Biomarkers and prognostic tools in the surgical management of colorectal liver metastases

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University, to be publicly defended on the 20th of February 2026, at 09.00 in Lecture Hall 1, Skåne University Hospital in Lund.

Faculty opponent
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Title: Biomarkers and prognostic tools in the surgical management of colorectal liver metastases

Abstract

Background: Colorectal liver metastases (CRLM) represent a major challenge in oncologic surgery, with surgical resection offering the only potential for cure. However, patient selection and prognostic assessment are complex due to the heterogeneity of tumor biology and response to treatment. Advances in molecular biomarkers and imaging techniques have shown promise in improving prognostic accuracy and guiding individualized treatment strategies.

Aims: The primary objectives of this thesis are: (I) to systematically review and analyze prognostic biomarkers that have been assessed through immunohistochemistry in CRLM; (II) to evaluate the prognostic significance of known and exploratory tumor characteristics in colon liver metastases (CLM); (III) to investigate the correlation between tumor regression grade on immunohistochemical analysis and radiologic findings using high-diffusion magnetic resonance imaging (MRI); and (IV) to assess the prognostic value of the expression of rho GTPase-activating protein 4 (ARHGAP4) in CLM.

Methods: A systematic review and meta-analysis were conducted to evaluate existing immunohistochemical biomarkers for CLM prognosis. A study was performed of patients undergoing liver resection for CLM at Skåne University Hospital, incorporating histopathological evaluation and immunohistochemical analysis of tumor samples. MRI-based diffusion imaging was utilized to assess tumor response to neoadjuvant chemotherapy. ARHGAP4 expression was examined via immunohistochemistry and correlated with survival outcomes.

Results and conclusions: The meta-analysis identified multiple biomarkers associated with prognosis in CRLM, although methodologies must be standardized before clinical application. Histopathological analysis revealed that lymphovascular invasion was a strong predictor of poor prognosis, whereas tumor regression grade and growth patterns had limited prognostic utility. MRI-based diffusion imaging could not be used reliably to differentiate between responding and non-responding tumors. ARHGAP4 overexpression was associated with worse rates of postoperative survival, and this finding indicated its potential as a novel prognostic biomarker. These findings highlight the need for integrated prognostic tools that combine histopathology and radiologic imaging to optimize clinical decision-making in CLM surgery.

Key words: Colorectal liver metastases, prognosis prediction, biomarkers, radiologic imaging

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All we have to decide is what to do with the time that is given to us –
Gandalf the grey

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IV. **Torén W**, Sasor A, Ansari D, Andersson R. ARHGAP4 as a prognostic biomarker for colon liver metastases after surgical resection. Anticancer Research 2024; 44:2597-2604

Thesis at a glance

Paper	Objective	Methods	Results/Conclusions
I	To systematically review and analyze prognostic biomarkers assessed through immunohistochemistry in colorectal liver metastases (CRLM).	A systematic review and meta-analysis of published studies was performed to evaluate immunohistochemical biomarkers for CRLM prognosis.	Several biomarkers were identified as associated with prognosis in CRLM, but standardization of methodologies is needed for clinical application.
II	To evaluate the prognostic significance of known and exploratory tumor characteristics by immunohistochemistry in CLM.	Patients at Skåne University Hospital who were undergoing liver resection for CLM were studied, and the tumor samples were analyzed histopathologically and immunohistochemically.	Classical immunohistochemical traits were found to be predictors of poor prognosis; novel markers had limited prognostic utility.
III	To investigate, through the use of high-diffusion MRI, the correlation between the grade of tumor regression on immunohistochemical analysis and radiologic findings.	MRI-based diffusion imaging was utilized to assess tumor response to neoadjuvant chemotherapy, compared to histopathological findings.	MRI-based diffusion imaging could not be used reliably to differentiate between responding and non-responding tumors. This highlighted the need for better imaging tools to estimate treatment response.
IV	To assess the prognostic value of expression of rho GTPase-activating protein 4 (ARHGAP4) in CLM.	ARHGAP4 expression in tumor samples from resected CLM patients was immunohistologically analyzed.	ARHGAP4 overexpression was associated with worse rates of postoperative survival, suggesting its potential as a novel prognostic biomarker.

Abbreviations

ADC	Apparent diffusion coefficient
AJCC	American Joint Committee on Cancer
ARHGAP4	Rho GTPase-activating protein 4
CAP	College of American Pathologists
CEA	Carcinoembryonic antigen
CI	Confidence interval
CLM	Colon liver metastases
CRC	Colorectal cancer
CRLM	Colorectal liver metastases
CT	Computed tomography
dMMR	Deficient mismatch repair
FLR	Future liver remnant
FOLFIRI	Irinotecan, leucovorin, and fluorouracil
FOLFOX	Oxaliplatin, leucovorin, and fluorouracil
FOLFOXIRI	Fluorouracil, leucovorin, oxaliplatin, and irinotecan
HR	Hazard ratio
IMA	Inferior mesenteric artery
KRAS	Kirsten rat sarcoma viral oncogene
ML	Machine learning
MMR	Mismatch repair
MRI	Magnetic resonance imaging
MSI-H	Microsatellite instability-high
OS	Overall survival
SMA	Superior mesenteric artery
TMA	Tissue microarray
TNM	Tumor, node, metastasis
TRG	Tumor regression grade

Abstract

Background: Colorectal liver metastases (CRLM) represent a major challenge in oncologic surgery, with surgical resection offering potential for cure. However, patient selection and prognostic assessment are complex due to the heterogeneity of tumor biology and response to treatment. Advances in molecular biomarkers and imaging techniques have shown promise in improving prognostic accuracy and guiding individualized treatment strategies.

Aims: The primary objectives of this thesis are: (I) to systematically review and analyze prognostic biomarkers that have been assessed through immunohistochemistry in CRLM; (II) to evaluate the prognostic significance of known and exploratory histopathological tumor characteristics in colon liver metastases (CLM); (III) to investigate the correlation between tumor regression grade on immunohistochemical analysis and radiologic findings using high-diffusion MRI; and (IV) to assess the prognostic value of the expression of rho GTPase-activating protein 4 (ARHGAP4) in CLM.

Methods: A systematic review and meta-analysis were conducted to evaluate existing immunohistochemical biomarkers for CLM prognosis. A study was performed of patients undergoing liver resection for CLM at Skåne University Hospital, incorporating histopathological evaluation and immunohistochemical analysis of tumor samples. MRI-based diffusion imaging was utilized to assess tumor response to neoadjuvant chemotherapy. ARHGAP4 expression was examined via immunohistochemistry and correlated with survival outcomes.

Results and conclusions: Paper I identified multiple biomarkers that were associated with prognosis in CLM, although methodologies must be standardized before clinical application. In Paper II, it is shown that histopathological analysis did not identify novel histopathological traits to have prognostic utility after hepatectomy. Classical prognostic traits such as lymphovascular invasion were found to have prognostic value. In Paper III, it is explained that MRI-based diffusion imaging could not be used reliably to differentiate between responding and non-responding tumors in patients undergoing neoadjuvant chemotherapy before hepatectomy. Paper IV demonstrated that ARHGAP4 overexpression was associated with worse rates of postoperative survival, indicating its potential as a novel prognostic biomarker in patients undergoing resection for CRLM.

Populärvetenskaplig sammanfattning

Kan biomarkörer visa vägen för skräddarsydd kirurgi för levermetastaser?

Varje år drabbas tusentals människor av tjock- och ändtarmscancer, en av de vanligaste cancerformerna i världen. För en stor andel av dessa patienter sprider sig sjukdomen till levern. När detta sker blir behandlingen mer komplex, men inte hopplös.

Många som får tjocktarmscancer eller ändtarmscancer kan bli helt friska om tumören opereras bort i tid. Många personer drabbas dock av spridning av dottertumörer till levern. Levern är kroppens filter för blodet från tarmen, och därför är det vanligt att just den drabbas först.

När cancern har spridit sig dit blir behandlingen mycket mer komplicerad. Att operera i levern är tekniskt svårt och riskfyllt, eftersom organet har en mycket rik blodförsörjning och styr flera av kroppens viktigaste funktioner. Kirurgen måste ta bort tumörerna utan att skada för mycket av den friska vävnaden, vilket gör ingreppet mer krävande än en vanlig tarmoperation.

Samtidigt visar levermetastaser att tumören har utvecklat förmågan att sprida sig i kroppen, något som försämrar prognosen och ökar risken för återfall. Därför är just spridning till levern en av de största utmaningarna inom dagens cancerbehandling.

Den enda behandling som kan leda till bot hos dessa patienter är kirurgisk borttagning av metastaserna i levern, så kallad leverresektion. Resultaten har förbättrats dramatiskt de senaste decennierna. För 40 år sedan dog hälften av patienterna efter en leveroperation men idag är dödligheten under 1 % vid specialiserade centra. Trots dessa framsteg är återfall vanligt, och det är fortfarande svårt att avgöra i förväg vilka patienter som verkligen kommer att ha nytta av operationen och vilka som riskerar att genomgå ett riskfyllt ingrepp utan långsiktig vinst.

För att lösa detta dilemma behövs nya verktyg. En möjlig lösning ligger i så kallade biomarkörer vilket är molekylära signaler som kan ge ledtrådar om tumörens aggressivitet, förmåga att sprida sig och känslighet för behandling. Samtidigt har nya bilddiagnostiska metoder, som avancerad magnetkamera, givit en föreställning om mer träffsäkra och individanpassade beslut i vården.

Syftet med denna avhandling är att förbättra möjligheterna att förutse prognosen för patienter med tjock- och ändtarmscancer där personen drabbats av dottertumörer i levern, och därigenom bidra till mer individanpassad och effektiv behandling. Detta görs genom att kombinera traditionell patologi med modern teknik inom bilddiagnostik.

Mer specifikt syftar arbetet till att:

1. Sammanställa och analysera existerande kunskap om immunohistokemiska biomarkörer hos patienter med levermetastaser från kolorektal cancer, genom en systematisk översikt och metaanalys.
2. Undersöka om klassiska och nyupptäckta tumöregenskaper i vävnadsprover kan förutsäga överlevnad efter leverkirurgi, med särskilt fokus på tumörens mikroskopiska utseende och svar på cellgiftsbehandling.
3. Utvärdera kopplingen mellan bilddiagnostik och verklig tumörrespons, genom att jämföra bilder från magnetkameraundersökning före och efter behandling med vad som ses i mikroskop efter operation.
4. Studera den nya biomarkören ARHGAP4, och undersöka om höga nivåer av detta protein är kopplade till sämre överlevnad efter kirurgi – något som kan göra den till en möjlig framtida indikator för behandlingsbeslut.

Avhandlingen bygger på fyra delstudier:

Studie I är en systematisk översikt och metaanalys av tidigare forskning kring immunohistokemiska biomarkörer för dottertumörer i levern från just tjock- och ändtarmscancer. Ett flertal biomarkörer identifierades i en systematisk översikt och en så kallad meta-analys genomfördes där data från de inhämtade studierna sammanfattas i en gemensam statistisk analys. Metoderna för att mäta biomarkörerna varierade kraftigt mellan studierna. Vi visar i denna studie att biomarkörer har stor potential men att metoderna behöver standardiseras. För att kunna använda dessa markörer inom sjukvården krävs enhetliga och reproducerbara protokoll vid biomarkörsforskning.

Studie II-IV bygger på data från patienter som opererats för levermetastaser vid Skånes universitetssjukhus. I studie II har tumörvävnad analyserats i mikroskop för att undersöka både redan kända och kliniskt rutinmässigt använda egenskaper, samt nya egenskaper som kan ha betydelse för framtida forskning och behandling. Vi visar i denna studie att tumörens mikroskopiska egenskaper påverkar prognosen efter kirurgi. Lymf- och kärlinväxt är starkt kopplat till sämre överlevnad. Däremot visade tumörens tillbakagång efter cellgifter (tumörregressionsgrad) och dess tillväxtmönster ingen tydlig koppling till prognos.

Studie III inkluderar avancerad magnetkamerateknik för att utvärdera tumörens svar på cellgiftsbehandling. Vi visar här att magnetkamera-baserade mätningar inte kunde på ett tillförlitligt sätt skilja mellan tumörer som svarade väl på cellgiftsbehandling innan operation och de som enligt mikroskopisk undersökning hade stor effekt av cellgiftsbehandling. Detta pekar på behov av mer exakta röntgenmetoder för att bedöma behandlingsrespons.

Studie IV fokuserar på ARHGAP4, ett protein som visat sig vara kopplat till sämre överlevnad. Genom att mäta nivåerna av ARHGAP4 i bortopererade tumörer och

göra statistisk analys där vi jämför nivåerna av denna biomarkör i tumörer med hur länge personer klarat sig efter operation av levermetastaser från tjocktarmstumörer kunde vi i våra analyser se att det finns underlag att tro att det finns samband mellan ARHGAP4 nivåer och överlevnad efter operation. Vi visar här att ARHGAP4 är en möjlig ny prognostisk markör då nivåerna i tumörvävnad var kopplat till överlevnad efter operation.

Introduction

From myth to modernity: a historical perspective on liver surgery

The liver's ability to heal itself has fascinated people for thousands of years. Possibly the earliest story about this comes from ancient Greek mythology.

Around 700BC, the poet Hesiod told the tale of Prometheus, a clever trickster who defied Zeus by stealing fire from Mount Olympus and giving it to humanity. Fire changed everything. It allowed people to cook food, forge weapons and build civilizations. But Zeus was furious. As punishment, he chained Prometheus to a mountain, where an eagle came every day to feast on his liver. Each night, his liver miraculously grew back, only for the torture to begin again the next morning [1, 2].

Today, the regenerative capacity of the liver is no longer a mythological tale, but rather a basis of modern hepatic surgery.

Around 300BC, a Greek physician made the first known attempt to describe the liver's anatomy. Herophilus studied the human body and wrote about the liver's structure [3]. Unfortunately, his work was lost to history until centuries later, around 150AD, another famous physician named Galen referenced Herophilus's findings and described the liver's lobes and blood vessels fundamentally as we know them today [4].

An English doctor named Francis Glisson made a major breakthrough in 1654. He wanted to understand how blood flowed through the liver, so he came up with a simple but brilliant experiment. He boiled a liver in water, which dissolved the soft tissue and exposed its network of blood vessels. Then, he injected colored milk into the vessels to trace the paths of blood flow [5].

Glisson's work was an important landmark that enabled us to understand the functional anatomy of the liver, but operating on the liver was a different challenge. For a long time, liver surgery was nearly impossible because of one major problem: bleeding.

Figure 1. Prometheus, bound to a mountain, his liver eaten by an eagle. Engraving by Cornelius Cort (1566). Source: Wellcome Collection.

Figure 2. James Hogarth Pringle, the surgeon with the mustache standing on the right, in the operating room of the Royal Infirmary in Glasgow (beginning of the twentieth century). Source: Photograph in the collection of the Royal College of Physicians and Surgeons of Glasgow.

Things started to change in the 19th century, when two key medical discoveries revolutionized surgery: anesthesia and germ theory. The use of anesthesia made it possible to perform long and complex procedures, while the discovery of bacteria led to the application of sterilization techniques that prevented deadly infections. With these advancements, new attempts at liver surgery began [6, 7].

In the 1880s, doctors in Germany performed the first gallbladder removal (cholecystectomy) and soon after, the first planned liver resection [8]. However, the control of bleeding was still a major issue. In 1908, a Scottish surgeon named James Hogarth Pringle came up with a brilliant and lifesaving surgical technique. He discovered that by temporarily squeezing the portal ligament, which contains the main blood supply to the liver, he could drastically reduce bleeding. This method, now known as the Pringle maneuver, is routinely used in modern surgery of the liver [9].

Throughout the twentieth century, surgeons kept pushing the limits of what was possible. In 1951, Swedish scientist Carl-Herman Hjortsjö mapped out the liver's complex system of bile ducts and their correlation to blood. Around the same time, Japanese surgeons pioneered a new approach: removal of parts of the liver based on

its natural segments. Yet, even with these advances, liver surgery was still extremely dangerous [9].

In the 1960s, the odds of surviving a major liver resection were grim; approximately 50% of patients did not survive the postoperative period [9]. By the 1980s, the use of better techniques had lowered the risk of early postoperative death, but the mortality rate was still around 10% [9]. Then, in the 1990s, everything changed. Advances in surgical techniques, imaging, and post-op care brought mortality rates down to just 1% in leading hospitals around the world [10].

Today, liver surgery is safer than ever and performed regularly in hospitals worldwide. What once seemed impossible – removal of large portions of the liver only to see it grow back – has become routine. Yet the journey is not over. Scientists and surgeons continue to push the boundaries of what they can do to save human life with surgical interventions of the liver. This thesis aims to make a modest contribution to the safety of liver surgery by exploring advancements in prognosis prediction.

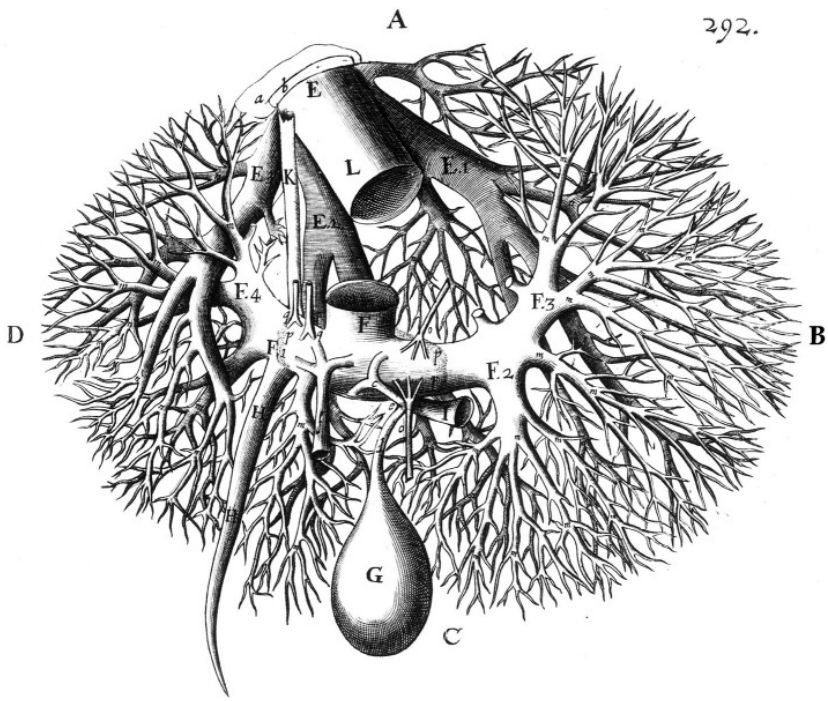


Figure 3. Illustration of the liver from Glisson's book, *Anatomia Hepatis* (1654), showing the internal blood vessels and bile ducts.

Evolution of modern liver surgery

Over the past few decades, liver surgery has undergone profound transformation. Traditionally, resections for colorectal liver metastases (CRLM) involved large anatomical hepatectomies, often requiring removal of entire lobes to secure tumor-free resection margins. While these procedures achieved oncologic clearance in selected cases, they were associated with higher rates of perioperative morbidity and mortality than are seen today [9]. The extensive sacrifice of functional parenchyma limited physiological reserve, so that repeat hepatectomy in cases of recurrence was frequently impossible.

Over time, there was a shift from large lobar resections toward parenchymal-sparing surgery, and thus toward atypical ("non-anatomical") resections [11]. The driving principle was preservation of maximal future liver remnant (FLR) to reduce the risk of postoperative liver failure, especially in patients with underlying liver injury from chemotherapy, steatosis or fibrosis. Crucially, this shift expanded the number of patients eligible for surgery and enabled repeat resections in cases of recurrent disease, which is a common scenario in CRLM [12-14]. This paradigm shift also broadened surgical eligibility. Patients who were previously considered unresectable or managed palliatively could now undergo radical surgery through atypical, parenchymal-sparing resections. By expanding the surgical repertoire in this way, more patients became eligible for curative-intent treatment [15, 16].

A further major transition came with the introduction of minimally invasive surgery. Laparoscopic approaches were initially reserved for small, superficial lesions in the anterior segments of the liver. However, accumulating experience and technical refinements have expanded their use to complex procedures, including major hepatectomies in specialist centers. Robotic systems have added further momentum to this shift, providing enhanced dexterity and facilitating precise dissection in challenging anatomical locations [17-20].

For patients, the benefits of minimally invasive techniques are substantial. Compared with open surgery, laparoscopic and robotic resections lead to reduced intraoperative blood loss, due to both the tamponading effect of pneumoperitoneum and the greater refinement of the procedure. Blood loss has also decreased with the use of modern energy devices [20-23]. Furthermore, patients experience less postoperative pain, resulting in lower analgesic requirements and facilitating earlier mobilization. Recovery is typically faster, with shorter hospital stays and earlier return not only to daily activities but also to systemic therapy when indicated. In addition, complication rates are generally lower, with fewer wound infections, less postoperative ileus, and an overall reduction in morbidity, all without compromising oncologic safety [24-26].

In parallel, the role of local ablative therapies delivered percutaneously, laparoscopically, or during open procedures, has expanded. While resection remains

the gold standard for resectable disease, ablation is a valuable option in patients with small or deep-seated lesions, limited remnant volume, or comorbidities that preclude extended resections. Combination strategies that involve resection and ablation permit the clearance of tumors while functional parenchyma is preserved [27-29].

Patient outcomes have improved profoundly in this era of surgical refinement. Perioperative mortality rates have fallen below 1% at high-volume centers, while morbidity has declined significantly due to the use of refined techniques and enhanced recovery after surgery programs [25]. Long-term outcomes have also improved: the five-year overall survival (OS) rate now approaches approximately 50% in resected patients [30-33]. Repeat hepatectomy, once rarely feasible, is now routinely performed and confers survival rates comparable with those achieved at first resection [12]. The incorporation of minimally invasive approaches has also improved the quality of patients' lives by reducing surgical trauma and hastening recovery, thereby broadening the appeal and feasibility of surgical treatment for patients who might previously have been deemed unfit [17, 19, 20, 23, 25, 26].

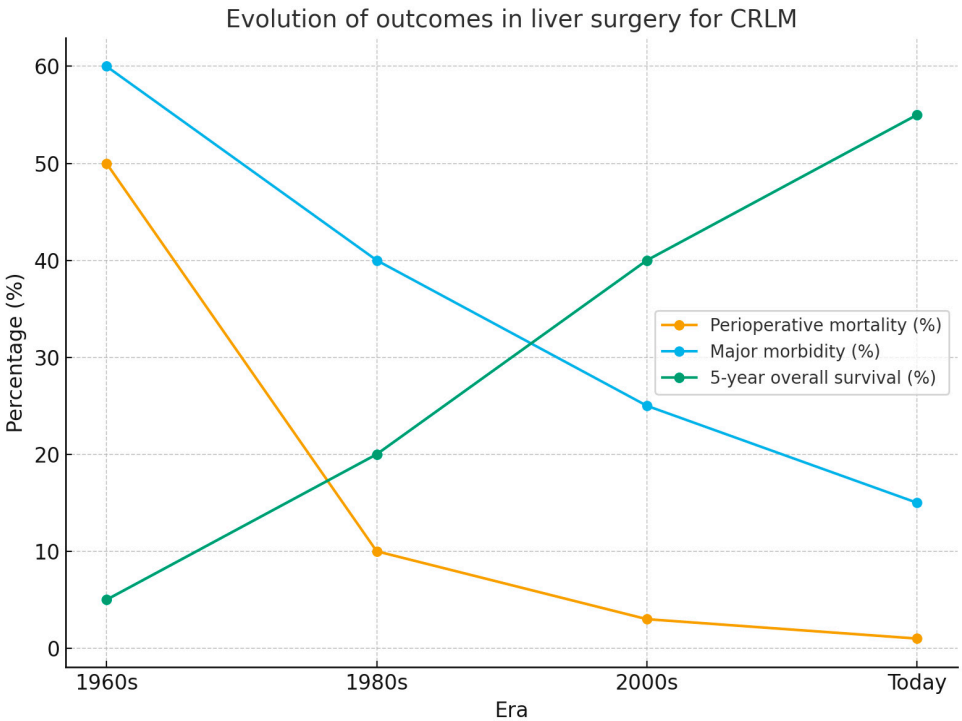


Figure 4. Illustration of the changes over time in rates of perioperative mortality, major morbidity and five-year OS after liver surgery for CRLM.

Anatomy: a guide to surgical landmarks

The colon extends from the terminal ileum to the anal canal, weaving through both the intraperitoneal and retroperitoneal spaces. It is crucial to understand its blood supply for both surgical planning and intraoperative decision-making.

Arterial supply of the gastrointestinal tract

The abdominal aorta has three major branches that supply the gastrointestinal tract:

1. Celiac artery – the first major branch, responsible for supplying blood to the esophagus, stomach, proximal duodenum, pancreas, liver, and spleen [34]. It splits into three main arteries:
    - Left gastric artery – divides into an esophageal and a stomach branch and supplies consequent organs.
    - Splenic artery – supplies the spleen but also branches into vessels that supply the pancreas and the stomach.
    - Common hepatic artery – its branches are the gastroduodenal and right gastric arteries, while the remaining branch, the hepatic artery proper, supplies the liver.
2. Superior mesenteric artery (SMA) – the second major branch, which supplies the intestines from the duodenum to the distal parts of the transverse colon. It crosses over the distal parts of the duodenum by traveling within the base of the small intestine mesentery [35]. Its branches are:
    - Ileocolic artery – supplies the appendix, cecum, and ascending colon.
    - Right colic artery – not always present; when it exists, it arises either from the SMA or as a branch of the ileocolic artery to supply the ascending colon.
    - Middle colic artery – a constant branch that supplies the transverse colon.
3. Inferior mesenteric artery (IMA) – the third major branch, dedicated to the left colon and rectum [36].
    - Left colic artery – branches to supply the left colic flexure and descending colon.
    - Sigmoidal arteries serve the sigmoid colon.
    - Superior rectal artery – marks the end of the IMA.

The marginal artery, an anastomotic network that connects these branches, ensures a continuous blood supply even in cases of arterial variation [34-36].

Venous drainage: the road to the liver

Venous drainage of the colon largely mirrors its arterial supply. The superior mesenteric vein runs alongside the SMA and eventually merges with the splenic vein to form the portal vein. The portal vein is a key vessel for the transportation of nutrient-rich blood from the intestinal tract to the liver [37].

The inferior mesenteric vein does not travel with its paired artery. Instead, it separates within the mesentery of the descending colon and drains into the splenic vein [38].

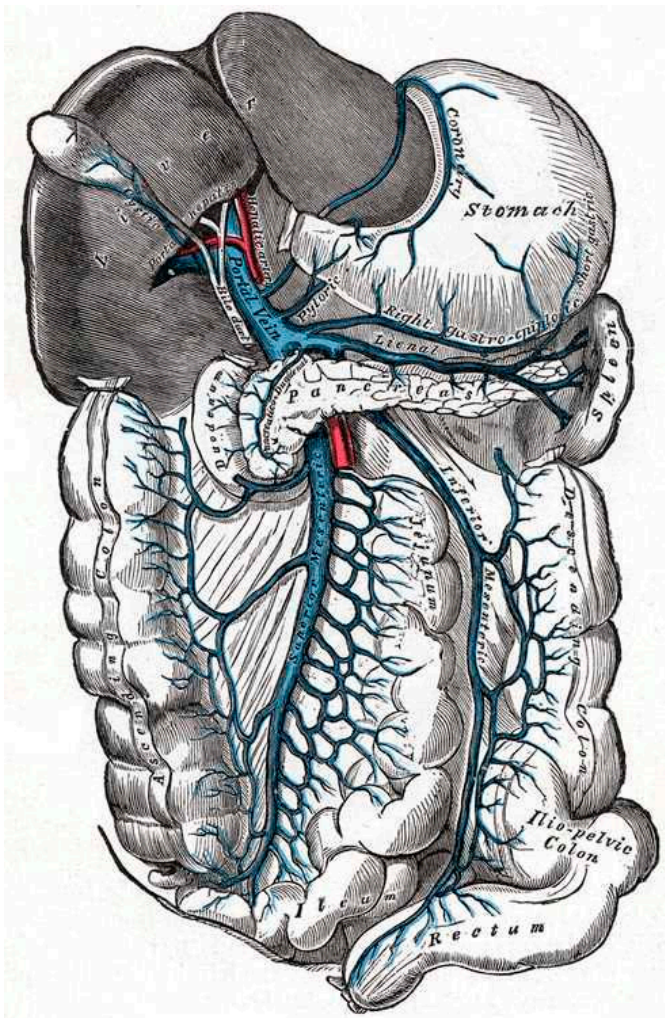


Figure 5. Anatomy of venous drainage from the gastrointestinal tract to the liver via the portal vein. Original work from Gray (1918), reproduced from *Gray's Anatomy,* 20th US edition, which is now in the public domain.

The portal vein

Unlike typical veins, the portal vein does not drain blood directly into the heart. Instead, it delivers blood from the intestines, pancreas, and spleen to the liver for metabolic processing. This supply facilitates both nutrient metabolism and detoxification [37]. This explains why the liver is the most common site for metastases from colorectal cancer, because malignant cells stream via the portal circulation, seeding tumor cells into the hepatic parenchyma.

Liver anatomy and surgical landmarks

The Brisbane 2000 terminology of liver anatomy and resections describes anatomic classifications accepted by the International Hepato-Pancreatico Biliary Association [39]. The Brisbane Classification describes internationally accepted terminology for anatomical and functional regions of the liver. This classification brings uniformity for hepatic interventionists in terms of anatomical landmarks. The classification is intended to unify anatomical and surgical terms, and to be anatomically correct, consistent, self-explanatory, linguistically correct, translatable, precise and concise.

First-order division: the liver is split into the right and left hemilivers, which are divided by the middle hepatic vein. Externally, this plane is approximated by Cantlie's line, a surface landmark that runs from the gallbladder to the inferior vena cava.

Second-order division: the right hepatic vein separates the right hemiliver into anterior and posterior segments. The left hepatic vein divides the left hemiliver into medial and lateral segments.

Third-order division: a horizontal plane at the portal vein bifurcation further subdivides the liver into eight Couinaud segments. Notably, segment I (caudate lobe) lies posterior to segment IV and has independent venous drainage.

Each hepatic segment receives its own branch of the portal vein, hepatic artery, and biliary duct.

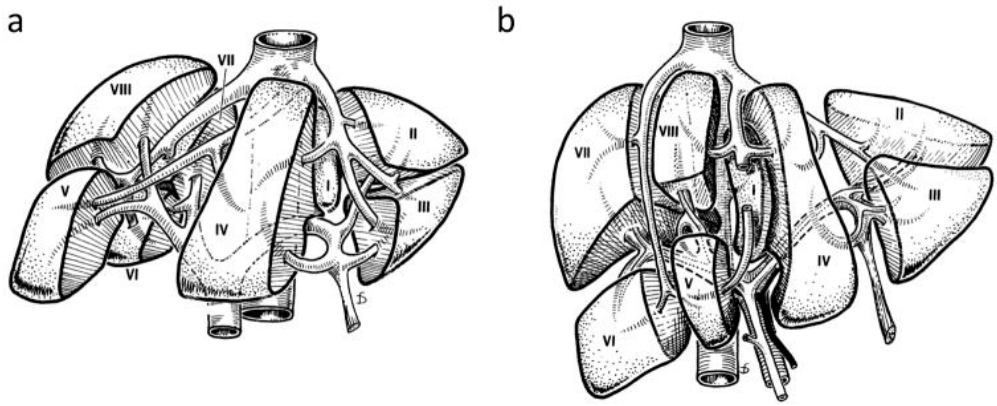


Figure 6. The functional division of the liver as seen *in vivo* (a) and *ex vivo* (b). Image reproduced from Jarnagin, *Blumgart's Surgery of the Liver, Biliary Tract and Pancreas* (5th ed.), Philadelphia, US: Elsevier, 2012. Reproduced with permission.

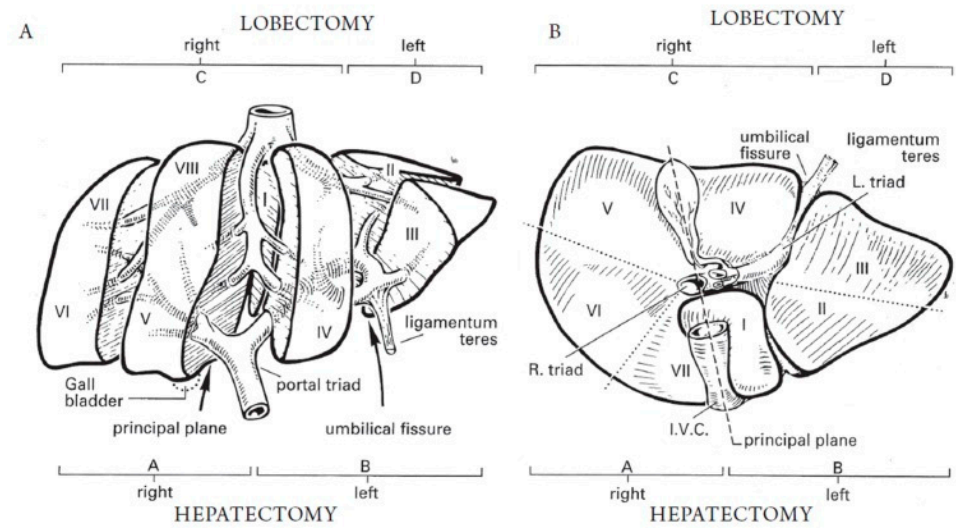


Figure 7. Exploded view (A) of the hepatic segmental anatomy defined by hepatic veins and portal triads, the basis of modern liver surgery. Inferior view (B) shows division into functional hemilivers by Cantlie's line and into anatomic lobes by the umbilical fissure. Brackets indicate segments included in common liver resections. Image reproduced from Blumgart & Belghiti, *Surgery of the Liver, Biliary Tract, and Pancreas*, (4th ed.), Philadelphia, PA: Saunders Elsevier; 2007. Reproduced with permission.

The biliary tree: the liver's drainage system

Bile, which is produced in the liver, drains through a system of ducts before it reaches the duodenum. Each segment of the liver has its own bile duct, which gradually merges with others into larger branches [40]:

- Right posterior sectoral duct (segments 6 and 7) and right anterior sectoral duct (segments 5 and 8) join to form the right hepatic duct.
- The left hepatic duct collects bile from segments 2, 3, and 4.
- The right and left hepatic ducts unite to form the common hepatic duct, which then joins the cystic duct from the gallbladder to form the common bile duct.
- The common bile duct merges with the pancreatic duct before emptying into the duodenum.

Surgical considerations: navigating variability

The vascular and biliary anatomy of the liver is highly variable, so preoperative imaging is critical for surgical planning. Having an individualized preoperative map of these structures minimizes operative surprises and decreases the risk of complications.

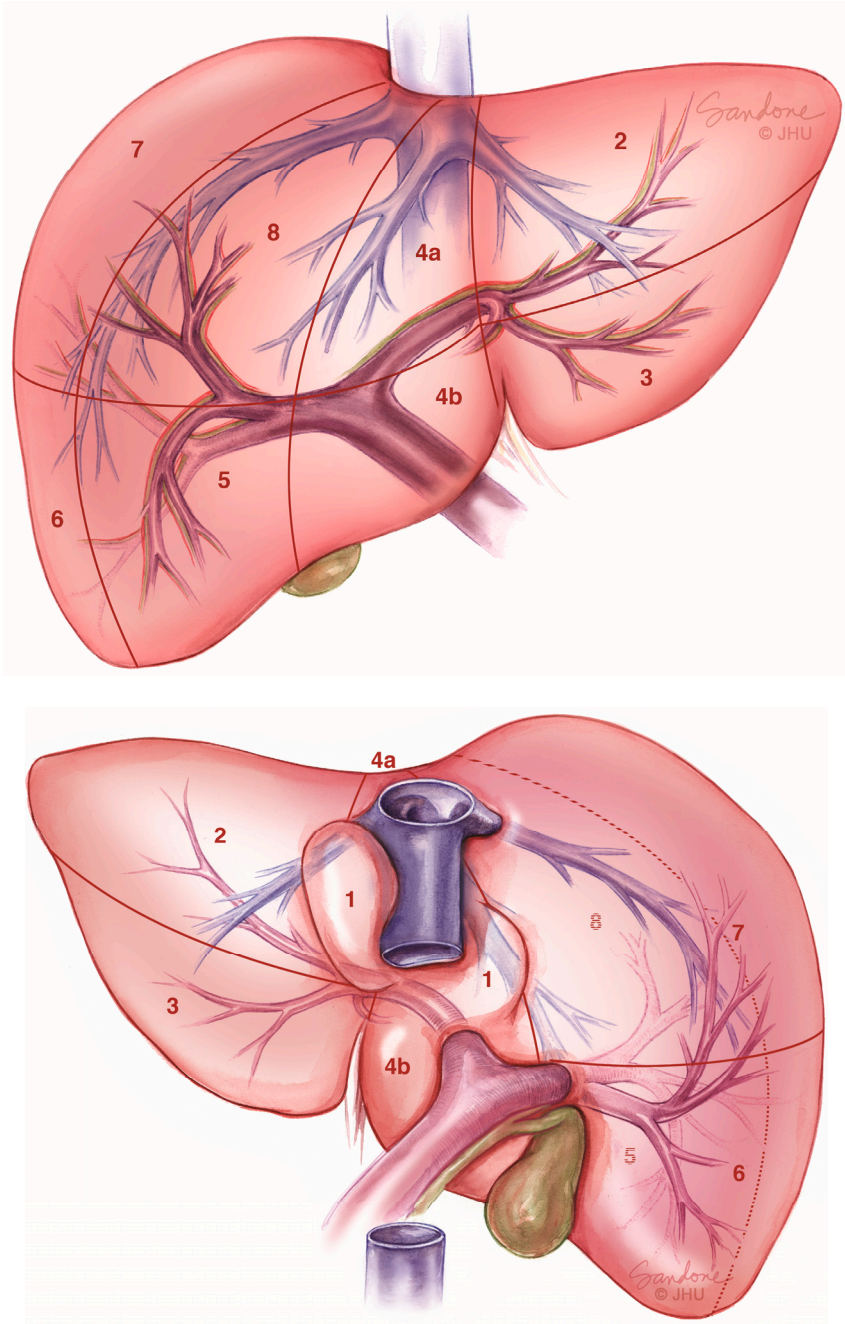


Figure 8. Intrahepatic vascular and biliary anatomy, anterior and posterior views. Illustration by Corinne Sandone © 2007 JHU AMM. Reproduced with permission.

Colorectal cancer: A growing health concern

Colorectal cancer (CRC) is one of the most common cancers worldwide; it ranks third in incidence, and numbers continue to rise [41]. The incidence is higher in Western countries, such as Sweden. Treatment of CRC requires specialists from primary care, radiology, endoscopy, pathology, surgery, and oncology to work together to provide the best possible care [42].

Some patients have no symptoms and are diagnosed through screening programs or because of accidental findings on radiologic images. A majority of cases, however, are diagnosed when people report suspicious symptoms. The most common symptoms include [43-45]:

- changes in bowel habits
- rectal bleeding combined with altered bowel habits
- an abdominal or rectal mass
- iron deficiency anemia
- weight loss
- abdominal pain.

Individuals who experience these symptoms (or are found to have abnormalities during screening or imaging) should be evaluated for CRC. In more severe cases, patients may present with emergencies such as bowel obstructions or perforations [46, 47].

The gold standard for diagnosing CRC is a histological examination of malignant tissue, which is usually obtained via biopsy during a colonoscopy or from a surgical specimen [48, 49]. The vast majority of colorectal tumors are adenocarcinomas [50].

Colonoscopy

Colonoscopy is the most effective way to diagnose CRC [51]. First and foremost, it enables identification of the tumor. The endoscopist can also secure biopsies and remove pre-cancerous polyps. Most CRCs appear as abnormal growths (endoluminal masses) that originate from the colon's inner lining. In some cases, advanced endoscopic techniques, such as mucosal or submucosal dissection, are used to remove large polyps [52]. If a lesion is found and removed, the site is tattooed to ensure that it can be accurately located in potential future surgery [53]. If a colonoscopy is not completed, perhaps due to an obstruction, computed tomography (CT) colonography can be used as an alternative; this method offers acceptable accuracy to detect larger lesions (10 mm or more in diameter) [54].

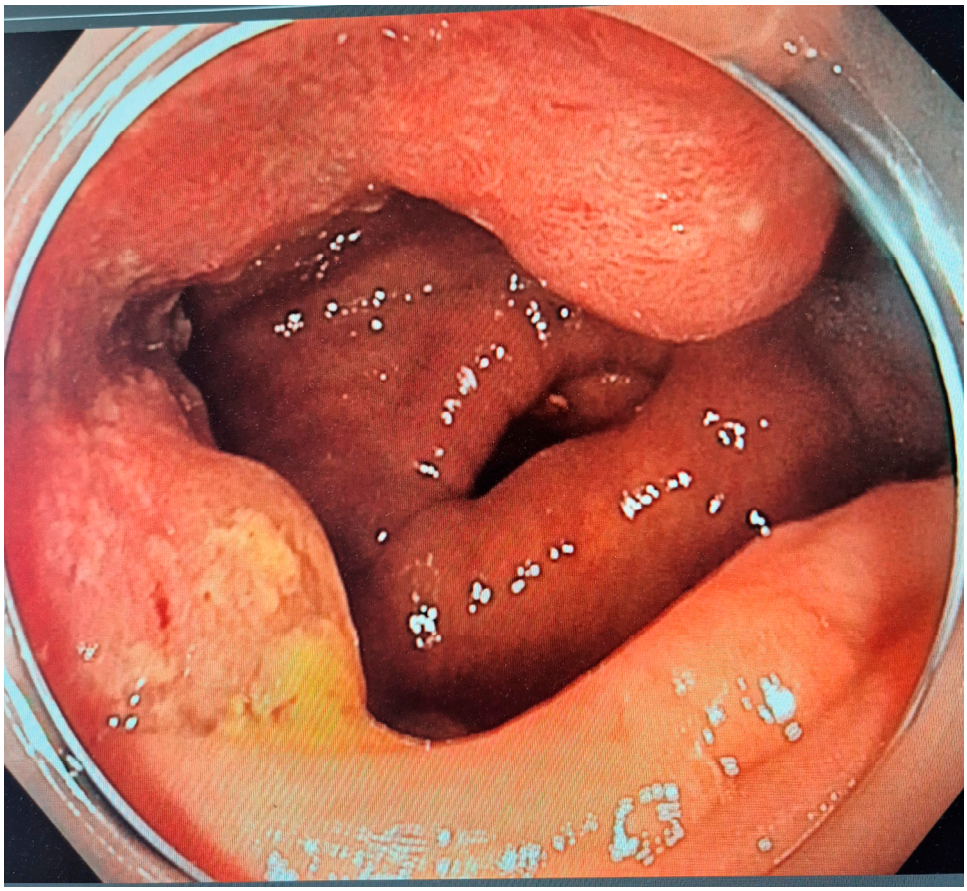


Figure 9. Primary tumor identified through colonoscopy. Malignant surface seen in the upper and left portions of the endoscopic view. The image was obtained by attending surgeon Dr David Wickström, with the patient's consent.

Radiologic imaging: assessing the extent of disease

Once CRC is diagnosed, imaging helps to determine the cancer stage and thereby to guide the choice of treatment [55]. Typical radiologic investigations include:

- CT scans: used to visualize metastases in the liver, lungs, peritoneum, or lymph nodes, and to assess the primary tumor's extent.
- MRI for rectal cancer: preferred for evaluation of local tumor extension [56].
- MRI for liver metastases: provides the best detection, especially in patients with fatty liver changes. The use of advanced triple-phase CT scans has also improved liver metastasis detection [57-59].

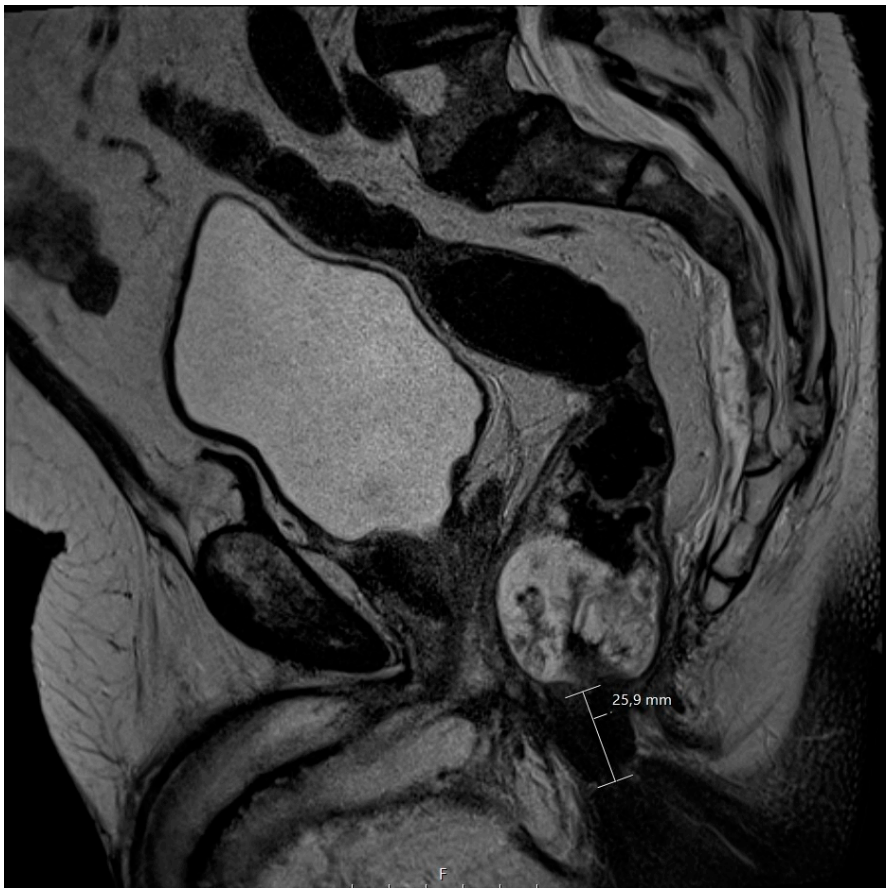


Figure 10. Primary tumor seen with MRI, localized just above the 25.9 mm marker. The image was obtained by attending surgeon Dr David Wickström, with the patient's informed consent.

The role of laboratory tests

Routine blood tests have limited diagnostic value for CRC. Many patients with CRC are iron-deficiently anemic, but this condition can arise from other sources [60, 61]. Absence of iron deficiency does not exclude CRC either [60, 61]. Carcinoembryonic antigen (CEA) and other serum markers are not recommended for CRC screening or diagnosis due to low sensitivity and specificity [62]. Elevated preoperative CEA levels (>5ng/mL) are useful, however, as predictors of poor prognosis, because elevated levels are correlated with worse outcomes [63]. Elevated preoperative CEA levels may return to normal after surgery, and this normalization of CEA levels tends to correlate with better outcomes [64]. In such cases, postoperative CEA measurement can serve as a tool to monitor for recurrent disease [65].

Colorectal cancer: an overview of the genetic and molecular landscape

CRC is not a single disease, as it develops due to a complex interplay of genetics, environmental influences, and molecular alterations [66, 67]. Understanding how CRC develops, the different pathways it follows, and what drives its progression is crucial for diagnosis and treatment. It is vital to gain an understanding of the genetics and molecular background of CRC in order to find and target biological markers as prognostic and predictive clinical tools for primary and metastatic disease.

Pathways of colorectal cancer development

Approximately 70% of CRC cases occur sporadically, meaning that they arise in individuals with no clear family history [68]. These cases typically develop after the age of 50 years and are often influenced by lifestyle factors such as diet, smoking, and obesity. While environmental exposures contribute, underlying genetic mutations remain the fundamental drivers of carcinogenesis.

At the opposite end of the spectrum are inherited CRC cases, which are relatively rare and account for approximately 10% of diagnoses [69]. These tumors are typically more aggressive than sporadic ones. Individuals with inherited CRC carry pathogenic germline mutations transmitted within families. A well-known example is Lynch syndrome, caused by mutations in DNA mismatch repair (MMR) genes, which result in high levels of microsatellite instability (MSI-H) and accelerated accumulation of DNA errors [69, 70]. Another example is familial adenomatous polyposis (FAP), in which mutations in the APC gene lead to the formation of hundreds to thousands of colorectal polyps, making malignant transformation almost inevitable without early intervention.

Between these extremes lies familial CRC, affecting individuals with strong family histories of the disease but no identifiable pathogenic germline mutation. This category represents an area of ongoing research, as investigators work to define the genetic factors that underlie this increased susceptibility [71].

Recent advances in genomic sequencing have identified numerous additional germline variants beyond the well-established hereditary syndromes that increase CRC risk [72]. As understanding of the genetic landscape expands, the distinction between inherited CRC syndromes and familial CRC becomes increasingly blurred.

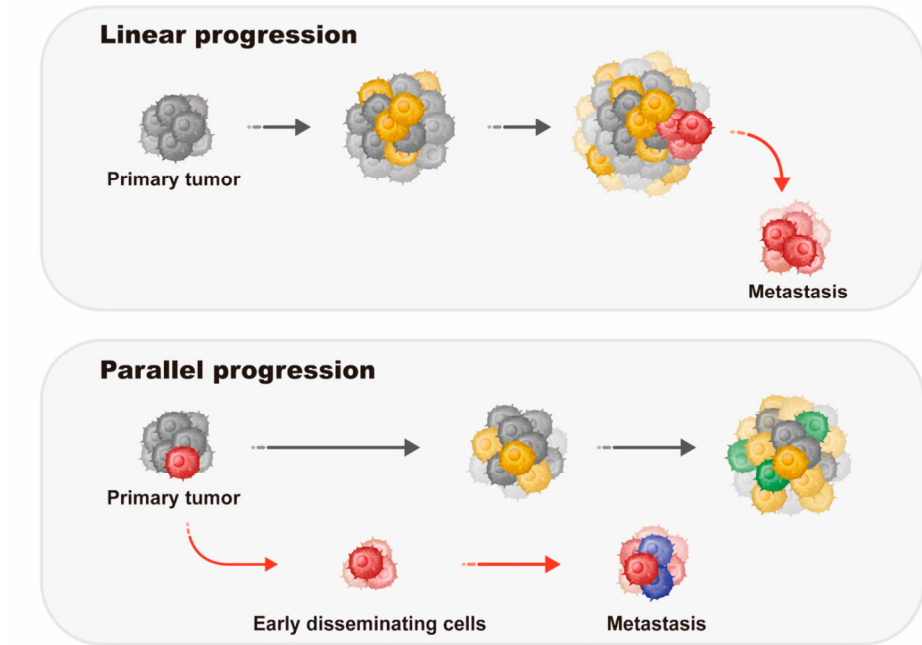


Figure 11. Two principal models have been proposed to explain metastatic spread of cancer: the linear and parallel progression models. In the linear model (A), metastatic cells appear relatively late in tumor development, once the primary tumor has acquired all the traits needed for successful dissemination and growth at distant sites. In the parallel model (B), tumor cells leave the primary lesion much earlier than in the linear model and continue to evolve independently. Image reproduced from Yang et al. (2022), *Genes,* 13(9), 1555, under the Creative Commons Attribution (CC BY) license.

From a normal cell to cancer

At its core, CRC is a disease of genomic instability. A normal, healthy colonocyte follows a tightly regulated cycle of growth, division, and programmed cell death. However, when key genes acquire pathogenic mutations, these regulatory mechanisms fail, leading to uncontrolled cellular proliferation. This marks the onset of the classic adenoma–carcinoma sequence [73, 74].

The earliest initiating event is often a mutation in the adenomatous polyposis coli (APC) gene, which disrupts the Wnt signaling pathway. APC functions as a gatekeeper of epithelial homeostasis; loss of its function allows cells to accumulate, forming small adenomatous polyps. While not all adenomas progress to cancer, additional genetic alterations promote further dysplasia. Mutations in the Kirsten rat sarcoma viral oncogene homolog (KRAS) are especially important in this process. Subsequent alterations in the tumor suppressor gene TP53 remove a critical barrier to malignant transformation, enabling progression from benign adenoma to invasive carcinoma [74].

CRC can also arise through an alternative pathway originating from serrated rather than adenomatous polyps. These tumors are driven predominantly by epigenetic changes, including promoter hypermethylation that silences tumor suppressor genes. Many serrated pathway tumors harbor mutations in the B-RAF protein kinase (BRAF) gene and are frequently associated with microsatellite instability–high (MSI-H) status, a feature with important therapeutic implications [75, 76].

Genetic markers and what they mean for patients

When it comes to predicting outcomes and selecting optimal treatment strategies, the genetic profile of a tumor provides critical insights. Microsatellite instability–high (MSI-H) status, for example, may indicate Lynch syndrome and also suggests that the patient is unlikely to benefit from traditional fluoropyrimidine-based chemotherapy such as 5-fluorouracil (5-FU) [77-79]. Conversely, the presence of KRAS or NRAS mutations means that epidermal growth factor receptor–targeted therapies, such as cetuximab and panitumumab, are ineffective, making molecular testing essential for appropriate treatment selection [80, 81].

Mismatch repair–deficient (MMR-d) tumors, although characteristic of Lynch syndrome, also occur in approximately 15% of sporadic CRCs. These cancers exhibit a distinct molecular signature, typically showing high burdens of mutations within repetitive DNA sequences due to defective repair mechanisms. Notably, MSI-H tumors generally confer a better prognosis overall and respond exceptionally well to immunotherapy. This breakthrough has transformed treatment for a subset of CRC patients [78, 82].

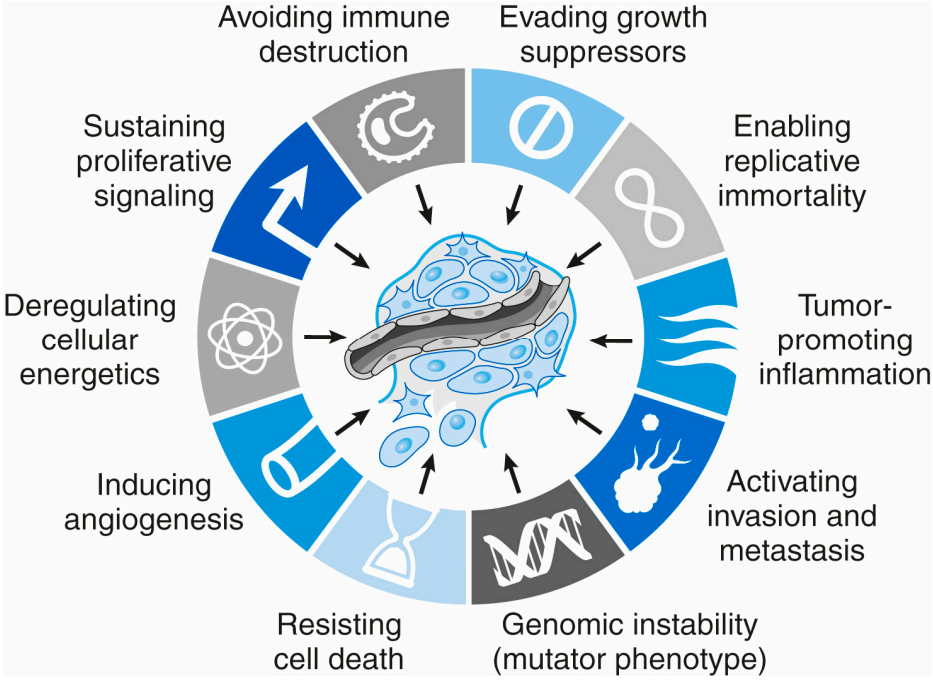


Figure 12. Cancer arises through key alterations in cells, which collectively develop into a malignant phenotype. These changes are referred to as the hallmarks of cancer. Image reproduced from Mitchell et al., *Pocket Companion to Robbins 10 Cotran Pathologic Basis of Disease,* (10th ed.) Philadelphia: Elsevier; Chapter 7, Neoplasia; p. 237-291. Copyright 2025 by Elsevier, Inc. Reproduced with permission.

CRC staging and the spread of disease

A tumor's genetic signature is only part of the story. Where the primary tumor is located and how far it has spread are the most important pieces of clinical information for prognosis. CRC follows predictable patterns of metastasis, with the liver being the most common site of distant spread due to the portal venous circulation [83, 84]. Rectal cancers, on the other hand, more frequently metastasize to the lungs because of their distinct venous drainage [85, 86].

The tumor, node, metastasis (TNM) staging system remains the gold standard for CRC classification and is used to guide treatment decisions. Early-stage tumors that are confined to the colon (T1-T2) have an excellent prognosis with surgery alone [87, 88]. Once lymph nodes become involved (N1-N2), adjuvant chemotherapy is often recommended to increase survival probability [87, 89]. The most significant jump in prognosis occurs once the cancer has spread to distant organs (M1); in many cases, this stage marks the transition from curable to potentially palliative treatment [90].

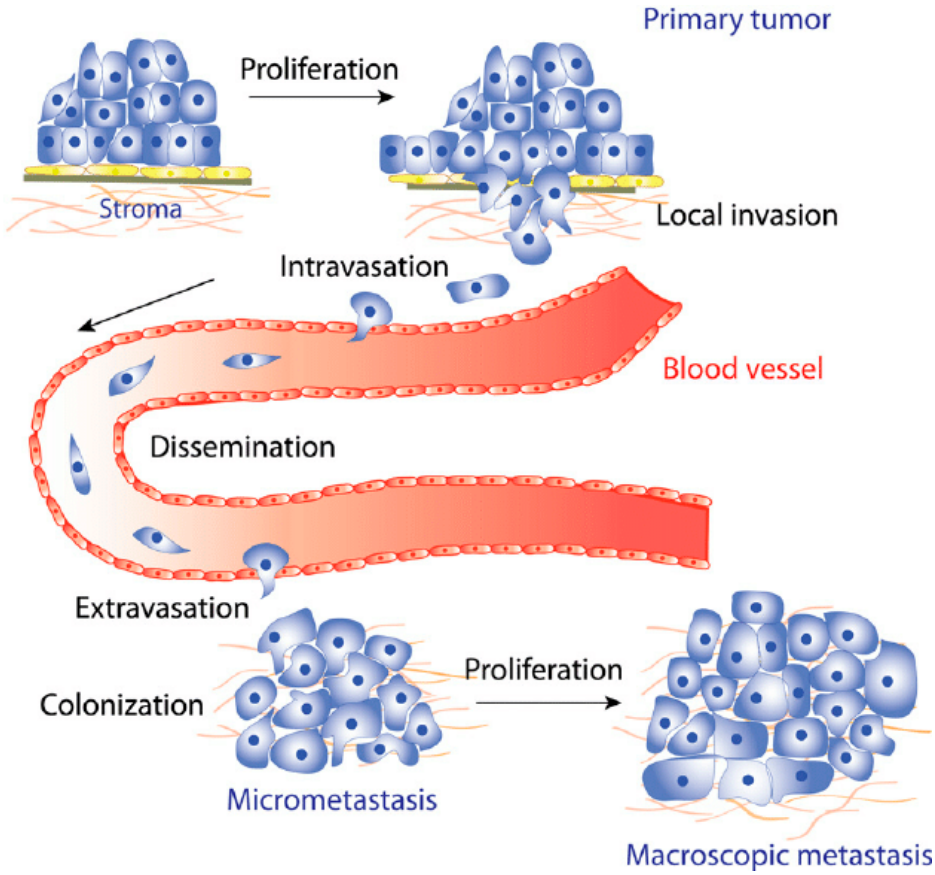


Figure 13. Schematic representation of the metastatic cascade, showing how cancer spreads step by step: the primary tumor grows, invades nearby tissue, enters the blood vessels (intravasation), travels through the circulation (dissemination), exits into distant tissue (extravasation), and eventually establishes new tumors as metastases. Image reproduced from Saxena & Christofori (2013), *Molecular Oncology,* 7(2), 283–296, under the Creative Commons Attribution (CC BY) license.

Principles of surgical resection

Approximately 80% of presenting colon cancers are confined to the colon wall or nearby lymph nodes [91] and, in these cases, surgery is the gold standard curative treatment. In early-stage cases in which the tumor arises within a polyp, endoscopic resection is a viable alternative if the lesion meets specific low-risk criteria, such as being well-differentiated, free of lymphovascular invasion, and having clear margins [42, 92, 93].

The primary objective of surgery for invasive colon cancer is the complete removal of the tumor along with its vascular pedicle and lymphatic drainage. In most cases,

bowel continuity is restored through primary anastomosis, although a temporary diverting colostomy or ileostomy may be required. An end-colostomy is an option for patients for whom the risk of low anterior resection syndrome is too high or for those with significant comorbidities that would mean they could not tolerate an anastomotic leak in a colorectal anastomosis [94-100].

Laparoscopic-assisted colectomy is generally preferred over open surgery when technically feasible. When performed by experienced surgeons, laparoscopic colectomy offers comparable oncologic outcomes and perioperative risks to those of open surgery, but promotes faster recovery and causes less surgical trauma [22, 97]. Regardless of surgical approach, resection should ensure a proximal and distal margin of at least 5-7 cm, with en bloc removal of the associated mesentery up to the origin of the primary feeding vessel. At least 12 lymph nodes should be included in the specimen for accurate staging [23, 101].

For locally advanced tumors that have invaded adjacent structures, multivisceral resection may be appropriate [102]. Most patients with resectable colon cancer undergo surgery first, followed by adjuvant systemic therapy as indicated. However, neoadjuvant therapy may be considered for those with borderline resectability [103].

For localized, unresectable colon cancers with deficient MMR (dMMR), immunotherapy is an emerging alternative to chemotherapy. Clinical trials are ongoing to better define its role in treatment [104-106].

Roles of adjuvant and neoadjuvant therapy

Adjuvant chemotherapy is intended to eliminate micrometastatic disease, thereby reducing recurrence risk and improving cure rates, particularly in stages III and IV colon cancer [107].

The exact role of neoadjuvant therapy in colon cancer remains somewhat debated [108]. While most patients with localized, resectable disease undergo upfront surgery, neoadjuvant therapy may be considered in particular cases that involve locally advanced tumors, anticipated margin difficulties, or inoperability [108, 109]. For locally advanced rectal cancer, neoadjuvant chemoradiotherapy is the standard approach before surgery. Accurate preoperative staging, including the use of rectal MRI and/or endoscopic ultrasound, is mandatory to determine the best treatment strategy [110]. Testing for dMMR status also helps to guide the selection of the best possible therapy [111].

Management of metastatic disease

For patients who present with stage IV colorectal cancer, treatment is individualized based on symptoms, complications, and metastatic lesion resectability. Surgery may provide a cure for patients with a few metastases in the liver or lungs [112].

Before liver resection is considered for CRLM, thorough preoperative imaging, typically in the form of CT of the abdomen and thorax and MRI of the liver, is essential. This is necessary to assess tumor burden, the distribution of liver tumors, and the extent of extrahepatic disease. A pelvic MRI is typically performed in cases of rectal cancer, as previously described. A liver biopsy may be warranted if the diagnosis is uncertain or if neoadjuvant therapy is planned. If chemotherapy is expected, small or deep-seated liver metastases should be marked with fiducial markers before treatment is started [112, 113].

Standard principles for typical clinical scenarios are listed next.

- **Unresectable liver metastases:** initial systemic chemotherapy is recommended, with surgery considered once metastases become clearly resectable. Liver resection is typically delayed for at least four to eight weeks after completion of chemotherapy.
- **Low-risk, resectable liver metastases:** patients with four or fewer lesions confined to one liver lobe and no RAS/BRAF mutations typically undergo surgery first, unless chemotherapy could significantly simplify the procedure.
- **High-risk, resectable liver metastases:** for patients with multiple liver lesions, suspected portal node involvement, extrahepatic metastases, or RAS/BRAF-mutated tumors, neoadjuvant chemotherapy is usually preferred before surgery.

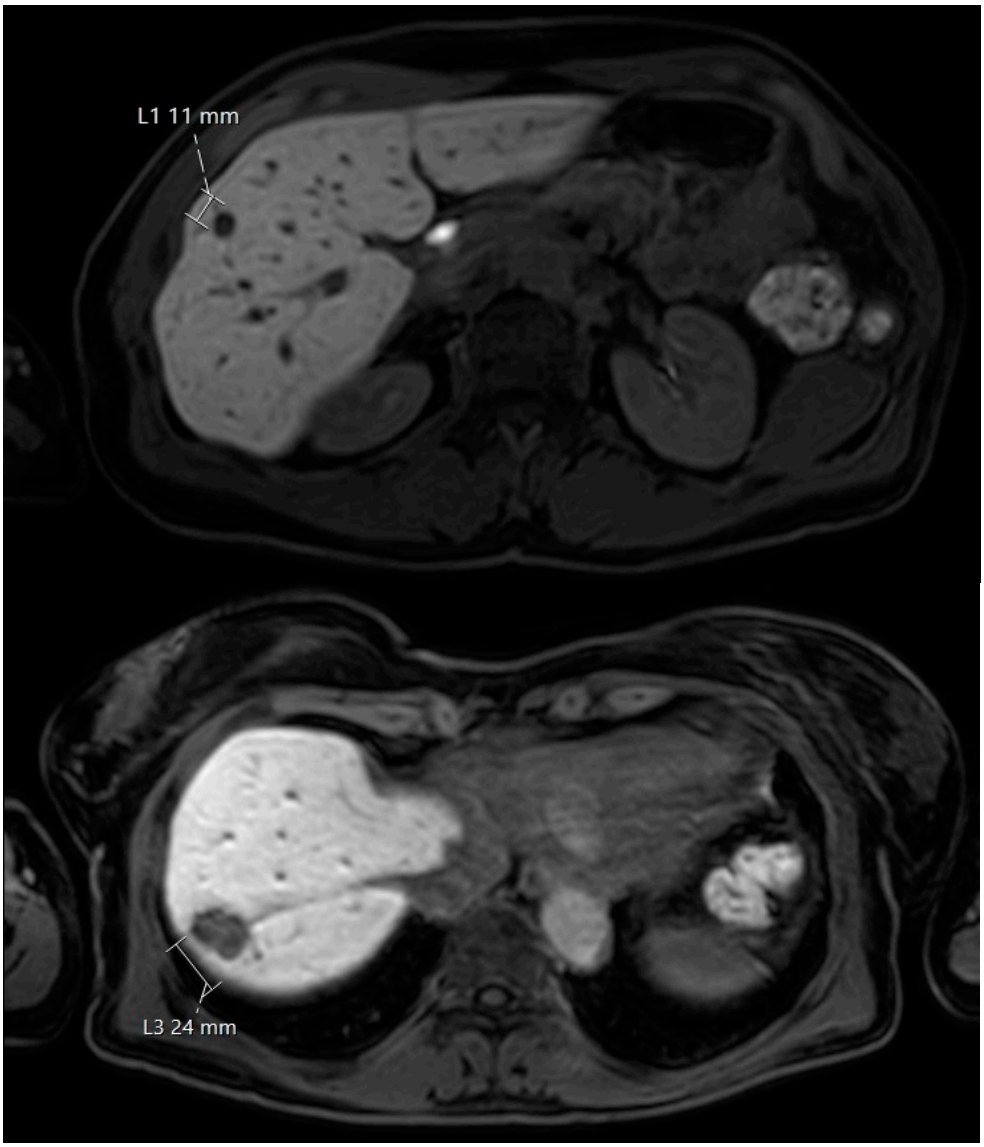


Figure 14. MRI demonstrates findings consistent with metastatic colorectal carcinoma. An 11 mm metastatic lesion is identified in hepatic segment IV (upper image). A second metastatic lesion of 24 mm is visualized in hepatic segment VII (lower image). The images were obtained by the attending surgeon, Dr Hanna Sternby, with the patient's informed consent.

Chemotherapy considerations

Common neoadjuvant chemotherapy regimens are FOLFOX (oxaliplatin, leucovorin, and fluorouracil), or FOLFOXIRI (fluorouracil, leucovorin, oxaliplatin, and irinotecan) for younger patients with good performance status. For patients who have been previously treated with FOLFOX, FOLFIRI (irinotecan, leucovorin, and fluorouracil) is an alternative [112, 113].

For resectable CRLM and dMMR tumors, cytotoxic chemotherapy is currently preferred over upfront immunotherapy in a general setting, although research is ongoing regarding this choice [112, 113].

CRLMs that are smaller than 2 cm in diameter and located at more than 1 cm depth within the liver are often not visible in images after systemic chemotherapy. Despite this, complete pathologic response is rare, so these lesions should still be surgically removed. Fiducial markers and perioperative ultrasound can help to locate tumors during surgery [112-114].

Following liver metastasectomy, adjuvant chemotherapy is recommended for most patients. If the patient has not received prior chemotherapy, an oxaliplatin-containing regimen is typically used. For those who have received neoadjuvant chemotherapy, a full six-month course (including preoperative and postoperative treatment) is generally advised unless prior adjuvant FOLFOX was administered within the previous year [112, 113].

Key considerations in operative planning

Surgical resection remains the standard curative option for CRLM. Advances in surgical techniques and perioperative care have significantly improved outcomes, with survival rates now approaching 50-60%. Procedure-related mortality is generally below 5% and lower than 1% in high-volume centers [112, 113, 115].

The primary objective of CRLM resection is to achieve negative surgical margins (>1 mm, ideally >10 mm). This achievement is particularly critical in RAS- or BRAF-mutated tumors, due to higher recurrence risks [112, 113].

Effective surgical planning is essential to ensure the preservation of hepatic blood supply, venous outflow, and biliary drainage while sufficient liver is retained to prevent postoperative liver failure. However, patient-related factors, such as significant comorbidities, may limit patients' eligibility for surgery.

To reduce the risk of liver insufficiency, approximately 30% of FLR should be preserved in patients with healthy livers. However, for those with compromised liver function, a larger FLR (around 40%) may be necessary. Patients with preexisting liver conditions, such as chemotherapy-induced liver injury, fibrosis, or steatosis, require a more cautious approach to minimize the risk of post-

hepatectomy liver failure. If the expected FLR is inadequate, preoperative portal vein embolization can be used to induce hypertrophy of the liver segments that will remain after surgery, thereby increasing the FLR [116, 117].

Surgical approach and timing in synchronous CRLM

For patients with synchronous CRLM (metastases are detected at the time of CRC diagnosis), three main surgical strategies are available [118, 119].

1. **Simultaneous resection** – best suited to carefully selected patients, particularly those with right-sided colon cancer and a limited liver metastatic burden.
2. **Classic (colorectal-first) approach** – recommended when the primary colorectal tumor is symptomatic and causes issues such as bleeding, obstruction, or perforation.
3. **Reverse (liver-first) approach** – prioritizes liver metastasectomy before colorectal resection to reduce the risk of disease progression in the liver.

One major advantage of the liver-first strategy is that its application helps to avoid delays in liver surgery and systemic oncologic treatment. Complications from colorectal surgery, such as anastomotic leakage, can significantly postpone the planned liver resection, and these delays may allow liver metastases to progress. By addressing the metastatic burden first, the liver-first approach ensures that systemic therapy can resume without such delays, and this is crucial in patients with aggressive disease biology. The choice of approach remains a topic of debate and depends on several factors, including tumor burden, patient condition, and institutional logistics. Simultaneous resection, for instance, requires the availability of both a colorectal and a liver surgeon in the same setting, which can be a logistical challenge, especially in centralized healthcare systems.

While surgical resection remains the gold standard for treating resectable CRLM, minimally invasive therapies such as ablation techniques are increasingly being used in selected patients [29, 120]. These modalities are particularly relevant when

- resection is not feasible due to anatomical constraints, insufficient FLR, or patient comorbidities;
- a multimodal approach is required, in which ablation is combined with surgery or systemic therapy to manage multiple lesions while liver function is preserved; or
- a repeatable, less-invasive option is needed to treat recurrence after resection.

Predicting individual outcomes

Prediction of individual outcomes in CRLM treatment is challenging due to disease heterogeneity [121]. Although several prognostic models have been developed to guide surgical decision-making, their accuracy in forecasting personalized prognosis and treatment response remains limited. Current scoring methods fail to capture the complexity of tumor biology and patient-specific variability.

There is a clear need for more sophisticated prognostic tools that integrate biomarkers, advanced imaging modalities, and analytics based on artificial intelligence (AI). Future research should prioritize the refinement of predictive models to enable truly individualized risk stratification, to optimize the timing of surgical interventions, and, in due course, to improve long-term outcomes for patients with CRLM.

Aims of the thesis

The treatment of CRLM has significantly improved in recent decades. Advances in perioperative care, the use of more effective chemotherapy regimens, and refinements in surgical techniques have all contributed to better outcomes for patients. However, accurate prediction of individual prognosis and identification of the best treatment approach for each patient remain challenges.

The overarching aim of the work that was done for this thesis was to explore different aspects of prognostic prediction in CLM and to highlight the need for more personalized treatment strategies. By contributing to this field, I hope to make a small but meaningful impact on the future care of patients affected by metastatic CRC.

Specific aims of the studies

I. To systematically review and meta-analyze prognostic biomarkers assessed by immunohistochemistry in CRLM.

II. To evaluate the prognostic significance of known and exploratory immunohistochemical tumor characteristics in CLM.

III. To correlate the tumor regression grade (TRG) observed in immunohistochemical analysis with radiologic findings from paired tumors, and to investigate whether high-diffusion MRI can serve as a predictive tool for chemotherapy response in CLM.

IV. To examine the potential role of rho GTPase-activating protein 4 (ARHGAP4) as a prognostic biomarker in CLM.

Materials and methods

Systematic review and meta-analysis

Systematic reviews are valuable because they synthesize all available evidence on a specific topic, allowing clinicians and researchers to gain a comprehensive understanding of the field. They follow a structured methodology, including a clearly defined research question, prespecified inclusion and exclusion criteria, and a comprehensive literature search. The collected data may be further analyzed quantitatively, for example through meta-analysis, to generate summary estimates that help inform clinical or research conclusions.

For Paper I, a systematic review and meta-analysis were conducted. We searched the PubMed database using the terms “colorectal,” “hepatic,” “liver,” “metastasis,” “metastases,” “prognosis,” “survival,” and “immunohistochem*.” To ensure that the process was thorough and transparent, we followed the guidelines regarding preferred reporting items for systematic reviews and meta-analyses (PRISMA) [122]. The initial search returned 1,073 articles. We also cross-checked references from these studies to find any additional relevant papers. Our search concluded on November 2, 2018.

To be included, studies had to have assessed immunohistochemical biomarkers in resected CRLM, followed the reporting recommendations for tumor marker prognostic guidelines, and analyzed data from at least 50 patients. If a study involved the use of tissue microarray (TMA) techniques, the authors had to provide detailed information on the methodology, including antibody selection, staining procedures, and interpretation [123]. We only included studies that reported OS rates in a multivariable analysis, with a hazard ratio (HR), a 95% confidence interval (CI), and a *p*-value. If a study was missing one of these parameters, we still considered it eligible for our study as long as enough data were available to estimate the missing values.

After review of all the studies, we identified 26 biomarkers from 25 papers that fitted our criteria. To better understand the role of these biomarkers, we grouped them according to the well-known "hallmarks of cancer" framework developed by Hanahan and Weinberg [124, 125]. These hallmarks describe how cancer cells grow, evade the immune system, resist cell death, invade new tissues, and more. We also included categories for deregulated metabolism, immune system control, and

genome instability. Since many biomarkers play multiple roles in cancer, we categorized them based on their most well-documented function in CRLM.

Study population

Study I

The study population in Paper I comprised patients included in previously published studies evaluating immunohistochemically assessed biomarkers in resected CRLM. All patients had undergone liver resection with curative intent for histologically confirmed adenocarcinoma. The included studies represented heterogeneous cohorts from multiple institutions and countries. Only studies that met the predefined inclusion criteria were included, namely evaluation of immunohistochemical biomarkers in resected CRLM, adherence to the REMARK quality criteria, and reporting of survival analyses with corresponding HRs, 95% CI, and *p*-values [123].

Study II

The study included all patients who had undergone liver surgery for CLM at Skåne University Hospital, Sweden, between 2006 and 2017. Data for individuals with mucinous adenocarcinoma were excluded, as were data from patients who died within 14 days of surgery. Deaths within 14 days of the operation were classified as surgery-related complications. Since the aim of this study was to investigate biomarkers and prognostic tools for long-term survival, the inclusion of patients who had died from operative complications would have reduced the validity of the analysis.

Patient information was gathered from medical records, with follow-up details obtained from the patient administrative system in Sweden and the Swedish population register. In addition, tumor samples from the included patients were collected from the regional biobank for further analysis.

The data collected covered key aspects such as patient demographics, tumor characteristics (including TNM stage, location, and presence of lymphovascular or perineural invasion), and treatment details, including chemotherapy and surgery dates. The study also tracked disease progression by recording recurrence and survival outcomes. All findings were reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [126].

The final analysis was of a study population of 260 patients.

Study III

This study included a selection of the patients involved in Study II. Patients who fulfilled the inclusion criteria for Study II and who had received preoperative chemotherapy were selected. To ensure consistency in imaging analysis, only patients who had MRI scans both before and after chemotherapy on the same 1.5 Tesla scanner with diffusion-weighted imaging that was acquired with b-values of 50, 400 and 800 mm^2/s were included.

Additional exclusion criteria were added to the study protocol. Patients without a documented pathological response were not included. Additionally, individual tumor lesions were excluded if they were smaller than 10 mm in diameter on imaging or if their MRI measurements were unreliable due to artifacts or poor image quality. Of 39 patients who were initially eligible, 12 were excluded due to lack of TRG assessment, mucinous histology, or imaging artifacts, leaving 27 patients with 49 measurable lesions for analysis.

Study IV

This study was based on the same study population as that used in Study II, but included an additional analysis of ARHGAP4. Nine patients were excluded during the ARHGAP4 staining process, which left a total study population of 251 patients.

Biobank and tumor preparation

For Papers II-IV, tumor samples from CLM resections at Lund University Hospital were assembled from the Regional Biobank Center, Southern Healthcare Region, Sweden (Regionalt Biobankscentrum, Södra Sjukvårdsregionen). These samples were then collected and prepared for evaluation following a standardized protocol. Resected tumor tissue was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. A senior pathologist, who was blinded to clinical data, reviewed the slides to confirm the presence of malignant cells that originated from the colon while excluding mucinous cancers. The histopathological evaluation included TRG, growth pattern classification, fibrosis, necrosis, the presence of pseudocapsules, and assessment of acellular mucin.

Histopathological examination and tumor growth pattern analysis

The regression grading of tumors followed the four-grade system devised by the American Joint Committee on Cancer/College of American Pathologists (AJCC/CAP) [127]:

- **TRG 0:** No residual tumor cells.
- **TRG 1:** Single cells or small groups of tumor cells.
- **TRG 2:** Residual cancer with a desmoplastic response.
- **TRG 3:** Minimal evidence of tumor response.

Tumor growth patterns were categorized using Vermeulen's classification [128], as explained here:

- **Desmoplastic:** tumor cells are completely separated from hepatocytes by a fibrous stroma.
- **Pushing:** a thin layer of reticulin fibers separates tumor cells from liver parenchyma, pushing the hepatocytes aside.
- **Replacing:** tumor cells directly replace liver parenchyma with no intervening fibrous layer, making direct contact with hepatocytes.
- **Mixed:** tumors exhibiting more than one growth pattern are classified accordingly.

A pseudocapsule was identified if a fibrous band was observed between the tumor and surrounding hepatocellular tissue, while acellular mucin was noted if mucin pools were present without neoplastic epithelium.

Tissue microarray and immunohistochemical processing

To facilitate biomarker analysis, TMA blocks were created from paraffin-embedded CLM specimens using an automated tissue arrayer (Minicore 3, Alphelys, Plaisir, France). Each sample was represented by four 2 mm-diameter cores taken from cancerous regions and embedded into recipient blocks. The prepared blocks were then cut into 4 μm sections for further processing.

Before immunohistochemical staining, tissue sections were incubated at 60°C for one hour, deparaffinized in xylene, and rehydrated through graded ethanol solutions. Antigens were retrieved through the use of EnVision FLEX Target Retrieval Solution (low pH, Dako) at 90°C for 20 minutes in an automated PT Link system (Dako, Glostrup, Denmark). The slides were then washed with phosphate-buffered saline and blocked for endogenous peroxidase activity using 0.3% hydrogen peroxide and 1% methanol in phosphate-buffered saline for 10 minutes.

After blocking with 5% goat normal serum, sections were incubated overnight at 4°C with a polyclonal rabbit anti-ARHGAP4 antibody (dilution 1:200). The next day, slides were treated with a biotinylated goat anti-rabbit secondary antibody

(Vector Technologies, cat no BA-1000, dilution 1:200) for one hour, and then incubated with an avidin/biotin complex (Vectastain Elite Kit, Vector Laboratories, cat no PK-6100) for 30 minutes. Visualization was achieved using the diaminobenzidine (DAB) chromogen (Vector Laboratories, cat no SK-4100) for five minutes, with hematoxylin used for nuclear counterstaining. Negative controls were included by omitting the primary antibody.

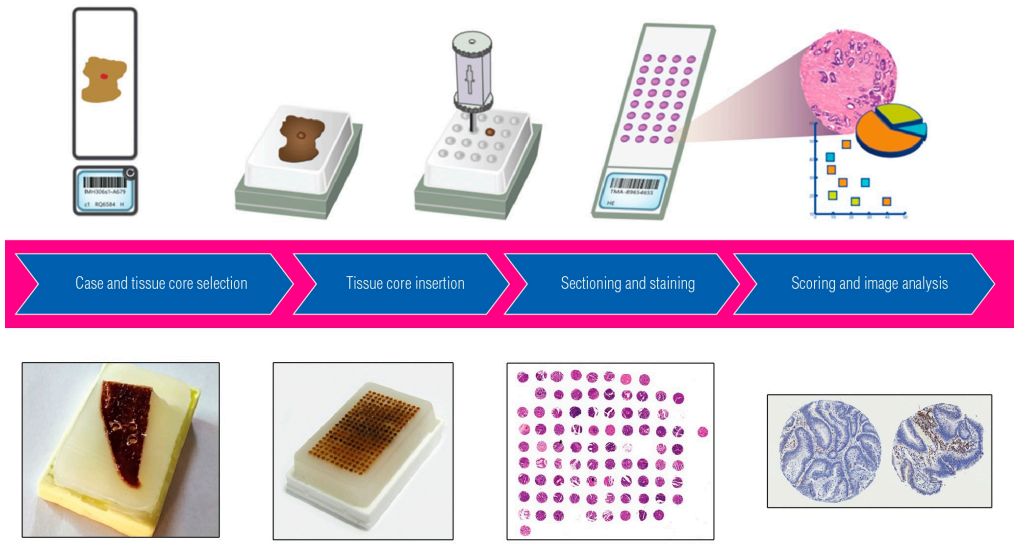


Figure 15. Workflow of TMA construction. The use of TMA allows the study of many tissue samples at once. Instead of cutting sections from hundreds of separate paraffin blocks, small tissue cores are assembled into a single recipient block. Sections cut from this block can then be stained and analyzed, so the use of this method offers an efficient approach to histological research. The process begins with selecting the areas of interest. Small cores are removed from these regions and placed in precise positions within the recipient block, with their location recorded. The recipient block is sectioned to produce slides made up of circular tissue samples. These slides are commonly used in immunohistochemistry to assess biomarker expression in large numbers of cases, for example, in cancer studies. Image reproduced from Lowe et al.. *Stevens & Lowe's Human Histology* (6th ed.), Amsterdam: Elsevier; Chapter 1, Histology; p. 1-14. Copyright 2025 Elsevier Inc. Reproduced with permission.

Immunohistochemical evaluation

The stained slides were scanned using the Aperio ScanScope system (Leica Biosystems, Germany), and ARHGAP4 expression was assessed by a senior pathologist who was blinded to clinical outcomes. Tumors were categorized as showing high or low ARHGAP4 expression based on staining intensity; weak or no staining was considered low, and moderate to strong staining was classified as high. A tumor was considered ARHGAP4-positive if at least 10% of epithelial cells exhibited a positive staining reaction. Positivity defined as staining in ≥10% of

tumor epithelial cells, is consistent with commonly used immunohistochemical frameworks.

This standardized approach to biobanking and tumor preparation ensured consistency in the histopathological and immunohistochemical analyses, allowing for reliable biomarker evaluation.

MRI methods

In Study III, we used diffusion-weighted MRI to explore whether tumor response to chemotherapy could be detected through imaging. All MRIs were performed on the same 1.5 Tesla scanner, both before and after neoadjuvant chemotherapy treatment to ensure consistency across patients. Diffusion-weighted images were acquired with *b*-values of 50, 400, and 800 mm²/s, so that we could calculate apparent diffusion coefficient (ADC) maps using a non-linear exponential model.

The imaging protocol included standard T1- and T2-weighted sequences, along with diffusion-weighted sequences. These were used to calculate ADC values, which reflect how water moves within tissues; this property can be altered by changes in tumor cellularity after chemotherapy.

To analyze the images, two independent readers who were blinded to the clinical and pathological data drew manually around each visible tumor. The goal was to include as much of the tumor as possible while avoiding clearly necrotic or cystic areas. If a lesion was smaller than 10 mm in diameter, poorly visible, or affected by motion artifacts, it was excluded from the study. ADC measurements were performed using an in-house MATLAB-based tool. For each lesion, two regions of interest were placed, one that covered the entire tumor at its equatorial plane and one along the periphery, In order to capture both global and marginal diffusion changes.

Only tumors that measured at least 10 mm in diameter were included to ensure reliable ADC measurements. The same imaging settings were used for each patient before and after treatment to minimize variation caused by technical factors.

By comparing pre- and post-treatment ADC values and tumor size, we aimed to determine whether MRI could reflect how much the tumor had responded to chemotherapy. Pathological response was graded according to the AJCC/CAP TRGs 0–3, in which TRGs 0–2 indicated pathological response and TRG 3 non-response [127]. These imaging results were then compared with the histological TRG, as assessed by the AJCC/CAP system after surgical resection.

Statistical analysis

Study I

This analysis was focused on studies that reported OS using multivariable models, including HR, 95% CI, and *p*-values. If a study did not provide all these details, it was included as long as enough published data were available to estimate the missing values.

In cases in which multiple studies had examined the same biomarker, a meta-analysis was performed to combine their findings. All additional calculations and meta-analyses were conducted using Review Manager (RevMan) version 5.3, which was developed by the Nordic Cochrane Center, the Cochrane Collaboration.

Study II

All statistical analyses were performed using R software (version 3.5.2). The primary outcome of the study was OS following surgery for CLM, while recurrence after surgery was assessed as a secondary outcome. Survival probabilities were visualized using Kaplan-Meier curves.

Associations between clinical or histopathological variables and survival outcomes were evaluated using Cox proportional hazards regression models, from which HRs and corresponding 95% CIs were calculated. Each HR was interpreted as a measure of relative risk over the study period.

A univariate analysis was performed first to identify potential prognostic variables. Variables that demonstrated *p*-values < 0.05 in univariate analysis were subsequently included in a multivariate Cox regression model to determine their independent association with survival outcomes. Statistical significance was defined as a two-sided *p*-value < 0.05.

Study III

Stata version 16.1 was used for statistical analysis in this study. Continuous data were summarized using medians and interquartile ranges, while categorical data were reported as frequencies.

To assess differences in ADC measurements among readers, the Wilcoxon signed-rank test for paired samples was used, along with a Bland-Altman plot to visualize any discrepancies. Comparisons of continuous data were conducted using either the Mann–Whitney U-test or the Wilcoxon signed-rank test, depending on whether the data were paired. For ordinal and categorical data, the chi-square test or Kruskal–Wallis test was applied.

The statistical analysis in this study was performed by the first author of the original article, Sam Eriksson.

Study IV

The statistical analysis in this study followed the same methodological framework as that used in Study II. All analyses were performed using R software (version 3.5.2). The primary outcome was OS after resection of CLM. Cox proportional hazards regression models were used to estimate HRs with 95% CIs. Variables with p-values < 0.05 in univariate analysis were included in a multivariate model to determine independent prognostic factors.

The key variable of interest was ARHGAP4 expression, which was dichotomized as low or high according to the immunohistochemical staining intensity. High expression was defined as positive staining in more than 10% of tumor epithelial cells. Survival outcomes were visualized with Kaplan-Meier curves.

All statistical test assumptions, including the proportional hazards assumption, were verified and satisfied. Statistical significance was defined as a two-sided p-value < 0.05.

Ethical considerations

All studies presented in this thesis were conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval was granted by Swedish ethical authorities for all studies (Dnr 2019-00203 and 2024-06150). All the research was conducted in accordance with Swedish law.

Main results

Paper I

From a systematic review of 25 articles, we identified 26 biomarkers that were independently significant as predictors of OS in patients with resected CRLM. These biomarkers span several oncogenic pathways, including those that regulate cell proliferation such as Ki67, insensitivity to anti-growth signals such as p53, ability to evade programmed cell death such as three-prime repair exonuclease 1 (TRX-1), limitless replicative potential such as human telomerase reverse transcriptase (hTERT), sustained angiogenesis such as cluster of differentiation (CD)34 and vascular endothelial growth factor A (VEGFA), the activation of invasion and metastasis such as thrombospondin 1 (TSP-1) and the metastasis suppressor KISS1, deregulated metabolism such as glucose transporter 1 (GLUT1/SLC2A1), immune evasion/suppression such as CD45RO, CD83 and CD68, and genome instability such as Aurora kinase A.

The full list of biomarkers, along with the number of supporting studies for each, is presented in Table 1. The process that was used to identify and select studies is summarized in Figure 16, which presents the PRISMA flowchart that outlines the number of records screened, excluded, and included in the final analysis.

CD34 and TSP-1 were evaluated in more than one study and were therefore suitable for inclusion in a meta-analysis. The pooled data, presented in forest plots (seen in Figure 17), showed statistically significant associations of CD34 markers with survival outcomes, indicating a potential prognostic value. For TSP-1, pooled data were not significantly associated with survival after surgery. The remaining biomarkers had not been assessed in more than one cohort at the time of our study. Many biomarkers were identified in analyses that involved relatively small patient populations, and none have undergone large-scale, prospective, multicenter validation, which would be necessary for clinical application.

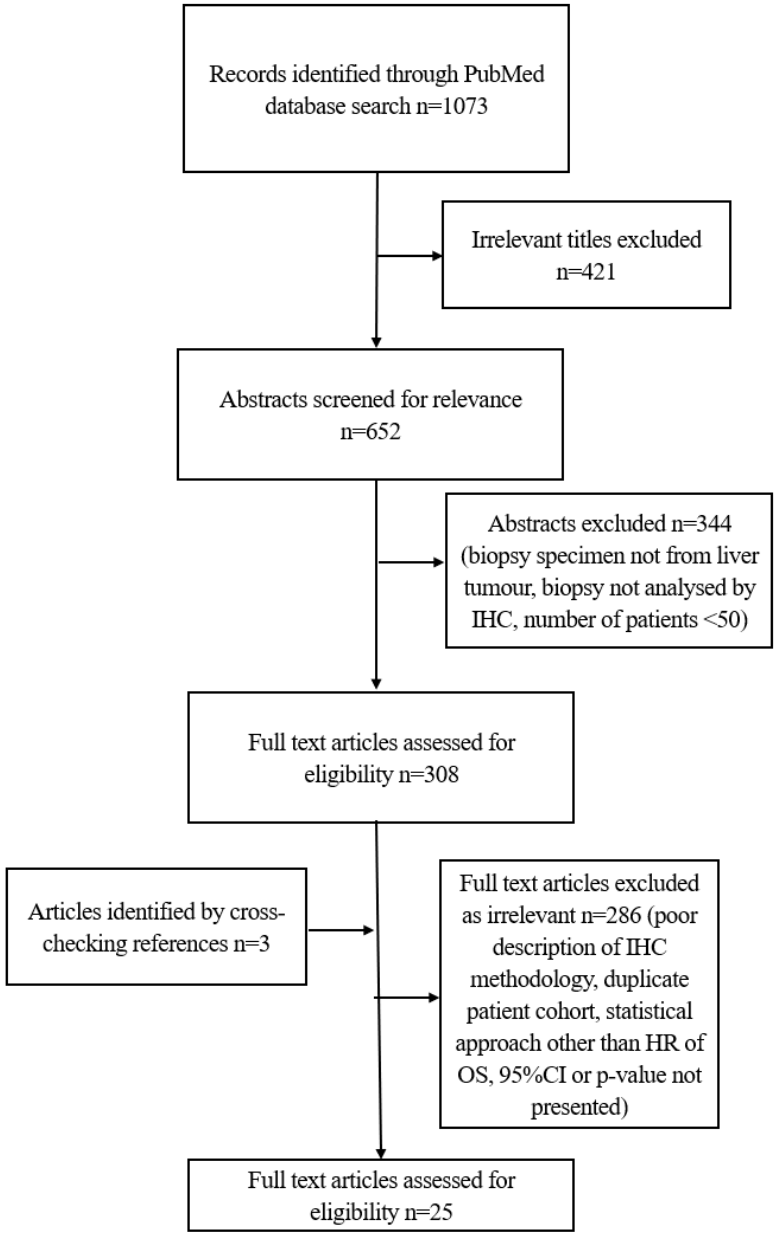
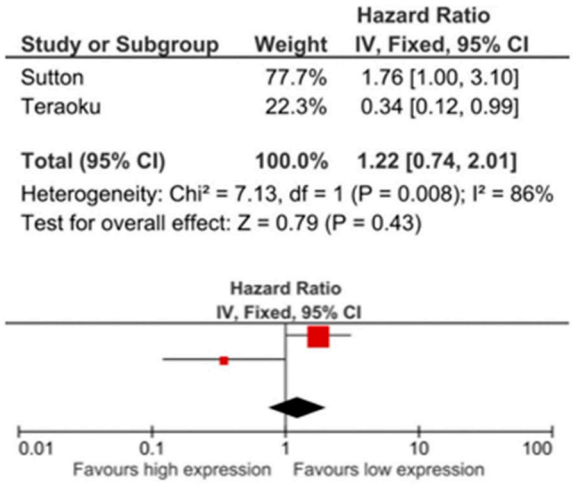


Figure 16. PRISMA flowchart illustrates the search strategy for articles to be systematically reviewed.

a)



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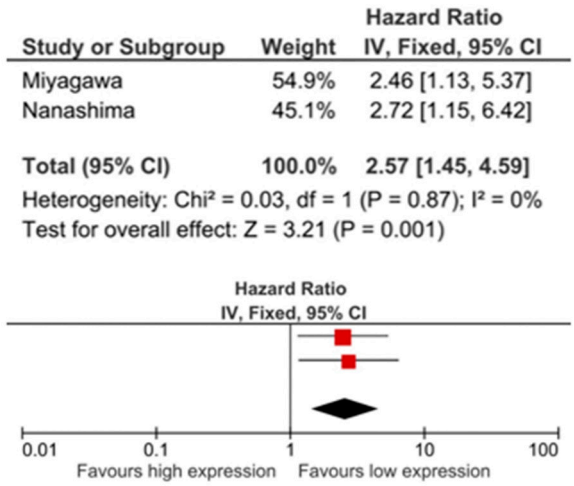

Figure 17. Forest plots of pooled data between expression of TSP-1, CD34 and survival. Association between TSP-1 expression and survival is seen in the upper illustration (a). Association between CD34 expression and survival is seen in the lower illustration (b). A fixed-effect model was used for meta-analysis.

Table 1. Biomarkers were identified in 25 articles that met the inclusion criteria applied in Study 1. They are categorized according to the hallmarks of cancer, as defined by Hanahan and Weinberg, with the addition of additional categories: deregulated metabolism, control of the immune system and genome instability. References numbered as in the original article (Paper I).

Biomarker	References	Year	N	HR (95% CI)	Detection ratea	*p*
Self-sufficiency in growth signals						
Ki-67	Ivanecz et al. [64]	2014	98	0.82 (0.68–0.98)	27/98 (28%)	0.038
EGFR	Goos et al. [120]	2014	323	1.54 (1.07–2.22)[c]	121/323 (37%)	0.02
RKIP	Kim et al. [77]	2012	68	0.19 (0.09–0.45)[c]	22/68 (32%)	0.014
Insensitivity to anti-growth signals						
p53	Nitti et al. [59]	1998	69	2.53 (1.84–3.22)	44/69 (64%)	0.008
Evading programmed cell death						
TRX-1	Noike et al. [142]	2008	84	0.41 (0.24–0.71)	37/84 (44%)	0.002
FAS/CD95	Onodera et al. [191]	2005	85	3.254 (1.00–10.49)	30/85 (35%)	0.048
Limitless replicative potential						
hTERT	Dômont et al. [40]	2005	201	2.03 (1.46–2.82)	86/201 (43%)	< 0.001
Sustained angiogenesis						
CD34	Miyagawa et al. [131]	2002	71	2.46 (1.13–5.37)	38/71 (54%)	0.023
	Nanashima et al. [132]	2009	139	2.71 (1.15–6.42)	69/139 (50%)	0.023
PTGS2/COX-2	Goos et al. [120]	2014	351	1.59 (1.14–2.26)[c]	85/351 (24%)	0.01
VEGFA	Goos et al. [198]	2016	335	1.50 (1.066–2.111)[c]	101/335 (30%)	0.02
Activating invasion and metastasis						
TSP-1	Sutton et al. [159]	2005	182	1.82 (1.00–3.10)	45/182 (25%)	0.01
	Teraoku et al. [160]	2016	94	0.38 (0.12–0.99)[c]	35/94 (63%)	< 0.05
CAV-1	Neofytou et al. [156]	2017	108	0.40 (0.21–0.78)[c]	61/108 (56%)	0.007
KISS1	Zhu et al. [172]	2015	55	0.20 (0.05–0.91)	19/55 (35%)	0.037
FRZB	Shen et al. [21]	2015	136	2.552 (1.86–3.64)	89/136 (65%)	< 0.001
Deregulated metabolism						
Glucose transporter 1 (GLUT1/SLC2A1)	Goos et al. [198]	2016	350	0.65 (0.51–0.863)[c]	179/350 (51%)	< 0.01
Immune evasion/suppression						
$MHC^{hi}CD3^{hi}$	Turcotte et al. [213]	2014	154	0.36 (0.20–0.67)	31/154 (20%)	0.001
CD3+CD8	Wang et al. [212]	2018	249	0.69 (0.59–0.80)	90/249 (36%)	< 0.001
CD45RO	Brunner et al. [211]	2014	201[b]	0.46 (0.28–0.73)[c]	155/201 (77%)	0.001
		2014	201[b]	0.25 (0.10–0.64)[c]	155/201 (77%)	0.004
pIgR	Liu et al. [179]	2014	136	2.673 (1.87–3.76)	86/136 (63%)	< 0.001
CD83	Miyagawa et al. [210]	2004	70	0.42 (0.23–0.76)[c]	44/70 (63%)	0.004
Tryptase	Suzuki et al. [209]	2015	135	17.3 (4.80–62)	73/135 (54%)	< 0.01
CD68	Miyagawa et al. [131]	2002	71	2.127 (1.01–4.50)	36/71 (51%)	0.049
Genome instability						
Aurora kinase A	Goos et al. [109]	2013	343	1.66 (1.08–2.54)[c]	115/243 (34%)	0.02
Other markers						
CD133	Yamamoto et al. [91]	2014	103	0.320 (0.13–0.81)	46/103 (45%)	0.016
APOBEC3G	Lan et al. [185]	2014	136	2.582 (1.83–3.63)	91/136 (67%)	< 0.001
CDX2	Shigematsu et al. [217]	2018	396	0.415 (0.26–0.66)	360/396 (91%)	< 0.001

Paper II

In this study, we explored key histopathological factors that influenced the outcomes for 260 patients who had undergone hepatectomy for CLM. OS was 4.6 years on average, but tumor characteristics played a crucial role in prognosis.

The inclusion and exclusion process that was used to determine the final study cohort is presented in Figure 18, which outlines each stage from the initial pool of patients to the 260 individuals who met the eligibility criteria. Survival data are presented in Table 2, in which outcomes after surgery are presented as univariate and multivariate OS.

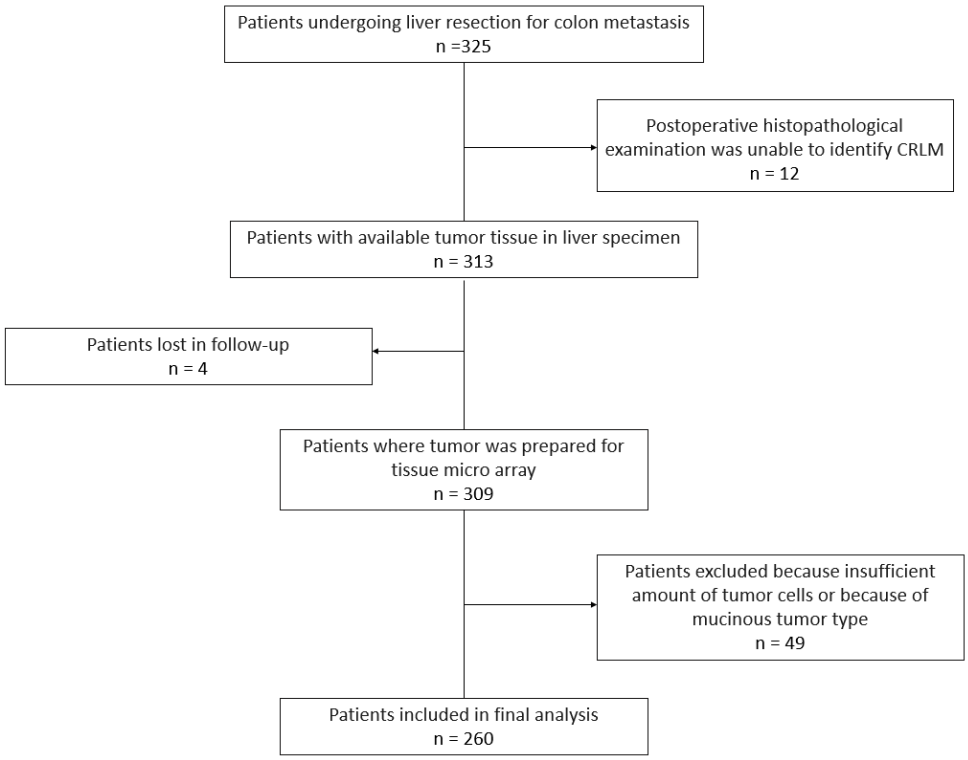

Figure 18. Flowchart shows patient inclusion and exclusion process for Study II.

The TRGs, which reflect how well tumors respond to chemotherapy, showed that most patients (69%) had moderate regression (TRG 2), while 29% had poor response (TRG 3), and only 2% had strong regression (TRG 1). Despite these differences in histological response, TRG was not significantly associated with survival rates after surgery, indicating that chemotherapy-induced tumor regression alone may not be a reliable predictor of long-term outcome in this patient population.

Lymphovascular invasion was observed in 35% of tumors, and perineural invasion in 25%. Both were linked to poorer OS in univariate analysis. However, only lymphovascular invasion retained statistical significance in multivariate analysis, and this finding underscored its independent prognostic value.

Presence of pseudocapsules (14% of tumors) was also evaluated but did not have a clear impact on survival after surgery. Tumor growth patterns were categorized as pushing, desmoplastic and replacement; however, no significant associations with OS were identified. Together, these findings suggest that although these histopathological features reflect tumor behavior, they are not reliable predictors of OS after tumor resection.

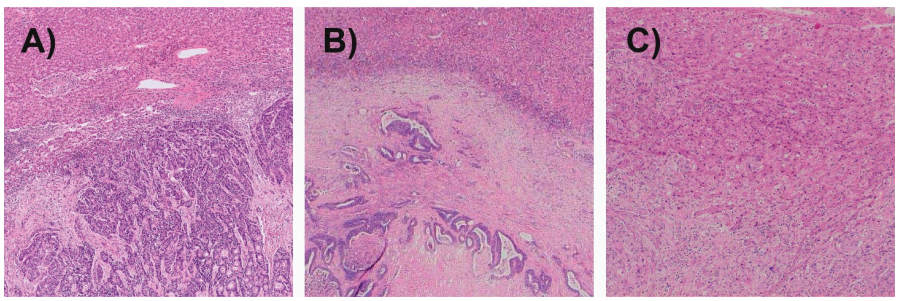

Figure 19. Representative illustrations of tumor growth patterns with standard histological stain (hematoxylin and eosin). (A) pushing growth pattern; (B) desmoplastic growth pattern; (C) replacement growth pattern.

Table 2. Survival data generated through use of Cox proportional hazard model.

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Right-sided primary tumor	1.3	0.93–1.86	.12			
Age >70	1.0	0.71–1.37	.93			
Gender (man)	1.0	0.75–1.50	.80			
Node positive primary tumor	1.2	0.85–1.80	.26			
>1 liver metastasis	1.1	0.75–1.50	.73			
Tumor size > 50 mm	1.4	0.95–2.04	.09			
Preoperative CEA >200	2.2	1.24–4.07	<.01*	2.1	1.13–3.92	.02*
Lung metastases at time of liver resection	1.9	1.18–3.00	<.01*	1.7	1.06–2.81	.03*
R0 resection (radical VS non-radical)	0.7	0.42–1.12	.13			
Synchronous (DFI < 12 months)	1.1	0.74–1.59	.66			
Acellular mucin	1.4	0.92–2.14	.11			
TRG 3 (vs 2)	0.85	0.59–1.24	.41			
Pattern C (vs B)	1.3	0.90–1.92	.16			
Pseudocapsule	0.95	0.59–1.55	.85			
Perineural invasion	1.7	1.20–2.51	<.01*	1.3	0.88–2.01	.17
Vascular invasion	1.7	1.23–2.4	<.01*	1.6	1.07–2.25	.02*

Paper III

In this study, we investigated whether MRI-based ADC measurements and changes in tumor size after neoadjuvant chemotherapy could serve as reliable indicators of histopathological treatment response in CLM. After application of the inclusion and exclusion criteria, 27 patients with a total of 49 measurable tumors were included in the analysis.

Following chemotherapy, ADC values increased in both histopathologically responding tumors (TRGs 0–2) and non-responding tumors (TRG 3). However, the degree of ADC increase did not allow for a clear distinction between these two groups. ADC values increased significantly after chemotherapy in both pathological responders (p=0.026) and non-responders (p=0.018), but the magnitude of change did not differ significantly between the groups (p=0.68). Tumor size was also reduced in both groups, with responding tumors showing a mean decrease of 35% compared with 28% in non-responders. Although responding tumors showed a greater average size reduction, the substantial overlap between groups indicates that size change alone is not a reliable indicator of response to chemotherapy.

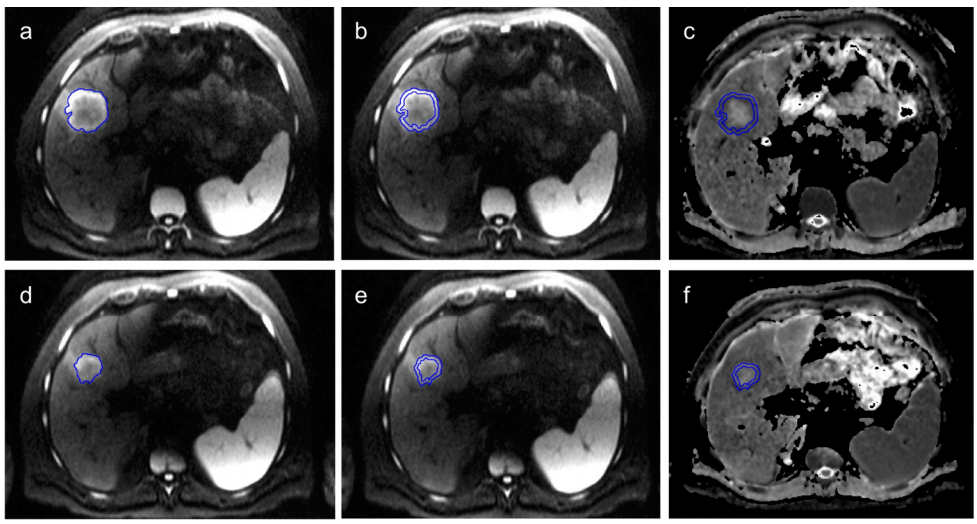

Figure 20. ADC measurements of a colorectal liver metastasis before (a–c) and after (d–f) chemotherapy. On pretreatment diffusion-weighted images (b = 800 mm²/s), regions of interest were drawn for the whole lesion (a) and periphery (b); the corresponding ADC map is shown in (c). Images taken after treatment show the same region of interest (d–f). The whole-lesion ADC increased from 1.10 to 1.21 ×10⁻³ mm²/s (10%), and peripheral ADC from 0.989 to 1.07 ×10⁻³ mm²/s (8%). The lesion shrank from 50 mm to 33 mm diameter, and pathology confirmed a complete response (TRG 0, no residual tumor cells).

When the tumors were evaluated according to the response evaluation criteria in solid tumors, several were classified as having a partial response despite showing ongoing microscopic cancer growth on pathological assessment [129]. This discrepancy underlines the limitations of these evaluation criteria to reflect accurately the true tumor regression at the cellular level.

Inter-reader agreement for ADC measurements was satisfactory, with no significant difference between readers (p=0.17).

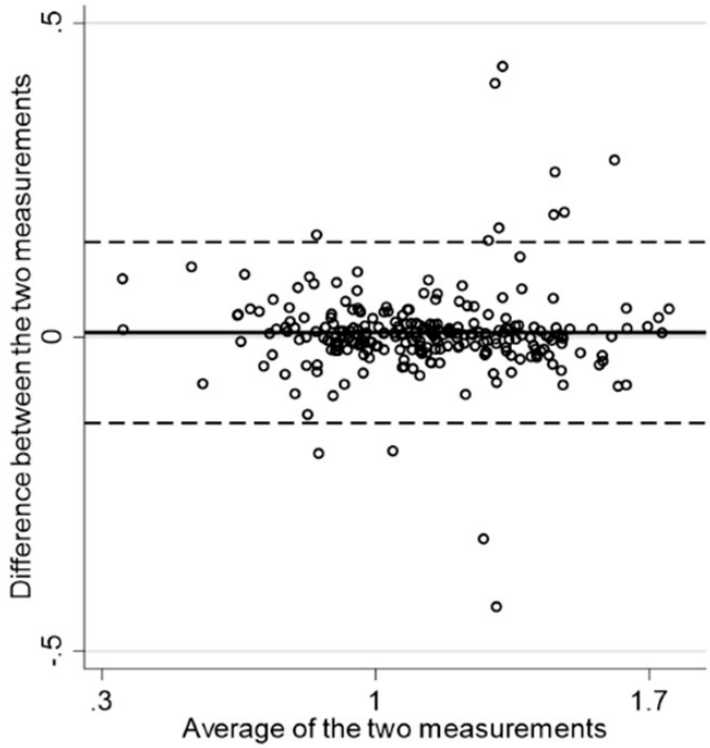

Figure 21. Bland-Altman plot showing inter-reader agreement for all ADC measurements (×10⁻³ mm²/s). The mean difference was 0.00723 ×10⁻³ mm²/s (solid line), with 95% limits of agreement ranging from –0.137 to 0.151 ×10⁻³ mm²/s (dashed lines).

Paper IV

In this study, the prognostic significance of ARHGAP4 expression, measured by immunohistochemistry, was assessed in tumor specimens from 251 patients who had undergone resection of CLM between 2006 and 2017 at Skåne University Hospital, Sweden. High ARHGAP4 expression was detected in 60% of patients, while 40% exhibited low expression levels.

Survival analysis revealed that high ARHGAP4 expression was significantly associated with worse OS in both univariate (HR=1.5) and multivariate (HR=1.5) analysis. In the multivariate analysis, three independent predictors of OS emerged: high ARHGAP4 expression, elevated preoperative CEA levels, and the detection of lymphovascular invasion on histopathology.

The inclusion and exclusion process that led to the final cohort of 251 patients is illustrated in Figure 22. Table 3 presents the results of the univariate and multivariate Cox regression analyses, and a summary of the prognostic value of ARHGAP4 and relevant clinicopathological variables. Visual representations of immunohistochemical micrographs showing representative ARHGAP4 staining are shown in Figure 23. Figure 24 presents Kaplan-Meier survival curves, in which the OS between high- and low-ARHGAP4 expression groups is compared. The figures demonstrate the prognostic impact of this marker.

Table 3. Survival data of 251 patients included in the final analysis, generated by using both univariate and multivariate Cox proportional hazard models.

	Univariate analysis			Multivariate analysis		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Right-sided primary tumor	1.3	0.93-1.86	0.12			
Age >70	1.0	0.71-1.37	0.93			
Sex (male)	1.0	0.75-1.50	0.80			
Node positive primary tumor	1.2	0.85-1.80	0.26			
>1 liver metastasis	1.1	0.75-1.50	0.73			
Tumor size >50 mm	1.4	0.95-2.04	0.09			
Preoperative CEA >200	2.2	1.24-4.07	<0.01*	2.0	1.06-3.70	0.03*
Lung metastases at time of liver resection	1.9	1.18-3.00	<0.01*	1.6	0.99-2.64	<0.05
R0 resection (radical vs. non-radical)	0.7	0.42-1.12	0.13			
Synchronous (DFI <12 months)	1.1	0.74-1.59	0.66			
Perineural invasion	1.7	1.20-2.51	<0.01*	1.3	0.85-1.96	0.17
Vascular invasion	1.7	1.23-2.4	<0.01*	1.5	1.06-2.23	0.02*
ARHGAP4 expression (high)	1.5	1.1-2.2	0.02*	1.5	1.00-2.11	<0.05*

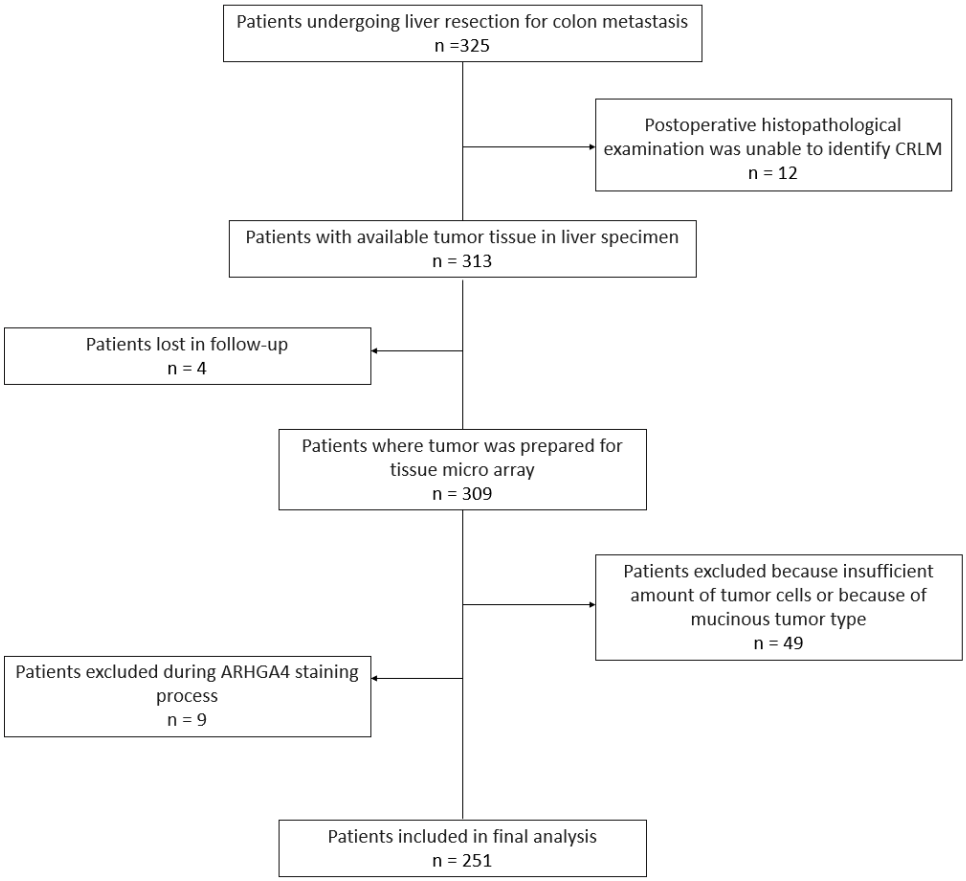

Figure 22. Flowchart representing the patient inclusion and exclusion process.

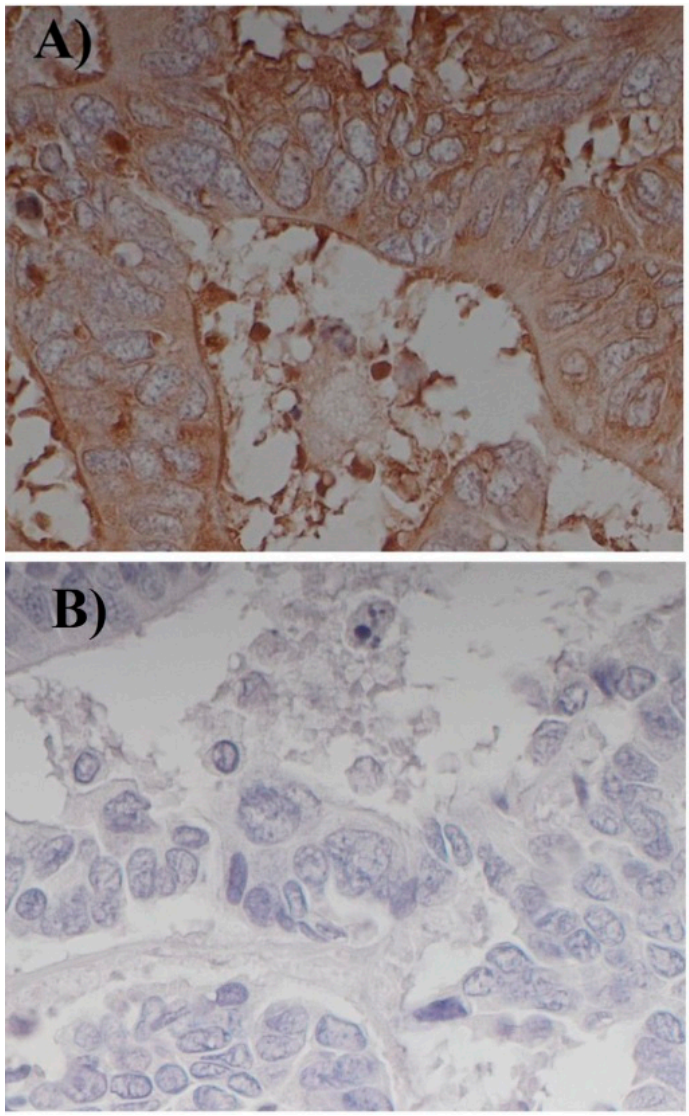

Figure 23. Representative images of high ARHGAP4 expression (A), defined as a minimum of 10% of tumor epithelial cells showing a positive staining reaction to ARHGAP4 antibodies; and of low ARHGAP4 expression (B), defined as less than 10% of tumor epithelial cells showing a positive staining reaction to ARHGAP4 antibodies.

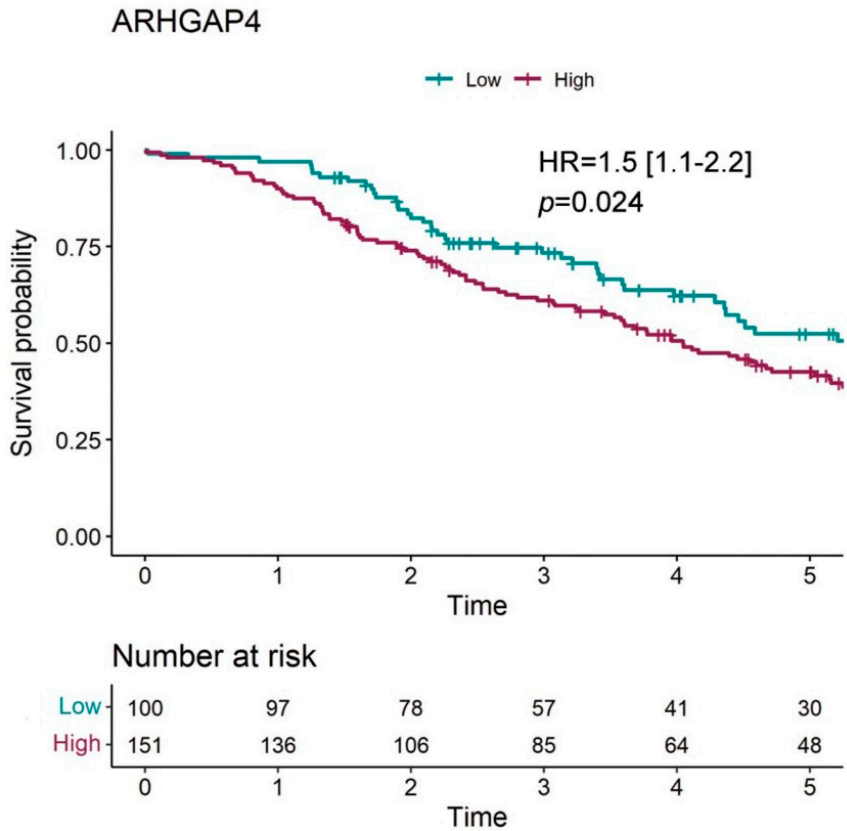

Figure 24. Kaplan-Meier curves representing patient survival probability in years in association with high or low levels of expression of ARHGAP4.

Discussion

Methodological considerations

The methodological approach in this thesis combined immunohistochemistry and radiological imaging in order to evaluate prognostic tools in CRLM. Each methodology served a specific aim but also brought technical and methodological limitations that must be considered when interpreting the results.

Immunohistochemistry

The application of immunohistochemistry was central to multiple studies in the included projects. It was used for original biomarker analysis in resected tumor tissue, and its use in previous studies was considered during the systematic synthesis of the literature. While immunohistochemistry is well-established in routine pathology, it poses several methodological challenges when applied to prognostic research [130].

In Paper IV, ARHGAP4 expression was considered positive when ≥10% of tumor epithelial cells demonstrated positive staining. This threshold was selected in accordance with commonly applied immunohistochemical scoring frameworks that are used for exploratory biomarkers in CRC, including markers such as human epidermal growth factor receptor 2 (HER2) and p53 [131, 132]. The use of a 10% cutoff provides a practical and reproducible distinction between positive and negative staining patterns and allows comparison with previous reports that had employed similar criteria. Nevertheless, it should be acknowledged that this approach is somewhat arbitrary [133]. Fixed percentage thresholds may not fully capture the biological or clinical relevance of marker expression. Instead, the use of data-driven methods to determine optimal cut-offs for novel biomarkers could be encouraged. Future studies with larger patient cohorts could apply such approaches to assess whether the 10% threshold optimally stratifies patients according to clinicopathological features or survival outcomes.

One of the key issues in this work was a lack of standardization, which is a point reinforced by the findings of Paper I [134]. Not only did cutoff methodologies vary between studies, but there were differences in antibody selection, antigen retrieval protocols, staining platforms, and scoring systems, which severely limited

reproducibility. This inconsistency in general undermines meta-analytic synthesis and obstructs clinical translation. Furthermore, scoring of immunohistochemical results remains semi-quantitative and inherently subjective. Results typically rely on a pathologist's interpretation. Even with standardized scoring systems (such as H-scores or Allred scores), inter-observer variability is a known confounder [132]. Digital pathology offers some promise in reducing this variability, but it was not implemented in these studies.

A practical limitation in these studies was that immunohistochemistry evaluates protein expression in a single tumor section, and therefore provides only a snapshot of a tumor, which in this type is often biologically heterogeneous. Observed results from one part of the tumor may not have reflected the tumor as a whole.

Despite these limitations, immunohistochemistry remains an essential bridge between molecular biology and clinical pathology. Future prognostic applications require harmonization of immunohistochemical methodology across research centers.

Diffusion-weighted MRI

In Study III, diffusion-weighted MRI was used to assess tumor response to neoadjuvant chemotherapy, and the imaging findings were compared with histopathological tumor regression. While the method is theoretically attractive due to its non-invasive nature and potential to reflect tumor cellularity, several limitations emerged.

The primary issue was poor correlation between ADC values and histopathological response. Part of the problem could be tumor heterogeneity. ADC values are influenced by many factors, including cellular density, fibrosis, necrosis, and interstitial edema [135]. As a result, diffusion-weighted MRI lacks the specificity that is required to evaluate response reliably in heterogeneous tumors such as CLM.

Another limitation was technical variability. ADC values are affected by scanner type, imaging parameters, and the post-processing techniques used, so they are difficult to standardize. Motion artifacts, especially in the liver, and low spatial resolution further hinder clinical application [136].

While diffusion-weighted MRI offers potential as part of a multiparametric imaging approach, it cannot be relied upon in isolation to assess treatment response. The full potential of diffusion-weighted MRI cannot be determined until more robust and specific imaging biomarkers are available.

Strengths and limitations

One of the most important strengths of the work performed for this thesis lies in its multimodal design, which combines immunohistochemistry and radiological imaging to address the challenge of prognostication and precision medicine. This approach reflects the complexity of real-world clinical decision-making, in which no single diagnostic or predictive modality can fully account for the biological and clinical heterogeneity of the disease. By integrating pathological, radiological, and computational data into a unified research framework, the work mirrors emerging strategies in precision oncology and offers a richer basis for risk stratification than has typically been achieved in single-modality studies.

The immunohistochemistry component benefited from the use of a standardized staining protocol and blinded evaluation, which reduced the likelihood of observer bias and increased reproducibility. In the broad CLM literature, methodological variation in antibody selection, staining procedures, and scoring systems often undermines the reliability of biomarker findings, as previously highlighted. The use of consistent methods and clear methodological descriptions in this thesis and the studies on which it is based represents a methodological strength. The inclusion of radiological features added a non-invasive prognostic layer, which could enable assessment of parameters that can be applied in the preoperative phase, before histopathology is available.

From a clinical perspective, the work benefits from a clearly defined patient cohort that was drawn from real-world practice and showed data linkage across pathology, imaging, and survival outcomes. Application of this comprehensive dataset increases the practical relevance of the findings and reduces the disconnect that sometimes exists between research data and clinical populations. The breadth of available variables, combined with detailed follow-up, strengthens the foundation for the analyses performed.

Despite these strengths, important limitations must be acknowledged. The retrospective design limited control over data quality and completeness. Retrospective studies depend on the accuracy and consistency of historical records, which may vary among cases and over time. In our research projects, although efforts were made to standardize data extraction, certain clinical variables were missing for some patients, particularly regarding details of postoperative adjuvant therapy. This incomplete data may have influenced multivariable analyses and the interpretation of prognostic relationships.

The immunohistochemical analyses, while standardized within the scope of this work, were subject to the broader limitations of the method. The methodological considerations are highlighted earlier in the discussion. To emphasize how these limitations applied in our research, it must be underlined that inter-laboratory variability in staining procedures cannot be excluded. Even within a standardized

protocol, there is inherent subjectivity in scoring, particularly in cases in which semi-quantitative systems are used. Tumor heterogeneity presented an additional challenge, as a single tissue section may not have accurately reflected the distribution of biomarker expression throughout the lesion. This raises the possibility of sampling bias and limits the generalizability of findings at the individual patient level.

The radiological component also had limitations. Imaging studies were reviewed and interpreted within a single center, using protocols and equipment that may have differed from those in other institutions. These variations may have influenced the visibility and characterization of prognostic features such as margin morphology or enhancement patterns. Inter-reader variability was another potential source of error, as subtle imaging features may have been assessed differently depending on the radiologist's experience and interpretation style. While the use of standardized review criteria may have reduced variability, it would not have eliminated it entirely, particularly given the inherently subjective nature of some features.

Finally, the thesis lacks prospective validation for the combined prognostic framework. Prospective studies are essential to assess how predictive tools perform in real-time decision-making and whether or not they influence outcomes after integration into clinical pathways. Until such validation is undertaken, the findings should be interpreted with caution, particularly in contexts that differ from the studies' settings.

Advances in prognostic biomarkers for CLM

Although surgery offers the chance of a cure, its success varies greatly among individuals, and predicting recurrence and survival outcomes is a complex challenge. Current biomarkers such as CEA are regularly used to estimate the possibility of recurrence and to guide treatment, but their accuracy is limited, particularly in early-stage disease [113].

Emerging biomarkers hold significant promise. In the future, analysis of the tumor's genetic and molecular landscape may lead to identification of signs of CLM even before clinical symptoms or imaging can detect them. This evolving field of biomarker discovery may reveal critical insights into tumor behavior and, therefore, help to make predictions of patient outcomes more reliable. One promising avenue is the development of multi-biomarker panels, which combine traditional markers such as CEA with genetic, epigenetic, and immune markers. This comprehensive approach could enhance diagnostic sensitivity and specificity, and ultimately improve the accuracy of prognostic predictions.

Despite exciting advances, several challenges remain in the validation of biomarkers for clinical use. One key obstacle is the challenge of translating a biomarker that appears predictive for CLM in the laboratory into clinical practice. The heterogeneity of both CRC and CLM complicates this task, as different tumor subtypes often exhibit varying molecular profiles. The variability in study designs also hampers efforts to standardize biomarker validation. With the study methods used in this thesis, we showcase a major challenge: a lack of study protocol standardization. Differences in antibody selection, staining techniques, and interpretation make it difficult to compare findings across studies. Both the National Cancer Institute and the European Organization for Research and Treatment of Cancer have emphasized the need for standardized protocols [137]. If we can achieve greater consistency, immunohistochemical biomarkers could become a powerful tool to guide treatment decisions and improve patient outcomes in CRLM.

To identify impactful biomarkers, large-scale, prospective clinical trials are essential to test rigorously and validate promising biomarkers across diverse patient populations. Also, regulatory challenges, the complexity of biomarker testing, and the high costs involved in the development of diagnostic tools slow their widespread integration into clinical practice.

Challenges in stratifying patients for surgical intervention

Stratifying patients with CRLM for surgical intervention is a delicate and multifactorial process. Not all patients are suitable candidates for surgery, but selecting those who are most likely to benefit is a significant challenge. A number of well-established clinical factors must be considered, such as the size, number, and location of metastatic lesions, along with the patient's liver function and overall health status.

Equally important are patient-related characteristics that can complicate decision-making. Age, presence of comorbidities such as cardiovascular disease or diabetes, nutritional status, and performance status all influence both surgical risks and the likelihood of recovery. Some patients may technically be eligible for resection based on tumor biology, but their overall frailty or limited physiological reserve may increase the risk of complications or impair their ability to withstand major surgery. Conversely, young or otherwise healthy patients may be offered surgery even in more advanced disease scenarios because they are better equipped to recover and tolerate further treatments. Balancing these biological and patient-related factors makes the decision highly individualized and often complex.

Advances in prognostic biomarkers for CLM surgery have the potential to provide valuable insights into the aggressiveness of the disease and the expected benefit of resection. Certain genetic mutations are associated with poorer prognosis and may influence whether surgery is likely to offer long-term survival benefits. Ongoing research is increasingly focused on the development of multi-biomarker panels to refine prognostic accuracy.

Machine learning (ML) tools are also gaining traction in research because their use can enhance outcome prediction by analyzing large, complex patient datasets. By integrating clinical characteristics, patient-related factors, and tumor genomics, ML algorithms can possibly identify patterns that are difficult to detect with conventional methods. These patterns can then be used to stratify patients more effectively, ensuring that those who are most likely to benefit are selected. Through continuous learning and adaptation, ML has the potential to improve the precision of clinical decision-making, optimizing both when and how surgical interventions are applied.

When combined, biomarker panels and ML tools offer a powerful approach to patient stratification. The use of ML can lead to the processing of large datasets of biomarker and patient-related information to identify the most predictive variables for surgical success. This integration may not only streamline patient selection but also help to personalize treatment strategies, making them more closely aligned with each patient's biological profile and overall health. In turn, this could improve surgical outcomes, reduce the number of unnecessary surgeries, and minimize risks for patients.

Clinical decision-making and personalized medicine

While patient stratification currently relies on clinical judgment supported by established prognostic factors, the growing field of personalized medicine is beginning to reshape how these decisions are made. Instead of solely evaluating clinical and patient-related characteristics, treatment planning is increasingly guided by molecular profiling, advanced imaging, and predictive modeling. Molecular insights into tumor biology, such as the presence of BRAF or dMMR mutations, are now recognized as powerful prognostic and predictive markers. These not only inform the probability of survival but may also influence whether or not surgical resection is likely to offer meaningful benefit.

Personalized medicine also extends to the surgical approach itself. Patients with distinct molecular features may be considered for more extensive resections or multimodal strategies that combine resection with local ablation or staged hepatectomy. Integration with systemic therapies is another key aspect; neoadjuvant treatment can be used to downstage disease and improve resectability, while

adjuvant therapies tailored to tumor biology can be used to reduce the chance of recurrence and prolong survival. The tumor biology can also be used as a predictor to guide the use of systemic treatment according to whether or not it will be beneficial and therefore to select the type of chemotherapy (or in some cases immunotherapy).

At the same time, the number of available treatment options for CLM patients continues to grow, creating additional complexity in clinical decision-making. This often requires several re-evaluations in multidisciplinary conferences, where surgeons, oncologists, radiologists, and pathologists reassess patient status in light of new treatments, updated imaging, or emerging biomarker data. Treatment guidelines also evolve rapidly as new research is published, meaning that best practice today may differ from recommendations only a few years ago. For healthcare systems, this dynamic landscape raises the challenge of ensuring that every patient receives the best possible treatment at the right time. It also prompts important questions about responsibility: should it be the surgeon, the oncologist, or the multidisciplinary team as a whole that ultimately ensures optimal care? These issues place new demands on collaboration, communication, and continuous learning within clinical teams.

The potential positive evolution of personalized medicine is tempered by several challenges. High costs, limited access to advanced molecular testing, and variability in data interpretation restrict its widespread adoption. Nevertheless, ongoing clinical trials are beginning to incorporate biomarker-driven patient selection. Current trend shifts suggest that such approaches can refine surgical and oncological decision-making and improve long-term outcomes. Looking ahead, the integration of biomarker panels and ML into everyday clinical workflows has the potential to shift CLM treatment from a largely reactive process to a more predictive and individualized one. This evolution may ultimately help to optimize surgical interventions, minimize the use of unnecessary procedures, and move the field closer to truly personalized cancer care.

From bench to bedside: can we implement precision?

The promise of precision medicine in CRLM that tailors surgical and oncological care to each patient is undeniable. However, there’s a question we do not ask often enough: can these innovations actually be used in real-life clinical settings and at what cost?

It is one thing to identify a biomarker such as ARHGAP4 in a research lab, and another to turn that discovery into something that every hospital pathologist can reliably test for. For a biomarker to make a real difference, we need more than just scientific excitement. We have highlighted that future research needs standard

protocols, validated cut-offs, and accessible technology that works in the same way in Malmö as it does in Mumbai. Without that, even the most promising biomarker risks being stuck in the academic bubble, never reaching the patient it could help.

Then there is the issue of cost. Health systems are under pressure, and new tests or predictive models cannot just be "better"; they must also be efficient enough. A good biomarker ideally should prevent unnecessary surgeries, help to avoid overtreatment, or measurably improve survival. If it does not change the outcome (or costs more than it saves), it is unlikely to be adopted, no matter how statistically significant the *p*-values are.

So how do we bridge this gap between innovation and implementation? We need to go beyond accuracy metrics and start to ask questions like: *Will this tool actually change clinical decisions? Is it feasible in everyday practice?* Perhaps most importantly: *Does it improve life for the patient?*

Precision medicine should not be a luxury item. For it truly to transform care in CRLM, future research must focus not just on discovering new tools, but also on making them practical, affordable, and equitable. A tool is only as useful as our ability to use it where it matters most: in the clinic, with the patient in front of us.

Future research directions

The findings of this thesis highlight several important avenues for further investigation that are necessary to strengthen the evidence base and move toward clinical implementation of multimodal prognostic tools in CRLM.

A key priority is prospective validation. While the retrospective design of the present work provided a valuable foundation, prospective studies are essential to determine how the proposed prognostic framework performs when applied in real time to patients undergoing evaluation and treatment for CRLM. Such validation would enable assessment not only of predictive accuracy but also of feasibility, clinician uptake, and potential influence on treatment decision-making. A well-designed prospective trial could also address gaps in the current dataset, such as standardized recording of recurrence patterns, adjuvant therapy regimens, and patient-reported outcomes.

Collaboration across multiple centers will be essential to achieve these goals. A multicenter design would provide large and diverse patient populations, improve statistical power for subgroup analyses, and enhance the generalizability of the findings. It would also allow for systematic comparison of imaging protocols, pathology processing, and treatment pathways among institutions, and hence provide insight into the sources of heterogeneity observed in prognostic studies. Multicenter work could be facilitated through national or international hepatobiliary

cancer center collaborations that would ensure harmonized methodologies and centralized data analysis.

Integration of molecular and genomic profiling represents another important direction. Advances in high-throughput sequencing and transcriptomic analysis have revealed a complex landscape of genetic alterations in CRLM that may have prognostic and therapeutic significance. Combining these molecular insights with histopathological, radiological, and clinical variables could yield more precise prognostic models that not only stratify patients by risk but also identify candidates for targeted therapies. Future studies could explore the incremental value of genomic markers in multimodal prediction models and evaluate both their prognostic performance and their cost-effectiveness in routine practice.

Finally, as ML models evolve, efforts should be focused on improving their interpretability. One of the main barriers to clinical adoption of AI tools is the "black box" nature of their decision-making processes. Future research should incorporate explainable AI techniques so that clinicians can understand which features most strongly influence a model's predictions. This transparency would increase trust in the technology, facilitate regulatory approval, and support shared decision-making with patients. Additionally, incorporating uncertainty estimation into AI models would help clinicians to gauge the confidence level of individual predictions and to decide when further diagnostic evaluation is warranted.

Conclusions

The major conclusions from the four articles included in this thesis are as follows:

I. A number of prognostic biomarkers for resected CRLM can be identified in published literature, but most have been evaluated in retrospective settings of small patient cohorts. Standardized methodology to research biomarkers in large multicenter settings is needed to make biomarkers available as clinically useful prediction tools.

II. Although certain histopathological factors, such as lymphovascular invasion, play key roles in prognosis, others, including tumor regression, growth pattern, and pseudocapsule presence, may have limited value as prognostic biomarkers after hepatectomy due to CLM.

III. The use of diffusion-weighted MRI did not reveal any differences in lesion-ADCs between pathologically responsive and non-responsive resected CLM. The true impact of TRG has yet to be determined. These findings suggest that traditional imaging markers may not tell the full story, and this finding emphasizes the need for more precise tools to assess true tumor viability in CLM patients.

IV. In resected CLM specimens, the biomarker ARHGAP4 serves as an independent predictive factor of OS. ARHGAP4 is a novel prognostic biomarker, and more research is necessary before its role as a prognostic biomarker can be determined.

Future perspectives

One of the biggest challenges in the treatment of CRLM is predicting how each patient will respond to given treatment. As clinicians, we rely on risk scores, imaging, and pathology reports to estimate prognosis, but the truth is, no model can perfectly predict an individual's outcome. Some patients defy expectations, living far beyond what statistics suggest, while others experience early recurrence despite receiving the best possible treatment. This uncertainty makes it difficult to guide patients through one of the most stressful periods of their lives.

A major reason for this unpredictability is the complexity of the disease itself. No two cases of CRLM are exactly alike. Clinical courses and tumors themselves behave differently depending on genetic mutations, immune responses, and prior treatments. Traditional prognostic tools, such as clinical risk scores, were developed based on large patient cohorts, but they often fail when applied to an individual sitting in front of us in the clinic.

Adding to this challenge is the evolving landscape of treatment. Advances in systemic therapies, including targeted agents and immunotherapy, have improved survival rates for some patients, but response rates remain highly variable. While some individuals experience dramatic tumor shrinkage, others see no benefit at all. As of the current era, we lack reliable biomarkers to predict who will respond to given therapy and who will not. This uncertainty forces us to decide on treatments based on probability rather than precision, and this situation can feel frustrating for both clinicians and patients.

Another major issue is the hidden threat of microscopic disease. Even after complete resection, tiny clusters of tumor cells may remain undetectable and cause recurrence months or years later. Currently studied prognostic biomarkers are still far from incorporation into routine practice. Until we have better ways to track residual disease, we remain one step behind in truly personalizing treatment and surveillance.

This is why precision medicine is so critical for the future of oncologic surgery. If we could accurately predict how a tumor would behave in a specific patient and treatment setting, we could emerge from generalized treatment strategies and move toward truly individualized care. Molecular profiling and advanced imaging hold promise, but we need more research to bring these tools into everyday clinical practice.

Until then, we continue to make the best decisions possible with the information we have, knowing that despite all our efforts, cancer remains unpredictable.

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References

1. Power C, Rasko JE. Whither Prometheus' liver? Greek myth and the science of regeneration. Ann Intern Med. 2008;149(6):421-6.
2. van Gulik TM, van Gulik MM, Koning HH. [Prometheus and liver regeneration: the dissection of a myth]. Ned Tijdschr Geneeskd. 2018;162.
3. Bay NS, Bay BH. Greek anatomist Herophilus: the father of anatomy. Anat Cell Biol. 2010;43(4):280-3.
4. Sjöstrand NO. [The medical illustration as the expression of illusion and imagination--the liver as an example from history]. Sven Med Tidskr. 2007;11(1):17-51.
5. van Gulik TM. Liver anatomy by Francis Glisson. Hepatobiliary Surg Nutr. 2022;11(4):502-3.
6. Greene NM. Anesthesia and the development of surgery (1846-1896). Anesth Analg. 1979;58(1):5-12.
7. Nakayama DK. Antisepsis and Asepsis and How They Shaped Modern Surgery. Am Surg. 2018;84(6):766-71.
8. Hardy KJ. Carl Langenbuch and the Lazarus Hospital: events and circumstances surrounding the first cholecystectomy. Aust N Z J Surg. 1993;63(1):56-64.
9. Banerjee A, Hariharan D. History of liver surgery. Clin Liver Dis (Hoboken). 2024;23(1):e0237.
10. Belghiti J, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. J Am Coll Surg. 2000;191(1):38-46.
11. Alvarez FA, Sanchez Claria R, Oggero S, de Santibañes E. Parenchymal-sparing liver surgery in patients with colorectal carcinoma liver metastases. World J Gastrointest Surg. 2016;8(6):407-23.
12. Ardito F. Possibility of repeat surgery for recurrence following two-stage hepatectomy for colorectal liver metastases: impact on patient outcome. Hepatobiliary Surgery and Nutrition. 2019;9(1):83-5.
13. Wang K, Liu Y, Hao M, Li H, Liang X, Yuan D, Ding L. Clinical outcomes of parenchymal-sparing versus anatomic resection for colorectal liver metastases: a systematic review and meta-analysis. World J Surg Oncol. 2023;21(1):241.
14. Ivey GD, Johnston FM, Azad NS, Christenson ES, Lafaro KJ, Shubert CR. Current Surgical Management Strategies for Colorectal Cancer Liver Metastases. Cancers (Basel). 2022;14(4).

15. Moris D, Dimitroulis D, Vernadakis S, Papalampros A, Spartalis E, Petrou A, et al. Parenchymal-sparing Hepatectomy as the New Doctrine in the Treatment of Liver-metastatic Colorectal Disease: Beyond Oncological Outcomes. Anticancer Research. 2017;37(1):9-14.
16. Gold JS, Are C, Kornprat P, Jarnagin WR, Gönen M, Fong Y, et al. Increased use of parenchymal-sparing surgery for bilateral liver metastases from colorectal cancer is associated with improved mortality without change in oncologic outcome: trends in treatment over time in 440 patients. Ann Surg. 2008;247(1):109-17.
17. Wei Chieh AK, Chan A, Rotellar F, Kim K-H. Laparoscopic major liver resections: Current standards. International Journal of Surgery. 2020;82:169-77.
18. Kamarajah SK, Bundred J, Manas D, Jiao L, Hilal MA, White SA. Robotic versus conventional laparoscopic liver resections: A systematic review and meta-analysis. Scandinavian Journal of Surgery. 2021;110(3):290-300.
19. Schmelzle M, Krenzien F, Schöning W, Pratschke J. Laparoscopic liver resection: indications, limitations, and economic aspects. Langenbeck's Archives of Surgery. 2020;405(6):725-35.
20. Sucher E, Sucher R. Minimally invasive liver surgery: a field is maturing. Laparoscopic Surgery. 2019;3.
21. Liang Y, Lin C, Zhang B, Cao J, Chen M, Shen J, et al. Perioperative outcomes comparing laparoscopic with open repeat liver resection for post-hepatectomy recurrent liver cancer: A systematic review and meta-analysis. International Journal of Surgery. 2020;79:17-28.
22. Chiu CC, Lin WL, Shi HY, Huang CC, Chen JJ, Su SB, et al. Comparison of Oncologic Outcomes in Laparoscopic versus Open Surgery for Non-Metastatic Colorectal Cancer: Personal Experience in a Single Institution. J Clin Med. 2019;8(6).
23. Deijen CL, Vasmel JE, de Lange-de Klerk ESM, Cuesta MA, Coene P-PLO, Lange JF, et al. Ten-year outcomes of a randomised trial of laparoscopic versus open surgery for colon cancer. Surgical Endoscopy. 2017;31(6):2607-15.
24. Knitter S, Andreou A, Kradolfer D, Beierle AS, Pesthy S, Eichelberg A-C, et al. Minimal-Invasive Versus Open Hepatectomy for Colorectal Liver Metastases: Bicentric Analysis of Postoperative Outcomes and Long-Term Survival Using Propensity Score Matching Analysis. Journal of Clinical Medicine. 2020;9(12):4027.
25. Brustia R, Slim K, Scatton O. Enhanced recovery after liver surgery. Journal of Visceral Surgery. 2019;156(2):127-37.
26. Willems E, D'Hondt M. Laparoscopic liver resection for colorectal liver metastases: perspectives from patients. Laparoscopic Surgery. 2019;3.
27. Wach MM, Washburn LA, Alvikas J, Qu LT, Zhang M, Etherington MS, et al. Combined hepatic resection and ablation for high burden of colorectal liver metastases demonstrates safety and durable survival. HPB. 2024;26(3):362-9.
28. Song H, Li Z, Zhang S, Ling X, Chen L, Wang Y, et al. Laparoscopic resection combined with ablation for multiple colorectal liver metastases: a multicentre propensity-matched analysis. Surgical Endoscopy. 2025.

29. Amygdalos I, Hitpass L, Schmidt F, Josephs G, Bednarsch J, Berres M-L, et al. Survival after combined resection and ablation is not inferior to that after resection alone, in patients with four or more colorectal liver metastases. Langenbeck's Archives of Surgery. 2023;408(1):343.

30. Misiakos EP, Karidis NP, Kouraklis G. Current treatment for colorectal liver metastases. World J Gastroenterol. 2011;17(36):4067-75.

31. Su YM, Liu W, Yan XL, Wang LJ, Liu M, Wang HW, et al. Five-year survival post hepatectomy for colorectal liver metastases in a real-world Chinese cohort: Recurrence patterns and prediction for potential cure. Cancer Med. 2023;12(8):9559-69.

32. Villard C, Abdelrafee A, Habib M, Ndegwa N, Jorns C, Sparrelid E, et al. Prediction of survival in patients with colorectal liver metastases- development and validation of a prognostic score model. European Journal of Surgical Oncology. 2022;48(12):2432-9.

33. Buisman FE, Giardiello D, Kemeny NE, Steyerberg EW, Höppener DJ, Galjart B, et al. Predicting 10-year survival after resection of colorectal liver metastases; an international study including biomarkers and perioperative treatment. European Journal of Cancer. 2022;168:25-33.

34. Ahluwalia N, Nassereddin A, Arbor TC, Futterman B. Anatomy, Abdomen and Pelvis: Celiac Trunk. StatPearls 2025. Treasure Island (FL): StatPearls Publishing.35. Shaikh H, Wehrle CJ, Khorasani-Zadeh A. Anatomy, Abdomen and Pelvis: Superior Mesenteric Artery. StatPearls 2025. Treasure Island (FL): StatPearls Publishing.

36. Skinner D, Wehrle CJ, Van Fossen K. Anatomy, Abdomen and Pelvis: Inferior Mesenteric Artery. StatPearls 2025. Treasure Island (FL): StatPearls Publishing.

37. Harkins JM, Ahmad B. Anatomy, Abdomen and Pelvis, Portal Venous System (Hepatic Portal System). StatPearls 2025. Treasure Island (FL): StatPearls Publishing.

38. Shetty AS, Fraum TJ, Ludwig DR, Itani M, Rajput MZ, Strnad BS, et al. Imaging of the Inferior Mesenteric Vasculature. Radiographics. 2024;44(11):e240047.

39. Strasberg SM, Belghiti J, Clavien PA, Gadzijev E, Garden JO, Lau WY, et al. The Brisbane 2000 Terminology of Liver Anatomy and Resections. HPB. 2000;2(3):333-9.

40. Hundt M, Wu CY, Young M. Anatomy, Abdomen and Pelvis: Biliary Ducts. StatPearls 2025. Treasure Island (FL): StatPearls Publishing.

41. Roshandel G, Ghasemi-Kebria F, Malekzadeh R. Colorectal Cancer: Epidemiology, Risk Factors, and Prevention. Cancers (Basel). 2024;16(8).

42. Lu D, Dong C, Wang K, Ye C, Chen L, Yuan Y, Hu H. Updates of CSCO guidelines for colorectal cancer version 2025. Chin J Cancer Res. 2025;37(3):297-302.

43. Thompson MR, O'Leary DP, Flashman K, Asiimwe A, Ellis BG, Senapati A. Clinical assessment to determine the risk of bowel cancer using Symptoms, Age, Mass and Iron deficiency anaemia (SAMI). Br J Surg. 2017;104(10):1393-404.

44. Schult AL, Botteri E, Hoff G, Randel KR, Dalén E, Eskeland SL, et al. Detection of cancers and advanced adenomas in asymptomatic participants in colorectal cancer screening: a cross-sectional study. BMJ Open. 2021;11(7):e048183.

45. Skalitzky MK, Zhou PP, Goffredo P, Guyton K, Sherman SK, Gribovskaja-Rupp I, et al. Characteristics and symptomatology of colorectal cancer in the young. Surgery. 2023;173(5):1137-43.

46. Decker KM, Lambert P, Nugent Z, Biswanger N, Samadder J, Singh H. Time Trends in the Diagnosis of Colorectal Cancer With Obstruction, Perforation, and Emergency Admission After the Introduction of Population-Based Organized Screening. JAMA Netw Open. 2020;3(5):e205741.

47. Pajola M, Fugazzola P, Cobianchi L, Frassini S, Ghaly A, Bianchi C, Ansaloni L. Surgical Emergencies in Rectal Cancer: A Narrative Review. J Clin Med. 2024;14(1).

48. Hu W, Li C, Rahaman MM, Chen H, Liu W, Yao Y, et al. EBHI: A new Enteroscope Biopsy Histopathological H&E Image Dataset for image classification evaluation. Physica Medica. 2023;107:102534.

49. Chlorogiannis DD, Verras GI, Tzelepi V, Chlorogiannis A, Apostolos A, Kotis K, et al. Tissue classification and diagnosis of colorectal cancer histopathology images using deep learning algorithms. Is the time ripe for clinical practice implementation? Prz Gastroenterol. 2023;18(4):353-67.

50. Benedix F, Schmidt U, Mroczkowski P, Gastinger I, Lippert H, Kube R. Colon carcinoma--classification into right and left-sided cancer or according to colonic subsite?--Analysis of 29,568 patients. Eur J Surg Oncol. 2011;37(2):134-9.

51. Gimeno-García AZ, Quintero E. Role of colonoscopy in colorectal cancer screening: Available evidence. Best Practice & Research Clinical Gastroenterology. 2023;66:101838.

52. Yilmaz S, Gorgun E. Endoscopic Mucosal Resection and Endoscopic Submucosal Dissection. Clin Colon Rectal Surg. 2024;37(5):277-88.

53. Nahid M, Shrestha AK, Imtiaz MR, Basnyat PS. Endoscopic tattooing for colorectal lesions: impact on quality of care and patient outcomes. Ann R Coll Surg Engl. 2020;102(8):594-7.

54. Johnson CD, Chen MH, Toledano AY, Heiken JP, Dachman A, Kuo MD, et al. Accuracy of CT colonography for detection of large adenomas and cancers. N Engl J Med. 2008;359(12):1207-17.

55. Kijima S, Sasaki T, Nagata K, Utano K, Lefor AT, Sugimoto H. Preoperative evaluation of colorectal cancer using CT colonography, MRI, and PET/CT. World J Gastroenterol. 2014;20(45):16964-75.

56. dePrisco G. MRI Local Staging and Restaging in Rectal Cancer. Clin Colon Rectal Surg. 2015;28(3):194-200.

57. Ghuman SS, Kochhar R, Mahajan H, Buxi TBS, Gupta A, Arora A, et al. CT and MR Imaging in Colorectal Carcinoma: A Tool for Diagnosis, Staging, Response Evaluation, and Follow-Up. South Asian J Cancer. 2024;13(4):236-40.

58. Maino C, Vernuccio F, Cannella R, Cortese F, Franco PN, Gaetani C, et al. Liver metastases: The role of magnetic resonance imaging. World J Gastroenterol. 2023;29(36):5180-97.

59. van Kessel CS, Buckens CF, van den Bosch MA, van Leeuwen MS, van Hillegersberg R, Verkooijen HM. Preoperative imaging of colorectal liver metastases after neoadjuvant chemotherapy: a meta-analysis. Ann Surg Oncol. 2012;19(9):2805-13.

60. Read AJ, Waljee AK, Sussman JB, Singh H, Chen GY, Vijan S, Saini SD. Testing Practices, Interpretation, and Diagnostic Evaluation of Iron Deficiency Anemia by US Primary Care Physicians. JAMA Netw Open. 2021;4(10):e2127827.

61. Almilaji O, Parry SD, Docherty S, Snook J. Evidence for improved prognosis of colorectal cancer diagnosed following the detection of iron deficiency anaemia. Sci Rep. 2021;11(1):13055.

62. Lee TH, Kim JS, Baek SJ, Kwak JM, Kim J. Diagnostic Accuracy of Carcinoembryonic Antigen (CEA) in Detecting Colorectal Cancer Recurrence Depending on Its Preoperative Level. J Gastrointest Surg. 2023;27(8):1694-701.

63. Baqar AR, Wilkins S, Staples M, Angus Lee CH, Oliva K, McMurrick P. The role of preoperative CEA in the management of colorectal cancer: A cohort study from two cancer centres. Int J Surg. 2019;64:10-5.

64. Pu H, Xie P, Chen Y, Zhao Y, Ye X, Lu G, et al. Relationship Between Preoperative and Postoperative Serum Carcinoembryonic Antigen and Prognosis of Patients with Stage I-III Rectal Cancer: A Retrospective Study of a Multicentre Cohort of 1022 Rectal Cancer Patients. Cancer Manag Res. 2021;13:2643-51.

65. Nicholson BD, Shinkins B, Pathiraja I, Roberts NW, James TJ, Mallett S, et al. Blood CEA levels for detecting recurrent colorectal cancer. Cochrane Database Syst Rev. 2015;2015(12):Cd011134.

66. Nguyen HT, Duong HQ. The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. Oncol Lett. 2018;16(1):9-18.

67. Li Q, Geng S, Luo H, Wang W, Mo YQ, Luo Q, et al. Signaling pathways involved in colorectal cancer: pathogenesis and targeted therapy. Signal Transduct Target Ther. 2024;9(1):266.

68. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467-80.

69. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138(6):2073-87.e3.

70. Grover S, Syngal S. Risk assessment, genetic testing, and management of Lynch syndrome. J Natl Compr Canc Netw. 2010;8(1):98-105.

71. de la Chapelle A. Genetic predisposition to colorectal cancer. Nat Rev Cancer. 2004;4(10):769-80.

72. Rebuzzi F, Ulivi P, Tedaldi G. Genetic Predisposition to Colorectal Cancer: How Many and Which Genes to Test? Int J Mol Sci. 2023;24(3).

73. Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. Br J Surg. 2002;89(7):845-60.

74. Smit WL, Spaan CN, Johannes de Boer R, Ramesh P, Martins Garcia T, Meijer BJ, et al. Driver mutations of the adenoma-carcinoma sequence govern the intestinal epithelial global translational capacity. Proc Natl Acad Sci U S A. 2020;117(41):25560-70.

75. De Palma FDE, D'Argenio V, Pol J, Kroemer G, Maiuri MC, Salvatore F. The Molecular Hallmarks of the Serrated Pathway in Colorectal Cancer. Cancers (Basel). 2019;11(7).

76. Nakanishi Y, Diaz-Meco MT, Moscat J. Serrated Colorectal Cancer: The Road Less Travelled? Trends in Cancer. 2019;5(11):742-54.

77. Jo WS, Carethers JM. Chemotherapeutic implications in microsatellite unstable colorectal cancer. Cancer Biomark. 2006;2(1-2):51-60.

78. Li K, Luo H, Huang L, Luo H, Zhu X. Microsatellite instability: a review of what the oncologist should know. Cancer Cell International. 2020;20(1):16.

79. Tomasello G, Ghidini M, Galassi B, Grossi F, Luciani A, Petrelli F. Survival benefit with adjuvant chemotherapy in stage III microsatellite-high/deficient mismatch repair colon cancer: a systematic review and meta-analysis. Scientific Reports. 2022;12(1):1055.

80. Dienstmann R, Salazar R, Tabernero J. Overcoming Resistance to Anti-EGFR Therapy in Colorectal Cancer. Am Soc Clin Oncol Educ Book. 2015:e149-56.

81. Zhou J, Ji Q, Li Q. Resistance to anti-EGFR therapies in metastatic colorectal cancer: underlying mechanisms and reversal strategies. J Exp Clin Cancer Res. 2021;40(1):328.

82. Ros J, Baraibar I, Saoudi N, Rodriguez M, Salvà F, Tabernero J, Élez E. Immunotherapy for Colorectal Cancer with High Microsatellite Instability: The Ongoing Search for Biomarkers. Cancers (Basel). 2023;15(17).

83. Vatandoust S, Price TJ, Karapetis CS. Colorectal cancer: Metastases to a single organ. World J Gastroenterol. 2015;21(41):11767-76.

84. Wang Y, Zhong X, He X, Hu Z, Huang H, Chen J, et al. Liver metastasis from colorectal cancer: pathogenetic development, immune landscape of the tumour microenvironment and therapeutic approaches. Journal of Experimental & Clinical Cancer Research. 2023;42(1):177.

85. Hugen N, van de Velde CJH, de Wilt JHW, Nagtegaal ID. Metastatic pattern in colorectal cancer is strongly influenced by histological subtype. Ann Oncol. 2014;25(3):651-7.

86. Robinson JR, Newcomb PA, Hardikar S, Cohen SA, Phipps AI. Stage IV colorectal cancer primary site and patterns of distant metastasis. Cancer Epidemiol. 2017;48:92-5.

87. Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology. 2020;31(10):1291-305.

88. Chakrabarti S, Peterson CY, Sriram D, Mahipal A. Early-stage colon cancer: Current treatment standards, evolving paradigms, and future directions. World J Gastrointest Oncol. 2020;12(8):808-32.

89. Labianca R, Nordlinger B, Beretta GD, Mosconi S, Mandalà M, Cervantes A, Arnold D. Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2013;24 Suppl 6:vi64-72.

90. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Ann Oncol. 2012;23(10):2479-516.

91. Guo L, Wang L, Cai L, Zhang Y, Feng X, Zhu C, et al. Global Distribution of Colorectal Cancer Staging at Diagnosis: An Evidence Synthesis. Clin Gastroenterol Hepatol. 2024.

92. Xue L, Williamson A, Gaines S, Andolfi C, Paul-Olson T, Neerukonda A, et al. An Update on Colorectal Cancer. Current Problems in Surgery. 2018;55(3):76-116.

93. Quénéhervé L, Pioche M, Jacques J. Curative criteria for endoscopic treatment of colorectal cancer. Best Practice & Research Clinical Gastroenterology. 2024;68:101883.

94. Carchman EH, Kalady MF. Colon Cancer Surgical Treatment: Principles of Colectomy. In: Steele SR, Hull TL, Hyman N, Maykel JA, Read TE, Whitlow CB, editors. The ASCRS Textbook of Colon and Rectal Surgery. Cham: Springer International Publishing; 2022. p. 451-62.

95. Weiser MR, Garcia-Aguilar J. Chapter 166 - Basic Principles of the Operative Treatment of Colorectal Cancer. In: Yeo CJ, editor. Shackelford's Surgery of the Alimentary Tract, 2 Volume Set (Eighth Edition). Philadelphia: Elsevier; 2019. p. 1981-91.

96. Kim NK, Kim YW, Han YD, Cho MS, Hur H, Min BS, Lee KY. Complete mesocolic excision and central vascular ligation for colon cancer: Principle, anatomy, surgical technique, and outcomes. Surgical Oncology. 2016;25(3):252-62.

97. Ueno H, Hase K, Shiomi A, Shiozawa M, Ito M, Sato T, et al. Optimal bowel resection margin in colon cancer surgery: prospective multicentre cohort study with lymph node and feeding artery mapping. The Lancet Regional Health – Western Pacific. 2023;33.

98. Croese AD, Lonie JM, Trollope AF, Vangaveti VN, Ho Y-H. A meta-analysis of the prevalence of Low Anterior Resection Syndrome and systematic review of risk factors. International Journal of Surgery. 2018;56:234-41.

99. Konishi T, You YN. Complete Mesocolic Excision and Extent of Lymphadenectomy for the Treatment of Colon Cancer. Surgical Oncology Clinics. 2022;31(2):293-306.

100. George J, Rockall T. Surgery for colorectal cancer. Surgery - Oxford International Edition. 2023;41(1):35-40.

101. Wong SL. Lymph node counts and survival rates after resection for colon and rectal cancer. Gastrointest Cancer Res. 2009;3(2 Suppl):S33-5.

102. Kitano Y, Hayashi H, Matsumoto T, Kinoshita S, Sato H, Shiraishi Y, et al. Borderline resectable for colorectal liver metastases: Present status and future perspective. World J Gastrointest Surg. 2021;13(8):756-63.

103. Guo M, Jin N, Pawlik T, Cloyd JM. Neoadjuvant chemotherapy for colorectal liver metastases: A contemporary review of the literature. World J Gastrointest Oncol. 2021;13(9):1043-61.

104. Gögenur I, Justesen TF, Tarpgaard LS, Bulut M, Hansen TF, Jensen LH, et al. Neoadjuvant Pembrolizumab in Stage I-III Deficient Mismatch Repair Colon Cancer: A Clinical Trial. Ann Surg. 2024.

105. Emiloju OE, Sinicrope FA. Neoadjuvant Immune Checkpoint Inhibitor Therapy for Localized Deficient Mismatch Repair Colorectal Cancer: A Review. JAMA Oncol. 2023;9(12):1708-15.

106. Cui HX, Yang XQ, Zhao GY, Wang FJ, Liu X. The neoadjuvant immunotherapy for non-metastatic mismatch repair-deficient colorectal cancer: a systematic review. Front Immunol. 2025;16:1540751.

107. Tatsuta K, Sakata M, Kojima T, Booka E, Kurachi K, Takeuchi H. Updated insights into the impact of adjuvant chemotherapy on recurrence and survival after curative resection of liver or lung metastases in colorectal cancer: a rapid review and meta-analysis. World Journal of Surgical Oncology. 2025;23(1):56.

108. Morton D, Seymour M, Magill L, Handley K, Glasbey J, Glimelius B, et al. Preoperative Chemotherapy for Operable Colon Cancer: Mature Results of an International Randomized Controlled Trial. J Clin Oncol. 2023;41(8):1541-52.

109. Liang Z, Li Z, Yang Q, Feng J, Xiang D, Lyu H, et al. The role of neoadjuvant chemotherapy in patients with locally advanced colon cancer: A systematic review and meta-analysis. Front Oncol. 2022;12:1024345.

110. Horvat N, El Homsi M, Miranda J, Mazaheri Y, Gollub MJ, Paroder V. Rectal MRI Interpretation After Neoadjuvant Therapy. J Magn Reson Imaging. 2023;57(2):353-69.

111. Ozer M, Vegivinti CTR, Syed M, Ferrell ME, Gonzalez Gomez C, Cheng S, et al. Neoadjuvant Immunotherapy for Patients with dMMR/MSI-High Gastrointestinal Cancers: A Changing Paradigm. Cancers (Basel). 2023;15(15).

112. Morris VK, Kennedy EB, Baxter NN, Benson AB, 3rd, Cercek A, Cho M, et al. Treatment of Metastatic Colorectal Cancer: ASCO Guideline. J Clin Oncol. 2023;41(3):678-700.

113. Cervantes A, Adam R, Roselló S, Arnold D, Normanno N, Taïeb J, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. Annals of Oncology. 2023;34(1):10-32.

114. Passot G, Odisio BC, Zorzi D, Mahvash A, Gupta S, Wallace MJ, et al. Eradication of Missing Liver Metastases After Fiducial Placement. J Gastrointest Surg. 2016;20(6):1173-8.

115. Egeland C, Rostved AA, Schultz NA, Pommergaard HC, Daugaard TR, Thøfner LB, et al. Morbidity and mortality after liver surgery for colorectal liver metastases: a cohort study in a high-volume fast-track programme. BMC Surg. 2021;21(1):312.

116. Arntz P, Olthof PB. Assessment of liver function before major hepatectomy. Br J Surg. 2023;110(12):1588-9.

117. Bilhim T, Böning G, Guiu B, Luz JH, Denys A. CIRSE Standards of Practice on Portal Vein Embolization and Double Vein Embolization/Liver Venous Deprivation. Cardiovasc Intervent Radiol. 2024;47(8):1025-36.

118. Giuliante F, Viganò L, De Rose AM, Mirza DF, Lapointe R, Kaiser G, et al. Liver-First Approach for Synchronous Colorectal Metastases: Analysis of 7360 Patients from the LiverMetSurvey Registry. Ann Surg Oncol. 2021;28(13):8198-208.

119. Lillemoe HA, Vauthey J-N. Surgical approach to synchronous colorectal liver metastases: staged, combined, or reverse strategy. Hepatobiliary Surgery and Nutrition. 2019;9(1):25-34.

120. Kron P, Linecker M, Jones RP, Toogood GJ, Clavien PA, Lodge JPA. Ablation or Resection for Colorectal Liver Metastases? A Systematic Review of the Literature. Front Oncol. 2019;9:1052.

121. Kokkinakis S, Ziogas IA, Llaque Salazar JD, Moris DP, Tsoulfas G. Clinical Prediction Models for Prognosis of Colorectal Liver Metastases: A Comprehensive Review of Regression-Based and Machine Learning Models. Cancers (Basel). 2024;16(9).

122. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst Rev. 2021;10(1):89.

123. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). European Journal of Cancer. 2005;41(12):1690-6.

124. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.

125. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.

126. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 2007;4(10):e296.

127. Amin MB ES, Greene FL, Byrd DR, Brookland RK, Washington MK, et al. AJCC Cancer Staging Manual. 8th ed.: Springer; 2017.

128. Vermeulen PB, Colpaert C, Salgado R, Royers R, Hellemans H, Van Den Heuvel E, et al. Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. J Pathol. 2001;195(3):336-42.

129. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47.

130. Kim SW, Roh J, Park CS. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. J Pathol Transl Med. 2016;50(6):411-8.

131. Valtorta E, Martino C, Sartore-Bianchi A, Penaullt-Llorca F, Viale G, Risio M, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. Modern Pathology. 2015;28(11):1481-91.

132. Zlobec I, Steele R, Michel RP, Compton CC, Lugli A, Jass JR. Scoring of p53, VEGF, Bcl-2 and APAF-1 immunohistochemistry and interobserver reliability in colorectal cancer. Mod Pathol. 2006;19(9):1236-42.

133. Zlobec I, Steele R, Terracciano L, Jass JR, Lugli A. Selecting immunohistochemical cut-off scores for novel biomarkers of progression and survival in colorectal cancer. J Clin Pathol. 2007;60(10):1112-6.

134. Torén W, Ansari D, Andersson R. Immunohistochemical investigation of prognostic biomarkers in resected colorectal liver metastases: a systematic review and meta-analysis. Cancer Cell International. 2018;18(1):217.

135. Caruso M, Stanzione A, Prinster A, Pizzuti LM, Brunetti A, Maurea S, Mainenti PP. Role of advanced imaging techniques in the evaluation of oncological therapies in patients with colorectal liver metastases. World J Gastroenterol. 2023;29(3):521-35.

136. Wang YXJ, Huang H, Zheng CJ, Xiao BH, Chevallier O, Wang W. Diffusion-weighted MRI of the liver: challenges and some solutions for the quantification of apparent diffusion coefficient and intravoxel incoherent motion. Am J Nucl Med Mol Imaging. 2021;11(2):107-42.

137. O'Connor JPB, Aboagye EO, Adams JE, Aerts HJWL, Barrington SF, Beer AJ, et al. Imaging biomarker roadmap for cancer studies. Nature Reviews Clinical Oncology. 2017;14(3):169-86.

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