starlinger P, Laengle J, Birner P, Klepetko W, Pils D, Streubel B, Hoetzenecker K: Mutational profile of colorectal cancer lung metastases and paired primary tumors by targeted next generation sequencing: implications on clinical outcome after surgery. J Thorac Dis 2018, 10:6147-57.
[18] Riihimaki M, Thomsen H, Sundquist K, Hemminki K: Colorectal cancer patients: what do they die of? Frontline Gastroenterol 2012, 3:143-9.
[19] van der Geest LG, Lam-Boer J, Koopman M, Verhoef C, Elferink MA, de Wilt JH: Nationwide trends in incidence, treatment and survival of colorectal cancer patients with synchronous metastases. Clin Exp Metastasis 2015, 32:457-65.
[20] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018, 68:394-424.
[21] Riihimaki M, Hemminki A, Sundquist J, Hemminki K: Patterns of metastasis in colon and rectal cancer. Sci Rep 2016, 6:29765.
[22] Mitry E, Guiu B, Cosconea S, Jooste V, Faivre J, Bouvier AM: Epidemiology, management and prognosis of colorectal cancer with lung metastases: a 30-year population-based study. Gut 2010, 59:1383-8.
[23] Nordholm-Carstensen A, Krarup PM, Jorgensen LN, Wille-Jorgensen PA, Harling H, Danish Colorectal Cancer G: Occurrence and survival of synchronous pulmonary metastases in colorectal cancer: a nationwide cohort study. Eur J Cancer 2014, 50:447-56.
[24] Vidarsdottir H, Moller PH, Jonasson JG, Pfannschmidt J, Gudbjartsson T: Indications and surgical outcome following pulmonary metastasectomy: a nationwide study. Thorac Cardiovasc Surg 2012, 60:383-9.
[25] Ge Y, Lei S, Cai B, Gao X, Wang G, Wang L, Wang Z: Incidence and prognosis of pulmonary metastasis in colorectal cancer: a population-based study. Int J Colorectal Dis 2020, 35:223-32.
[26] Manfredi S, Lepage C, Hatem C, Coatmeur O, Faivre J, Bouvier AM: Epidemiology and management of liver metastases from colorectal cancer. Ann Surg 2006, 244:254-9.
[27] Cavallaro P, Bordeianou L, Stafford C, Clark J, Berger D, Cusack J, Kunitake H, Francone T, Ricciardi R: Impact of Single-organ Metastasis to the Liver or Lung and Genetic Mutation Status on Prognosis in Stage IV Colorectal Cancer. Clin Colorectal Cancer 2020, 19:e8-e17.
[28] Kondo H, Okumura T, Ohde Y, Nakagawa K: Surgical treatment for metastatic malignancies. Pulmonary metastasis: indications and outcomes. Int J Clin Oncol 2005, 10:81-5.
[29] Internullo E, Cassivi SD, Van Raemdonck D, Friedel G, Treasure T, Group EPMW: Pulmonary metastasectomy: a survey of current practice amongst members of the European Society of Thoracic Surgeons. J Thorac Oncol 2008, 3:1257-66.
[30] Cheang MY, Herle P, Pradhan N, Antippa P: Video-assisted thoracoscopic surgery versus open thoracotomy for pulmonary metastasectomy: a systematic review. ANZ J Surg 2015, 85:408-13.
[31] Ripley RT, Downey RJ: Pulmonary metastasectomy. J Surg Oncol 2014, 109:42-6.

[32] Vogelsang H, Haas S, Hierholzer C, Berger U, Siewert JR, Prauer H: Factors influencing survival after resection of pulmonary metastases from colorectal cancer. Br J Surg 2004, 91:1066-71.

[33] Renaud S, Seitlinger J, Lawati YA, Guerrera F, Falcoz PE, Massard G, Ferri L, Spicer J: Anatomical Resections Improve Survival Following Lung Metastasectomy of Colorectal Cancer Harboring KRAS Mutations. Ann Surg 2018.

[34] Shiono S, Okumura T, Boku N, Hishida T, Ohde Y, Sakao Y, Yoshiya K, Hyodo I, Mori K, Kondo H: Outcomes of segmentectomy and wedge resection for pulmonary metastases from colorectal cancer. Eur J Cardiothorac Surg 2017, 51:504-10.

[35] Pfannschmidt J, Klode J, Muley T, Dienemann H, Hoffmann H: Nodal involvement at the time of pulmonary metastasectomy: experiences in 245 patients. Ann Thorac Surg 2006, 81:448-54.

[36] Welter S, Jacobs J, Krbek T, Krebs B, Stamatis G: Long-term survival after repeated resection of pulmonary metastases from colorectal cancer. Ann Thorac Surg 2007, 84:203-10.

[37] Hamaji M, Cassivi SD, Shen KR, Allen MS, Nichols FC, Deschamps C, Wigle DA: Is lymph node dissection required in pulmonary metastasectomy for colorectal adenocarcinoma? Ann Thorac Surg 2012, 94:1796-800.

[38] Renaud S, Alifano M, Falcoz PE, Magdeleinat P, Santelmo N, Pages O, Massard G, Regnard JF: Does nodal status influence survival? Results of a 19-year systematic lymphadenectomy experience during lung metastasectomy of colorectal cancer. Interact Cardiovasc Thorac Surg 2014, 18:482-7.

[39] Renaud S, Falcoz PE, Olland A, Schaeffer M, Reeb J, Santelmo N, Massard G: The intrathoracic lymph node ratio seems to be a better prognostic factor than the level of lymph node involvement in lung metastasectomy of colorectal carcinoma. Interact Cardiovasc Thorac Surg 2015, 20:215-21.

[40] Embun R, Rivas de Andres JJ, Call S, de Olaiz Navarro B, Freixinet JL, Bolufer S, Jarabo JR, Pajuelo N, Molins L, Spanish Group of Lung Metastases of Colo-Rectal Cancer of the Spanish Society of P, Thoracic S: Causal Model of Survival After Pulmonary Metastasectomy of Colorectal Cancer: A Nationwide Prospective Registry. Ann Thorac Surg 2016, 101:1883-90.

[41] Pfannschmidt J, Hoffmann H, Dienemann H: Reported outcome factors for pulmonary resection in metastatic colorectal cancer. J Thorac Oncol 2010, 5:S172-8.

[42] Gonzalez M, Poncet A, Combescure C, Robert J, Ris HB, Gervaz P: Risk factors for survival after lung metastasectomy in colorectal cancer patients: a systematic review and meta-analysis. Ann Surg Oncol 2013, 20:572-9.

[43] Zellweger M, Abdelnour-Berchtold E, Krueger T, Ris HB, Perentes JY, Gonzalez M: Surgical treatment of pulmonary metastasis in colorectal cancer patients: Current practice and results. Crit Rev Oncol Hematol 2018, 127:105-16.

[44] Salah S, Watanabe K, Welter S, Park JS, Park JW, Zabaleta J, Ardissone F, Kim J, Riquet M, Nojiri K, Gisabella M, Kim SY, Tanaka K, Al-Haj Ali B: Colorectal cancer pulmonary oligometastases: pooled analysis and construction of a clinical lung metastasectomy prognostic model. Ann Oncol 2012, 23:2649-55.

[45] Riquet M, Foucault C, Cazes A, Mitry E, Dujon A, Le Pimpec Barthes F, Medioni J, Rougier P: Pulmonary resection for metastases of colorectal adenocarcinoma. Ann Thorac Surg 2010, 89:375-80.

[46] Khattak MA, Martin HL, Beeke C, Price T, Carruthers S, Kim S, Padbury R, Karapetis CS: Survival differences in patients with metastatic colorectal cancer and with single site metastatic disease at initial presentation: results from South Australian clinical registry for advanced colorectal cancer. Clin Colorectal Cancer 2012, 11:247-54.

[47] Prasanna T, Karapetis CS, Roder D, Tie J, Padbury R, Price T, Wong R, Shapiro J, Nott L, Lee M, Chua YJ, Craft P, Piantadosi C, Sorich M, Gibbs P, Yip D: The survival outcome of patients with metastatic colorectal cancer based on the site of metastases and the impact of molecular markers and site of primary cancer on metastatic pattern. Acta Oncol 2018, 57:1438-44.

[48] Tsitsias T, Toufektzian L, Routledge T, Pilling J: Are there recognized prognostic factors for patients undergoing pulmonary metastasectomy for colorectal carcinoma? Interact Cardiovasc Thorac Surg 2016, 23:962-9.

[49] Blackmon SH, Stephens EH, Correa AM, Hofstetter W, Kim MP, Mehran RJ, Rice DC, Roth JA, Swisher SG, Walsh GL, Vaporciyan AA: Predictors of recurrent pulmonary metastases and survival after pulmonary metastasectomy for colorectal cancer. Ann Thorac Surg 2012, 94:1802-9.

[50] Cho JH, Kim S, Namgung M, Choi YS, Kim HK, Zo JI, Shim YM, Kim J: The prognostic importance of the number of metastases in pulmonary metastasectomy of colorectal cancer. World J Surg Oncol 2015, 13:222.

[51] Onaitis MW, Petersen RP, Haney JC, Saltz L, Park B, Flores R, Rizk N, Bains MS, Dycoco J, D'Amico TA, Harpole DH, Kemeny N, Rusch VW, Downey R: Prognostic factors for recurrence after pulmonary resection of colorectal cancer metastases. Ann Thorac Surg 2009, 87:1684-8.

[52] Bolukbas S, Sponholz S, Kudelin N, Eberlein M, Schirren J: Risk factors for lymph node metastases and prognosticators of survival in patients undergoing pulmonary metastasectomy for colorectal cancer. Ann Thorac Surg 2014, 97:1926-32.

[53] Yokoyama S, Mitsuoka M, Kinugasa T, Hashiguchi T, Matsumoto R, Murakami D, Nishi T, Yoshiyama K, Kashihara M, Takamori S, Akagi Y: Survival after initial lung metastasectomy for metastatic colorectal cancer in the modern chemotherapeutic era. BMC Surg 2017, 17:54.

[54] Meimarakis G, Spelsberg F, Angele M, Preissler G, Fertmann J, Crispin A, Reu S, Kalaitzis N, Stemmler M, Giessen C, Heinemann V, Stintzing S, Hatz R, Winter H: Resection of pulmonary metastases from colon and rectal cancer: factors to predict survival differ regarding to the origin of the primary tumor. Ann Surg Oncol 2014, 21:2563-72.

[55] Iida T, Nomori H, Shiba M, Nakajima J, Okumura S, Horio H, Matsuguma H, Ikeda N, Yoshino I, Ozeki Y, Takagi K, Goya T, Kawamura M, Hamada C, Kobayashi K, Metastatic Lung Tumor Study Group of J: Prognostic factors after pulmonary metastasectomy for colorectal cancer and rationale for determining surgical indications: a retrospective analysis. Ann Surg 2013, 257:1059-64.

[56] Hwang MR, Park JW, Kim DY, Chang HJ, Kim SY, Choi HS, Kim MS, Zo JI, Oh JH: Early intrapulmonary recurrence after pulmonary metastasectomy related to colorectal cancer. Ann Thorac Surg 2010, 90:398-404.
[57] Kim CH, Huh JW, Kim HJ, Lim SW, Song SY, Kim HR, Na KJ, Kim YJ: Factors influencing oncological outcomes in patients who develop pulmonary metastases after curative resection of colorectal cancer. Dis Colon Rectum 2012, 55:459-64.
[58] Watanabe K, Nagai K, Kobayashi A, Sugito M, Saito N: Factors influencing survival after complete resection of pulmonary metastases from colorectal cancer. Br J Surg 2009, 96:1058-65.
[59] Zampino MG, Maisonneuve P, Ravenda PS, Magni E, Casiraghi M, Solli P, Petrella F, Gasparri R, Galetta D, Borri A, Donghi S, Veronesi G, Spaggiari L: Lung metastases from colorectal cancer: analysis of prognostic factors in a single institution study. Ann Thorac Surg 2014, 98:1238-45.
[60] Yedibela S, Klein P, Feuchter K, Hoffmann M, Meyer T, Papadopoulos T, Gohl J, Hohenberger W: Surgical management of pulmonary metastases from colorectal cancer in 153 patients. Ann Surg Oncol 2006, 13:1538-44.
[61] Borasio P, Gisabella M, Bille A, Righi L, Longo M, Tampellini M, Ardissone F: Role of surgical resection in colorectal lung metastases: analysis of 137 patients. Int J Colorectal Dis 2011, 26:183-90.
[62] Iizasa T, Suzuki M, Yoshida S, Motohashi S, Yasufuku K, Iyoda A, Shibuya K, Hiroshima K, Nakatani Y, Fujisawa T: Prediction of prognosis and surgical indications for pulmonary metastasectomy from colorectal cancer. Ann Thorac Surg 2006, 82:254-60.
[63] Pfannschmidt J, Muley T, Hoffmann H, Dienemann H: Prognostic factors and survival after complete resection of pulmonary metastases from colorectal carcinoma: experiences in 167 patients. J Thorac Cardiovasc Surg 2003, 126:732-9.
[64] Zabaleta J, Iida T, Falcoz PE, Salah S, Jarabo JR, Correa AM, Zampino MG, Matsui T, Cho S, Ardissone F, Watanabe K, Gonzalez M, Gervaz P, Emparanza JI, Abraira V: Individual data meta-analysis for the study of survival after pulmonary metastasectomy in colorectal cancer patients: A history of resected liver metastases worsens the prognosis. Eur J Surg Oncol 2018, 44:1006-12.
[65] Renaud S, Romain B, Falcoz PE, Olland A, Santelmo N, Brigand C, Rohr S, Guenot D, Massard G: KRAS and BRAF mutations are prognostic biomarkers in patients undergoing lung metastasectomy of colorectal cancer. Br J Cancer 2015, 112:720-8.
[66] Schweiger T, Nikolowsky C, Starlinger P, Traxler D, Zimmermann M, Birner P, Hegedus B, Dome B, Bergmann M, Mildner M, Klepetko W, Hoetzenecker K, Ankersmit HJ: Stromal expression of heat-shock protein 27 is associated with worse clinical outcome in patients with colorectal cancer lung metastases. PLoS One 2015, 10:e0120724.
[67] Schweiger T, Starkl V, Glueck O, Glogner C, Traxler D, Jedamzik J, Liebmann-Reindl S, Birner P, Streubel B, Klepetko W, Hoetzenecker K: Clinical impact of c-MET expression and mutational status in patients with colorectal cancer lung metastases. Eur J Cardiothorac Surg 2016, 49:1103-11; discussion 11.

[68] Nanji S, Karim S, Tang E, Brennan K, McGuire A, Pramesh CS, Booth CM: Pulmonary Metastasectomy for Colorectal Cancer: Predictors of Survival in Routine Surgical Practice. Ann Thorac Surg 2018, 105:1605-12.
[69] Menna C, Berardi G, Tierno SM, Andreetti C, Maurizi G, Ciccone AM, D'Andrilli A, Cassiano F, Poggi C, Diso D, Venuta F, Rendina EA, Ibrahim M: Do Repeated Operations for Recurrent Colorectal Lung Metastases Result in Improved Survival? Ann Thorac Surg 2018, 106:421-7.
[70] Okumura T, Boku N, Hishida T, Ohde Y, Sakao Y, Yoshiya K, Higashiyama M, Hyodo I, Mori K, Kondo H: Surgical Outcome and Prognostic Stratification for Pulmonary Metastasis From Colorectal Cancer. Ann Thorac Surg 2017, 104:979-87.
[71] Pages PB, Serayssol C, Brioude G, Falcoz PE, Brouchet L, Le Pimpec-Barthes F, Thomas PA, Bernard A: Risk factors for survival and recurrence after lung metastasectomy. J Surg Res 2016, 203:293-300.
[72] Hirosawa T, Itabashi M, Ohnuki T, Yamaguchi N, Sugihara K, Kameoka S, Japanese Society for Cancer of the C, Rectum Study Group for Pulmonary Metastases from Colorectal C: Proposal of a new prognostic staging system for pulmonary metastases from colorectal cancer. Surg Today 2015, 45:576-84.
[73] Hunt SL, McKay A, Kelly LM, Kirk AJ: A case series of pulmonary resection for metastatic colorectal cancer in a UK regional thoracic center. Future Oncol 2015, 11:35-6.
[74] Cho S, Song IH, Yang HC, Jheon S: Prognostic factors of pulmonary metastasis from colorectal carcinoma. Interact Cardiovasc Thorac Surg 2013, 17:303-7.
[75] Karim S, Nanji S, Brennan K, Pramesh CS, Booth CM: Chemotherapy for resected colorectal cancer pulmonary metastases: Utilization and outcomes in routine clinical practice. Eur J Surg Oncol 2017, 43:1481-7.
[76] Park HS, Jung M, Shin SJ, Heo SJ, Kim CG, Lee MG, Beom SH, Lee CY, Lee JG, Kim DJ, Ahn JB: Benefit of Adjuvant Chemotherapy After Curative Resection of Lung Metastasis in Colorectal Cancer. Ann Surg Oncol 2016, 23:928-35.
[77] Subbiah IM, Blackmon SH, Correa AM, Kee B, Vaporciyan AA, Swisher SG, Eng C: Preoperative chemotherapy prior to pulmonary metastasectomy in surgically resected primary colorectal carcinoma. Oncotarget 2014, 5:6584-93.
[78] Zhang C, Tan Y, Xu H: Does adjuvant chemotherapy improve the prognosis of patients after resection of pulmonary metastasis from colorectal cancer? A systematic review and meta-analysis. Int J Colorectal Dis 2019, 34:1661-71.
[79] Nakajima J, Iida T, Okumura S, Horio H, Asamura H, Ozeki Y, Ikeda N, Matsuguma H, Chida M, Otsuka H, Kawamura M, Metastatic Lung Tumor Study Group of J: Recent improvement of survival prognosis after pulmonary metastasectomy and advanced chemotherapy for patients with colorectal cancer. Eur J Cardiothorac Surg 2017, 51:869-73.
[80] Brandi G, De Lorenzo S, Nannini M, Curti S, Ottone M, Dall'Olio FG, Barbera MA, Pantaleo MA, Biasco G: Adjuvant chemotherapy for resected colorectal cancer metastases: Literature review and meta-analysis. World J Gastroenterol 2016, 22:519-33.

[81] Treasure T, Farewell V, Macbeth F, Monson K, Williams NR, Brew-Graves C, Lees B, Grigg O, Fallowfield L, PulMi CCTG: Pulmonary Metastasectomy versus Continued Active Monitoring in Colorectal Cancer (PulMiCC): a multicentre randomised clinical trial. Trials 2019, 20:718.

[82] Jawed I, Wilkerson J, Prasad V, Duffy AG, Fojo T: Colorectal Cancer Survival Gains and Novel Treatment Regimens: A Systematic Review and Analysis. JAMA Oncol 2015, 1:787-95.

[83] Rodriguez-Fuster A, Belda-Sanchis J, Aguilo R, Embun R, Mojal S, Call S, Molins L, Rivas de Andres JJ, Gecmp Ccr S: Morbidity and mortality in a large series of surgical patients with pulmonary metastases of colorectal carcinoma: a prospective multicentre Spanish study (GECMP-CCR-SEPAR). Eur J Cardiothorac Surg 2014, 45:671-6.

[84] Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL: Genetic alterations during colorectal-tumor development. N Engl J Med 1988, 319:525-32.

[85] Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 1990, 61:759-67.

[86] Punt CJ, Koopman M, Vermeulen L: From tumour heterogeneity to advances in precision treatment of colorectal cancer. Nat Rev Clin Oncol 2017, 14:235-46.

[87] Harada S, Morlote D: Molecular Pathology of Colorectal Cancer. Adv Anat Pathol 2020, 27:20-6.

[88] Mendelaar PAJ, Smid M, van Riet J, Angus L, Labots M, Steeghs N, Hendriks MP, Cirkel GA, van Rooijen JM, Ten Tije AJ, Lolkema MP, Cuppen E, Sleijfer S, Martens JWM, Wilting SM: Whole genome sequencing of metastatic colorectal cancer reveals prior treatment effects and specific metastasis features. Nat Commun 2021, 12:574.

[89] Sottoriva A, Kang H, Ma Z, Graham TA, Salomon MP, Zhao J, Marjoram P, Siegmund K, Press MF, Shibata D, Curtis C: A Big Bang model of human colorectal tumor growth. Nat Genet 2015, 47:209-16.

[90] Guinney J, Dienstmann R, Wang X, de Reynies A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa EMF, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S: The consensus molecular subtypes of colorectal cancer. Nat Med 2015, 21:1350-6.

[91] Loree JM, Pereira AAL, Lam M, Willauer AN, Raghav K, Dasari A, Morris VK, Advani S, Menter DG, Eng C, Shaw K, Broaddus R, Routbort MJ, Liu Y, Morris JS, Luthra R, Meric-Bernstam F, Overman MJ, Maru D, Kopetz S: Classifying Colorectal Cancer by Tumor Location Rather than Sidedness Highlights a Continuum in Mutation Profiles and Consensus Molecular Subtypes. Clin Cancer Res 2018, 24:1062-72.

[92] Thanki K, Nicholls ME, Gajjar A, Senagore AJ, Qiu S, Szabo C, Hellmich MR, Chao C: Consensus Molecular Subtypes of Colorectal Cancer and their Clinical Implications. Int Biol Biomed J 2017, 3:105-11.

[93] Okita A, Takahashi S, Ouchi K, Inoue M, Watanabe M, Endo M, Honda H, Yamada Y, Ishioka C: Consensus molecular subtypes classification of colorectal cancer as a predictive factor for chemotherapeutic efficacy against metastatic colorectal cancer. Oncotarget 2018, 9:18698-711.

[94] Kim MJ, Lee HS, Kim JH, Kim YJ, Kwon JH, Lee JO, Bang SM, Park KU, Kim DW, Kang SB, Kim JS, Lee JS, Lee KW: Different metastatic pattern according to the KRAS mutational status and site-specific discordance of KRAS status in patients with colorectal cancer. BMC Cancer 2012, 12:347.

[95] Tie J, Lipton L, Desai J, Gibbs P, Jorissen RN, Christie M, Drummond KJ, Thomson BN, Usatoff V, Evans PM, Pick AW, Knight S, Carne PW, Berry R, Polglase A, McMurrick P, Zhao Q, Busam D, Strausberg RL, Domingo E, Tomlinson IP, Midgley R, Kerr D, Sieber OM: KRAS mutation is associated with lung metastasis in patients with curatively resected colorectal cancer. Clin Cancer Res 2011, 17:1122-30.

[96] Cejas P, Lopez-Gomez M, Aguayo C, Madero R, de Castro Carpeno J, Belda-Iniesta C, Barriuso J, Moreno Garcia V, Larrauri J, Lopez R, Casado E, Gonzalez-Baron M, Feliu J: KRAS mutations in primary colorectal cancer tumors and related metastases: a potential role in prediction of lung metastasis. PLoS One 2009, 4:e8199.

[97] Schweiger T, Hegedus B, Nikolowsky C, Hegedus Z, Szirtes I, Mair R, Birner P, Dome B, Lang G, Klepetko W, Ankersmit HJ, Hoetzenecker K: EGFR, BRAF and KRAS status in patients undergoing pulmonary metastasectomy from primary colorectal carcinoma: a prospective follow-up study. Ann Surg Oncol 2014, 21:946-54.

[98] Renaud S, Guerrera F, Seitlinger J, Costardi L, Schaeffer M, Romain B, Mossetti C, Claire-Voegeli A, Filosso PL, Legrain M, Ruffini E, Falcoz PE, Oliaro A, Massard G: KRAS exon 2 codon 13 mutation is associated with a better prognosis than codon 12 mutation following lung metastasectomy in colorectal cancer. Oncotarget 2017, 8:2514-24.

[99] Seligmann JF, Fisher D, Smith CG, Richman SD, Elliott F, Brown S, Adams R, Maughan T, Quirke P, Cheadle J, Seymour M, Middleton G: Investigating the poor outcomes of BRAF-mutant advanced colorectal cancer: analysis from 2530 patients in randomised clinical trials. Ann Oncol 2017, 28:562-8.

[100] Taylor CR: An exaltation of experts: concerted efforts in the standardization of immunohistochemistry. Hum Pathol 1994, 25:2-11.

[101] Shah AA, Frierson HF, Jr., Cathro HP: Analysis of immunohistochemical stain usage in different pathology practice settings. Am J Clin Pathol 2012, 138:831-6.

[102] Cartun RW, Taylor CR, Dabbs DJ: Techniques of Immunohistochemistry: Principles, Pitfalls, and Standardization, 2018.

[103] Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998, 4:844-7.

[104] Nocito A, Kononen J, Kallioniemi OP, Sauter G: Tissue microarrays (TMAs) for high-throughput molecular pathology research. Int J Cancer 2001, 94:1-5.

[105] Matoso A, Singh K, Jacob R, Greaves WO, Tavares R, Noble L, Resnick MB, Delellis RA, Wang LJ: Comparison of thyroid transcription factor-1 expression by 2 monoclonal antibodies in pulmonary and nonpulmonary primary tumors. Appl Immunohistochem Mol Morphol 2010, 18:142-9.
[106] Ordonez NG: Value of thyroid transcription factor-1 immunostaining in tumor diagnosis: a review and update. Appl Immunohistochem Mol Morphol 2012, 20:429-44.
[107] Comperat E, Zhang F, Perrotin C, Molina T, Magdeleinat P, Marmey B, Regnard JF, Audouin J, Camilleri-Broet S: Variable sensitivity and specificity of TTF-1 antibodies in lung metastatic adenocarcinoma of colorectal origin. Mod Pathol 2005, 18:1371-6.
[108] Kadota K, Nitadori J, Rekhtman N, Jones DR, Adusumilli PS, Travis WD: Reevaluation and reclassification of resected lung carcinomas originally diagnosed as squamous cell carcinoma using immunohistochemical analysis. Am J Surg Pathol 2015, 39:1170-80.
[109] Penman D, Downie I, Roberts F: Positive immunostaining for thyroid transcription factor-1 in primary and metastatic colonic adenocarcinoma: a note of caution. J Clin Pathol 2006, 59:663-4.
[110] Xu B, Thong N, Tan D, Khoury T: Expression of thyroid transcription factor-1 in colorectal carcinoma. Appl Immunohistochem Mol Morphol 2010, 18:244-9.
[111] NordiQC: Thyroid transcription factor-1 (TTF-1): assessment run 58 (2020). https://www.nordiqc.org/epitope.php?id=75, 2020.
[112] Travis WD BE, Burke AP, et al (eds). : WHO classification of tumours of the lung, pleura, thymus and heart, 4th edition. 4th ed: Lyon: IARC Press, 2015.
[113] Yatabe Y, Dacic S, Borczuk AC, Warth A, Russell PA, Lantuejoul S, Beasley MB, Thunnissen E, Pelosi G, Rekhtman N, Bubendorf L, Mino-Kenudson M, Yoshida A, Geisinger KR, Noguchi M, Chirieac LR, Bolting J, Chung JH, Chou TY, Chen G, Poleri C, Lopez-Rios F, Papotti M, Sholl LM, Roden AC, Travis WD, Hirsch FR, Kerr KM, Tsao MS, Nicholson AG, Wistuba I, Moreira AL: Best Practices Recommendations for Diagnostic Immunohistochemistry in Lung Cancer. J Thorac Oncol 2019, 14:377-407.
[114] Bae JM, Kim JH, Park JH, Park HE, Cho NY, Kang GH: Clinicopathological and molecular implications of aberrant thyroid transcription factor-1 expression in colorectal carcinomas: an immunohistochemical analysis of 1319 cases using three different antibody clones. Histopathology 2018, 72:423-32.
[115] Ye J, Hameed O, Findeis-Hosey JJ, Fan L, Li F, McMahon LA, Yang Q, Wang HL, Xu H: Diagnostic utility of PAX8, TTF-1 and napsin A for discriminating metastatic carcinoma from primary adenocarcinoma of the lung. Biotech Histochem 2012, 87:30-4.
[116] Alkushi A, Irving J, Hsu F, Dupuis B, Liu CL, Rijn M, Gilks CB: Immunoprofile of cervical and endometrial adenocarcinomas using a tissue microarray. Virchows Arch 2003, 442:271-7.
[117] Graham AD, Williams AR, Salter DM: TTF-1 expression in primary ovarian epithelial neoplasia. Histopathology 2006, 48:764-5.

[118] Siami K, McCluggage WG, Ordonez NG, Euscher ED, Malpica A, Sneige N, Silva EG, Deavers MT: Thyroid transcription factor-1 expression in endometrial and endocervical adenocarcinomas. Am J Surg Pathol 2007, 31:1759-63.
[119] Zhang PJ, Gao HG, Pasha TL, Litzky L, Livolsi VA: TTF-1 expression in ovarian and uterine epithelial neoplasia and its potential significance, an immunohistochemical assessment with multiple monoclonal antibodies and different secondary detection systems. Int J Gynecol Pathol 2009, 28:10-8.
[120] Robens J, Goldstein L, Gown AM, Schnitt SJ: Thyroid transcription factor-1 expression in breast carcinomas. Am J Surg Pathol 2010, 34:1881-5.
[121] Sakurai A, Sakai Y, Yatabe Y: Thyroid transcription factor-1 expression in rare cases of mammary ductal carcinoma. Histopathology 2011, 59:145-8.
[122] Lim TK, Teo C, Giron DM, Chong YL, Cheng C, Tan PH: Thyroid transcription factor-1 may be expressed in ductal adenocarcinoma of the prostate:a potential pitfall. J Clin Pathol 2007, 60:941-3.
[123] Galloway M, Sim R: TTF-1 staining in glioblastoma multiforme. Virchows Arch 2007, 451:109-11.
[124] Kristensen MH, Nielsen S, Vyberg M: Thyroid transcription factor-1 in primary CNS tumors. Appl Immunohistochem Mol Morphol 2011, 19:437-43.
[125] Zamecnik J, Chanova M, Kodet R: Expression of thyroid transcription factor 1 in primary brain tumours. J Clin Pathol 2004, 57:1111-3.
[126] Surrey LF, Frank R, Zhang PJ, Furth EE: TTF-1 and Napsin-A are expressed in a subset of cholangiocarcinomas arising from the gallbladder and hepatic ducts: continued caveats for utilization of immunohistochemistry panels. Am J Surg Pathol 2014, 38:224-7.
[127] Turner BM, Cagle PT, Sainz IM, Fukuoka J, Shen SS, Jagirdar J: Napsin A, a new marker for lung adenocarcinoma, is complementary and more sensitive and specific than thyroid transcription factor 1 in the differential diagnosis of primary pulmonary carcinoma: evaluation of 1674 cases by tissue microarray. Arch Pathol Lab Med 2012, 136:163-71.
[128] Xu B, Abourbih S, Sircar K, Kassouf W, Aprikian A, Tanguay S, Brimo F: Diagnostic and prognostic role of immunohistochemical expression of napsin-A aspartic peptidase in clear cell and papillary renal cell carcinoma: a study including 233 primary and metastatic cases. Appl Immunohistochem Mol Morphol 2014, 22:206-12.
[129] Fadare O, Desouki MM, Gwin K, Hanley KZ, Jarboe EA, Liang SX, Quick CM, Zheng W, Parkash V, Hecht JL: Frequent expression of napsin A in clear cell carcinoma of the endometrium: potential diagnostic utility. Am J Surg Pathol 2014, 38:189-96.
[130] Skirnisdottir I, Bjersand K, Akerud H, Seidal T: Napsin A as a marker of clear cell ovarian carcinoma. BMC Cancer 2013, 13:524.
[131] Ao MH, Zhang H, Sakowski L, Sharma R, Illei PB, Gabrielson E, Askin F, Li QK: The utility of a novel triple marker (combination of TTF1, napsin A, and p40) in the subclassification of non-small cell lung cancer. Hum Pathol 2014, 45:926-34.
[132] Bishop JA, Sharma R, Illei PB: Napsin A and thyroid transcription factor-1 expression in carcinomas of the lung, breast, pancreas, colon, kidney, thyroid, and malignant mesothelioma. Hum Pathol 2010, 41:20-5.

[133] Ye J, Findeis-Hosey JJ, Yang Q, McMahon LA, Yao JL, Li F, Xu H: Combination of napsin A and TTF-1 immunohistochemistry helps in differentiating primary lung adenocarcinoma from metastatic carcinoma in the lung. Appl Immunohistochem Mol Morphol 2011, 19:313-7.

[134] King JE, Thatcher N, Pickering CA, Hasleton PS: Sensitivity and specificity of immunohistochemical markers used in the diagnosis of epithelioid mesothelioma: a detailed systematic analysis using published data. Histopathology 2006, 48:223-32.

[135] Albadine R, Schultz L, Illei P, Ertoy D, Hicks J, Sharma R, Epstein JI, Netto GJ: PAX8 (+)/p63 (-) immunostaining pattern in renal collecting duct carcinoma (CDC): a useful immunoprofile in the differential diagnosis of CDC versus urothelial carcinoma of upper urinary tract. Am J Surg Pathol 2010, 34:965-9.

[136] Bishop JA, Teruya-Feldstein J, Westra WH, Pelosi G, Travis WD, Rekhtman N: p40 (DeltaNp63) is superior to p63 for the diagnosis of pulmonary squamous cell carcinoma. Mod Pathol 2012, 25:405-15.

[137] Bayrak R, Haltas H, Yenidunya S: The value of CDX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than CDX2 antibody. Diagn Pathol 2012, 7:9.

[138] Chu P, Wu E, Weiss LM: Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol 2000, 13:962-72.

[139] Paner GP, Annaiah C, Gulmann C, Rao P, Ro JY, Hansel DE, Shen SS, Lopez-Beltran A, Aron M, Luthringer DJ, De Peralta-Venturina M, Cho Y, Amin MB: Immunohistochemical evaluation of novel and traditional markers associated with urothelial differentiation in a spectrum of variants of urothelial carcinoma of the urinary bladder. Hum Pathol 2014, 45:1473-82.

[140] Barbareschi M, Murer B, Colby TV, Chilosi M, Macri E, Loda M, Doglioni C: CDX-2 homeobox gene expression is a reliable marker of colorectal adenocarcinoma metastases to the lungs. Am J Surg Pathol 2003, 27:141-9.

[141] Park SY, Kim BH, Kim JH, Lee S, Kang GH: Panels of immunohistochemical markers help determine primary sites of metastatic adenocarcinoma. Arch Pathol Lab Med 2007, 131:1561-7.

[142] Saad RS, Cho P, Silverman JF, Liu Y: Usefulness of Cdx2 in separating mucinous bronchioloalveolar adenocarcinoma of the lung from metastatic mucinous colorectal adenocarcinoma. Am J Clin Pathol 2004, 122:421-7.

[143] Nottegar A, Tabbo F, Luchini C, Brunelli M, Bria E, Veronese N, Santo A, Cingarlini S, Gilioli E, Ogliosi C, Eccher A, Montagna L, Pedron S, Doglioni C, Cangi MG, Inghirami G, Chilosi M: Pulmonary Adenocarcinoma With Enteric Differentiation: Immunohistochemistry and Molecular Morphology. Appl Immunohistochem Mol Morphol 2018, 26:383-7.

[144] Liu H, Shi J, Wilkerson ML, Lin F: Immunohistochemical evaluation of GATA3 expression in tumors and normal tissues: a useful immunomarker for breast and urothelial carcinomas. Am J Clin Pathol 2012, 138:57-64.

[145] Miettinen M, McCue PA, Sarlomo-Rikala M, Rys J, Czapiewski P, Wazny K, Langfort R, Waloszczyk P, Biernat W, Lasota J, Wang Z: GATA3: a multispecific but potentially useful marker in surgical pathology: a systematic analysis of 2500 epithelial and nonepithelial tumors. Am J Surg Pathol 2014, 38:13-22.

[146] Clark BZ, Beriwal S, Dabbs DJ, Bhargava R: Semiquantitative GATA-3 immunoreactivity in breast, bladder, gynecologic tract, and other cytokeratin 7-positive carcinomas. Am J Clin Pathol 2014, 142:64-71.

[147] Ordonez NG: Value of PAX 8 immunostaining in tumor diagnosis: a review and update. Adv Anat Pathol 2012, 19:140-51.

[148] Derry JM, Kerns JA, Francke U: RBM3, a novel human gene in Xp11.23 with a putative RNA-binding domain. Hum Mol Genet 1995, 4:2307-11.

[149] Danno S, Nishiyama H, Higashitsuji H, Yokoi H, Xue JH, Itoh K, Matsuda T, Fujita J: Increased transcript level of RBM3, a member of the glycine-rich RNA-binding protein family, in human cells in response to cold stress. Biochem Biophys Res Commun 1997, 236:804-7.

[150] Wellmann S, Buhrer C, Moderegger E, Zelmer A, Kirschner R, Koehne P, Fujita J, Seeger K: Oxygen-regulated expression of the RNA-binding proteins RBM3 and CIRP by a HIF-1-independent mechanism. J Cell Sci 2004, 117:1785-94.

[151] Ehlen A, Brennan DJ, Nodin B, O'Connor DP, Eberhard J, Alvarado-Kristensson M, Jeffrey IB, Manjer J, Brandstedt J, Uhlen M, Ponten F, Jirstrom K: Expression of the RNA-binding protein RBM3 is associated with a favourable prognosis and cisplatin sensitivity in epithelial ovarian cancer. J Transl Med 2010, 8:78.

[152] Jogi A, Brennan DJ, Ryden L, Magnusson K, Ferno M, Stal O, Borgquist S, Uhlen M, Landberg G, Pahlman S, Ponten F, Jirstrom K: Nuclear expression of the RNA-binding protein RBM3 is associated with an improved clinical outcome in breast cancer. Mod Pathol 2009, 22:1564-74.

[153] Jonsson L, Bergman J, Nodin B, Manjer J, Ponten F, Uhlen M, Jirstrom K: Low RBM3 protein expression correlates with tumour progression and poor prognosis in malignant melanoma: an analysis of 215 cases from the Malmo Diet and Cancer Study. J Transl Med 2011, 9:114.

[154] Salomonsson A, Micke P, Mattsson JSM, La Fleur L, Isaksson J, Jonsson M, Nodin B, Botling J, Uhlen M, Jirstrom K, Staaf J, Planck M, Brunnstrom H: Comprehensive analysis of RNA binding motif protein 3 (RBM3) in non-small cell lung cancer. Cancer Med 2020, 9:5609-19.

[155] Jonsson L, Gaber A, Ulmert D, Uhlen M, Bjartell A, Jirstrom K: High RBM3 expression in prostate cancer independently predicts a reduced risk of biochemical recurrence and disease progression. Diagn Pathol 2011, 6:91.

[156] Olofsson SE, Nodin B, Gaber A, Eberhard J, Uhlen M, Jirstrom K, Jerkeman M: Low RBM3 protein expression correlates with clinical stage, prognostic classification and increased risk of treatment failure in testicular non-seminomatous germ cell cancer. PLoS One 2015, 10:e0121300.

[157] Hjelm B, Brennan DJ, Zendehrokh N, Eberhard J, Nodin B, Gaber A, Ponten F, Johannesson H, Smaragdi K, Frantz C, Hober S, Johnson LB, Pahlman S, Jirstrom K, Uhlen M: High nuclear RBM3 expression is associated with an improved prognosis in colorectal cancer. Proteomics Clin Appl 2011, 5:624-35.

[158] Siesing C, Sorbye H, Dragomir A, Pfeiffer P, Qvortrup C, Ponten F, Jirstrom K, Glimelius B, Eberhard J: High RBM3 expression is associated with an improved survival and oxaliplatin response in patients with metastatic colorectal cancer. PLoS One 2017, 12:e0182512.

[159] Karnevi E, Dror LB, Mardinoglu A, Elebro J, Heby M, Olofsson SE, Nodin B, Eberhard J, Gallagher W, Uhlen M, Jirstrom K: Translational study reveals a two-faced role of RBM3 in pancreatic cancer and suggests its potential value as a biomarker for improved patient stratification. Oncotarget 2018, 9:6188-200.

[160] Melling N, Simon R, Mirlacher M, Izbicki JR, Stahl P, Terracciano LM, Bokemeyer C, Sauter G, Marx AH: Loss of RNA-binding motif protein 3 expression is associated with right-sided localization and poor prognosis in colorectal cancer. Histopathology 2016, 68:191-8.

[161] Cousin S, Grellety T, Toulmonde M, Auzanneau C, Khalifa E, Laizet Y, Tran K, Le Moulec S, Floquet A, Garbay D, Robert J, Hostein I, Soubeyran I, Italiano A: Clinical impact of extensive molecular profiling in advanced cancer patients. J Hematol Oncol 2017, 10:45.

[162] Yohe S, Thyagarajan B: Review of Clinical Next-Generation Sequencing. Arch Pathol Lab Med 2017, 141:1544-57.

[163] Bernardo L Rapoport GT, Fernando Schmitt, Nayler aSJ: Comprehensive Genomic Profiling. Oxford, UK: S. Karger Publishers Ltd, 2020. p. 109.

[164] Lindquist KE, Karlsson A, Leveen P, Brunnstrom H, Reutersward C, Holm K, Jonsson M, Annersten K, Rosengren F, Jirstrom K, Kosieradzki J, Ek L, Borg A, Planck M, Jonsson G, Staaf J: Clinical framework for next generation sequencing based analysis of treatment predictive mutations and multiplexed gene fusion detection in non-small cell lung cancer. Oncotarget 2017, 8:34796-810.

[165] Pestinger V, Smith M, Sillo T, Findlay JM, Laes JF, Martin G, Middleton G, Taniere P, Beggs AD: Use of an Integrated Pan-Cancer Oncology Enrichment Next-Generation Sequencing Assay to Measure Tumour Mutational Burden and Detect Clinically Actionable Variants. Mol Diagn Ther 2020.

[166] Prabhakaran S, Woo WLW, Xing G, Moffat D, Hussey M, Henderson DW, Klebe S: The Incidence of Labelling of Non-Lung Adenocarcinomas With Antibodies Against TTF-1 and Diagnostic Implications. Appl Immunohistochem Mol Morphol 2020, 28:471-6.

[167] Dettmer M, Kim TE, Jung CK, Jung ES, Lee KY, Kang CS: Thyroid transcription factor-1 expression in colorectal adenocarcinomas. Pathol Res Pract 2011, 207:686-90.

[168] Laurent E, Begueret H, Bonhomme B, Veillon R, Thumerel M, Velasco V, Brouste V, Hoppe S, Fournier M, Grellety T, MacGrogan G: SOX10, GATA3, GCDFP15, Androgen Receptor, and Mammaglobin for the Differential Diagnosis Between Triple-negative Breast Cancer and TTF1-negative Lung Adenocarcinoma. Am J Surg Pathol 2019, 43:293-302.

[169] Chang A, Amin A, Gabrielson E, Illei P, Roden RB, Sharma R, Epstein JI: Utility of GATA3 immunohistochemistry in differentiating urothelial carcinoma from prostate adenocarcinoma and squamous cell carcinomas of the uterine cervix, anus, and lung. Am J Surg Pathol 2012, 36:1472-6.
[170] Hattori Y, Yoshida A, Yoshida M, Takahashi M, Tsuta K: Evaluation of androgen receptor and GATA binding protein 3 as immunohistochemical markers in the diagnosis of metastatic breast carcinoma to the lung. Pathol Int 2015, 65:286-92.
[171] Warth A, Muley T, Herpel E, Meister M, Herth FJ, Schirmacher P, Weichert W, Hoffmann H, Schnabel PA: Large-scale comparative analyses of immunomarkers for diagnostic subtyping of non-small-cell lung cancer biopsies. Histopathology 2012, 61:1017-25.
[172] Koh J, Go H, Kim MY, Jeon YK, Chung JH, Chung DH: A comprehensive immunohistochemistry algorithm for the histological subtyping of small biopsies obtained from non-small cell lung cancers. Histopathology 2014, 65:868-78.
[173] Cadioli A, Rossi G, Costantini M, Cavazza A, Migaldi M, Colby TV: Lung cancer histologic and immunohistochemical heterogeneity in the era of molecular therapies: analysis of 172 consecutive surgically resected, entirely sampled pulmonary carcinomas. Am J Surg Pathol 2014, 38:502-9.
[174] Noh S, Shim H: Optimal combination of immunohistochemical markers for subclassification of non-small cell lung carcinomas: A tissue microarray study of poorly differentiated areas. Lung Cancer 2012, 76:51-5.
[175] Sterlacci W, Fiegl M, Hilbe W, Auberger J, Mikuz G, Tzankov A: Clinical relevance of neuroendocrine differentiation in non-small cell lung cancer assessed by immunohistochemistry: a retrospective study on 405 surgically resected cases. Virchows Arch 2009, 455:125-32.
[176] Szade J, Majewska HI, Zaczek AJ, Biernat W: High-grade non-small cell lung carcinoma: a comparative analysis of the phenotypic profile in small biopsies with the corresponding postoperative material. Pol J Pathol 2019, 70:100-8.
[177] Zhang Y, Zheng D, Li Y, Pan Y, Sun Y, Chen H: Comprehensive investigation of clinicopathologic features, oncogenic driver mutations and immunohistochemical markers in peripheral lung squamous cell carcinoma. J Thorac Dis 2017, 9:4434-40.
[178] Terry J, Leung S, Laskin J, Leslie KO, Gown AM, Ionescu DN: Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell carcinomas in small tumor samples. Am J Surg Pathol 2010, 34:1805-11.
[179] Ordonez NG: The diagnostic utility of immunohistochemistry in distinguishing between epithelioid mesotheliomas and squamous carcinomas of the lung: a comparative study. Mod Pathol 2006, 19:417-28.
[180] Werling RW, Yaziji H, Bacchi CE, Gown AM: CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. Am J Surg Pathol 2003, 27:303-10.
[181] Kim JH, Choi YD, Lee JS, Lee JH, Nam JH, Choi C: Utility of thyroid transcription factor-1 and CDX-2 in determining the primary site of metastatic adenocarcinomas in serous effusions. Acta Cytol 2010, 54:277-82.

[182] Yatabe Y, Koga T, Mitsudomi T, Takahashi T: CK20 expression, CDX2 expression, K-ras mutation, and goblet cell morphology in a subset of lung adenocarcinomas. J Pathol 2004, 203:645-52.
[183] De Lott LB, Morrison C, Suster S, Cohn DE, Frankel WL: CDX2 is a useful marker of intestinal-type differentiation: a tissue microarray-based study of 629 tumors from various sites. Arch Pathol Lab Med 2005, 129:1100-5.
[184] Dennis JL, Hvidsten TR, Wit EC, Komorowski J, Bell AK, Downie I, Mooney J, Verbeke C, Bellamy C, Keith WN, Oien KA: Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. Clin Cancer Res 2005, 11:3766-72.
[185] Inamura K, Satoh Y, Okumura S, Nakagawa K, Tsuchiya E, Fukayama M, Ishikawa Y: Pulmonary adenocarcinomas with enteric differentiation: histologic and immunohistochemical characteristics compared with metastatic colorectal cancers and usual pulmonary adenocarcinomas. Am J Surg Pathol 2005, 29:660-5.
[186] Kriegsmann M, Harms A, Longuespee R, Muley T, Winter H, Kriegsmann K, Kazdal D, Goeppert B, Pathil A, Warth A: Role of conventional immunomarkers, HNF4-alpha and SATB2, in the differential diagnosis of pulmonary and colorectal adenocarcinomas. Histopathology 2018, 72:997-1006.
[187] Wakejima R, Inamura K, Ninomiya H, Nagano H, Mun M, Okumura S, Okubo K, Ishikawa Y: Mucinous lung adenocarcinoma, particularly referring to EGFR-mutated mucinous adenocarcinoma. Pathol Int 2020, 70:72-83.
[188] Herawi M, De Marzo AM, Kristiansen G, Epstein JI: Expression of CDX2 in benign tissue and adenocarcinoma of the prostate. Hum Pathol 2007, 38:72-8.
[189] Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, Aranda Aguilar E, Bardelli A, Benson A, Bodoky G, Ciardiello F, D'Hoore A, Diaz-Rubio E, Douillard JY, Ducreux M, Falcone A, Grothey A, Gruenberger T, Haustermans K, Heinemann V, Hoff P, Kohne CH, Labianca R, Laurent-Puig P, Ma B, Maughan T, Muro K, Normanno N, Osterlund P, Oyen WJ, Papamichael D, Pentheroudakis G, Pfeiffer P, Price TJ, Punt C, Ricke J, Roth A, Salazar R, Scheithauer W, Schmoll HJ, Tabernero J, Taieb J, Tejpar S, Wasan H, Yoshino T, Zaanan A, Arnold D: ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol 2016, 27:1386-422.
[190] Van Cutsem E, Cervantes A, Nordlinger B, Arnold D, Group EGW: Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014, 25 Suppl 3:iii1-9.
[191] Aberg T, Malmberg KA, Nilsson B, Nou E: The effect of metastasectomy: fact or fiction? Ann Thorac Surg 1980, 30:378-84.
[192] Pfannschmidt J, Dienemann H, Hoffmann H: Surgical resection of pulmonary metastases from colorectal cancer: a systematic review of published series. Ann Thorac Surg 2007, 84:324-38.
[193] van Dorp M, Beck N, Steup WH, Schreurs WH: Surgical treatment of pulmonary metastases in the Netherlands: data from the Dutch Lung Cancer Audit for Surgery. Eur J Cardiothorac Surg 2020, 58:768-74.

[194] Ishimaru K, Kawai K, Nozawa H, Sasaki K, Murono K, Emoto S, Ishii H, Anzai H, Sonoda H, Yamauchi S, Sugihara K, Ishihara S, And other members of the University of Tokyo Study G: Hazard function analysis of metastatic recurrence after colorectal cancer surgery-A nationwide retrospective study. J Surg Oncol 2021, 123:1015-22.
[195] Mizuno N, Kato Y, Izumi Y, Irimura T, Sugiyama Y: Importance of hepatic first-pass removal in metastasis of colon carcinoma cells. J Hepatol 1998, 28:865-77.
[196] Hughes ES, Cuthbertson AM: Recurrence after curative excision of carcinoma of the large bowel. JAMA 1962, 182:1303-6.
[197] Koch M, Weitz J, Kienle P, Benner A, Willeke F, Lehnert T, Herfarth C, von Knebel Doeberitz M: Comparative analysis of tumor cell dissemination in mesenteric, central, and peripheral venous blood in patients with colorectal cancer. Arch Surg 2001, 136:85-9.
[198] Renaud S, Seitlinger J, St-Pierre D, Garfinkle R, Al Lawati Y, Guerrera F, Ruffini E, Falcoz PE, Massard G, Ferri L, Spicer J: Prognostic value of neutrophil to lymphocyte ratio in lung metastasectomy for colorectal cancer. Eur J Cardiothorac Surg 2019, 55:948-55.
[199] Shiono S, Ishii G, Nagai K, Murata Y, Tsuta K, Nitadori J, Kodama T, Ochiai A: Immunohistochemical prognostic factors in resected colorectal lung metastases using tissue microarray analysis. Eur J Surg Oncol 2006, 32:308-9.
[200] Pfannschmidt J, Bade S, Hoheisel J, Muley T, Dienemann H, Herpel E: Identification of immunohistochemical prognostic markers for survival after resection of pulmonary metastases from colorectal carcinoma. Thorac Cardiovasc Surg 2009, 57:403-8.
[201] Inoue M, Ohta M, Iuchi K, Matsumura A, Ideguchi K, Yasumitsu T, Nakagawa K, Fukuhara K, Maeda H, Takeda S, Minami M, Ohno Y, Matsuda H, Thoracic Surgery Study Group of Osaka U: Benefits of surgery for patients with pulmonary metastases from colorectal carcinoma. Ann Thorac Surg 2004, 78:238-44.
[202] Nelson DB, Tayob N, Mitchell KG, Correa AM, Hofstetter WL, Sepesi B, Walsh GL, Vaporciyan AA, Swisher SG, Antonoff MB, Roth JA, Rice DC, Vauthey JN, Mehran RJ: Surgical margins and risk of local recurrence after wedge resection of colorectal pulmonary metastases. J Thorac Cardiovasc Surg 2019, 157:1648-55.
[203] Andre T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, Bonetti A, Clingan P, Bridgewater J, Rivera F, de Gramont A: Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol 2009, 27:3109-16.
[204] Schmoll HJ, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, Hill M, Hoersch S, Rittweger K, Haller DG: Capecitabine Plus Oxaliplatin Compared With Fluorouracil/Folinic Acid As Adjuvant Therapy for Stage III Colon Cancer: Final Results of the NO16968 Randomized Controlled Phase III Trial. J Clin Oncol 2015, 33:3733-40.
[205] Kelland L: The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer 2007, 7:573-84.
[206] Alcindor T, Beauger N: Oxaliplatin: a review in the era of molecularly targeted therapy. Curr Oncol 2011, 18:18-25.

[207] de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A: Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000, 18:2938-47.

[208] Petrella F, Chieco P, Solli P, Veronesi G, Borri A, Galetta D, Gasparri R, Spaggiari L: Which factors affect pulmonary function after lung metastasectomy? Eur J Cardiothorac Surg 2009, 35:792-6.

[209] Vignot S, Lefebvre C, Frampton GM, Meurice G, Yelensky R, Palmer G, Capron F, Lazar V, Hannoun L, Miller VA, Andre F, Stephens PJ, Soria JC, Spano JP: Comparative analysis of primary tumour and matched metastases in colorectal cancer patients: evaluation of concordance between genomic and transcriptional profiles. Eur J Cancer 2015, 51:791-9.

[210] Imamura Y, Lochhead P, Yamauchi M, Kuchiba A, Qian ZR, Liao X, Nishihara R, Jung S, Wu K, Nosho K, Wang YE, Peng S, Bass AJ, Haigis KM, Meyerhardt JA, Chan AT, Fuchs CS, Ogino S: Analyses of clinicopathological, molecular, and prognostic associations of KRAS codon 61 and codon 146 mutations in colorectal cancer: cohort study and literature review. Mol Cancer 2014, 13:135.

[211] Han CB, Li F, Ma JT, Zou HW: Concordant KRAS mutations in primary and metastatic colorectal cancer tissue specimens: a meta-analysis and systematic review. Cancer Invest 2012, 30:741-7.

[212] Fujiyoshi K, Yamamoto G, Takahashi A, Arai Y, Yamada M, Kakuta M, Yamaguchi K, Akagi Y, Nishimura Y, Sakamoto H, Akagi K: High concordance rate of KRAS/BRAF mutations and MSI-H between primary colorectal cancer and corresponding metastases. Oncol Rep 2017, 37:785-92.

[213] Bhullar DS, Barriuso J, Mullamitha S, Saunders MP, O'Dwyer ST, Aziz O: Biomarker concordance between primary colorectal cancer and its metastases. EBioMedicine 2019, 40:363-74.

[214] Watanabe T, Kobunai T, Yamamoto Y, Matsuda K, Ishihara S, Nozawa K, Iinuma H, Shibuya H, Eshima K: Heterogeneity of KRAS status may explain the subset of discordant KRAS status between primary and metastatic colorectal cancer. Dis Colon Rectum 2011, 54:1170-8.

[215] Choi JY, Choi S, Lee M, Park YS, Sung JS, Chang WJ, Kim JW, Choi YJ, Kim J, Kim DS, Lee SH, Seok J, Park KH, Kim SH, Kim YH: Clinical Implication of Concordant or Discordant Genomic Profiling between Primary and Matched Metastatic Tissues in Patients with Colorectal Cancer. Cancer Res Treat 2020, 52:764-78.

[216] Zou SM, Li WH, Wang WM, Li WB, Shi SS, Ying JM, Lyu N: The gene mutational discrepancies between primary and paired metastatic colorectal carcinoma detected by next-generation sequencing. J Cancer Res Clin Oncol 2018, 144:2149-59.

[217] Stein MK, Williard FW, Xiu J, Tsao MW, Martin MG, Deschner BW, Dickson PV, Glazer ES, Yakoub D, Shibata D, Grothey AF, Philip PA, Hwang JJ, Shields AF, Marshall JL, Korn WM, Lenz HJ, Deneve JL: Comprehensive tumor profiling reveals unique molecular differences between peritoneal metastases and primary colorectal adenocarcinoma. J Surg Oncol 2020, 121:1320-8.

[218] Giardina T, Robinson C, Grieu-Iacopetta F, Millward M, Iacopetta B, Spagnolo D, Amanuel B: Implementation of next generation sequencing technology for somatic mutation detection in routine laboratory practice. Pathology 2018, 50:389-401.
[219] Fisher KE, Zhang L, Wang J, Smith GH, Newman S, Schneider TM, Pillai RN, Kudchadkar RR, Owonikoko TK, Ramalingam SS, Lawson DH, Delman KA, El-Rayes BF, Wilson MM, Sullivan HC, Morrison AS, Balci S, Adsay NV, Gal AA, Sica GL, Saxe DF, Mann KP, Hill CE, Khuri FR, Rossi MR: Clinical Validation and Implementation of a Targeted Next-Generation Sequencing Assay to Detect Somatic Variants in Non-Small Cell Lung, Melanoma, and Gastrointestinal Malignancies. J Mol Diagn 2016, 18:299-315.
[220] Prieto-Potin I, Carvajal N, Plaza-Sanchez J, Manso R, Auz-Alexandre CL, Chamizo C, Zazo S, Lopez-Sanchez A, Rodriguez-Pinilla SM, Camacho L, Longaron R, Bellosillo B, Somoza R, Hernandez-Losa J, Fernandez-Soria VM, Ramos-Ruiz R, Cristobal I, Garcia-Foncillas J, Rojo F: Validation and clinical application of a targeted next-generation sequencing gene panel for solid and hematologic malignancies. PeerJ 2020, 8:e10069.
[221] Torhorst J, Bucher C, Kononen J, Haas P, Zuber M, Kochli OR, Mross F, Dieterich H, Moch H, Mihatsch M, Kallioniemi OP, Sauter G: Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001, 159:2249-56.