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Entwicklung eines klinischen präoperativen Scores für die Behandlung von Patienten mit hepatischen Metastasen bei kolorektalem Karzinom im oligometastatischen Stadium

"Development of a clinical preoperative score for the treatment of patients with liver metastases from colorectal cancer in oligometastatic stage"

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I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources. As well I declare that I have not submitted a dissertation without success and not passed the oral exam. The present dissertation (neither the entire dissertation nor parts) has not been presented to another examination board.

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Preamble

The results of this work were published the 20th of April 2022 by the ESMO Open Journal of the European Society for Medical Oncology:

"Predictive preoperative clinical score for patients with liver-only oligometastatic colorectal cacer".

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On the 14th of July 2023, this publication was awarded with the **CCC WERA Allianz Publication Award 2023**.

On the 13th of October 2023, at the annual meeting of the German, Austrian, and Swiss societies for hematology and oncology (DGHO). S. Neumaier et al. presented in an oral abstract a validation study of the preoperative predictive score: *"Augsburg Score: ein neuer präoperativer klinischer Risikoscore beim oligometastatischen kolorektalen Karzinom – eine monozentrische, retrospektive Studie".*

1. Introduction

1.1. Colorectal carcinoma

1.1.1. Definition and classification

Colorectal cancer (CRC) arises from epithelial cells of the colorectal mucosa progressing to invade the lumen and bowel wall, potentially involving neighbour organs. Over 90% of CRC are adenocarcinomas. Less frequent histological subtypes include neuroendocrine, squamous cell, adenosquamous, spindle cell and undifferentiated carcinomas. (1, 2)

The development of colorectal cancer is a gradual process, typically initiated by a precancerous precursor lesion (polyp). This process involves a progressive accumulation of a series of genetic mutations and chromosomal instability over an estimated period of 10-15 years. These mutations disrupt the function of genes that normally control cell growth (tumor suppressor genes) and promote uncontrolled growth (oncogenes), ultimately leading to uncontrolled proliferation and tumor formation. (3, 4) Increasing evidence supports that Cancer Stem Cells (CSC) are the cells of origin and driving force behind tumour progression and metastasis. These CSCs are believed to originate from the base of the colonic crypts and play a crucial role in the initiation and maintenance of tumours. (5, 6)

CRC-carcinogenesis occur through two well-defined precursor lesion pathways: the traditional adenoma-carcinoma pathway, which constitutes 70-90% of cases, and the serrated neoplasia pathway, accounting for 10-20%. The traditional pathway, also known as the chromosomal instability sequence (CIN), is typified by extensive chromosomal aberrations. These aberrations include gains and losses of chromosomal material, as well as translocations. The development of CIN typically begins with a mutation in the *APC* gene, followed by the activation of the *RAS* oncogene or the loss of function in the tumor suppressor genes *SMAD4* and *TP53*. (3) In contrast to the CIN pathway, the serrated neoplasia pathway is characterized by mutations in *RAS* and *RAF* genes, and a phenomenon known as epigenetic instability. This epigenetic instability manifests as the CpG island methylator phenotype (CIMP), where specific tumor suppressor genes become silenced through methylation. (7) In less than 5% of cases, CRC occurs in the context of Lynch Syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC). Lynch syndrome represents the most prevalent hereditary cause of CRC. This autosomal dominant syndrome arises from germline mutations in DNA mismatch repair (MMR) genes, most commonly *MLH1*, *MSH2*, *MSH6*, *or PMS2*. Germline mutations in MMR genes impair the cell's ability to correct errors during DNA replication, leading to the accumulation of insertions or deletions in repetitive DNA sequences (microsatellites). Such microsatellite instability (MSI) is a hallmark feature of Lynch syndrome-associated tumors. Lynch syndrome diagnosis relies on the revised Amsterdam Criteria. Individuals with this

syndrome are predisposed not only to colorectal cancer but also to other malignancies, including endometrial, ovarian, gastric, and urinary tract cancers. (8, 9)

Colon cancer may manifest as single tumour, or with multiple (synchronous) lesions. The macroscopic appearance depends on the growth pattern and the developmental phase at the time of diagnose. Carcinomas may exhibit predominantly intraluminal growth (exophytic), or diffusely infiltrative/ (linitis plastica), with some showing subtle endophytic features involving the colorectal wall circumferentially, resulting in lumen constriction. Typically, exophytic carcinomas are located proximally, while those arising from the transverse and descending colon are generally endophytic and annular. Tumors can also penetrate through the muscularis propria, infiltrating adjacent structures. Local tumor spread is influenced by the anatomic site. For instance, advanced rectal carcinomas are more likely to extend into pelvic organs like the urinary bladder and vagina, whereas more proximal tumors are associated with infiltration of the peritoneal cavity, leading to peritoneal carcinomatosis. Additionally, distant metastasis can occur through lymphatic or vascular infiltration. (4)

1.1.2. Clinical manifestation

Symptoms are commonly non-specific for colon cancer and most frequently associated with relatively large tumours and advanced diseases. The wide range of signs and symptoms, which also depend on the location and stage of the primary tumour, include changes in bowel habit, abdominal pain, weight loss, weakness, iron deficiency and anaemia, rectal bleeding. Only 40% of patients present with localized disease. (4, 8, 10)

1.1.3. Epidemiology and risk factors

CRC ranks as the third most common cancer in men and the second most common in women worldwide. It represents about 10% of all cancer types globally. Men have a 25% higher risk of incidence compared to women, although this varies by geographical region. Developed countries tend to have the highest incidence rates. Projections suggest that the incidence of CRC will continue to rise, with an estimated 2.5 million new cases anticipated by 2035. This increase is attributed to lifestyle modifications and the effects of 'Westernization', including factors such as obesity, physical inactivity, alcohol intake, high-calorie diets which includes animal fat, and excessive meat consumption, and cigarette smoking. Ranking as the fourth most common cause of cancer death globally, CRC claims more than 600,000 lives worldwide annually. Despite its prevalence, mortality rates from CRC have been declining in some regions. In Europe, for example, the death rate from CRC has decreased over time, with current estimates suggesting 15-20 deaths per 100,000 individuals. However, CRC remains a

serious disease, with overall 5-year survival rates ranging from 30% to 60% for both men and women. (3, 4, 8, 10)

Risk factors for colon cancer development can be categorized as lifestyle-related and genetical. Age is the primary non-modifiable risk factor for sporadic (non-hereditary) CRC. Around 70% of patients diagnosed are over 65 years old, with a median age of onset at 67. While less than 15% of cases occur in individuals younger than 50, a concerning trend of increasing incidence in this population has been observed, with an estimated annual rise of approximately 2% since 1990. Male sex is also strongly associated with higher risk of CRC. Family history plays a role, with up to 20% of patients having a positive family history. The risk increases with the number and closeness of affected relatives. Hereditary syndromes such as the familial adenomatous polyposis coli (FAP, 1%), Lynch-associated syndromes (HNPCC, 2%–4%), Turcot, Peutz–Jeghers and *MUTYH*-associated polyposis syndrome contribute to roughly 5% of all CRC cases. Another constitutional risk factor for CRC constitutes long standing inflammatory bowel disease (3, 4, 8, 11-13).
There are several modifiable lifestyle factors that contribute to an increased risk of CRC, which include: smoking, excessive alcohol consumption, obesity, and red/ processed meat intake. (14-17) These factors are believed to promote colon cancer development through various mechanisms, such as the production of harmful substances like heterocyclic amines, increased levels of bile acids in the stool, generation of reactive oxygen species that damage cells, and possibly hyperinsulinemia. The most compelling evidence of diet-related risk factors was provided by studies that found a decreased risk of CRC associated with high consumption of folate acide by vegetable and fiber. (4) Data have also suggested a link between gut microbiota dysbiosis and colon cancer carcinogenesis. In this context, some bacterial species are highligthed, such as Fusobacterium nucleatum, Bacteroides fragilis, Poststreptococcus species, among others. (18-20)

A medical and/ or family history of adenoma, colon cancer, chronic inflammatory bowel diseases (such as Crohn’s disease and ulcerative colitis), and a well-defined hereditary cancer syndrome, are strong predisponing factors for CRC development. Therefore, these patients require more intensive screening to detect precancerous lesions or early-stage CRC. Identification of the mentioned factors is crucial, as it allows for implementation of a surveillance strategy to prevent CRC, optimal treatment in case of incidental diagnosis and proper advice for relatives at risk. It's important to note that up to 90% of colorectal cancer deaths could be prevented with effective strategies. This includes early diagnosis through national screening programs, prevention of cancer development by removing suspected lesions through polypectomy. In addition to screening and polypectomy, lifestyle modifications

such as a healthy diet and regular physical activity can also significantly impact CRC risk. (3, 4)

1.1.4. Diagnosis, risk assessment and stadification

To ensure an accurate diagnosis of the primary tumour and determine the stage of CRC, a complete work-up is essential. A total colonoscopy is the gold standard for diagnostic confirmation of colon cancer. Occasionally, an urgent tomour resection can be indicated in the presence of bowel obstruction or life-threatening gastrointestinal bleeding. In these cases, colonoscopy of the remaining bowel segments should be performed to rule out synchronous tumours. This work-up includes also a thorough assessment of the patient´s baseline characteristics. Clinical examination with comprehensive physical examination and laboratory tests, including complete blood count, coagulation, liver, and kidney functions tests must be carried out before establishing a definitive treatment approach. These data are essential for the initial assessment of patient´s baseline performance, clinical conditions and comorbidities as well as potential cancer-related complications. (10)

Pre-operative meassurement of carcinoembryonic antigen (CEA) serum levels is considered helpful for diagnosis but most importantly, it will help in the early detection of recurrent/ metastatic disease during the post-treatment follow-up period. (21) CEA level determination has also prognostic relevance, since a detectable postoperative serum CEA is associated with worse outcome. (22)

The extension of the disease will determine whether a primary tumour resection should be performed, or if systemic therapy should be started (in the case of primary unresectable metastases). Synchronous metastases are found in up to 20% of a newly diagnosed CRC. The most frequent organs involved are the liver, followed by peritoneum, lung and lymph nodes. The method of choice for disease stage assessment, and for evaluation of the presence of distant metastases is the computer tomography (CT) of the thoracic, abdominal, and pelvic cavities with intravenous contrast administration. For evaluation of surrounding structures in locally advanced tumours, or to better define unclear liver lesions previously detected by CT scan, the contrast-enhanced magnetic resonance imaging (MRI) is the preferred test. [^{18}F]2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET), with or without integrated CT (PET-CT), offers no significal advantage over CT scans alone for therapeutic purposes. Therefore, its routine use for staging localized CRC is not recommended. (10)

For CRC, histological analysis of tumor tissue remains the gold standard for both disease staging and treatment planning. (23) The pathologically determined tumor stage serves as a

crucial prognostic indicator, predicting tumor behavior and patient outcomes. (4) Following surgical resection, a detailed pathological report is generated. This report analyzes the extent of tumor invasion through the bowel wall, involvement of lymph nodes, and potential spread to nearby tissues. Additional pathological reports include the assessment of biopsies from suspicious liver or peritoneal nodes identified intraoperatively. A report should standardly include (10):

- morphological description of the specimen,
- definition of tumour site and size,
- presence or absence of macroscopic tumour perforation,
- angiogenesis,
- histological type and grade,
- extension of tumour into the bowel wall and adjacent organs,
- distance of cancer from resected margins,
- presence or absence of tumour deposits,
- lymphovascular and/or perineural invasion,
- presence of tumour budding,
- site and number of removed regional lymph nodes and their possible infiltration by cancer cells,
- involvement of other organs if submitted (either removed or biopsied),
- mismatch repair (MMR)/microsatellite instability (MSI) status of the tumour.

The first effective staging system for CRC was introduced by Cuthbert E. Dukes, published in 1932 and stood the test of time because of its simplicity and its role in influencing therapeutic strategies (24). This work was at that time a breakthrough in the understanding of locally advanced CRC. However, currently the pathological stage is reported following guidelines of the Union for International Cancer Control (UICC) tumour, node, metastasis (TNM) classification, 8th edition (25) (**Table 1).**

Table 1. UICC TNM classification 8th edition

UICC Stage	T	N	M
0	Tis	N0	M0
I	T1, T2	N0	M0
IIA	T3	N0	M0
IIB	T4a	N0	M0
IIC	T4b	N0	M0

IIIA	T1-2	N1 (1-3 LN)	M0
	T1	N2a (4-6 LN)	M0
IIIB	T3-4	N1 (1-3 LN)	M0
	T2-3	N2a (4-6 LN)	M0
	T1-2	N2b (> 6 LN)	M0
IIIC	T4a	N2a (4-6 LN)	M0
	T3-T4a	N2b (> 6 LN)	M0
	T4b	N1-2	M0
IVA	Any T	Any N	M1a (1 organ, excluding peritoneum)
IVB	Any T	Any N	M1b (>1 organ, excluding peritoneum)
IVC	Any T	Any N	M1c (Peritoneum with or without other organ involvement)

Adapted from DGHO (Onkopedia Letilinie Kolonkarzinom)(26)

Besides the classic histological assessment of the tumor including grading and the TNM staging, the value of immune- and tumour-based markers has increasingly gained recognition. Mismatch repair testing not only identifies Lynch syndrome but also offers predictive information for adjuvant fluoropyrimidine-based therapy. Additionally, it holds the potential to identify patients with metastatic CRC who could benefit from immunotherapy. The presence of *RAS* and *RAF* mutations also plays an established role as prognostic and predictive markers. (3) Other biomarkers, such as the Immunoscore and postoperative circulating tumor DNA (ctDNA), have shown promise in assessing recurrence risk and can be used to further personalize adjuvant treatment decisions in challenging cases. (10) For example, the Immunoscore has recently been validated in a large prospective cohort of over 2500 patients as a robust predictor of time to recurrence, overall survival (OS), and disease-free survival (DFS), independent of patient age, sex, microsatellite instability (MSI), and other known prognostic factors. (27)

Monitoring ctDNA is emerging as a promising tool to identify patients at high risk of recurrence following primary tumor resection, as observed in studies including stage III patients by Tie et al. (28) The same author showed that postoperative ctDNA guided decision of an adjuvant oxaliplatin therapy was non-inferior to standard management of stage II CRC with the result of reduced chemotherapy exposition with ctDNA-guided management. (29)

Furthermore, based on gene expression, CRC has been classified into four molecular subtypes known as consensus molecular subtypes (CMS) 1–4. Each subtype (CMS1: MSI immune, CMS2: canonical, CMS3: metabolic, CMS4: mesenchymal) is characterized by unique genes or pathways implicated in its development. This classification serves as a basis

for more precise clinical stratification and the development of targeted interventions based on specific subtypes. (30) For instance, right-sided colorectal cancers are more commonly associated with specific molecular subtypes, such as MSI-immune and metabolic, supporting clinical observations that tumor sidedness may be relevant to disease behavious. (31) CRC tumors originating from the right and left sides of the colon have distinct developmental origins. This difference translates into variations in their clinical and molecular characteristics, including incidence rates and the composition of the gut microbiome. Compared to left-sided tumors, right-sided colorectal cancers tend to be mucinous, associated with an inflammatory response, exhibit mismatch repair deficiency (MMR), leading to microsatellite instability-high (MSI-H) status, and more likely harbor *RAS* and *BRAF* mutations. Left-sided CRCs, on the other hand, more frequently displays chromosomal alterations, higher expression of EGFR, *HER2/neu* amplification, and aberrant *EGFR* signalling pathways. (31-33) Several studies that have explored the influence of tumor location on CRC prognosis have consistently reported that the primary tumor location is a significant risk factor for survival. Patients with right-sided colon cancers tend to have a poorer prognosis compared to those with left-sided tumors. (34-38)

1.1.5 Biology of CRC metastases

Metastasis isn't a random occurrence. Within the same primary tumor, there are distinct tumor cell subpopulations with varying abilities to metastasize. Metastases occur when tumor cells capable of participating in all stages of a complex metastasis cascade selectively disseminate. The cells from vascularized primary tumors must undergo several steps to metastasize:

1. Invasion: Tumor cells invade lymphatic and vascular structures surrounding the primary tumor.
2. Intravasation: They enter the bloodstream by crossing into blood vessels.
3. Survival in circulation: Tumor cells must survive interactions with blood components and immune cells while circulating in the bloodstream.
4. Extravasation: Upon reaching distant organ sites, they exit the bloodstream and infiltrate the surrounding tissue.

These processes are essential for tumor cells to establish metastases at distant sites in the body. (39)

Angiogenesis

Angiogenesis is the process through which new blood vessels develop around a solid tumor. It plays a critical role in facilitating the growth of most primary tumors and their subsequent spread. In adults, while physiological angiogenesis continues at a reduced rate, it primarily supports vascular maintenance, wound healing, and menstrual cycling. Tumors can initially obtain nutrients and oxygen through simple diffusion when they are small, up to a size of about 1–2 mm. However, further growth beyond this size requires the development of a vascular supply through angiogenesis. Even a small tumor mass consisting of 100–300 transformed cells can initiate angiogenesis to support its growth by attracting new blood vessels.(40)

Neovascularization, the formation of new blood vessels, is a critical process for both physiological tissue growth and tumor development. In the context of colorectal cancer (CRC), this process is driven by the recruitment of circulating endothelial precursor cells from the bone marrow. A complex interplay of growth factors within the tumor microenvironment orchestrates this neovascularization. Vascular endothelial growth factor (VEGF) plays a central role, specifically targeting endothelial cells for activation. Other factors, such as basic fibroblast growth factor (bFGF) and matrix metalloproteinases (MMPs), exert broader effects on the surrounding stroma. Notably, the tumor itself, adjacent tissues, and infiltrating immune cells like macrophages and fibroblasts can all contribute to the production of these pro-angiogenic factors. (40)

The vascular endothelial growth factor pathway is recognized as the master regulator of angiogenesis in various malignancies. Because of its central importance, VEGF presents an appealing target for therapeutic intervention strategies. (41) Numerous studies have highlighted the critical role of VEGF in the development of hepatic metastases originating from CRC. Interestingly VEGF expression is upregulated in response to hypoxic conditions generated by the growing primary tumor. Subsequently, VEGF levels decrease when the liver parenchyma becomes well-vascularized, indicating that VEGF may play a role in initiating hepatic metastasis but may not be essential for maintaining it. (40)

VEGF-A, the initial VEGF identified, has been a model for the advancement of antiangiogenesis as a treatment approach. In 1989, Napoleone Ferrara and colleagues made a significant breakthrough by isolating and cloning VEGF-A, paving the way for research into antiangiogenesis therapies. Their subsequent work in 1993 demonstrated that blocking VEGF-induced angiogenesis with specific antibodies dramatically suppressed tumor growth in animal models. Further development led in 1997 to the design of the first humanized anti-VEGF monoclonal antibody approved for clinical use (Bevacizumab). (42)

Proteinases and tumor cell invasion

Metastatic tumor cells must acquire invasive ability as a cardinal feature to build metasases. Tumor cells must possess the ability to disrupt the basement membrane matrix in order to invade and penetrate lymphatic and blood vessels, allowing them to spread beyond the primary tumor site.

Alterations in the structure of the basal membrane have been described to be involved in human and experimental colorectal carcinomas. (39) In this context, Proteolytic enzymes particularly serine, cysteine, aspartic, and metalloproteinases (MMPs), play a crucial role in this process. These enzymes degrade the extracellular matrix, facilitating the detachment of tumor cells from their primary site and enabling invasion into surrounding tissues and blood vessels. Among MMPs, MMP-7 and Urokinase plasminogen activator (uPA) have been particularly linked to liver metastasis in colorectal cancer. (40)

MMPs constitute a family of 25 zinc-dependent secretory proteolytic enzymes that are believed to exert a crucial role in colorectal cancer progression by influencing tumor differentiation, remodeling, invasion, and metastasis. Their expression and secretion are regulated by various factors, including interleukins (IL-1, IL-4, IL-6), growth factors, and TNF-a. uPA, a serine protease, plays a critical role alongside MMPs. uPA bind and activate Urokinase plasminogen activator receptor (uPAR), which in turn converts plasminogen to plasmin, an enzyme that degrades the extracellular matrix (ECM) and activates pro-MMPs present in the extracellular space. (40) Elevated expression of uPAR in CRC has been correlated with poorer survival outcomes. Ahmed et al. performed studies on mouse models and cell lines and could demonstrate that reducing uPAR expression by mRNA modification can inhibit liver metastasis in mouse models and decrease cancer cell invasion and motility. Additionally, this reduction can lead to lower levels of uPA and inactive MMPs. (43)

Adhesion molecules

Once circulating tumor cells reach other organs, they need to adhere to the endothelium before they can extravasate and invade into the parenchyma. This process goes beyond physical lodging in the vascular bed and requires specific adhesion to structures on the endothelial cell surface. (39) Adhesion molecules play a crucial role in interacting with external stimuli for metastasis development. These induces several intracellular pathways associated with motility and survival in a local environment. Some of the adhesion molecules include integrins, cadherins, selectins, immunoglobulins, and hyaluronate binding proteins.

Integrins are a family of cell surface receptors that play a critical role in cell adhesion and migration. In the context of CRC liver metastasis, integrins mediate the attachment of cancer

cells to the endothelium of blood vessels in the liver. Integrins are capable of binding to various extracellular matrix (ECM) molecules such as laminin, collagen, fibrinogen, and vitronectin. (40) Kitayama et al. showed that laminin facilitates the tethering of cancer cells in a human colorectal cancer cell model. This process was facilitated by the preferential binding of certain integrin subtypes to laminin, rather than to fibronectin or vitronectin. Moreover, tethering of cancer cells was partially inhibited by mAbs targeting integrin subunits. Interestingly, laminin is not typically expressed on the luminal surface of blood vessels, but rather on the underlying basement membrane, which makes it challenging for cancer cells to bind to. Staining experiments have revealed that laminin is also strongly expressed by epithelial cells in the portal and hepatic veins of the liver. (44) This localized expression of laminin may create a favorable microenvironment for CRC cells, allowing the binding to portal and hepatic vessels and potentially facilitate the development of hepatic metastasis.

Osteopontin (OPN) is a phosphoglycoprotein that can bind integrin and induce integrin-mediated cell survival, motility, and anti-apoptotic intracellular pathways. A pooled gene expression profiling approach using human colorectal cancer cells from a series of clinical stages identified OPN as a predictive marker for colorectal cancer progression. The tumor/ normal colonic tissue osteopontin expression ratios ranged from 15-fold for cancers and 27-fold for liver metastasis, additionally linking OPN expression with advanced tumor stage and making it a potential marker for assessing risk of future metastasis. (45, 46)

Carcinoembryonic antigen (CEA), a membrane glycoprotein that is normally present on fetal gastrointestinal and liver cells, exhibits aberrant expression in various malignancies. Notably, colorectal cancer (CRC) demonstrates particularly high CEA levels. Previous studies have acknowledged the role for CEA in facilitating liver metastasis, the most common site of distant spread in CRC patients. One important underlying mechanism includes an enhancement in cell adhesion properties, for example by promoting the adhesion of disseminated CRC cells to the liver sinusoidal endothelium. Interestingly, in vitro studies have demonstrated that colon cancer cells with inherently low metastatic potential can exhibit enhanced liver metastasis after DNA-transfection coding for CEA. (47-49)

All this data confirms that CEA is involved in hepatic metastasis, whereby this association positions CEA as a valuable clinical tool. Not only does CEA serve as a biomarker for disease progression and prognosis, but research by Gangopadhyay et al. indicate that CEA actively contributes to a permissive microenvironment within the liver, facilitating the survival and proliferation of metastatic colon cancer cells. CEA was found to induce the release of several cytokines, including IL-1b, IL-6, and TNF-a after binding to its receptor on hepatic Kupffer cells. (40, 50)

Cell survival and microenvironment

The capacity of cancer cells to survive in a new local environment is another critical factor in the development of metastasis. This concept forms the foundation of the "seed and soil" hypothesis, first proposed by Paget in 1889. Metastatic "seeds" tend to grow preferentially in an organ environment that provides a suitable "soil" in some manner. Colon cancer cells, like many other tumors, demonstrate organotropic properties, with the liver and lungs being the most frequent sites of distant metastases. This preference goes beyond factors like physical proximity or blood flow patterns, indicating a more complex biological interaction between cancer cells and the target organ. (39) Normal cells that are away from the primary site, lack adhesion capabilities, or are not able to evade the immune system, resulting in cell death.
Numerous molecular factors have been identified that confer colorectal cancer cells with the ability to survive and thrive in the hepatic environment [more extensively reviewed by Rudmik et al. (40)]. Recent research has highlighted the intriguing role of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in this process. TRAIL, also referred to as Apo-2 ligand, a member of the TNF (tumor necrosis factor) family, selectively triggers apoptosis in tumor cells while sparing healthy tissues. It is speculated that TRAIL may act as an internal safety control, particularly in tumors with *APC* deletions, making tumor cells more susceptible to NK destruction. (40) In human colorectal cancer specimens, high expression of TRAIL in the primary tumor correlated with a substantially longer disease-free survival compared to tumors with low TRAIL expression. (51) Takeda et al. demonstrated that hepatic metastasis formation is enhanced in a colon cancer murine model after administration of anti-TRAIL antibodies. Also, TRAIL -/- mice showed an increased susceptibility to develop liver metastasis. (52, 53)
Interferon-beta (IFN-b) is a cytokine that has been associated with anti-metastatic effects on human CRC cells. (40) IFN-beta secreted from adenocarcinoma cells was shown to stimulate inducible nitric oxide synthase (iNOS) leading to production of nitric oxide (NO), which in turn leads to suppression of tumor growth and metastasis; iNOS, was shown to be markedly downregulated in IFN-beta resistant, highly metastatic colon cancer cell lines. Xie et al. demonstrated complete regression of murine colon cancer tumors and metastases with the transfection of a functional iNOS gene into a highly metastatic cell line that had at baseline low expression of iNOS. (54)

Healthy cells rely on DNA repair mechanisms to maintain genetic stability and prevent uncontrolled growth. Researchers investigated this concept in the context of CRC metastasis to the liver. They studied a human colon cancer cell line known to spread to the liver and found it resistant to the effects of IFN-beta. Interestingly, treating these cells with increasing doses of IFN-beta reversed their metastatic behavior, making them non-metastatic. DNA microarrays analyses showed downregulation of genes involved in apoptosis in the metastastic cell lines.

These genes included *BRCA-1*, a DNA repair protein, and *ATM*, a tumor suppressor. Notably, treatment with IFN-beta restored the activity of these genes. (55)

Insulin like growth factor receptor (IGFR-1) is another molecular factor affected by IFN-beta, and its expression has been linked with progression of colorectal carcinoma and may favor hepatic metastasis. (56)

CXCR4 is a receptor protein on cell surfaces that interacts with CXCL12, a chemokine also known as stromal cell-derived factor (SDF-1), has been found to be expressed on colonic epithelial cells as well as various types of carcinomas. Upon binding SDF-1, CXCR4 initiates a cascade of intracellular signaling events that culminate in cellular motility and invasion. Zeelenberg et al. investigated the hypothesis that CXCR4 contributes to metastasis in CRC. Their findings suggest a critical role for CXCR4 in promoting the spread of cancer cells to the liver and lungs. In their experiments, they inhibited the function of CXCR4 in CRC cells by transfecting them with SDF-1 (CXCL12), the ligand for CXCR4. Consequently, metastasis of these cells to the liver and lungs was significantly reduced, with some cases showing complete inhibition. (40, 57)

Finally, c-Met, a transmembrane protein tyrosine kinase receptor predominantly found in epithelial tissues, has hepatocyte growth factor/scatter factor (HGF/SF) as its primary ligand. It has been observed that c-Met is elevated in 70% of CRC metastases relative to the primary tumor. (58) Binding of HGF to c-Met induces the activation of an intracellular signaling cascade that triggers various cellular processes including mitogenesis (cell division), motility (cell movement), morphogenesis (tissue formation), and cell survival. (59)

In summary, the spread of cancer cells to distant organs, is a complex and selective process in advanced colorectal cancer. Within the primary tumor in colon cancer, only a subset of malignant cells acquires the necessary genetic advantages like enhanced adhesion, immune system evasion, and the ability to survive in a foreign microenvironment. This is why not all advanced colorectal cancers develop metastases. (40)

1.1.6. Management of colorectal carcinoma

1.1.6.1. Management of localised colorectal carcinoma

Certain early-stage cancers can be effectively cured with localized treatment alone. Notably, the incidence of early colorectal cancer has risen due to widespread screening programs utilizing colonoscopy. After an endoscopic diagnosis, malignant polyps can be removed, enabling a detailed assessment of high-risk characteristics and evaluation of margins by the pathologist. (3) Endoscopic resection has emerged as the preferred treatment for early

colorectal neoplasia, and complete *en bloc* endoscopic resection should be performed whenever the morphological structure of the polyp allows for it. (10, 60) Furthermore, multiple studies have indicated that endoscopic removal is not only safer but also more cost-effective compared to surgery. (61)

Endoscopic resection is suitable for hyperplastic or adenomatous polyps and non-invasive (pTis, i.e., intraepithelial or intramucosal) adenocarcinomas. However, for invasive carcinomas (pT1), the treatment strategy will be influenced by the polyp morphology and the presence of histological features that predict adverse outcomes, such as lymphatic or venous invasion, grade 3 differentiation, or significant (grade > 1) tumor budding. (10) If any unfavorable factor is present in a sessile or flat polyp with a pT1 carcinoma, surgical resection is required in patients deemed suitable for surgery. The objective of surgical resection is to completely remove the lesion, including lymph node removal to accurately assess the risk. In cases where surgery is not feasible, surveillance colonoscopy within 6 months after polyp removal is advised. (10)

Endoscopic evaluation of the lesion's nature and resectability should be conducted at centers with a high level of proficiency and with high patient volume (at least over 50 endoscopic submucosal dissections per year). (62)

1.1.6.2. Management of local infiltrative colorectal carcinoma

Colon cancer that has infiltrated the wall and adjacent structures cannot be removed through colonoscopy and necessitates surgical intervention aimed at extensive resection of the affected segment and corresponding lymphatic drainage. The surgical resection should include at least a 5 cm colonic segment, althoug the extent may vary based on the blood supply and distribution of regional lymph nodes. When technical expertise is available, laparoscopic colectomy can be safely carried out since it provides similar oncological outcomes with reduced morbidity and improved tolerance. (10) Surgery for rectal cancer is notably more intricate and largely influenced by the accessibility and anatomical characteristics of the pelvis. Total mesorectal excision is the established oncological procedure, and the extent of resection additionally depends on the involvement of the sphincter complex and adjacent structures. (3)

1.1.6.2.1 Adjuvant treatment

Decisions regarding the indication for adjuvant treatment must be made following comprehensive discussions with the patient about the risks and benefits of available options. The decision to recommend systemic adjuvant treatment is guided by the risk of recurrence, with the aim of extending the relapse-free interval and improving overall patient survival. This

risk must be assessed considering the expected benefits but also potential complications from the given adjuvant chemotherapy. (10)

Assessing the risk of relapse after colon cancer resection involves integrating clinicopathological features of the tumor along with molecular markers. For instance, factors like microsatellite instability (MSI) and *SMAD4* expression have been identified as independent prognostic indicators for disease-free survival (DFS). (63) Nevertheless, TNM staging remains the most important histological criterion for assessing the risk following surgery for CRC. (10) According to the TNM staging, the 5-year survival rates following surgical resection alone are as follows: 99% for stage I, 68%-83% for stage II, and 45%-65% for stage III disease. (25)

Other major and minor prognostic parameters to consider for assessing the risk of stage II colorectal cancer and determining the need for adjuvant chemotherapy include (10):

Major prognostic parameters:

- < 12 lymph nodes sampling
- pT4 stage including perforation

Minor prognostic parameters:

- high grade tumour
- vascular invasion
- perineural invasion
- tumour presentation with obstruction
- high preoperative CEA levels.

Adjuvant systemic therapy with single-agent 5-fluorouracil (5-FU) offers a modest but significant reduction in mortality risk (3-5%) in high-risk stage II and by 10-15% in stage III CRC. Adding oxaliplatin to the regimen further improves survival rates by 4-5%. (10) MSI/ MMR deficiency status has emerged as a valuable tool for guiding treatment decisions regarding adjuvant chemotherapy alongside traditional clinical factors, in addition to its implications for Lynch syndrome diagnosis. Patients with tumors exhibiting MSI/dMMR generally have a more favorable prognosis and a lower anticipated benefit from chemotherapy. A subset of stage II patients with MSI/MMR (approximately 10-15%) have very low risk of recurrence. For these patients, the potential benefits of 5-FU chemotherapy are minimal, and it is recommended to avoid adjuvant chemotherapy altogether. (64, 65)

Counterindications for adjuvant chemotherapy have to be carefully assessed, such as Eastern Cooperative Oncology Group (ECOG) performance status > 2, uncontrolled infection, severe liver and renal dysfunction and heart failure [New York Heart Association (NYHA) III and IV]. (10)

Dihydropyrimidine dehydrogenase (DPD) is the primary enzyme responsible for the metabolism of fluoropyrimidines. DPD's function is crucial, as it acts as the rate-limiting enzyme in this process. Genetic polymorphisms can cause deficiencies in DPD function in approximately 3-5% of patients, resulting in increased fluoropyrimidine toxicity that can be life-threatening. (66) Therefore, testing for DPD polymorphism is mandatory before initiating 5-FU based chemotherapy. Depending on the level of deficiency, the doses of 5-FU should be reduced up to 50% or not given at all. (10, 66)

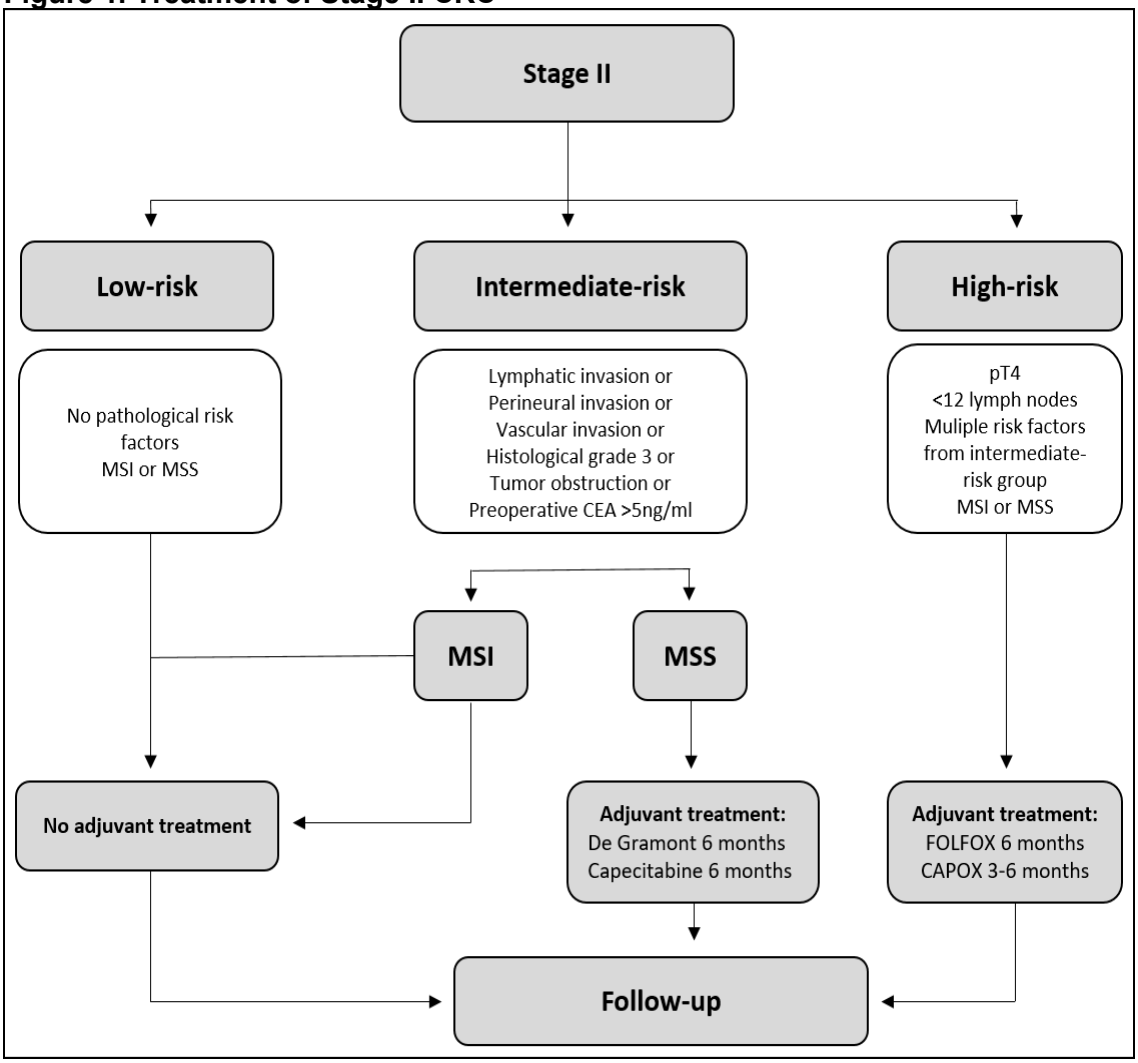
Adjuvant treatment for stage II CRC

Some patients with stage II CRC will not require additional treatment after surgery, whereas other do benefit from adjuvant chemotherapy. For low-risk stage II patients, based on factors linke TNM stage and absence of concerning features, close follow-up is an option, whereas chemotherapy is recommended for those with a high risk of recurrence to improve long-term outcomes. The “de Gramont” (5-FU, folinic acid) is the only established treatment regimen that has shown efficacy in this context by reducing recurrence rates. (67) Capecitabine (an orally administered alternative) represents a viable option for patients who might not be suitable for placement of a central line, which is often needed for the de Gramont regimen. An expert panel of the European Society for Medical Oncology (ESMO) suggests considering oxaliplatin-based therapy for specific high-risk groups. This groups include: patients with pT4 and/or fewer than 12 lymph nodes, or those with a combination of several risk factors. The rationale for using oxaliplatin is based on evidence suggesting potential benefits for these specific high-risk patient subsets. (10, 68) **(Figure 1)**

Very recently, the Dynamic trial (Tie et al., NEJM 2022) investigated the use of postoperative ctDNA for decision-making regarding adjuvant chemotherapy in stage II CRC. (29) The researchers evaluated whether an approach guided by ctDNA could decrease the use of adjuvant chemotherapy while maintaining recurrence risk. Patients with stage II colon cancer were randomly assigned in a 2:1 ratio to have their treatment decisions informed by either ctDNA results or conventional clinicopathological features. In the ctDNA-guided management approach, a positive ctDNA result after surgery led to the administration of oxaliplatin-based or 5-FU chemotherapy, while ctDNA-negative patients did not receive treatment. Key endpoints included recurrence-free survival at 2 years and the frequency of adjuvant

chemotherapy utilization. Among the 455 randomized patients, 302 were allocated to ctDNA-guided management while 153 were assigned to standard management. A lower proportion of patients in the ctDNA-guided group (15%) received adjuvant chemotherapy compared to the standard management group. (28%) In the assessment of 2-year recurrence-free survival, ctDNA-guided management demonstrated noninferiority to standard management, with rates of 93.5% and 92.4%, respectively.

Figure 1. Treatment of Stage II CRC



Adapted from ESMO guidelines 2022(69)
MSI: microsatellite instability, **MSS:** microsatellite stable, **CEA:** carcinoembriogen antigen, **de Gramont:** folinic acid ü 5-fluorouracil, **FOLFOX:** folinic acid + 5-fluorouracil + oxaliplatin, **CAPOX:** capecitabine + oxaliplatin

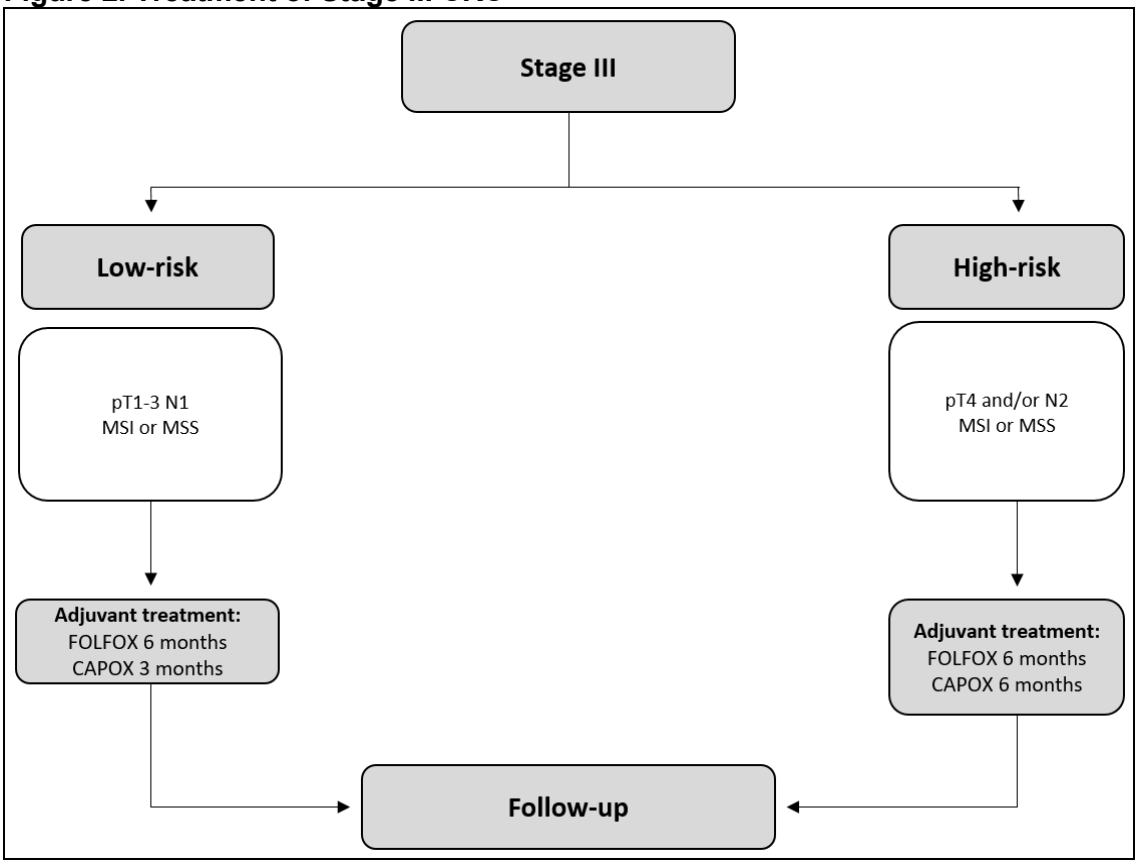
Adjuvant treatment for stage III CRC

In stage III CRC, the current standard of care for adjuvant therapy is a combination of 5-FU and oxaliplatin. This is based on compelling evidence from three pivotal clinical trials (MOSAIC, NSABP C-07, and XELOXA). These trials compared the effectiveness of 5-FU/oxaliplatin regimens against fluoropyrimidine alone, which was the previous standard of care. The results

favored the combination therapy, demonstrating a substantial improvement in DFS for patients receiving 5-FU and oxaliplatin. The positive impact of adding oxaliplatin extends across all age groups, including elderly patients above 80 years old, as demonstrated in a retrospective analysis of a Canadian database, including patients over 80 years of age, which addressed concerns that elderly individuals might not tolerate the combination therapy. A pooled analysis of four randomized trials (involving 1 886 patients) has demonstrated that treatment with oxaliplatin in combination with capecitabine (the oral form of 5-FU) is beneficial across all age groups and can be considered as a treatment option. In another analysis, patients younger than 70 years experienced a greater benefit and significantly lower rates of toxicity. (10, 70, 71)

Thus, the combination of 5-FU and oxaliplatin (FOLFOX and CAPOX) has become the the established adjuvant therapy for stage III CRC, providing substantial benefits in terms of disease-free survival for patients across various age groups. Irinotecan, cetuximab, and bevacizumab have not shown clinical efficacy in the localized setting and therefore should not be used as adjuvant treatments in this context. (10) **(Figure 2)**

Figure 2. Treatment of Stage III CRC



Adapted from ESMO guidelines 2022(69)
MSI: microsatellite instability, **MSS:** microsatellite stable, **CEA:** carcinoembriogen antigen, **de Gramont:** folinic acid ü 5-fluorouracil, **FOLFOX:** folinic acid + 5-fluorouracil + oxaliplatin, **CAPOX:** capecitabine + oxaliplatin

1.1.6.3. Management of metastatic colorectal carcinoma (mCRC)

A significant proportion (approximately 20% to 50%) of patients treated for localized CRC will experience recurrence over time. Another concerning statistic is that roughly 15-30% of patients present with metastases at the time of initial diagnosis. (69)

The likelihood of developing metastases increases with stages and is as follows (8):

- Stage I (lymph node–negative with tumor extending up to the muscularis propria): less than 10%.
- Stage II (lymph node–negative with tumor extending through the muscularis propria or into other structures): 10% to 20%.
- Stage III (lymph node–positive): 25% to 50%.

Survival rates from diagnosis among patients presenting with stage IV CRC:

- 1-year survival: approximately 70-75%
- 3-year survival: approximately 30%-35%,
- 5-year survival: fewer than 20%

Before initiating any therapy, a clinical or biological suspicion of metastatic colorectal cancer (mCRC) should always be confirmed by appropriate radiological imaging and histological examination of the metastatic lesions. Additional valuable modalities include abdominopelvic ultrasonography with specific contrast enhancers or magnetic resonance imaging (MRI). MRI and PET-CT scans may be useful to differentiate metastases from benign lesions, especially when CT findings are inconclusive. MRI is the preferred imaging modality for cases of mCRC that may be suitable for local treatment, as it provides accurate characterization of the number and precise location of metastases. (69)

The most frequent sites of metastases in colorectal cancer are the liver, lungs, peritoneum, and distant lymph nodes. Given the prevalence of metastasis in specific organs like the liver and lungs, imaging studies should prioritize these areas during routine follow-up for patients with colon cancer. Bone and central nervous system metastases are infrequent, rendering bone scans and brain imaging unnecessary for patients without symptoms suggestive of metastases in these sites. An FDG-PET offer a unique advantage in certain situations, especially in patients exhibiting elevated tumour markers without apparent metastatic disease, or to assess the feasibility of surgical intervention with curative intent by delineating the extent of metastatic disease in cases of potentially resectable metastases. (69)

Upon diagnosis of metastatic colon cancer, a thorough assessment is essential. This evaluation includes a detailed patient history, a comprehensive physical examination, and a panel of blood tests. The blood work typically includes a complete blood count to assess blood

cell levels and a comprehensive metabolic panel to evaluate organ function. Additionally, measurement of CEA levels is recommended, and carbohydrate antigen (CA 19-9) may also be included. (69)

To personalize treatment for patients with mCRC, molecular profiling of tumor tissue has become a crucial step. This analysis helps identify patients who may benefit from targeted therapies. Testing for mismatch repair (MMR) status and mutations in specific genes, including *KRAS* (exons 2, 3, and 4) and *BRAF*, is recommended for all mCRC patients at the time of diagnosis. (69) Because mutations in the *RAS* gene can significantly reduce the effectiveness of anti-epidermal growth factor receptor (EGFR) monoclonal antibody (mAb) therapy, testing for these mutations is mandatory before initiating treatment for metastatic colon cancer. (72, 73) While the *BRAF* p.V600E mutation is a known negative prognostic factor in mCRC, it also holds promise for targeted therapy approaches. Therefore, assessing this mutation status alongside *RAS* mutation testing is crucial for a comprehensive evaluation of treatment options for mCRC patients. (69, 74) Evaluation of deficient mismatch repair (dMMR)/microsatellite instability (MSI) status has emerged as a valuable tool during the initial molecular workup of metastatic mCRC. This testing can guide clinician decision-making in selecting patients who may benefit from immune checkpoint blockade (ICB) therapy. (69, 75, 76) For patients with mCRC whose tumors test wild-type (wt) for RAS mutations, evaluating for human epidermal growth factor receptor 2 (HER2) amplification is recommended. This assessment, typically performed using immunohistochemistry (IHC) or fluorescence in-situ hybridization (FISH), helps identify patients who may potentially benefit from HER2-targeted therapy. (77) **See Table 2: drug match for genomic alterations in mCRC**

Table 2. Drug match for genomic alterations in mCRC

Biomarker or genomic alteration	Method of detection	Drug match
MMI or dMMR	PCR or IHC	Pembrolizumab in first-line treatment
RAS* mutations** **Including any mutation at exon 2, 3, 4 in *KRAS* and *NRAS	dPCR or NGS	Cetuximab or panitumumab (EGFR inhibitors) should be avoided
***BRAF* V600E mutations**	Sanger sequencing, dPCR or NGS	Encorafenib-cetuximab in second or further lines of treatment
HER2 (*ERBB2*) amplification	IHC, ISH, or NGS	HER2 blockade*** - Trastuzumab + lapatinib - Transtuzumab + Pertuzumab - Trastuzumab deruxtecan - Trastuzumab + Tucatinib - Trastuzumab + Pyrotinib
NTRK fusions	Sanger sequencing or NGS	NTRK inhibitors (larotrectinib, entrectinib)

Adapted from ESMO guidelines 2022 (69)

*** RAS wt

*** Double Blockade

1.1.6.4 Management of advanced and unresectable metastatic colorectal cancer

When determining the most appropriate initial treatment for patients with unresectable mCRC, several key factors must be considered. These factors encompass the patient's clinical presentation, including any urgent symptoms they may be experiencing. Additionally, the tumor characteristics, including histology and molecular analysis, are crucial considerations. Finally, a comprehensive assessment of the patient's overall health is vital, incorporating factors such as age, performance status, presence of other medical conditions (comorbidities), and socioeconomic background. (69)

Tumor biology plays a critical role in both predicting a patient's prognosis and tailoring the most effective treatment approach. As discussed earlier, a tumor's mutational status serves as a cornerstone for treatment decisions. Additionally, the location of the primary tumor, particularly if proximal to the splenic flexure, can influence prognosis. Tumors in this location are often associated with shorter survival due to several factors: the inherent nature of the tumor itself, the potential for earlier spread to metastatic sites, and a potentially reduced response to chemotherapy and monoclonal antibody (mAb) therapy (discussed later in “First line treatment”). (69)

A crucial step in the treatment planning process is establishing clear goals with the patient from the beginning. This discussion should comprehensively address potential treatment-related issues, including both the expected toxicities and the impact on the patient's quality of life (QoL). (69) **Table 3 resumes the drivers for first line treatment in mCRC**

Table 3. Drivers for first line treatment in mCRC

Tumor characteristics	Patient characteristics	Treatment characteristics
Clinical presentation - Tumor burden - Tumor localisation Tumor biology - *RAS* mutational status - *BRAF* mutational status - MSI/dMMR status	Age Performance status Organ function Comorbidities Patient´s expectation and preference	Toxicity profile Flexibility of treatment administration Socioeconomic factors Quality of life

Adapted from ESMO guidelines 2022(69)
MSI: microsatellite instability, **dMMR:** deficient mismatch repair

For treatment stratification, it must first be determined whether the patient is suitable for treatment. (78) Age itself should not be the sole factor when considering a patient for systemic combined therapy. Patients with a good performance status and no significant comorbidities may be suitable candidates for this treatment approach, regardless of their age. For frail patients that will not tolerate combination therapies, the goal is maintaining QoL and palliate symptoms following a best supportive care (BSC) concept. For these patients, treatment

options may include well-tolerated therapies such as single-agent fluoropyrimidine or combinations incorporating targeted agents like bevacizumab or anti-EGFR mAbs, for patients with left-sided *RAS* wild-type tumors. In contrast, treatment goal for fit patients is disease control, improvement of symptoms and a prolonged survival in palliative intention. (69)

First-line treatment

Cytotoxic agents: the treatment backbone of mCRC is 5-FU. Extensive clinical trials, encompassing both first-line and second-line treatment settings, have investigated various combinations centered on fluoropyrimidines. These combinations have utilized different administration methods for 5-FU, including intravenous bolus injection and continuous infusion, as well as the oral prodrug capecitabine. Notably, these administration approaches are considered to have comparable efficacy. (69) The addition of oxaliplatin and/or irinotecan to 5-FU have improved response rates and survival. (79, 80) FOLFOX and FOLFIRI, both regimens containing fluoropyrimidines, demonstrate comparable effectiveness as first-line treatment options for mCRC. Capecitabine is more commonly combined with oxaliplatin (CAPOX) due to its favorable tolerability profile compared to the FOLFIRI regimen, which incorporates irinotecan. While CAPIRI, combining capecitabine with irinotecan, exists, its use is less frequent due to potentially increased toxicity compared to FOLFIRI. (81) The addition of irinotecan to the FOLFIRI regimen, creating FOLFOXIRI, has been shown to improve response rates and overall survival for patients with metastatic colon cancer. However, the increased efficacy of FOLFOXIRI comes at the cost of potentially greater side effects. Therefore, this regimen is typically reserved for patients who are considered medically fit and have minimal pre-existing comorbidities to ensure they can tolerate the more intense treatment. (69, 82)

Biological targeted agents: Two anti-EGFR mAbs are approved for the treatment of mCRC as monotherapy or in addition to chemotherapy: cetuximab is a chimeric anti-EGFR mAb (73), and panitumumab a humanised anti-EGFR mAb (83). Both antibodies were better when added to chemotherapy. (84-87)

Mutations in the *RAS* gene are known to significantly reduce the effectiveness of anti-EGFR mAb therapy; (72, 73) consequently, testing for these mutations is mandatory before initiating treatment with anti-EGFR mAbs. In other words, the presence of a *RAS* mutation precludes the use of anti-EGFR mAbs in a treatment plan for mCRC. (69) A retrospective analysis revealed that only patients with tumors lacking *KRAS* exon 2 mutations experienced a significant clinical benefit when adding cetuximab to FOLFIRI treatment. These benefits included a reduced risk of disease progression, improved OS, and increased response rates compared to patients receiving FOLFIRI alone. (86)

Selection of patients for anti-EGFR monoclonal antibody (mAb) therapy in mCRC hinges on two critical factors: *RAS* mutation status and primary tumor location. Clinical trials have shown a significant benefit for patients with left-sided *RAS* wt tumors who receive anti-EGFR mAbs. These patients experience improved response rates and achieve longer PFS and OS. Conversely, a large retrospective analysis encompassing over 2 000 patients with *RAS* mutational data from six randomized trials found no improvement in PFS or OS for patients with right-sided tumors who received the anti-EGFR mAbs cetuximab or panitumumab. (35)

Bevacizumab, a targeted monoclonal antibody specifically targeting vascular endothelial growth factor receptor A (VEGFR-A), stands out as the sole antiangiogenic therapy demonstrating improved outcomes when combined with chemotherapy for first-line treatment of mCRC. Studies have confirmed that adding bevacizumab to capecitabine significantly increases PFS compared to using capecitabine alone. (88) This befenit was confirmed in the AVEX phase III trial including elderly (> 70 years) patients. (89) Bevacizumab in addition to an irinotecan- and an oxaliplatin-based chemotherapy was also superior versus placebo in two different randomized trials. (90, 91) In the Japanese TRICOLORE phase III trial, oral 5-FU and Irinotecan plus bevacizumab was non-inferior when compared to FOLFOX6 or CAPOX and bevacizumab. (92)

The FIRE-3 trial directly addressed the question of which targeted therapy might offer a greater advantage when combined with chemotherapy in the first-line setting for *KRAS* wild-type (exon 2) mCRC. This trial compared two treatment regimens: FOLFIRI plus bevacizumab and FOLFIRI plus cetuximab. The study found no significant difference between the two arms in terms of overall response rates or PFS. Interestingly, however, the FIRE-3 trial did observe an improvement in OS for patients who received FOLFIRI plus cetuximab compared to those receiving FOLFIRI plus bevacizumab. (93) Further solidifying the findings from FIRE-3, a combined analysis incorporating both FIRE-3 and CRYSTAL trials specifically confirmed the OS benefit observed in patients with left-sided tumors who received FOLFIRI plus cetuximab. (94)

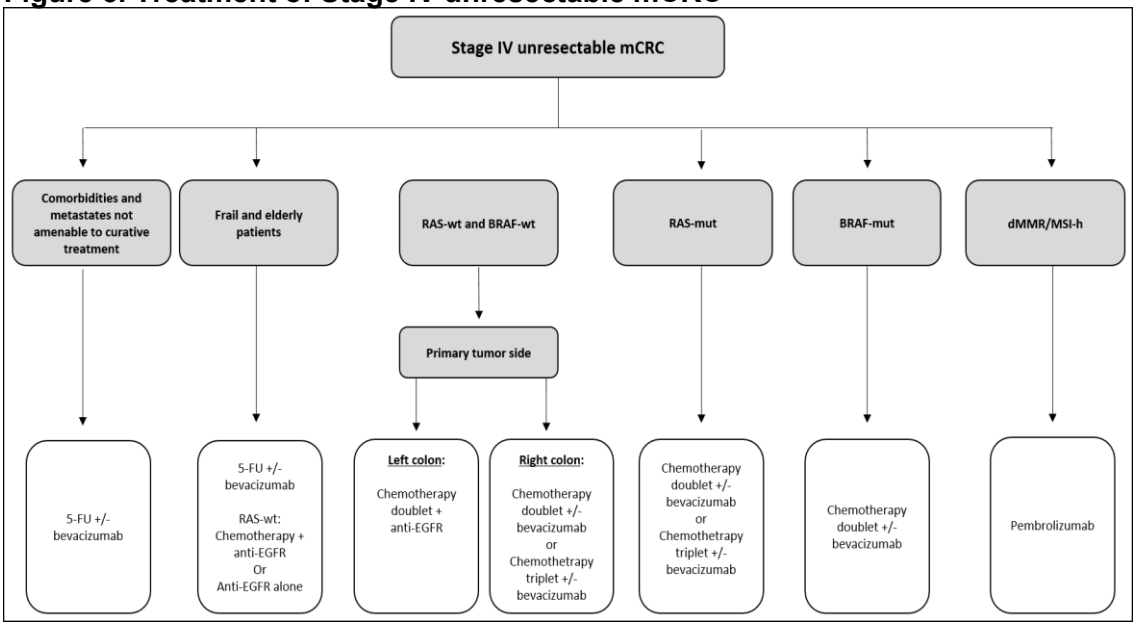
The CALGB/SWOG 80405 trial further supports the influence of tumor location. While this trial did not observe a significant difference in overall survival between the cetuximab and bevacizumab arms, a subsequent exploratory analysis revealed a trend. Patients with left-sided tumors treated with cetuximab exhibited improved OS and PFS, whereas those with right-sided tumors appeared to experience a longer OS with bevacizumab. These findings collectively suggest a diminished efficacy of anti-EGFR therapies, such as cetuximab, in right-sided mCRC. (95, 96)

In conclusion, tumor location alongside *KRAS* mutational status plays a critical role in guiding targeted therapy selection for patients with *KRAS* wild-type mCRC receiving first-line chemotherapy. Patients with left-sided tumors may benefit from FOLFIRI or FOLFOX plus cetuximab, while bevacizumab may be a more suitable option for right-sided tumors.

Pembrolizumab, a programmed cell death protein 1 (PD-1) blockade immunotherapy, has emerged as a promising first-line treatment option for patients with mCRC harboring deficiencies in mismatch repair (dMMR) and microsatellite instability-high (MSI-H). This finding is supported by a pivotal phase III clinical trial involving 307 treatment-naive patients. The trial compared pembrolizumab administered until disease progression or a maximum of two years to standard FOLFOX or FOLFIRI-based chemotherapy regimens, with or without bevacizumab or cetuximab, according to investigator's choice. Pembrolizumab demonstrated superiority in terms of PFS, with a median PFS of 16.5 months compared to 8.2 months for standard therapy. Additionally, patients receiving pembrolizumab reported a significant improvement in quality of life (QoL). Importantly, treatment related adverse events were significantly higher in the chemotherapy arm. (76, 97)

Altogether, these findings underscore the importance of incorporating tumor location and molecular biomarkers into treatment decision-making for this patient population. **Figure 3 shows an algorithm for the first line therapy in unresectable mCRC.**

Figure 3. Treatment of Stage IV unresectable mCRC



Adapted from ESMO guidelines 2022(69)
wt: wild type, **mut:** mutated, **dMMR:** deficient mismatch repair, **MSI-h:** microsatellite instability (high), **EGFR:** epidermal growth factor receptor
5-FU: 5-Fluorouracil**, Doublet:** FOLFOX (Folinic acid + 5-FU + Oxaliplatin), FOLFIRI (Folinic acid + 5-FU + Irinotecan), **C**APOX (Capecitabine + Oxaliplatin)
Triplet: FOLFOXIRI (Folinic acid + 5-FU + Oxaliplatin + Irinotecan)

1.1.6.5. Management of resectable & potentially resectable mCRC

Surgical resection of metastases from CRC have been performed now since over three decades. Reported 5-year survival rates range between 20-45%. (98)

In addition to surgical resection, minimally invasive ablative techniques like thermal ablation (TA) or stereotactic body radiotherapy (SBRT) can be employed in two key scenarios for mCRC (69):

- Adjuvant therapy: These techniques can be used in conjunction with surgery to achieve a complete eradication of visible tumors, potentially improving long-term outcomes.
- Alternative to resection: For patients who are deemed unfit for surgery due to frailty or challenging tumor location, ablative techniques may offer a viable alternative to achieve local tumor control.

Traditionally, the number, size, and bilobar distribution of liver metastases were considered limitations for surgical resection. However, advancements in surgical techniques and patient care allow for resection even in these scenarios, provided a sufficient functional liver remnant remains after surgery. This remnant liver tissue, typically at least 30% of the original volume, is crucial to maintain adequate liver function post-operatively. Not less important are oncological criteria for the decision of local ablative treatment and include prognostic factors that influence DFS or curability potential. (69)

While upfront surgical resection of liver metastases from mCRC is a possibility, several tumor characteristics influence treatment planning. These factors include:

- Timing of metastasis: Synchronous metastases (identified at the time of initial cancer diagnosis) may warrant a different approach compared to metachronous metastases (identified later).
- Tumor aggressiveness: Cancers exhibiting high aggressiveness may benefit from initial systemic therapy to control the disease before surgery.
- Presence of extrahepatic disease: If the cancer has spread beyond the liver (extrahepatic disease), systemic therapy is often the first-line approach to achieve disease control before considering surgery (referred in the surgical jargon as „proof of time“).

While biological factors such as *RAS* and *BRAF* mutations, and dMMR/MSI status can influence survival in mCRC, achieving an R0 resection of all metastatic lesions appears to be associated with comparable survival outcomes across all prognostic risk groups. This suggests

that the success of complete surgical resection (R0) may supersede the negative prognostic impact of some genetic markers in mCRC. (69)

A randomized clinical trial explored the use of perioperative FOLFOX therapy in patients with technically easy resectable mCRC of the liver. The study observed a statistically significant improvement in DFS for patients who received FOLFOX (administered for 3 months pre- and post-surgery) compared to those who did not receive any chemotherapy. (99) Conversely, a separate randomized trial investigating the addition of cetuximab to standard FOLFOX chemotherapy yielded unexpected results. This study found that patients who received the combination therapy (cetuximab plus FOLFOX) experienced a shorter DFS compared to those who received FOLFOX alone. (100) These findings underscore the critical role of tailoring postoperative chemotherapy regimens for mCRC liver metastases based on individual patient characteristics and tumor biology. Not all chemotherapy approaches are equally effective, and some targeted therapies, like cetuximab in this case, may even have a detrimental impact on DFS. Further research is needed to optimize treatment strategies and identify patient selection criteria for maximizing the benefit of postoperative chemotherapy in mCRC patients with liver involvement.

For patients with initially unresectable mCRC, a promising strategy known as conversion therapy offers the potential for surgical resection. This approach involves administering systemic therapy (chemotherapy or targeted agents) to shrink tumors and improve the chance of successful surgical removal with curative intent. (69) Following successful conversion therapy with systemic agents to achieve resectable mCRC, careful consideration should be given to the timing of surgical intervention. Ideally, surgery should be performed 3-4 weeks after the completion of chemotherapy, with or without anti-EGFR monoclonal antibodies. If bevacizumab was included in the conversion therapy regimen, a longer waiting period is necessary. Surgery should be scheduled at least 5 weeks after the last dose of bevacizumab. The rationale behind this timing is to minimize unnecessary chemotherapy exposure; early surgery after achieving resectability helps avoid prolonged exposure to chemotherapy, which can increase liver toxicity and potentially lead to higher post-operative complications. On the other side, allowing sufficient time for recovery after bevacizumab is crucial because this drug can impair wound healing. Overall, a well-timed surgical approach following conversion therapy is essential to optimize treatment outcomes and minimize complications in patients with extensive mCRC. (101)

Summarizing, selection of the optimal treatment strategy for mCRC patients with liver metastases will relies on a careful evaluation of oncological criteria: in patients with favourable oncological criteria with characteristics like metachronous lesions, a limited number of

metastases, unilobar disease, and no extrahepatic disease may benefit most from upfront surgical resection immediate surgery. Conversely, in patients with unfavourable oncological criteria with features like synchronous lesions, multiple metastases, bilobar liver involvemet, or spread beyond the liver may be better suited for perioperative chemotherapy, preferably including a fluoropyrimidine (e.g. capecitabine) and oxaliplatin. While some studies have explored the use of chemotherapy after surgery, there is a lack of strong randomized trial evidence to support its routine use. Therefore, it is not currently considered the standard of care in this setting. (69)

Resection of lung metastases showed up to 40% 5-year survival rates in selected populations. (102) Likewise, lung and liver metastases resection in conjunction has shown a survival benefit. (103)

A systematic review and meta-analysis involving patients with peritoneal metastasis from various studies, primarily from single specialized centers experienced in peritonectomy procedures, investigated the effectiveness of complete cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC). The analysis yielded encouraging results, suggesting that this approach may lead to prolonged survival for patients with peritoneal metastasis. (104) Despite the encouraging findings from the systematic review and meta-analysis, these observations have not yet been definitively confirmed in large, randomized phase III clinical trials. As a result, CRS+HIPEC cannot be universally recommended as the standard of care for all patients with peritoneal metastasis at this time. (105)

Resection of selected other single metastases, including ovariectomy, and lymphadenectomy have also been demonstrated to render survival benefit in patient series. (106)

1.2. Oligometastases

1.2.1. Definition and general considerations

The presence of distant metastases continues to be a significant contributor to cancer-related mortality. Historically, the frequent observation of disseminated metastases established a paradigm that metastatic cancer inherently represents a widespread systemic disease. (107) The TNM staging system classifies cancers with distant metastasis as Stage IV, traditionally defining an advanced (end) stage of disease. However, this classification does not necessarily translate treatment into a purely palliative approach. Recent advancements in cancer research and treatment have transformed the outlook for patients with metastatic disease. In patients with mCRC, systemic therapies remain a mainstay, including cytotoxic chemotherapy,

hormonal therapy, and targeted immunotherapies. Nevertheless, the goal of treatment in this collective has shifted beyond mere symptom management. (108)

Clinical aspects of oligometastases

In 1995, a pivotal contribution to our understanding of metastatic cancer came from Hellman and Weichselbaum at the University of Chicago. Their work introduced the concept of oligometastases, proposing a new paradigm for how cancer progression might develop. This hypothesis challenged the prevailing view of metastasis as an inevitable progression to widespread disease. Instead, Hellman and Weichselbaum suggested that cancer metastases exist on a spectrum, with oligometastases representing a transitional state between a localized tumor and a fully disseminated cancer. (109)

The core idea behind oligometastases is that some patients may have a limited number of detectable metastases, potentially creating a window of opportunity for curative intervention. This concept contradicted the prevailing dogma that clinical apparent metastases represent the manifestation of a few detectable lesions from a widespread occult disease, and therefore, local treatment of these lesions would result in a useless effort. (110)

However, over the past few decades, a growing body of clinical data has demonstrated promising long-term survival outcomes following aggressive local treatment of oligometastases in specific patient subsets (detailed later). These positive results are particularly notable for patients with limited number of metastases, certain primary tumor types, and early-stage primary tumors. (108, 110, 111)

For therapeutic purposes, the correct definition of oligometastatic disease and identification of patients in this stage is of vital relevance, as local ablative treatment strategies should be based on the possibility of eradicating all metastases, either initially or after systemic therapy. (109, 111)

In summary, the oligometastatic state of cancer is an (still) inconsistently defined a transitional stage in cancer progression, characterized by a limited number of metastases in specific locations. (110) While the exact definition remains to be determined, the concept emphasizes a potentially manageable burden of metastatic disease. A traditional clinical definition of oligometastatic disease according to the ESMO guidelines is (69):

- one to five metastatic lesions, occasionally more if complete eradication is possible,
- up to two metastatic sites,

- controlled primary tumour (optionally resected),
- all metastatic sites must be safely treatable by local treatment.

Biological aspects of oligometastases

At present, the diagnosis of oligometastasis relies primarily on radiological imaging and a physician's clinical expertise. While the role of biological factors in diagnosis is being explored, the current definition focuses on the number and location of detectable metastases. (112-114) Although *oligometastatic clones* are not directly identified, normally, primary tumors exhibit intratumoral heterogeneity, characterized by distinct subpopulations of cancer cells with varying metastatic potential. (108) Advanced genomic analyses, including next-generation sequencing (NGS) and high-resolution single nucleotide polymorphism (SNP) and copy number analyses, provide compelling evidence that within the heterogeneous tumor microenvironment, clones harboring specific genetic advantages are likely the ones driving distant metastasis. Navin et al. employed whole genome amplification (WGA) on single cells isolated from breast tumors for high-resolution analysis of genetic diversity. Their study suggested a monoclonal origin of metastases, indicating that a single clone may have expanded to form both the primary tumor and the distant metastases. (115) Fidler et al. identified melanoma cells in murine models with a particular malignant behaviour according to the target metastasized organ with cells from brain metastases. Notably, cells from brain metastases exhibited a slower metastatic potential compared to those from visceral metastases. This finding suggests that brain metastases might originate from a distinct subpopulation of cells within the primary tumor, supporting the concept of clonal heterogeneity within the primary tumor. (116)

Yachida et al. employed next-generation sequencing (NGS) technology to analyze the clonal relationships between primary tumors and metastases in seven patients with oligometastastic pancreatic cancer. Their work provided further significant evidence supporting the concept of oligometastases. (117) Quantitative analysis of genetic mutations revealed a significant time gap between the initial tumor mutation and the emergence of metastatic ability. Their findings suggest that at least ten years were necessary before the birth of a cancer cell with metastatic potential, followed by an additional five years for the development of full metastatic competence. This study highlights the temporal nature of metastatic clone evolution, implying that oligometastatic clones may arise chronologically before polymetastatic clones during tumor progression. Adding to the evidence supporting oligometastases, Wuttig et al. conducted a study on patients with renal cell carcinoma. Their analysis of tumor samples identified distinct gene expression patterns associated with either a limited number (fewer than 8) or a high number (more than 16) of lung metastases. This suggests a potential link between specific

genetic profiles and the development of either limited or numerous number of metastases in this cancer type. (118)

While mutated cancer stem cells are recognized as a driving force in tumor formation and progression, they do not operate in isolation. The tumor microenvironment (TME) plays a critical role, consisting of various cell types recruited from surrounding normal tissues and the bone marrow. These include stromal cells and immune system subpopulations. The TME actively contributes to and sustains the development of the hallmarks of cancer, as described later. This supportive effect occurs at different scales, mediated by the reciprocal interactions between neoplastic cells and the diverse cellular components within the TME. Stromal cells within the TME have been shown to promote cancer cell hyperproliferation in various contexts. This support is achieved through the release of paracrine and juxtacrine signaling molecules that stimulate cancer cell growth. (119)

Furthermore, nearly all solid tumors harbor infiltrates of a complex and dynamic population of immune cells. These infiltrates include myeloid-derived cells and lymphoid lineage cells. The specific composition and activation state of this immune infiltrate vary depending on the tumor type, location (tissue/organ), and stage of malignancy. Interestingly, some immune cells within the TME can paradoxically promote tumor growth. These cells may secrete growth factors like epidermal growth factor (EGF), transforming growth factor-β (TGF-β), fibroblast growth factors (FGFs), and various interleukins (ILs). Additionally, factors like tumor necrosis factor-α (TNF-α), chemokines, histamine, and heparins can also contribute to tumor progression in certain contexts. Moreover, these immune cells express various classes of proteolytic enzymes, including metalloproteases, serine proteases, and cysteine proteases, enabling them to modify the structure and function of the extracellular matrix, a process typically associated with tissue repair following injury. However, in the setting of chronic exposure to mitogenic signaling molecules provided by immune cells in the tumor microenvironment, they can supply evolving neoplastic cells with signals that support their sustained proliferation. (119-121)

These observations are supported by solid biological rationale. Fidler et al. (122) proposed a multi-step model for cancer metastasis, highlighting a series of sequential and interconnected events. This model emphasizes the following key stages:

- loss of cellular adhesion,
- increased motility and invasiveness of the primary tumor,
- intravasation and survival within the circulatory system,
- extravasation into new organs,
- and ultimately, successful colonization of these distant sites.

Each step within this metastatic cascade can act as a rate-limiting event. Failure or insufficiency at any stage can halt the entire process. The ultimate outcome of metastasis hinges on a complex interplay between the intrinsic properties of the tumor cells and the response mounted by the host. (122)

The hallmarks of cancer, originally proposed by Hanahan and Weinberg in 2000 and subsequently updated in 2011, represent a comprehensive framework for understanding tumorigenesis and progression. These hallmarks encompass ten distinct capabilities acquired by cancer cells, categorized into two broad groups: functional capabilities and enabling characteristics (123, 124).

- Functional Capabilities:
    - Genome instability and mutation
    - Resisting cell death
    - Deregulating cellular metabolism
    - Sustaining proliferative signaling
    - Evading growth suppressors
    - Avoiding immune destruction
    - Activating invasion and metastasis
    - Inducing or accessing vasculature
- Enabling Characteristics:
    - Enabling replicative immortality
    - Tumor-promoting inflammation

The evolving understanding of cancer biology has led to the proposal of additional emerging hallmarks alongside the established ten. These novel concepts highlight the complexity of tumorigenesis and progression (125):

- Unlocking phenotypic plasticity: This hallmark emphasizes the ability of cancer cells to adopt diverse phenotypes, potentially facilitating adaptation to various microenvironments and therapeutic pressures.
- Non-mutational epigenetic reprogramming: This concept focuses on how alterations in gene expression patterns, independent of mutations, can contribute to cancer development.
- Polymorphic microbiomes: The composition of microbial communities within or near tumors may influence disease progression.
- Senescent cells: While senescent cells normally function as tumor suppressors, their accumulation within the tumor microenvironment can paradoxically promote

tumorigenesis under certain conditions. Senescent cells represent an intriguing area of research in cancer biology. While not yet classified as a hallmark, their complex role within the tumor microenvironment is being actively explored.

The precise order and extent to which cancer cells acquire the hallmarks of cancer can vary significantly during tumor progression. This dynamic interplay between hallmark acquisition may influence the emergence of oligometastatic tumors. While the exact sequence remains under investigation, the specific combination and timing of hallmark activation might be a key factor in determining the development of a limited number of metastases. As Weichselbaum and Hellman wrote: “there may be primary tumor cells with a limited capability in one or more of the necessary biological requirements for metastasis, thus the origin of oligometastases”. (111)

Treating oligometastatic disease offers a potential biological advantage: it can prevent the further evolution of genetically unstable clones and subsequent metastatic spread. This translates clinically into two key benefits: improved overall disease control and the ability to delay the need for potentially toxic systemic therapies. (110)

1.2.1.1. Oligometastastic colorectal cancer

Mounting evidence suggests that patients with mCRC confined solely to the liver represent a unique group that may significantly benefit from a more aggressive treatment approach. (110) Surgical resection of liver metastases in this population has yielded promising results, with studies demonstrating 5-year overall survival (OS) rates ranging from 39% to 47% and 10-year OS rates between 17% and 28%. These figures are considerably higher compared to outcomes observed in patients receiving systemic therapy alone. (108, 126)

Some evidence suggests a potential link between tumor genotype and eligibility for hepatic resection in metastatic cancer. Tumors harboring *BRAF* p.V600E mutations, typically associated with a more aggressive phenotype, are rarely observed in patients undergoing liver resection. This observation may indicate that the aggressive nature of *BRAF*-mutated tumors often precludes the development of a limited metastatic state amenable to local treatment approaches. (127, 128)

Pitroda et al. conducted a study on liver metastases and identified distinct molecular profiles associated with long-term patient survival. (107) Their research revealed three distinct molecular subtypes of metastatic CRC that exhibited heterogeneous clinical outcomes. One particular subtype, characterized by enrichment of the epithelial-mesenchymal transition (EMT) and *KRAS* signaling pathways, was distinguished by high degree of immune cell

infiltration within the metastases, an abundance of molecular signatures associated with interferon alpha and gamma signaling, and activation of the p53 tumor suppressor pathway. Compared to the other two subtypes, this EMT/KRAS-enriched subtype displayed a significant overexpression of genes associated with both the innate and adaptive immune response. These overexpressed genes included those crucial for T cell activation and the communication between antigen-presenting cells (APCs) and T cells. This suggests a potentially more immunologically active microenvironment within these specific metastases. At the histological level, metastases exhibited dense peritumoral infiltration of CD3-positive and CD8-positive lymphocytes extending intratumorally. The exclusive mutational landscape of this subtype included *NRAS*, *CDK12*, and *EBF1* mutations. Notably, amplification of *VEGFA*, a finding observed in other subtypes, was less prevalent here. This group of patients experienced lower rates of metastatic recurrence or death after hepatic resection compared to the other subtypes. Additionally, in cases of recurrence, these patients were more likely to develop a limited number of metastases. (107)

The compelling findings from this study strongly supports the concept of a distinct biological and molecular basis driving the development and clinical behaviour of colorectal oligometastases. This research sheds light on the potential identification of patients harboring potentially curable metastatic disease, paving the way for more personalized treatment strategies.

1.2.1.2. Management of oligometastatic colorectal carcinoma

Prior to initiating local treatment (LT) for oligometastases, a comprehensive imaging evaluation is crucial. This often involves a combination of contrast-enhanced CT scans, MRIs, ultrasounds, and PET scans to assess the full extent of systemic disease and the depth of local infiltration within the involved organs. A comprehensive multidisciplinary patient assessment and counseling are crucial. Several aspects influence the selection of the most suitable LT for oligometastases, including the size, number, and anatomic location of the metastatic lesions, the anticipated success rate of achieving complete eradication of the disease, the degree of invasiveness associated with the chosen LT technique and the available local expertise in its application, careful assessment of patient frailty, their projected life expectancy, and their individual treatment preferences. (69)

Both LT modalities, surgical and non-surgical approaches, can be integrated into the therapeutic armamentarium for managing oligometastases. While traditionally employed for curative intent in patients with limited disease, the role of LT is expanding to encompass various other clinical contexts (e.g. in the context of limited mCRC).

Typically, surgery is employed for curative purposes; however, in certain cases, tumor characteristics (such as localization) and/or patient factors may restrict the use of surgical approaches. Therefore, non-surgical LT emerges as a valuable alternative when surgical intervention is contraindicated. In scenarios with uncertain prognosis or following successful response to systemic therapy in more extensive disease, non-surgical LT can offer several potential advantages. These include halting further tumor dissemination, potentially delaying the need for systemic treatment, or even eliminating its necessity altogether. LT (surgical and nonsurgical) is appropriate as an initial intervention for slow-growing tumors. Additionally, LT serves as a valuable tool for post-systemic therapy consolidation aiming to delay or even or interrupt further systemic treatment. (69)

For the management of oligometastases, first, induction chemotherapy is typically employed. Here, a favorable response or disease stabilization serves as a strong indicator of positive treatment outcomes, therefore justifying proceeding to an invasive LT. However, for patients presenting with favourable prognosis and limited number of metastases, upfront LT is the established standard of care. The concept of oligo-progressive disease describes a scenario of a very limited recurrence or lack of response under systemic treatment. In such cases, LT emerges as a potential therapeutic option. This behavior of limited metastasis could be seen as a result of intra-tumor heterogeneity. In this context, LT targets the non-responsive cell clones. By eliminating these resistant clones, LT may enable the continuation of effective systemic therapy.

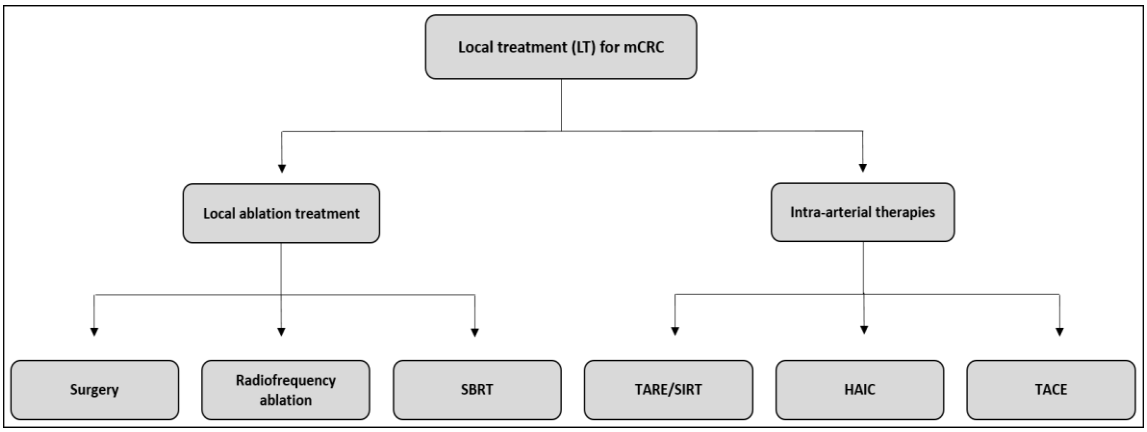
When feasible, achieving complete tumor eradication with surgical R0 resection and/ or A0 ablation should be the primary goal of treatment. In cases of oligometastatic disease with solitary organ involvement (most commonly the liver or lung) or a limited number of organs, complete resection of all metastatic lesions has been shown to yield long-term survival rates, with even the potential for cure, in 20-45% of patients. (69)

Due to the absence of randomized controlled trials directly comparing surgical and non-surgical management of oligometastases, surgery has historically remained as the standard treatment approach for resectable lesions. However, other strategies such as radiotherapy and thermal ablation (TA) have shown promise in achieving complete eradication of small metastases. These minimally invasive options serve as valuable alternatives in scenarios where surgical intervention is not feasible. (129-133)

Patients harboring liver and lung metastases have been observed to have a better prognosis compared to those with involvement of other organ sites. Notably, limited lung metastases are often associated with slower tumor growth and prolonged patient survival. This observation has led to the exploration of a "watch and wait" strategy, incorporating regular surveillance imaging, as a viable approach in select cases. (134, 135)

In cases of more widespread metastatic disease, LT is unlikely to be curative on its own. However, as stated before, LT can play a significant role in extending DFS rates. (136) In this setting, LT integrates into a multimodal treatment strategy, aiming to achieve well-controlled metastatic disease. This approach may allow for the potential discontinuation of systemic therapy, ultimately contributing to improved long-term disease control and potentially even translating into a prolonged OS. (69) **Modalities for LT are summarised in Figure 4**.

Figure 4. Modalities for LT in mCRC



Adapted from ESMO guidelines 2022(69)
SBRT: stereotactic body radiotherapy, **TARE:** transarterial radioembolization**, SIRT:** selective internal radiotherapy**, HAIC:** hepatic arterial infusion chemotherapy, **TACE:** transarterial chemoembolization.

1.3. Preoperative scores for survival prediction in potentially resectable mCRC

The current reliance on solely radiographic imaging for diagnosing oligometastases and determining potential curability represents a significant limitation. The characterization of metastases by imaging—detailing their number, size, and organ involvement—represents the disease's stage and tumor burden at a specific moment in the cancer's progression. This information is valuable for determining whether to pursue local treatment of the metastases. However, this approach does not provide insights into the risk of relapse or the subsequent behavior of the tumor following treatment. As a major challenge in the management of metastatic CRC, it is important to acknowledge that significant portion of patients undergoing LT (surgery, TA, radiotherapy) will eventually experience disease recurrence, with some exhibiting particularly aggressive progression. Therefore, developing reliable predictive and prognostic scoring systems to distinguish patients most likely to achieve long-term disease control through local therapy is of paramount importance for optimizing clinical practice.

Fong et al. conducted a pivotal study identifying five independent clinical parameters as significant predictors for OS in patients with metastatic CRC. These parameters included nodal

status of the primary tumor, disease-free interval from diagnosis to discovery of metastases, number of metastases, CEA level, and tumor size. This work established the Clinical Risk Score. (137) Building upon this foundation, Rees et al. developed the Basingstoke Predictive Index, a score incorporating both pre-operative and post-operative variables such as tumor differentiation grade, extrahepatic metastases, and resection margins. (138) Both scoring systems effectively stratified patients into low-risk groups, with reported median DFS exceeding 6 years and median cancer-specific survival exceeding 7 years. Malik et al. further highlighted the potential role of the systemic inflammatory response to the tumor (IRT) as a prognostic factor. (139)

As the field of metastatic CRC management continues to evolve, two recent studies have explored novel risk stratification tools. The 'Metro ticket' score calculates a tumor burden score based on size and number of lesions, while the 'Genetic and Morphological Evaluation' (GAME) score integrates multiple variables, including the tumor burden score, to predict patient outcomes. These advancements hold promise for improving risk assessment in patients with liver metastases. (140, 141)

2. Scientific question, aims and goals

Prior attempts to identify patients with oligometastatic CRC using scoring systems have yielded mixed results in terms of accurately predicting long-term survival and guiding treatment decisions. (142-144) While the recently proposed 'Metro ticket' and 'GAME' scores demonstrate good performance, their applicability in routine clinical practice is limited. These scores require information that is not always readily available in daily clinical practice, hindering their widespread adoption. (140, 141) Consequently, a critical need remains for the development of more robust scoring systems capable of effectively differentiating true oligometastases from cases harboring occult, systemic disease. Such a tool would be crucial in refining patient selection for curative-intent local therapy.

In this multicenter retrospective analysis, we investigated the role of known and novel prognostic factors in predicting outcomes for patients with CRC who underwent surgical resection of liver metastases. We aimed to re-evaluate the established inflammatory response to the tumor (IRT) (139) as a prognostic factor and explore the potential of readily available clinical parameters to improve outcome prediction. Additionally, the study examined the influence of tumor sidedness (primary tumor location) on survival in the oligometastatic setting. With a focus on clinical utility, we developed and validated a simple, novel preoperative risk model for OS in oligometastatic CRC. This model aimed to identify patients with a favorable prognosis who would benefit most from surgical resection of liver metastases.

3. Patients and methods

3.1. Patient cohort and data collection

This retrospective multicenter study collected clinical and therapeutic data from patients with CRC who underwent surgical resection of liver metastases at participating institutions. Data were obtained from routine medical records at the University Hospital Augsburg, University Hospital Regensburg, Katharinen-Hospital Stuttgart, and 13 additional peripheral centers in Germany. A centralized data repository was established at the Tumor Center Regensburg, Institute for Quality Assurance and Health Services Research, University of Regensburg.

Inclusion criteria were established to ensure a homogenous study population. Only patients who underwent surgical resection of liver metastases pathologically confirmed as colon adenocarcinoma were included. Additionally, patients were excluded if they:

- Died from postoperative complications (not tumor progression).
- Had extrahepatic or peritoneal metastases.
- Lacked a preoperative C-reactive protein (CRP) value within 30 days of surgery.
- Presented with evidence of concurrent infectious complications.

CRP levels were used as a marker of the inflammatory response to the tumor (IRT). A CRP level ≥1 mg/dl was considered positive for IRT, while a value below 1 mg/dl indicated the absence of IRT. Finally, the study incorporated analysis of mutations in exons 2, 3, and 4 of the *KRAS* and *NRAS* genes from tumor samples.

We classified tumors based on their anatomical origin. Tumors in the ascending colon and transverse colon were categorized as 'right-sided', while those located in the left colic flexure, descending colon, sigma, and upper rectum were classified as 'left-sided'.

To assess metastatic spread, the number of liver metastases, presence of extrahepatic metastases, and lymph node involvement were evaluated preoperatively using imaging techniques such as CT, MRI, or ultrasound. These findings were subsequently confirmed by pathological examination of tissue samples and documented in the patients' medical records.

All patients initially received curative-intent treatment for the primary tumor, aiming for complete eradication of the primary tumor.

A variety of perioperative radio/chemotherapy regimens administered either before surgery (neoadjuvant) or after surgery (adjuvant) were considered. Chemotherapeutic agents included 5-fluorouracil (5-FU) or its prodrug capecitabine, alone or combined with oxaliplatin (FUFOX,

FOLFOX, CAPOX), irinotecan (FOLFIRI, Capecitabine/ Irinotecan), or a combination of all three (FOLFIRINOX/ FOLFOX-IRI). Additionally, anti-angiogenic agents like bevacizumab and anti-EGFR antibodies (cetuximab/ panitumumab) were incorporated into some regimens, based on tumor board decisions. The specific type, duration, and dosage of these regimens were tailored to each patient, considering factors like toxicity, pre-existing medical conditions, age, and patient preference.

Data collection and analyses were performed following good clinical practice guidelines and practice according to the terms of the Declaration of Helsinki, and the Bavarian Hospitals Act. Patient data were anonymized before analysis to protect patient confidentiality.

3.2. Statistical analyses

Differences of variable distribution between the two sets were tested with chi-square test for categorical and t-test for continuous variables. To enable a non-continuous scoring of age, this variable was split according to the threshold showing the best performance in a univariable regression among all possible values. The study assessed two main outcomes: overall survival (OS) and disease-free survival (DFS). OS was defined as the time between liver surgery and death from any cause. DFS was defined as the time between surgery and any confirmed recurrence of the disease detected by imaging tests. Patients who remained alive and disease-free at last follow-up were censored from the analysis. The cut-off date for survival analyses was 31 December 2019. The reverse Kaplan-Meier method was used to calculate follow-up time for each patient.

The entire dataset was divided into a training set (TC) and a validation set (VC) to evaluate the performance of a newly developed prognostic model. Following consultation with a statistician, patient data from the University Hospital Augsburg and the 13 peripheral centers were designated as the training set. Patients from the University Hospital Regensburg and Katharinen-Hospital Stuttgart comprised the validation set. This approach ensured a random distribution of data points across the two cohorts while minimizing potential biases introduced by center-specific practices.

Based on the TC, a separate univariable proportional hazards model was fitted for both outcomes using each clinical variable to determine appropriate variables for a multivariable model. Variables demonstrating P values <0.15 in univariable analyses were selected for inclusion in a multivariable proportional hazard model using backward selection based on P values. We assesed Cox model assumptions of proportional hazards via Schoenfeld residuals. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) were calculated for each final model.

Variables that remained in both final models were assigned scores based in the rounded quotient of their HR and the smallest HR of the model as score. Each variable present in the final model contributed 1 point to the score. Patients in the TC were stratified in to five different risk groups based on the cumulative score derived from the selected variables. For each risk group, OS and DFS were estimated by Kaplan-Meier curves, with significance assessed using the log-rank test. This score was thereafter validated in an independent VC to assess its performance and reliability.

Finally, we conducted a comparative analysis of the outcomes derived from the novel scoring system with those obtained using the scoring system proposed by Malik et al. for risk stratification. (139) This comparison aimed to evaluate the performance and effectiveness of the new scoring system in relation to a previous method that also considered systemic inflammatory response to the tumor (IRT), within the context of risk assessment and prognostication.

All tests were performed two sided on a significance level of 5% using statistical computing program R version 4.0.2 (R Foundation, Vienna, Austria) and IBM SPSS Statistic 25 (New York, NY).

4. Results

4.1. Patients' characteristics

Clinical records of 1537 patients were initially assessed for eligibility, of whom 1025 patients did not meet the inclusion criteria and were subsequently excluded from analyses. The remaining cohort comprised 512 patients with metastatic CRC who underwent surgical resection of de novo liver metastases between 2006 and 2016 at 16 different hospitals across Germany. Among these patients, 322 (62.9%) were treated at 3 high-volume centers, while 190 received treatment at 13 peripheral clinics **(see the consort diagram in figure 5)**. Additionally, 30 (5.9%) patients underwent combined surgical and thermo-ablation procedures.

Median age at the time of liver resection in the entire cohort was 66 years (range: 27-89), 159 (31%) patients were female. The primary tumor side was left in 379 (74%) patients. The median number of liver metastases was 2 (range: 1-14), 242 (47%) had a solitary metastasis. Three-hundred five (60%) patients had synchronous disease. The primary tumor had a positive nodal status in 329 (64%) patients. A *KRAS* mutational status was informative for for 204 patients. *KRAS* was mutated in 68 (13%). An inflammatory response to the tumor (IRT) was detected in 114 (22.3%) cases.

Preoperative chemotherapy was given for 169 (33%) patients, and postoperative chemotherapy for 259 (50%). Preoperative, the most common used chemotherapy protocol was FOLFOX (48%), followed by FOLFIRI (27%), 5-FU (15%), Capecitabin (3%), CAPOX (2%), FOLFOXFIRI (2%), FUFOX (2%), FOLFIRINOX, Irinotecan, Capecitabine/ Irinotecan (all 0,6%, respectively). Postoperative protocols used were FOLFOX (58%), FOLFIRI or Capecitabin (13%, respectively), 5-FU (10%), CAPOX (3%), FUFOX (2%), Capecitabin/ Irinotecan (1%), FOLFOXFIRI (1%). One hundred (20%) patients received an anti-EGFR or anti-VEGF monoclonal antibody. Reasons for not administering chemotherapy were in decreasing order of frequency: tumor board decision, patient´s preference, age, comorbidity, limiting toxicity. Common causes for not receiving a chemotherapy in the synchronous situation were simultaneous surgical resection of the primary tumor and metastases and a “liver first” approach, that lead to early disease progression or operative complications with subsequent delay with the beginning of chemotherapy until futher progression of the disease. The most common reason in the metachronous situation was the tumor board decision. An R0 resection margin status of liver metastases was achieved in 411 (80%).

Analysis of patient characteristics revealed no significant difference between the TC (n = 282) and the VC (n = 230) in terms of sex, synchronous vs metachronous disease, primary tumor side, lymph node involvement in the primary tumor, the presence of IRT, positive resection margins, *KRAS* mutational status, and administration of perioperative chemotherapy. However, the TC did differ in terms of age at the time of surgery. The median age in the TC was 68 years, with 86 patients (31%) older than 72 years. In comparison, the VC had a median age of 65 years, with 51 patients (22%) exceeding 72 years old (p-values <0.001 and 0.044, respectively). Additionally, the VC included a higher proportion of patients with multiple liver metastases (p=0.041). A more detailed breakdown of patient characteristics is provided in **Table 4.**

Patients were followed for a median duration of 81.2 months. The follow-up period was slightly longer in the TC with a median of 83.2 months compared to the VC with a median of 70.3 months. The median OS for the entire cohort was 60.4 months (95% CI 52.2-68.5 months). The median DFS was 17.0 months (95% CI 14.3-19.8 months).

4.2. Univariable analyses of OS and DFS

Univariable analyses were conducted within the TC to evaluate the independent prognostic impact of selected clinical parameters on DFS and OS. Detailed results are presented in **table 5**. The impact of the number of resected metastases on outcome was evaluated using the presence of one liver metastasis as reference for the HR. The presence of > 1 liver metastases

resulted in a significantly increased risk for reduced DFS (HR 1.5) and OS (HR 2.1). This elevated risk remained relatively constant for patients with more than two metastases (HR 1.4 for DFS; HR 2.1 for OS). Consequently, the scoring system incorporated a binary categorization of solitary versus multiple liver metastases (number of metastases > 1). We observed that age at the time of surgery, initially assessed as a continuous variable, displayed a progressively increasing hazard ratio for the endpoint OS with advancing age. To facilitate integration into the scoring system, a categorical age variable was established based on the cut-off point yielding the most statistically significant result in the univariable analysis (lowest P value). Age > 72 years emerged as a significant independent risk factor for OS ($p < 0.001$; HR 1.7). The HR of age increased more than two-fold (HR 2.9) for OS in patients older than 80 years. In contrast, age did not exert a significant influence on DFS.

Univariable analysis also revealed a significant impact of two tumor biology-related variables: primary tumor side and IRT on both endpoints DFS and OS. Patients with left-sided primary tumors exhibited a significant improved median OS (65.2 months, 95% CI: 55.6-74.8 months) compared to those with right-sided tumors (41.1 months, 95% CI: 25.8-56.4 months) ($p=0.009$). This result correlated with DFS, with a median DFS of 19.7 months (95% CI: 15.5-23.9 months) for left-sided tumors and 10.8 months (95% CI: 5.9-15.6 months) for right-sided tumors ($p=0.002$).

Similarly, the presence of IRT emerged as a significant negative prognostic factor. Patients without IRT had a superior median OS (72.7 months, 95% CI: 63.6-81.7 months) compared to those with IRT (28.2 months, 95% CI: 18.4-38.0 months) ($p<0.0001$). This was the case for DFS as well, with a median DFS of 20.4 months (95% CI: 16.4-24.3 months) observed in patients without IRT and a median of 10.8 months (95% CI: 8.6-13.0 months) for patients with IRT ($p<0.0001$).

Additionally, the detection of an affected lymph node by the primary tumour and synchronous disease presentation emerged as significant negative prognostic factors for OS ($p = 0.021$ and 0.014, respectively). Conversely, these factors did not exert a significant influence on DFS ($p = 0.149$ and 0.143, respectively).

Positive resection margin, male sex, and mutated *KRAS* status were identified as significant predictors for DFS ($p = 0.003$, 0.016, and 0.016, respectively) but did not significantly impact OS.

4.3. Multivariable analyses of OS and DFS

For OS, five variables showed significance with a p value < 0.05: a positive IRT (p < 0.001; HR 1.92; 95% CI 1.35 - 2.75), right-sided primary tumor (p = 0.008; HR 1.63; 95% CI 1.14 - 2.34), multiple liver metastases (p <0.001; HR 1.75; 95% CI 1.27 - 2.42), node-positive primary tumor (p = 0.026; HR 1.49; 95% CI 1.05 - 2.13), and age > 72 years at the time of surgery (p <0.001; HR 1.72; 95% CI 1.24 - 2.44).

For DFS, four variables resulted significant with a p value < 0.05: IRT (p = 0.002; HR 1.74; 95% CI 1.23 - 2.47), right-sided primary tumor (p = 0.014; HR 1.56; 95% CI 1.09 - 2.21), multiple liver metastases (p = 0.016; HR 1.46; 95% CI 1.07 - 1.98), and male sex (p = 0.035; HR 1.44; 95% CI 1.03 - 2.03). Multivariable analyses of OS and DFS are shown in **Table 6**.

Figure 5. Consort diagram

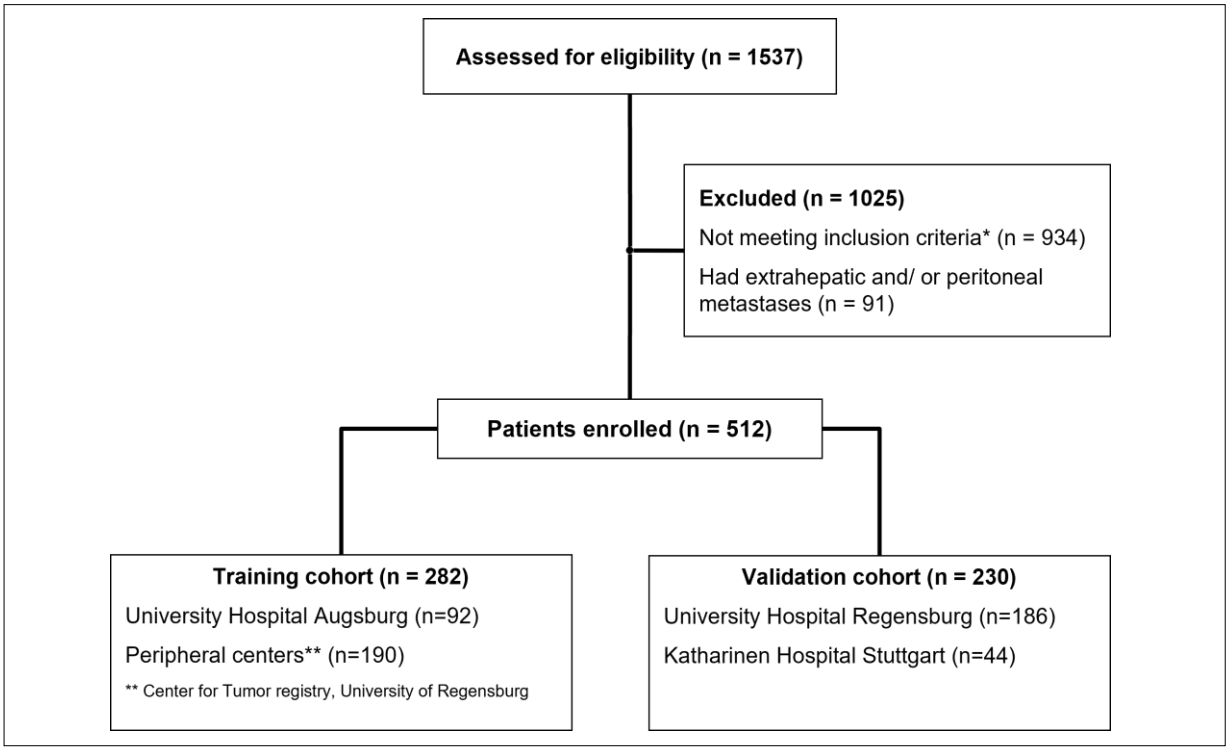


Figure 5. Consort diagram of patients enrolled in the study.
*Patients with liver metastases from CRC not treated in curative intention with surgical resection of liver metastases (e.g., only RFA, only chemotherapy), documented to die from postoperative complications (not from tumor progression), postoperative histological diagnosis other than adenocarcinoma of the colon (NET, GIST, SCC), patients with FAP, incomplete data for CRP values, CRP values older than 30 days before liver surgery, concurrent infectious disease or inflammation due to complications of tumor progression, extrahepatic metastases, diffuse peritoneal metastases.

Table 4. Patients characteristics

Variables		Training (n=282)	Validation (n=230)	Total (%) (n=512)	P value
Sex	Female	92 (32.6 %)	67 (29.1 %)	159 (31.1 %)	0.451
	Male	190 (67.4 %)	163 (70.9 %)	353 (68.9 %)	
Median age at time of surgery (range)		68y (31-89)	65y (27-88)	66y (27-89)	<0.001
Age at time of surgery	<72 years	196 (69.5 %)	179 (77.8 %)	375 (73.2 %)	0.044
	>72 years	86 (30.5 %)	51 (22.2 %)	137 (26.8 %)	
Inflammatory response to tumor (IRT)	No IRT	217 (77.0 %)	181 (78.7 %)	398 (77.7 %)	0.715
	IRT	65 (23.0 %)	49 (21.3 %)	114 (22.3 %)	
Primary tumor side	Left	215 (76.2 %)	164 (71.3 %)	379 (74.0 %)	0.244
	Right	67 (23.8 %)	66 (28.7 %)	133 (26.0 %)	
Median number of liver metastases		1 (1-9)	2 (1-14)	2 (1-14)	0.009
Solitary vs multiple liver metastases	Solitary	146 (51.8 %)	96 (41.7 %)	242 (47.3 %)	0.041
	Multiple	136 (48.2 %)	131 (57.0 %)	267 (52.1 %)	
	Missing data	0 (0.0 %)	3 (1.3 %)	3 (0.6 %)	
Node positive primary tumor	Negative	94 (33.3 %)	74 (32.2 %)	168 (32.8 %)	0.816
	Positive	179 (63.5 %)	150 (65.2 %)	329 (64.3 %)	
	Missing data	9 (3.2 %)	6 (2.6 %)	15 (2.9 %)	
Synchronous vs metachronous disease	Metachronous	110 (39.0 %)	97 (42.2 %)	207 (40.4 %)	0.525
	Synchronous	172 (61.0 %)	133 (57.8 %)	305 (59.6 %)	
KRAS	Wildtype	91 (32.3 %)	45 (19.6 %)	136 (26.6 %)	0.875
	Mutated	44 (15.6 %)	24 (10.4 %)	68 (13.3 %)	
	Missing data	147 (52.1 %)	161 (70.0 %)	308 (60.2 %)	
Chemotherapy (Ctx)					
Preoperative	Yes	88 (31.2%)	81 (35.2%)	169 (33.0%)	0.247
	No	191 (67.7%)	139 (60.4%)	330 (64.5%)	
	Missing data	3 (1.1%)	10 (4.3%)	13 (2.5%)	
Postoperative	Yes	142 (50.4%)	117 (50.9%)	259 (50.6%)	0.641
	No	120 (42.6%)	90 (39.1%)	210 (41%)	
	Missing data	20 (7.1%)	23 (10%)	43 (8.4%)	
Preoperative or postoperative (Perioperative Ctx)	-Yes (p*re/ post or both)*	184 (65.2%)	162 (70.4%)	346 (67.6%)	0.187
	-No Ctx (pre and post)	86 (30.5%)	55 (23.9%)	141 (27.5%)	
	-Missing data	12 (4.3%)	13 (5.5%)	25 (4.9%)	
Resection margin status	R0	227 (80.5 %)	184 (80.0 %)	411 (80.3 %)	0.336
	R1	25 (8.9 %)	28 (12.2 %)	53 (10.4 %)	
	Missing data	30 (10.6 %)	18 (7.8 %)	48 (9.4 %)	

Note: Variables with significant differences between training and validation cohorts were age at time of surgery and number of liver metastases.

Table 5. Univariable analyses of OS and DFS

Variables	Significance (log rank)	
	Overall survival	**Disease-free survival**
Female vs male sex	0.225	0.016
Age at time of surgery (> 72 years)	< 0.001	0.400
Inflammatory response to tumor (IRT)	<0.001	< 0.001
Left vs right-sided primary tumor	0.015	0.016
Solitary vs Multiple Metastases	< 0.001	0.005
Negative vs positive nodal status (primary tumor)	0.021	0.149
Metachronous vs synchronous disease	0.014	0.143
Perioperative chemotherapy (no vs yes)	0.558	0.398
Resection margin status (R0 vs R1)	0.082	0.003
KRAS-mutated (no vs yes)	0.055	0.016

Note: Preoperative variables with p-value <0.15 were included in a multivariable model.

Table 6. Multivariable analyses of OS and DFS

Variables	Overall survival		Disease-free survival	
	Significance	Hazard ratio (CI 95%)	Significance	Hazard ratio (CI 95%)
Inflammatory response to the tumor	**< 0.001**	**1.92 (1.35 - 2.75)**	0.002	1.74 (1.23 - 2.47)
Right-sided primary tumor	**0.008**	**1.63 (1.14 - 2.34)**	0.014	1.56 (1.09 - 2.21)
Solitary vs multiple liver metastases	**< 0.001**	**1.75 (1.27 - 2.42)**	0.016	1.46 (1.07 - 1.98)
Node positive primary tumor	**0.026**	**1.49 (1.05 - 2.13)**	---	---
Age at time of therapy (> 72y)	0.001	1.72 (1.24 - 2.44)	---	---
Male sex	---	---	0.035	1.44 (1.03 - 2.03)

Note: Inflammatory response to tumor, right-sided primary tumor, multiple metastases (>1) and node positive primary tumor were variables that composed the preoperative risk score.

4.4. Predictive score for patients undergoing local treatment in oligometastatic CRC

Four variables emerged as independent predictors of OS in multivariable analyses: IRT (HR 1.92), right-sided primary tumor (HR 1.63), multiple liver metastases (HR 1.75), and node-positive primary tumor (HR 1.49). These factors were incorporated into a scoring system to predict patient outcomes. Age, while significant, was excluded because it was not considered a cancer-specific risk factor. All variables that were significant for OS, except for node-positive primary tumor and age, were also significant for DFS. Male sex was only significant for DFS.

Each risk factor identified in the analysis contributed 1 point to the scoring system when present. Differential weighting of individual factors was not considered necessary, because all of them shared comparable HRs ranging between 1.49 and 1.92. This model of patient stratification according to the cumulative number of their positive risk factors resulted in five distinct risk groups (0, 1, 2, 3, and 4 risk factors).

Based on these risk factors, analysis of the entire cohort (n=512) revealed the following distribution across these risk groups: in the TC, 35 patients and in the VC, 29 patients had 0 risk factors (12.5% of the total cohort). Ninety-two patients in the TC and 60 in the VC had 1 risk factor (30% of the entire cohort). Ninety-six patients in the TC and 80 in the VC had 2 risk factors (34% of the entire cohort). Forty-five patients in the TC and 45 in the VC had 3 risk factors (17% of the entire cohort). The number of patients presenting all 4 risk factors was low (5 and 7 in the TC and VC, respectively; representing < 5% of the entire cohort).

Kaplan-Meier survival analyses based on our score significantly distinguished OS ($p < 0.0001$) between all risk groups in both cohorts (TC and VC). The median OS for the lowest risk group (0 risk factors) in the TC was 133.8 months [95% CI 81.2 months - not reached (nr)] and was not reached in the VC (95% CI 95.2 months - nr). The highest risk group (all four risk factors present) had a median OS of 14.3 months (95% CI 10.5 months - nr) in the TC and 16.6 months (95% CI 14.6 months - nr) in the VC **(Figure 6, Table 7)**.

Regarding the impact of the score on DFS **(shown in figure 7 and table 8)**, median DFS in the group of patients without risk factors was not reached (CI 95% 22.1 – nr) in the TC and 80.2 months (CI 95% 60.0 – nr) in the VC. In contrast, median DFS in the groups of patients with all four risk factors was 9.3 months (CI 95% 4.2 - nr) in the TC and 3.7 months (CI 95% 2.9 – nr) in the VC.

Figure 6. Kaplan-Meier analyses of overall survival

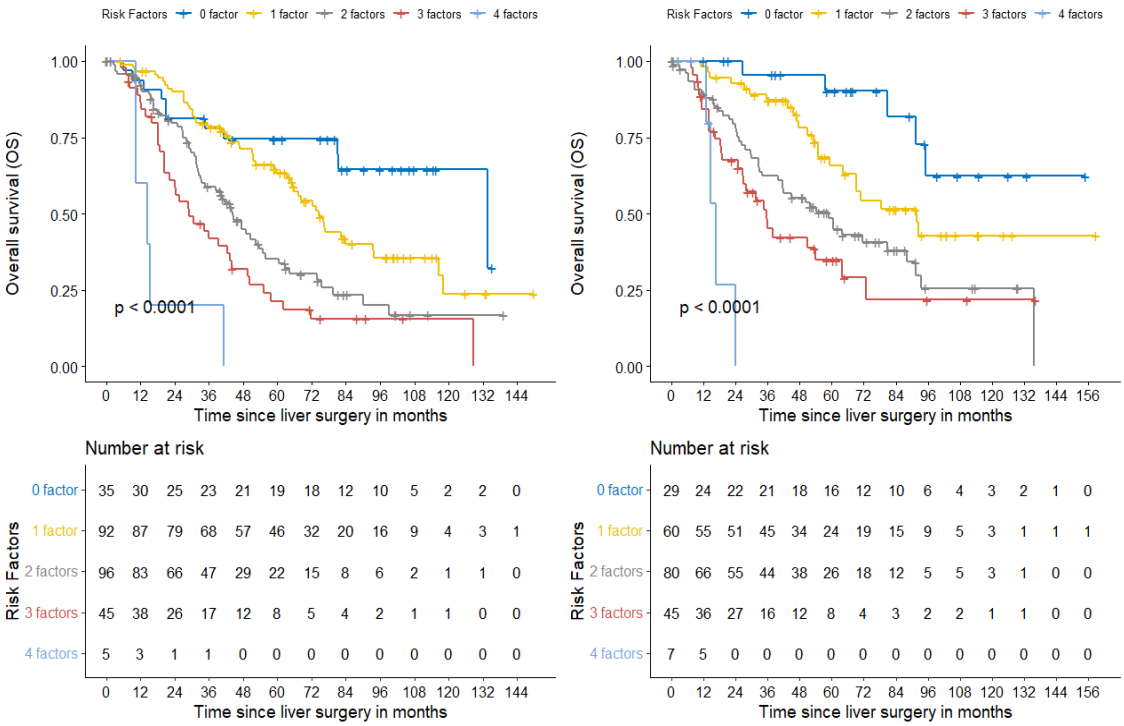


Figure 6. Stratification of patients according to the number of risk factors. Overall survival for the training (left) and validation cohort (right) are shown.

Table 7. Predictive preoperative score for oligometastatic colorectal cancer

Risk group (Definition)	Number of patients (Training / Validation)	Median OS in months (p < 0.0001)	
		Training (CI 95%)	Validation (CI 95%)
0 risk factors	35 / 29	133.8 (81.2 - nr)	Not reached (95.2 - nr)
1 risk factor	92 / 60	74.4 (65.3 - 93.7)	91.6 (69.0 - nr)
2 risk factors	96 / 80	44.4 (34.7 - 54.9)	58.8 (41.5 - 91.4)
3 risk factors	45 / 45	29.0 (22.1 - 44.0)	35.7 (26.8 - 72.7)
4 risk factors	5 / 7	14.3 (10.5 - nr)	16.6 (14.6 - nr)

Figure 7. Kaplan-Meier analyses of disease-free survival

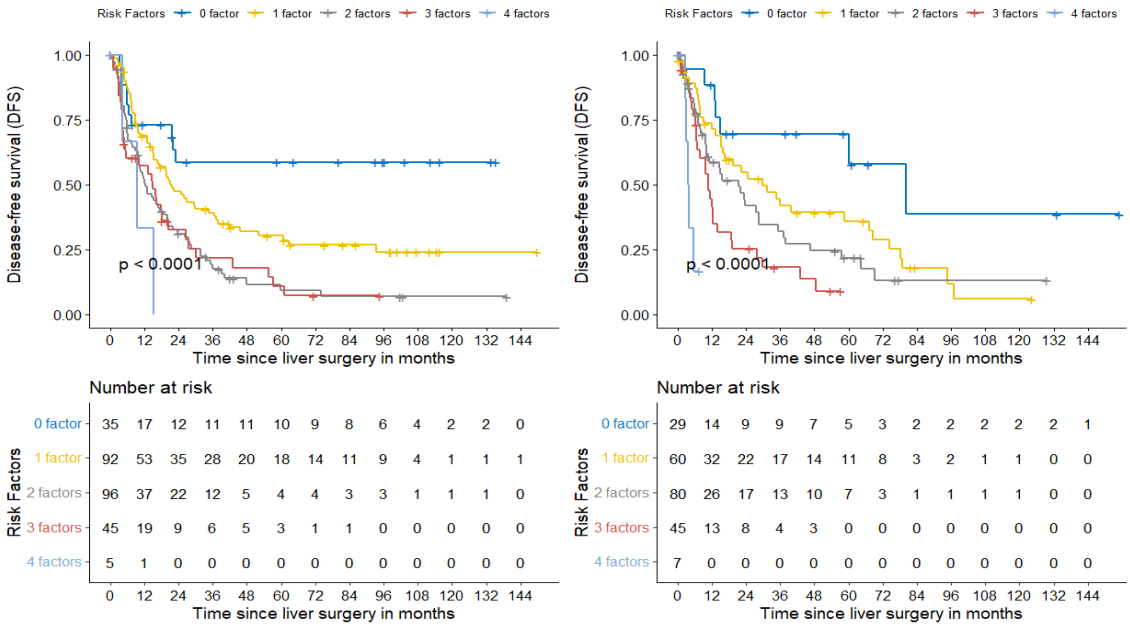


Figure 7. Stratification of patients according to the number of risk factors. DFS for the training cohort (left) and validation cohort (right) are shown.

Table 8. Predictive preoperative score for oligometastatic colorectal cancer

Risk group (Definition)	Number of patients (Training / Validation)	Median DFS (months) (p <0.0001)	
		Training (CI 95%)	Validation (CI 95%)
0 risk factors	35 / 29	Not reached (22.1 - nr)	80.2 (60.0 - nr)
1 risk factor	92 / 60	21.7 (15.1 - 37.2)	29.7 (15.9 - 68.4)
2 risk factors	96 / 80	12.4 (10.1 - 20.2)	21.5 (10.0 - 35.2)
3 risk factors	45 / 45	15.0 (5.3 - 26.7)	10.7 (6.7 - 18.7)
4 risk factors	5 / 7	9.3 (4.2 - nr)	3.7 (2.9 - nr)

We then compared our score with the previously published system by Malik et al. (139) Patients in the validation set (VC) were stratified into three risk groups (score 0, 1, and 2) based on the Malik system's criteria (presence of inflammatory response and number of metastases > 8). This allowed for a direct comparison of survival outcomes between the two scoring methods. The analysis revealed that our novel scoring system identified a subgroup of patients within the Malik system's low-risk category (no inflammation and less than eight

metastases) with a significantly improved overall survival (OS). Overall survival (OS) for the lowest risk group (absence of IRT and < 8 metastases) was 67.3 months (95% CI 57.0 - 77.6 months) **(Figure 8).** This suggests that our system may offer a more refined risk stratification within this particular patient group.

It's important to note that the presence of 8 or more metastases was uncommon in our study, resulting in a very small group of patients assigned to the Malik system's highest risk category. To address censored data and ensure sufficient sample size for statistical analysis of performance calculation between both scores, we categorized patients into high-risk and low-risk groups based on our own scoring system and excluded those with less than 12 months of follow-up.

In this analysis, our scoring system achieved a Harrell's c-index of 0.676 for predicting OS, compared to 0.616 for the Malik score when applied to our patient cohort. The higher c-index indicates better discrimination between patients with different prognoses.

Figure 8. Comparison with another score

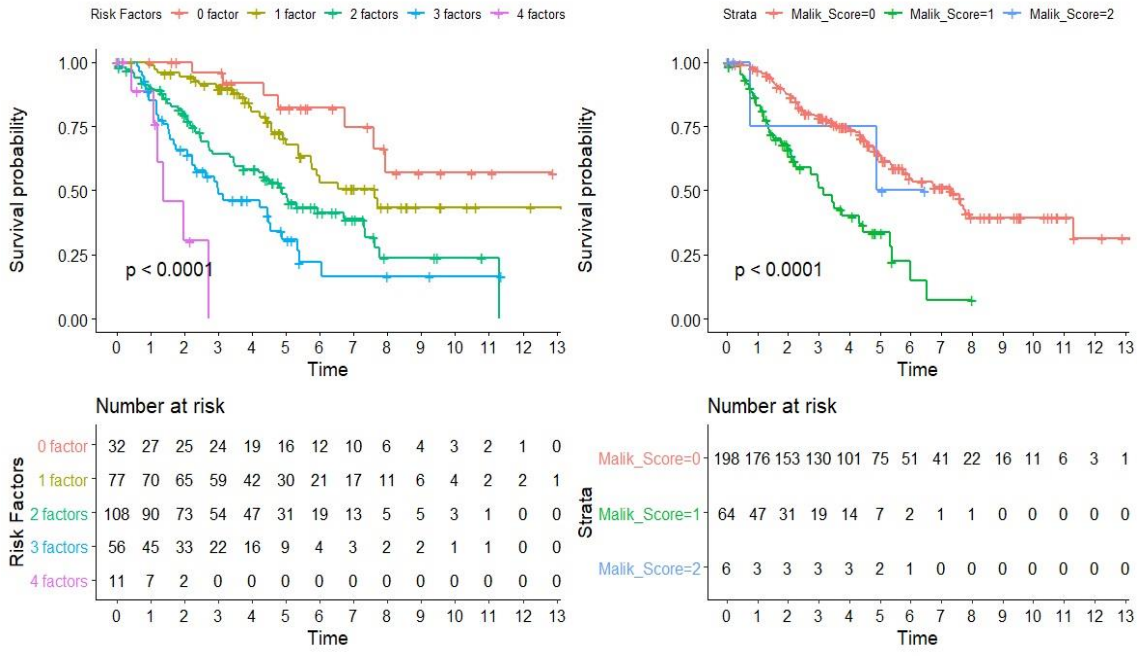


Figure 8. Direct comparison of the preoperative clinical score to the score developed by Malik et al.

5. Discussion

This multicenter, retrospective analysis investigated the clinical characteristics and survival outcomes of 512 patients with colorectal cancer (CRC) who underwent curative-intended surgical resection of liver-only metastases. The study included patients diagnosed with de novo liver metastases at 16 German hospitals between 2006 and 2016. The median patient age was 66 years, and 47% presented with solitary liver metastases. Preoperative and postoperative chemotherapy were administered to 33% and 50% of patients, respectively. With a median follow-up of 81 months (almost 7 years), the median OS for the entire cohort was 60 months (5 years). The 5-year survival rate was 33%.

To define the oligometastatic stage of cancer and predict which patients will benefit most likely from surgical resection of metastases, predictive biomarkers are essential. In this study, we identified preoperative cancer-specific risk factors for survival in patients that had undergone surgical resection of liver metastases from CRC and developed a predictive preoperative clinical score. We identified a training cohort (TC) and validated the score in a separate validation cohort (VC). There were differences regarding age and number of liver metastases. The VC included younger patients (median age 65 vs 68 years), and patients with higher number of liver metastases (median number 2 vs 1). There might have been an interaction between these two variables, since the VC included patients treated more recently, potentially reflecting a shift towards operating on younger patients with more metastases in recent years (median year of liver resection 2011 vs 2010). The VC also comprised patients from an academic institute, which might be more inclined to perform complex surgeries like liver resections as centre of reference (which otherwise wouldn´t have been performed at peripheral institutions). It's important to note that these baseline characteristic differences did not negatively impact survival in the VC vs the TC. In fact, patients in the VC exhibited better OS compared to the TC.

In multivariable analyses, we identified four significant risk factors influencing OS. Two risk factors: presence of an IRT and right-sided primary tumor are linked to the biology of the disease, while solitary versus multiple liver metastases and node-positive primary tumor additionally deliver information on the dynamics of the disease and evolution in time.

While age at the time of liver surgery emerged as a significant factor influencig OS in multivariable analyses, it did not impact DFS. This suggests that age is not a cancer-specific risk factor in this context. Consequently, age was excluded from the scoring system to maintain its focus on tumor-related characteristics. Additionally, the clinical relevance and validity of the score could be affected by patient age, as differences often exist between chronological age and biological age. It remains uncertain whether age would remain a significant risk factor after adjusting for life expectancy. Therefore, considering age at the time of diagnosis could

introduce bias and limit the applicability of the results to younger or healthier patient populations.

We examined the impact of the identified significant risk factors on OS and DFS. However, the focus was set mainly on OS as the key outcome of interest given that one variable (node-positive primary tumor) was significant for OS but did not achieve significance for DFS, possibly due to a type II error. Additionally, OS is generally considered the most important endpoint in oncological studies, reflecting long-term patient outcomes. Nevertheless, DFS was significantly influenced by other identified risk factors that were significant for OS.

Based on previous evidence (described in the introduction), we assume that the tumor´s ability to invade and metastasize is also influenced by the diversity of the TME and activity of the stromal and tumor-associated immune cells alongside the inherent biology of the tumor. The complex interplay between tumor cells and the host generates a chronic inflammation, hence an inflammatory response to the tumor, which induces genomic instability within tumor cells, promoting their evolution. This environment creates favorable conditions for tumor growth, invasive potential, and angiogenesis, ultimately favoring neoplastic spread and metastasis. (119, 120, 145-147) Conversely, research has shown that high levels of T-cell infiltration were predictive of better outcome in CRC. (148, 149) These findings highlight the critical role of the TME in in cancer development and metastasis.

Our research, along with others, (139, 150) suggests that the proinflammatory immune response to the tumor triggers a systemic inflammatory response detectable by measuring serum CRP. CRP serves as a readily available and established systemic marker of inflammation. It is an acute-phase protein synthesized by the liver in response to pro-inflammatory cytokines, such as interleukin-1 (IL-1) and interleukin-6 (IL-6). CRP levels exhibit a rapid increase during inflammatory episodes and a subsequent decline upon resolution of the inflammatory stimulus. These characteristics make CRP a suitable biomarker for monitoring ongoing tumor activity. (151, 152) Several studies have demonstrated the prognostic value of CRP in gastrointestinal malignancies, including CRC. (153-157) Other potential inflammatory biomarkers exist, such as the neutrophil-to-lymphocytes ratio, specific cytokines (e.g. tumor necrosis factor-alfa, IL-6, IL-1B, CCL2), as well as the quantification of lymphocytes subpopulations in the tumor (Immunoscore). (158) CRP offers the distintic advantage of being easily accessible. CRP levels are routinely assessed as part of standard blood tests and are readily available in most patients' medical electronical records. This facilitates the practical integration of CRP into a clinical scoring system. The specific cut-off value of 1 mg/dl was established based on a prior study addressing a similar scientific question (139). This value aligns with cut-off points employed in other published CRC studies (155, 156)

Extensive clinical data suggests that tumors originating from the right and left sides of the colon exhibit distinct clinical and molecular profiles. Right-sided tumors are more frequently associated with mutations in *RAS* and *BRAF* genes. However, we were unable to perform a comprehensive analysis of these mutations due to limited data availability for a subset of our patients. Consequently, the observed association between *KRAS* mutation and survival in univariate analysis might be underestimated due to incomplete data.

The four risk factors that showed significance for shorter OS in multivariate analyses were selected to construct a risk model. Patients were categorized into one of five risk groups based on their cumulative number of risk factors. This clinical score identified a subset of patients without any risk factors who exhibited very prolonged survival rates following surgical treatment of liver metastases (median OS > 11 years). Most importantly, this finding suggests that this group of patients (comprising 13% of the entire cohort) likely represents the true oligometastatic stage of CRC, characterized by a high potential for cure with surgical resection of liver metastases.

The scoring system also identified another risk group with a favorable prognosis, characterized by the presence of only one risk factor, yet showing prolonged survival rates despite some experiencing metastatic recurrence (median OS > 7 years, median DFS 30 months in the VC, respectively). This group of patients (30% of the entire cohort) clinically represent the states of *oligorecurrence* or *oligoprogression*. These scenarios imply a noncurable stage of tumor disease of limited metastatic potential, making them likewise suitable for surgical resection of metastases with or without additional systemic therapy achieving prolonged disease-free intervals. (112, 159)

Thus, in this large cohort of patients with hepatic metastases from CRC, we identified a favorable risk group (patients in the risk groups with 0 – 1 risk factors) that comprised more than one-third (43%) of the entire studied population. This substantial proportion of patients exhibited significantly prolonged long-term survival (median OS for both groups combined at least > 6 years) despite having a stage IV disease. The clinical course of this group of patients aligns with the definition of oligometastatic disease.

By contrast, the group of patients carrying a high number of risk factors (3-4) had a significantly shorter OS ($p < 0.0001$) compared to those with fewer risk factors. The median OS in the group of patients with 3 risk factors was < 36 months in both, the TC and the VC. Patients with all four risk factors had a very poor outcome, with only one patient alive 24 months after surgery in the VC. These findings suggest that patients with a high-risk score (3-4 risk factors) likely have tumors that represent a stage with biological and clinical characteristics beyond the oligometastatic state of cancer. These patients have already an advanced disease and probably are not good candidates for surgical resection in curable intention because of evident

non-oligometastatic situation. However, withholding surgery from this group of patients remains debatable due to limited alternative therapies. These ‘very-high-risk’ patients might benefit from participation in clinical trials exploring intensive combined pre- and postoperative chemotherapy regimens, optionally incorporating less invasive ablative techniques. (114)

Altoghether, this study demonstrates that our clinical scoring system score was able to effectively stratify patients in distinct risk groups based on readily available factors. This stratification provides valuable prognostic information and may be useful for decision making in daily clinical practice. However, it's important to acknowledge that incorporating biological and molecular characteristics of both the tumor and the patient will be a key step in the next future for refining this scoring system to further improve risk stratification and guide treatment personalization for patients with oligometastatic CRC.

We compared the performance of our novel scoring system with a previously published system for predicting survival after surgical resection of liver metastases from CRC which includes IRT and number of metastases as risk factors (139). Our system incorporates additional risk factors allowing for a better discrimination of patient subgroups. This is evident in our ability to distinguish a subgroup of IRT-negative patients, showing a remarkably longer OS in a direct comparison compared to the Malik et al. score, rendering our score with better performance according to Harrell´s c-index.

Similarly, Dupré et al. (150) published the ‘Liverpool score’ for CRC liver metastases treated in curative intention. Their score identifies risk groups based on four preoperative variables that overlap with factors included in our system. Notably, both scores acknowledge the importance of IRT, although defined using different methods (Dupré et al. use the neutrophil-to-lymphocyte ratio). This overlap highlights the relevance of these shared risk factors in predicting survival outcomes for CRC patients with liver metastases. A key strength of our scoring system lies in its ability to effectively stratify patients into distinct risk groups using a limited number of readily available clinical variables.

Two recent studies, namely the 'Metro ticket' and the 'GAME' score, have demonstrated valuable strategies in the current era of liver metastases treatment. Our approach enhances its potential for practical application in daily clinical practice compared to these scoring systems, since the practical use of these effective scores may be limited because some of the variables they incorporate are not readily accessible in routine clinical practice. (140, 141) Direct comparison with these scores was not feasible due to the unavailability of certain variables in our records.

Pitroda et al., (107) integrated RNA-based molecular subtyping with Fong's Clinical Risk Score (137) and also identified a subgroup of patients with metastatic CRC that clinically performed in concordance with a potentially curable oligometastatic state. These tumors were distinguished by gene signatures that correlated histologically with high levels of T-cell infiltrations, (113) which aligns with the findings of Galon et al. (who proposed the pathological-based Immunoscore). (148)

Here, we identified and validated four clinical risk factors relevant to oligometastatic CRC, two of which are linked to biological aspects implicated in the origin of oligometastases. In fact, considering IRT as significant predictive factor, we believe that our study, building on the work of Malik et al., (139) represents an important step toward identifiying specific biomarkers that help to precisely define this state of disease.

We emphasize on the practicability of our score, as it incorporates variables that are routinely available in clinical practice. Additionally, we highlight its robustness, supported by a multicenter analysis that include both high- and low-volume centers, as well as the successful reproduction of the score across heterogeneous cohorts.

Limiting factors include the retrospective nature of this study, the heterogeneity in patient's characteristics, and the number of cases censored from the analysis due to lost of follow up. It is also acknolewdgeable that the sensitivity of radiographic imaging has improved over time during the long time period of patient enrolment, potentially resulting in additional metastases being undetected by less sensitive CT scans in the earlier years of the study.

Our study employed a well-established cut-off value for CRP to define the presence of IRT. However, previous research suggests that this threshold might vary across patient populations and may not be universally applicable. (157) This variability could introduce bias in the definition of IRT. Additionally, CRP is a nonspecific marker of inflammation, and its value can be influenced by other causes of inflammation beyond the tumor itself, such as chemotherapy, obesity, smoking, or coronary heart disease, (157) variables for which this study have not adjusted.

A final potential limitation to consider is the impact of chemotherapy on the relapse incidence and long term outcome of patients. Perioperative chemo-therapy regimens in our study were diverse and tailored based on factors like synchronous/metachronous disease, age, performance status, comorbidities, and patient preferences. Patients with a higher risk of disease progression, indicated by nodal status, tumor size, and number of metastases were likely to receive more intensive chemotherapy, introducing a potential confounding factor. Despite these limitations, perioperative chemotherapy did not achieve statistical significance

in either univariable or multivariable analyses. Nevertheless, due to the retrospective nature of the study, this finding should be interpreted with caution.

Taken together, our preoperative clinical score includes variables that reflect biological aspects of the disease and are easily obtainable from medical records. The score is easy to apply and shows potential to be implemented in daily clinical practice for the identification of patients in the oligometastatic state of CRC who are likely to considerably benefit from local treatment.

6. Summary

We conducted a retrospective multicentre analysis on the outcome of patients with liver metastases from CRC that had undergone a surgical resection of their metastases in curative intention. In this study, 512 patients from 16 different hospitals were included.

In multivariable analyses of clinical variables collected from clinical records, we identified 4 significant risk factors for shorter overall survival: 1. inflammatory response to the tumor (defined as a CRP value of 1mg/dl or higher), 2. Multiple liver metastases (number of metastases >1), 3. Right sided primary tumor and 4. Node positive primary tumor. These variables were integrated into a preoperative score to predict the survival rates after resection of liver metastases. Each factor added 1 point to the score when present.

Using Kaplan-Meier analyses we calculated the survival of patients according to the cumulative score given by the presence or absence of the selected risk factors. We emphasized on OS, as this is the key endpoint in oncological studies. The score identified 5 well differentiated risk groups and, as a main finding, we observed a notably good outcome in patients that presented none or only 1 risk factor (altogether exhibiting and OS > 6 years). The clinical course of these patients (> 40% of the entire cohort) is consistent with the oligometastatic state of cancer, which is defined as an intermediate stage within the spectrum of a localized and a widespread metastatic disease, harbouring potential for curative treatment.

The score was first developed in a training cohort and thereafter validated in an independent validation cohort. Furthermore, we compared our score with two previously published scores that used similar clinical variables and conclude that our score is advantageous in the identificacion of patients with a very good prognosis.

Including easily obtainable variables, this preoperative score identifies oligometastatic CRC patients with prolonged survival rates that may be cured, and harbors potential to be implemented in daily clinical practice.

7. Outlook

The field of oncology is rapidly evolving, this is evident by current advances in molecular diagnostics and the emergence of new generation treatment options for solid tumors, such as immunotherapies, in particular for metastatic CRC.

To achieve more effective and less toxic treatments for our patients, in a near future, clinical scoring systems, like the one presented here, should integrate biological aspects of the tumor and potentially the patient´s host immune response. This comprehensive approach has the potential to guide treatment selection towards more personalized oncological treatment strategies.

Prospective validation of our scoring system is crucial for confirming its effectiveness in a controlled setting. Indeed, prospective trials addressing the treatment of patients with oligometastatic CRC are scarce. Future research should prioritize interventional trials considering biological factors and incorporating omics technologies for more holistic analysis. In line with these future directions, our research group is actively designing a clinical trial that explores the implementation of liquid biopsies as a novel tool for molecular diagnostics. This approach is intended to improve survival prediction and treatment outcomes in patients with oligometastatic CRC.

8. Summary in German language (Zusammenfassung)

Wir führten eine retrospektive multizentrische Analyse bei Patienten mit Lebermetastasen bei kolorektalem Karzinom (KRK) durch, die sich in kurativer Absicht einer chirurgischen Resektion ihrer Metastasen unterzogen hatten. In diese Studie wurden 512 Patienten aus 16 verschiedenen Krankenhäusern eingeschlossen.

In einer multivariablen Analyse von klinischer Variablen aus Datenakten, haben wir vier signifikante Risikofaktoren für ein kürzeres Gesamtüberleben identifiziert: 1. Inflammatorische Reaktion auf den Tumor (definiert als ein CRP-Wert von 1 mg/dl oder höher), 2. Multiple Lebermetastasen (Anzahl von Metastasen > 1), 3. Rechtsseitiger Primärtumor und 4. Lymphknotenpositiver Primärtumor. Diese Variablen wurden in einen präoperativen Score integriert, um die Überlebensraten der Patienten nach Resektion der Lebermetastasen vorherzusagen. Jeder Faktor erhöhte die Punktzahl um 1 Punkt, wenn er vorhanden war.

Mithilfe von Kaplan-Meier-Analysen haben wir das Überleben der Patienten anhand des kumulativen Scores berechnet, der sich aus dem Vorhandensein oder Fehlen der ausgewählten Risikofaktoren ergibt. Der Score identifizierte fünf gut differenzierte Risikogruppen, aber vor allem wir beobachteten ein besonders guter Outcome bei Patienten,

die keinen oder nur einen Risikofaktor aufwiesen (OS > 6 Jahre). Der klinische Verlauf dieser Patienten (> 40 % der gesamten Kohorte) stimmte mit dem oligometastasierten Krebsstadium überein, das als Zwischenstadium innerhalb des Spektrums einer lokalisierten und weit verbreiteten metastasierten Erkrankung definiert wird.

Der Score wurde zunächst in einer Trainingskohorte entwickelt und anschließend in einer unabhängigen Validierungskohorte validiert. Darüber hinaus verglichen wir unseren Score mit zwei zuvor veröffentlichten Scores, die ähnliche klinische Variablen verwendeten, und kamen zu dem Schluss, dass unser Score bei der Identifizierung von Patienten mit einer sehr guten Prognose von Vorteil ist.

Unter Einbeziehung leicht erhältlicher Variablen identifiziert dieser präoperative Score oligometastatische KRK-Patienten mit verlängerten Überlebensraten, die geheilt werden können, und birgt Potenzial für die Umsetzung in die tägliche klinische Praxis.

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Appendix

I. Abbreviations

CRC colorectal cancer

mCRC metastatic colorectal cancer

CR complete remission

ECM extracellular matrix

PR partial remission

HNPCC Hereditary nonpolyposis colon cancer

MMPs metaloproteinases

MMR mismatch repair

dMMR deficient mismatch repair

CEA carcinoembryonic antigen

CT computer tomography

MRI magnetic resonance imaging

FDG-PET Fluorodeoxyglucose positron emission tomography

UICC Union for International Cancer Control

TNM tumour, node, metastasis

TNF tumor necrosis factor

ctDNA circulating tumor DNA

OS overall survival

DFS disease-free survival

MSI microsatellite instable

MSI-H microsatellite instable- high

CMS consensus molecular subtypes

ECOG Eastern Cooperative Oncology Group performance status

ESMO European Society for Medical Oncology

EGFR epidermal growth factor receptor

VEGF-A vascular-epidermal growth factor A

MMP metaloproteinases

HER2 human epidermal growth factor receptor 2

DPD Dihydropyrimidine dehydrogenase

FISH fluorescence in-situ hybridisation

mAbs monoclonal antibodies

ICB immunce checkpoint blockade

5-FU 5- fluorouracil

FOLFOX 5-fluorouracil in combination with oxaliplatin and folinic acid

CAPOX capecitabine in combination with oxaliplatin

FOLFIRI 5-fluorouracil in combination with irinotecan and folinic acid

FOLFOXIRI 5-fluorouracil in combination with oxaliplatin, irinotecan and folinic acid

wt wild type

PCR polymerase chain reaction

IHC immunohistochemistry

iNOS inducible nitric oxide synthase

NO nitric oxide

NGS next generation sequencing

QoL quality of life

BSC best supportive care

PD-1 program death ligand 1

LT local treatment

TA thermal ablation

SBRT stereotactic body radiotherapy

HIPEC hyperthermic intraperitoneal chemotherapy

TACE transarterial chemoembolisation

TARE transarterial radioembolisation

SIRT selective internal radiotherapy

SNP single nucleotide polymorphisms

CRP C-reactive protein

IFN interferon

IRT inflammatory response to the tumor

TC training cohort

VC validation cohort

HR hazard ratio

CI confidence interval

IL interleukin

uPA Urokinase plasminogen activator

II. Figures

III. Tables

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