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RESEARCH PAPER

Meeting the biologic challenge of colorectal metastases

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Abstract An overview of colorectal cancer discussed (Philip Paty) the good outcome after primary management with local control in 90–95 % of colon and 85 % in rectal cancer patients with major progression to metastases and to death related to hematogenous dissemination. The major disease pathways include the APC, aneuploid pathway involving mutations of P53, KRAS, SMAD 4, or the CMP/MSI pathway, mismatched repair defect as characterized by Lynch syndrome, the major hereditary form which may also have KRAS and P53 mutations. The common sporadic colorectal cancers are MS1 high, with many patients having BRAF and KRAS mutations. The sentinel node biopsy in colorectal cancer surgery may provide more definitive staging and perhaps modification of the extent of resection with better outcome as suggested by Dr. Saha. The identification of sentinel lymph nodes outside of the planned bowel resection may increase the resection biologically indicated by the sentinel lymph node location leading to better outcome. In a small study by Dr. Saha, the operation was enhanced in 21 % by extending the length of bowel resection, which increased node recovery to 18.5 nodes versus 12 nodes with the more conventional resection, increasing nodal recovery, and positivity to 60 % with reduction to five year recurrence rate to 9 % versus 27 % with the conventional resection. A new (Swiss) technique for pathologic node examination, the OSNA (the One Step Nucleic Acid diagnostic system), was presented which demonstrated increased detection of micro-metastases in a focused pathology study of 22 patients (Zuber) to 11 out of 15 patients versus the 7 micro-metastases identified by the standard single slide per node, and compared to 14 out of 15 with an intensive multi-slide technique. This suggests value in pursuing OSNA study by other centers with relevant clinical trials to establish its true value. An analysis of liver resection for metastatic colorectal cancer (CRC) emphasized the value of 10-year follow-up (DeAngelica). The 10-year survival of 102 patients among 612 patients was 17 % (Memorial Sloan Kettering data). At the five-year point 99 of 102 survivors were NED and 86 have been free of disease since the resection. The usual five-year figure after hepatic resection reveals that one-third of five-year survivors die from recurrence of distant disease suggesting the value of longer term follow-up in these patients. An

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additional question reviewed related to the role of neoadjuvant systemic chemotherapy (with response rates in the 50 % range) to produce down staging of the hepatic metastases and allow one to retrieve these patients with possible residual disease. In a series of 116 patients who had hepatic resection of CRC metastases in presence of regional node metastases, post neoadjuvant chemotherapy (normally not candidates for resection) these patients were demonstrated to have a 95 % recurrence at median time of 9 months. This raises a cautionary note to the literature report of five-year survivals in the 20–30 % range for hepatic metastases in presence of extra hepatic disease. Such may reflect patient selection rather than a true measure of the biology of disease, and warrant clinical trial evaluation. Lastly, regional therapy and overall systemic therapy were addressed by Dr. Kemeny. The CALGB study of hepatic artery infusion (HAI) with FUDR, dexamethasone versus 5FU leucovorin showed an overall survival of 24.4 months with HAI versus 20 months with systemic therapy ($P = 0.0034$). An adjuvant trial of HAI at MSK in 156 patients showed an overall survival benefit at 2 year and recent long term 10yr follow-up showing a significant overall survival of 41 % with HAI versus 27 % with systemic therapy (5FU leucovorin). In the neoadjuvant Nordlinger trial for hepatic metastases, there was a significant outcome differences—the preoperative therapy group had 9.2 % increase of progression free survival versus the surgery alone group which suggests the value of combining neoadjuvant surgery in good risk liver resection candidates. Conclude the final lesson from this well presented mini symposium confirms the need for continued evaluation of the numerous discussion points by clinical trial.

Keywords Colorectal cancer · Signaling pathways · Sentinel lymph nodes liver metastasis · Regional therapy

Meeting the biologic challenge of colorectal metastases

Introduction: Harold J. Wanebo, MD, FACS

Overview—Introduction

This section presented an overview of the biologic challenges involving colorectal cancer metastases and includes the discussion of the origin and pathways of metastasizing colorectal cancer (Philip Paty). The sentinel node mapping in colon cancer (Sukamal Saha), the molecular investigation of the nodes in sentinel node metastases (Mark Zuber), and finishes with selecting the optimum surgical approach to liver metastases (Michael D'Angelica) and the Overall Therapies for liver metastases including regional therapy (Nancy Kemeny).

This session provided an overview of the major management approaches for metastatic colorectal cancer with emphasis on lymph node and liver metastases as a testable site for therapies and biology. In the overview for colorectal cancer it was emphasized by Dr. Paty that the local treatment is very good in achieving control in 90–95 % of resectable colon cancers and 85 % of resectable rectal cancers but emphasized that the progression to death is significantly related to distant hematogenous spread of the preceding peritoneal or invasion by lymphatic metastases. There are numerous theories regarding the mechanism of invasion and subsequent metastases related primarily to cancer cell type, i.e. high-grade cancers (such as signet ring cells) or whether the process is primarily related to pattern of spread, direct local or regional spread with subsequent metastases and distant disease or whether the more common events involve cells in lymphatic channels metastasizing to lymph nodes with subsequent blood borne metastases or whether there is hematogenous spread via direct vascular invasion. The Vogelstein model emphasizes sequential progression of cancer with accumulation of cellular transforming events with accumulation of tumor cell population and sub populations, which gain metastatic potential. This is counter-balanced by the Berrnard Fisher model which considers cells to be transformed early with invasive potential and capable of metastasizing from day one. The question of the molecular events preceding metastases include cellular mutations, gene amplifications or deletions, epigenetic methylation, acetylation, micro RNA disregulation, post translational changes and other transformative events. These were addressed by Dr. Paty as well as in the overview provided in the initial chapter. Some authors consider there to be two major pathways. The APC or the Aneuploid Pathways commonly involving mutations of P53, KRAS, SMAD4 and the other pathway being the CIMP or MSI pathway which contains a defect in mismatch repair, providing another spectrum of mutations. These generally result in right sided tumors involving older patients and generally have a good prognosis.

Lynch syndrome tumors are the major hereditary form of colon cancer of MSI origin. These patients have defects in mismatch repair but also have a cellular mutational status, which is chromosomal and can develop KRAS mutations and P53 mutations. The more common colon cancers are MSI high and do have a different group of mutations including BRAF mutations and KRAS mutations. Both tumors are MSI but have different mutational patterns with both having a good prognosis in general.

Dr. Paty also discussed tumor budding, which occurs at the invasive edge of the colon cancer and consists of stem cells and more invasive EMT transition cells, which accumulate at the edge where the tumor infiltrates into the surrounding tissue. These cells contain beta catenin and

tend to be high-grade and occur in higher staged tumors and correlate with lymph node metastases and poor prognosis. There is a large array of molecular changes preceding metastases which are well described in the initial chapter authored by Dr. LeGolvan and Dr. Wanebo. The evolution of sentinel node mapping in colon cancer was discussed by Dr. Saha. The discussions initially focused on changes in the NCCN guideline 2011 regarding colorectal cancer where a recent additional indication for chemo therapy stated that the finding of less than 12 nodes within the resected colorectal specimen became an additional indication for chemotherapy. Unfortunately this node recovery number is uncommonly achieved. According to Dr. Saha the average count in a large patient study, (over 400,000 patients examined) was 11. In North America, the average hospitals are demonstrating less than 12 nodes in the specimen. Even data from the NCI designated cancer centers shows data from 22 % of the patients still do not achieve a 12 node count.

Dr. Saha's group has been devoted to evaluating the use of sentinel node biopsy to enhance the nodal count as well as to increase the adequacy of resection of the colon site. In his series of sentinel node biopsies in the colon, they found at least one blue sentinel node in about 99 % of the colon cancer patients examined versus about 89 % of the patients in the rectal cancer group. The reduced number probably relates to the fact that chemoradiation probably reduces the nodal access in the rectum. Overall in about 50 % they found an average of three nodes by lymphatic mapping and believe this leads to better staging and improved resection. In colon cancer, they found nodal positivity in about 34 % with less in rectal cancer (80 % of the patients had received neoadjuvant radiation). This finding has been translated into increased resection of the primary site in about 21 % of the colorectal patients because of the finding of the sentinel node outside of the normal extent of the planned resection leading to a larger resection.

In a small study group of 160 patients examined for extent of resection related to the location of the sentinel node, they found that 16.5 nodes were removed with an overall positivity of 46 %. In 79 % of the patients, there was no change in operation required, with an average node recovery of about 16 per patient. However, in 21 % the operation was changed by extending the length of the colon resected and in these patients the average node recovered was about 18.5 with nodal positivity increased to 60 %. This is different from the group having the standard resection. In 1 in 5 patients the sentinel node sampling increased the upstaging from 42 to 60 %. They believe that this may translate into overall improvement and outcome. In those patients with a follow-up of 5 years, overall recurrence was 9 % compared to conventional 27 %. Although all positive patients received the same chemotherapy from the same medical oncology group the overall recurrences were significantly less in that group (9 %) with more selective extended resection based on location of node positivity compared to 37 % overall from that institution. The importance of node negative findings is also stressed. The recurrence in the group with sentinel node procedure with a 5 year follow-up was only 4.5 % compared to 22 % in the conventional resected patients. This type of data needs to be expanded. It compliments findings from European sources regarding detailed examinations of the nodes in specimens and is something that Dr. Saha suggested that sentinel node perhaps should be included in the surgical management of colorectal cancer patients.

Dr. Zuber's paper discusses the effect of a new technique for examining the nodes and nodal metastases (OSNA). This refers to the fact that in the standard pathologic examination, only 1 % of the lymph node tissue is examined by single slide sectioning in contrast to multi-level sectioning and immuno-histochemistry of the sentinel node. This is a very time consuming and costly process however. The OSNA system (The One Step Nucleic Diagnostic System) has been installed in Dr. Zubers clinic and provides better staging than the routine use of one slide per node. In an analysis of 307 nodes in 22 patients, median number of nodes harvested, was 30, and special examination was done of 13 nodes per patient with using special cuttings by the reference laboratory method (5 levels in each patient) as well as comparison to the OSNA for the detection of the CK19 RNA messenger. The standard sectioning technique demonstrated seven micro-metastases with no macro-metastases, no invasive cancer, no isolated tumor cells in the remaining 15 of total 22 patients examined. The multi slice reference technique demonstrated micro metastases in (8 total and 6 ITC) (isolated tumor cells) (14 path positive of 22 patients). By the new method of OSNA, all the macro-metastases were detected (8 patients) and there was only once case, which could not be confirmed by the ultra-staging technique or by RT PCR. There were three ITC's found. There were 10 node PNO patients compared to 15 PNCO by the other technique. On the basis of single node analysis the upstaging with one slide in the patients analyzed by OSNA, identified 4 of 15 patients which increased the staging in this very selected group. Thus upstaging by the OSNA technique identified four of 15 patients who were not identified by standard H&E pathology. Single slide study demonstrated 7 micromets only where as multi synchronizing (5 slides) identified 18 micromets. Overall, 11 of these 15 patients were ultra-staged (by the OSNA) exam of single slide), which provides a more defined tool for examining the nodal status. This was discussed in detail by Zuber (Table 1).

The optimum surgical approach for the liver metastases is reviewed in detail by Dr. D'Angelica who provides a

Table 1 Hepatic resection for metastatic colorectal cancer. Neoadjuvant/other therapy

Neoadjuvant	Phase	Patients	Resection (%)		Peri-op mortality	Surv med mos	
			RO	>3 segments		DFS	OS
Nordlinger	III	182	87 %	79 %	2 %	19	NR
Adam	II	493	97 %	52 %	NR	22	50
Blazer	II	35	80 %	NA	3 %	23	56 % 5 year
Gruenberger	II	56	93 %	36 %	0 %	25	76
Observational	19 studies	2,456	93 % (39–100 %)		68 % (23–97)	21	46
	10 series	4,310 patients	NR		NR	21	45

NR not resected

CR/PR radiologic response

Data abstracted from Chua et al. [3]

very detailed "worklist" as utilized in his institution, Memorial Sloan-Kettering in selecting and resecting hepatic metastases. The overview of this is provided in the Wanebo and LeGolvans initial paper but the fine tuned approach is discussed by Dr. D'Angelica who provides additional facts of life not commonly discussed. Of interest the 10 year survival in a 612 patient database was median of 44 months, with 102 (10 year) survivors, giving a 10 year cure of 17 %. In contrast among 5 year survivors (usual statistic used) one-third recurred (in same institutions). Thus, 5 year is to short of an observation time. One issue addressed was the question of neoadjuvant therapy. The patients that have this, represent a high risk group. In patients that have had neoadjuvant chemotherapy (116 patients database) having complete resections and (excluding R2 resections) 95 % of them have recurred in 9 months. This raises the question about the value of resection in patients that respond to aggressive chemotherapy for hepatic metastases. This is different than the management concept of patients with the primary metastatic disease, which is discussed in the initial overall review in which there is a planned effort in patients with singular hepatic metastases. This represents more of a reality check in patients that have more extensive metastases at the time of neoadjuvant chemotherapy. This type of discussion brings some reality to the management of this complex group of patients considering the variable and confusing data in the literature.

Dr. Kemeny provides a very thoughtful paper regarding overall therapy for liver metastases focus on additional regional therapy. She provides a rather complete manuscript on the overall management of hepatic metastases with the incorporation of regional therapy in addition to the more standard approaches for treating hepatic metastases. Her paper is very complete with very factual displayed summary sheets. The use of hepatic artery infusion (HAI) may certainly provide an additional highway to travel in an effort to increase survival in these patients although this technique is not commonly utilized in the oncologic community.

As a final commentary, the initial review by Drs. Wanebo and LeGolvan includes some of the recent therapeutic avenues as well as the molecular factors involved in the progression of colorectal cancer to metastases. It provides additional information regarding avenues of study, which might be of value for therapeutic approaches in the future.

The format for these presentations at this meeting provided the opportunity to not only hear the hard data but also to see the interchange among the different experts during the discussion which helps to shed additional light and also raises more questions to be addressed in future studies with hopeful improvement in outcome.

Biologic challenge of colorectal metastases (I)

Harold J. Wanebo, MD, FACS, and Mark P. LeGolvan, DO

Clinical overview

Colorectal cancer (CRC) is the third most common malignancy in the USA with 150,000 new cases in 2008 and 50,000 deaths [1]. Metastases to the liver are found in up to 25 % at primary diagnosis and occurs during the following disease course in 50–70 % at 3 years of follow-up. Surgical resection is currently the only therapy producing long term cure in CRC patients with hepatic metastases [2, 3]. Current multi-drug chemotherapy regimens for metastatic CRC have been shown to increase survival from only 6 months to greater than 18–20 months [4–10]. The addition of targeted agents has been shown to add benefit in appropriately selected patients. An updated review by Adam et al. [11] of >700 patients who

underwent resection following neoadjuvant chemotherapy for advanced liver metastases demonstrated that resection was possible in 13.5 % (95 patients) without perioperative mortality. At the time of publication, 92 % had completed a five year follow-up and had an overall survival of 35 % from the time of resection [11]. Subgroup analyses revealed five-year survival rates of 60 % for large tumors, 49 % for poorly treated responsive lesions, 34 % for multinodular disease and 19 % for liver metastases with extra hepatic disease [11]. This important study initiated by the Bismuth group actually precedes the current programs for managing hepatic metastases, that have blossomed out in recent years and provides creditability to use of neoadjuvant therapy in properly selected patients [11, 12, 14]. Currently the use of extended resection has been expanded to include multi lobe metastases, and extra hepatic deposits which were once thought of no benefit and the use of synchronous resections is becoming much more common. This is complemented by the continued evolution of neoadjuvant therapy for metastatic colorectal cancer [10].

Table 1 summarizes the outcome of neoadjuvant therapy with resection for hepatic metastases as summarized by Chua et al. [3]. There was 1 phase III study and three phase II studies involving 3,278 patients, with 19 observational studies in a total of 3,278 patients [3, 14–16]. In this large series radiologic responses were observed in 64 % with complete response in 4 % and partial responses in 52.5 %. The overall pathologic response rate was complete in 9 % and partial in 36 %. Median disease free survival was 21 months and the overall survival was 46 months. The rate of complete resection (RO) with clear margins was 93 % and major resections involving three segments were done in 68 % with perioperative mortality of 2 % and morbidity in 27 %. The involved chemotherapy combinations included Folfox, Folfiri and Xelox which were used in 16 of these series. Targeted therapies were added in five series one of which included Cetuximab and four with Bevacizumab. The randomized three-arm Phase III by Nordlinger compared neoadjuvant and adjuvant Folfox chemotherapy with surgery. In the patient group having preoperative therapy 83 % underwent partial hepatectomy [13]. This group experienced 9.2 % longer progression free survival ($P = 0.0025$) when compared to the patients who received surgery alone as treatment for the liver metastases.

In recent years, an increased effort has made use of targeted therapy for hepatic metastases either using angiogenesis inhibitors such as Bevacizumab or the use of EGFR inhibitors such as Cetuximab/Panitumamab as well as selected tyrosine kinase inhibitors. A series of studies have been done, which have shown that the addition of selected biologic therapies significantly adds to the effect of the other therapies (Reviewed by Wanebo and Berz [10]).

Angiogenesis inhibitor

Angiogenesis is regulated by vascular endothelial growth factor (VEGF) and their receptors VEGFRS. The VEGF family consists of five members VEGFA-E and placental growth factor (PIGF) [17]. These ligands bind to three VEGF receptors (VEGFR1, 2 and 3) forming VEGFR homodimers and heterodynes [17, 18]. VEGF signaling is modulated by variable affinity of ligands for specific receptors and co-receptors necropolis and heparan sulfate proteoglycans (HSPGs) [18]. The concept of treating tumors whose growth is heavily dependent on angiogenesis was an obvious rationale for developing angiogenesis inhibitors and led to early studies of potential effectiveness [8, 19]. Although anti-VEGF therapy alone had modest effects on tumor growth the combination of VEGF inhibitors with chemotherapy produced meaningful anti-tumor activity [8, 19, 20].

Bevacizumab (Bev), a humanized monoclonal antibody (moAb), targets VEGFA and has been approved for first and second line therapy of metastatic colorectal cancer (CRC). Bev added to bolus 5FU + leucovorin (LV) and irinotecan (IFL) increased 35 % time to progression by 11 months versus 6 months and OS 20 months versus 16 months in control group in the Hurwitz study [8].

EGFR inhibitors

The epidermal growth factor receptor (EGFR) is a member of ErbB family of tyrosine kinase receptors (EGFR) (ErbB-1/HER 1, ErbB-2, Her-2/neg, ErbB-3 (HERB), ErbB-4 (HER4)) [21]. These receptors (trans-membrane glycoprotein) contain an extra-cellular domain. The intra-cellular domain has tyrosine kinase activity and transduces downstream signals to proteins involved in tumor cell proliferation, invasion, migration and inhibition of apoptosis.

The EGFR receptor is activated when a relevant physiologic ligand EGF, transforming growth factor alpha (TGF-a), or amphiregulin binds to the extra cellular domain. The EGFR binding site is an attractive target for moAb therapy [27, 28]. The two monoclonal antibodies approved for the use in metastatic CRC are the chimeric human-mouse antibody, Cetuximab and the fully humanized compound, Panitumumab. Cetuximab and Panitumumab show only modest single-agent activity in a heavily pretreated patient population, but have demonstrated promising clinical utility in appropriate selected patients when combined with chemotherapy [21, 22]. The retrospective analysis of the CRYSTAL trial substantiated the hypothesis that Cetuximab as a EGFR-directed antibody when added to a FOLFIRI chemotherapy backbone only adds efficacy in the setting of Kras wild-type

colorectal tumors [23]. This observation was confirmed when the responses and survival were analyzed according to the Kras mutation status in other series [37]. In all trials that published the Kras mutation status subsets, only the patients with Kras wild-type configured tumors obtained benefit from the addition of Cetuximab or panitumumab to chemotherapy or Cetuximab/panitumumab single-agent therapy.

The Crystal study, which combined FOLFIRI chemotherapy with Cetuximab versus placebo[36], demonstrated an improved PFS (8.7 vs. 9.9 months) and OS (21 vs. 24.9 months) with the addition of Cetuximab in Kras WT patients. In patients with Kras-mutated tumors neither PFS (8.1 vs. 7.6) nor OS (17.7 vs. 17.5 months) were statistically significantly influenced by the addition of Cetuximab and had increased toxicities [24].

Similar results were seen in the OPUS study, a large European phase II study, combining FOLFOX4 with Cetuximab or placebo [7]. Although the addition of Cetuximab demonstrated a trend towards an increased objective response rate (36 vs. 46 %), the PFS with the addition of Cetuximab demonstrated only a positive trend in the patients with Kras WT configured tumors (7.2 vs. 7.7 months). In patients with Kras-mutated tumors the PFS was reduced from 8.6 months in patients treated with FOLFOX4 only versus 5.5 months in patients receiving FOLFOX4 with Cetuximab [7].

It also demonstrated that if a patient is KRAS wild type but also has Braf mutation, these patients are also non-responsive. Other negative inhibitors include loss of PTEN (a tumor inhibitor); about 30 % of patients with Kras wild type do not respond without an obvious molecular reason. The conclusion is that KRAS in the RAS pathway has a significant effect on anti tumor responses but there are other effects from AKT/P13K and mTOR molecular signals. We are learning more and more about these inhibitors and their benefits or negative influence in selected patients depending on the molecular background of the tumor [25–28].

The pattern of metastases is probably partially related to anatomic factors such as portal vein drainage of the gastrointestinal tract, and can be predicted by the extent of primary site invasion as recorded in TNM staging, but of equal or even greater impact may be the molecular biology of the carcinoma [29, 30].

Metastases

The metastatic cascade is orchestrated by a series of molecular steps which programs the developing cancer cell in the primary tumor to progress through a series of transformations which facilitate invasion and subsequent metastases. The steps involve [1] an epithelial–mesenchymal transition (EMT) with proteolysis of the basement membrane (BM) and extra-cellular matrix (ECM) [2]; dissociation of tumor cells from the bulk tumor by alteration of adhesive properties with suppression of anoikis (apoptosis with inappropriate loss of cell adhesion) [3]; local invasion and cell migration [4]; angiogenesis and intravasation [5]; viable vascular dissemination with immune evasion [6]; extravasation from vessels [7]; distant embolization with the establishment of and survival at a secondary anatomic site; and finally [8] an outgrowth of micro and macro metastases. Each stage contains many barriers that must be overcome in order for the successful metastasis of the malignant cell to be achieved [30].

Epithelial mesenchymal transition (EMT) is crucial for tumor cell invasion and mirrors similar reversible events in embryonic development. In tumorigenesis, aberrant reactivation of EMT at the invasive front causes loss of cell polarity, down regulation of epithelial proteins, and acquisition of a spindled morphology with induction of mesenchymal proteins including N-cadherin, vimentin, various matrix metalloproteinases (MMP) [31, 32]. The basement membrane (expressed in the main tumor mass) is frequently lost at the invasive front, but then may be re-expressed in the metastases [33]. Proteolysis of the basement membrane (BM) and extra cellular matrix (ECM) (made up of laminins, type IV collagen, nidogens and proteoglycans) occurs via proteolytic enzymes as well as reduced production of BM components [34, 35]. This is especially true of laminin-5, the most important BM component, due to reduced production at the invasive front of Lama3, a component of laminin-5 [36, 37].

Cell adhesion molecules (CAMs) normally maintain cell–cell and cell ECM adhesion and include a broad range of molecular families: cadherins, immunoglobulin super family CAMs; selectins and integrins. Deregulation of the cadherin-catenin system permits tumor cell detachment and the altered expression of various cell surface molecules on the colon cancer cell, as well as endothelial and hepatic sinusoidal cells facilitate tumor cell escape and hepatic metastases [19, 38]. The prognostic value of the cadherin-catenin system in CRC liver metastasis has been widely studied. Higher levels of E-cadherin mRNA in patients with CRC have been associated with greater overall survival, while absence is associated with reduced survival [40]. Immunohistochemical analysis of E-cadherin and B-catenin expression in patients with or without hepatic lesions indicates that reduced E-cadherin expression and increased cytoplasmic, as well as, nuclear translocation of B-catenin was more frequently observed in patient's with metastasis [41]. Low expression of B-catenin in CRC hepatic metastases is a marker of poor prognosis while increased nuclear expression at the invasive front is a powerful predictor of liver metastasis [42, 43]. Choi et al.

[44] has shown that loss of membranous E-cadherin and accumulation of nuclear B-catenin in the primary tumor was associated with liver metastases and that serum response factor (SRF), a modulator of e-cadherin/B-catenin, expression was increased, and membranous E-cadherin expression was also lost in the liver metastases.

Integrins, another member of the CAM family, are heterodimers, made up of a non-covalently bonded alpha and beta subunits and comprising 18 alpha and 8 beta subunits which provides for multiple pairing combinations creating receptor diversity with a family of 24 CAMs [45]. Integrins are expressed on endothelial and epithelial cells, leukocytes, platelets, and a variety of other normal and tumor cells. Integrins are involved in tumor cell arrest, adhesion, and migration within the liver vasculature [46]. Their expression tends to vary among normal colonic tissue, adenomas, primary tumor and metastatic sites. For example there is reduced or even loss of expression of the normal enterocyte integrins, alpha 2, alpha 6 and beta 1 in the adenoma to carcinoma sequence, as well as, increased expression of other integrins, such as alpha5beta1 in highly invasive cell lines [47, 48].

Invasion and metastases Loss of the BM facilitates the detachment of tumor cells, which promotes EMT. This loss may be secondary either to reduced production or secondary degradation of the ECM by proteolytic enzymes. A family of proteinases, the matrix metalloproteinases (MMPs), and their corresponding inhibitors, tissue inhibitors of metalloproteinase's (TIMPs), appear to exert the dominant effect [49].

MMPs are a family of zinc-dependent secretory proteolytic enzymes, whose expression and secretion are tightly regulated by interleukins, growth factors, and TNF-a. MMPs can degrade all constituents of connective tissue, and thus facilitate invasion [50]. They are grouped into collagenases gelatinizes, stromelysins (e.g., and matrilysins) according to their substrate specificity [49].

Numerous studies have shown higher expression of MMPs in CRC with suggested correlation between tumor stage, metastasis and prognosis [51]. MMP-7 appears to be one of the most important in CRC. It is over expressed in the majority of CRC, and its expression correlates with progression and hepatic metastasis [52, 53]. MMP-2 and MMP9 have also been extensively studied, due to their ability to hydrolyze the main component of the BM, type IV collagen. Two confirmatory studies have shown that both MMPs have increased expression in CRC with metastasis to the liver compared to CRC without metastasis [54, 55]. MMP-9 was also found to have prognostic value, with significantly higher levels associated with increased risk of metastasis and an unfavorable outcome [55]. The MMP/TIMP expression profile is readily distinguished between primary tumor and liver metastasis. In particular, MMP1, -2, -3, and -12 were significantly down regulated in the liver metastases. Thus, CRC cells colonizing the liver are biologically diverse from the cells of the primary tumor. This correlates with the clinical observation that synthetic MMP inhibitors are only effective when given early in the phase of tumor establishment, but not once metastatic disease is present [56]. In addition to the MMPs, there are a variety of other classes of proteinases that have been studied in relation the ECM degradation including urokinase plasminogen activator and heparinase [57, 58].

Cell migration c-Met, the primary ligand for hepatocyte growth factor/scatter factor (HGF/SF) is a transmembrane tyrosine kinase receptor that is found in epithelial tissue. The binding of HGF/SF to c-Met activates a signaling cascade that results in many down stream events, including mitogenesis, motility, morphogenesis, and survival. Its elevation has been identified in 70 % of colorectal metastasis versus primary cancers [59]. Many pathways have been implicated in the over-expression of c-Met, including the Wnt pathway, which is commonly activated in CRC, the Ras pathway, promoting migration and invasion and the phosphatidylinositol 3-kinase (P13 K)/AKT pathway, which suppresses apoptosis and promotes survival [32] and beta catenin accumulation [60]. c-Met mRNA expression has been shown to be high in tumors that later developed distant metastasis, a and was associated with a shorter metastasis free survival [61].

Growth factors and receptors Epidermal growth factor receptor (EGFR) is a member of the human epidermal growth factor receptor family, (HER)-erb2 family of receptor tyrosine kinases, and is involved in signaling pathways (including the RAS-RAF-MAPK and P13 K-AKT), affecting cellular growth, differentiation, proliferation and angiogenesis [62]. Its abnormal expression has been described in many tumors, with implications for prognosis, especially with the advent of anti-EGFR therapy. EGFR can be detected in approximately 60–80 % of CRC, but its exact role in the CRC metastatic cascade has not been completely elucidated as EGFR status itself, is variable both in testing and between primary CRC and their metastases, as recently reviewed in Siena et al. [62].

Ligands for EGFR include epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), and amphiregulin (AR). Yamada et al. performed an immunohistochemical study of AR, EGFR, and HER2 to evaluate their expression levels and prognostic relevance in CRC. Neither EGFR nor HER2 expression was significantly related to any of the clinic pathologic factors; however, AR positivity in primary lesions significantly correlated with liver metastases [63].

Table 2 Selected molecules predictive for liver metastasis in colorectal cancer

Role in the metastatic cascade	Gene/protein	Abnormality	Reference
Cell matrix/cell–cell interactions	E-cadherin	↓ Expression	21, 22
Cell matrix/cell–cell interactions and signalling pathways	Beta-cadherin	↑ Expression and nuclear translocation	22, 23
Cell matrix/cell–cell interactions	Serum response factor	↑ Expression	24
Invasion and metastasis	MMP2, MMP7, & MMP9	↑ Expression	32–35
Cell migration	c-met	↑ Expression	39
Angiogenesis	Angiogenesis 1 and 2	Imbalance in expression with Ang-2 expressed and Ang-1 infrequently expressed	48, 49

Angiogenesis

Angiogenesis is a critical step in the metastatic cascade, providing both a source for hematogenous dissemination and ensuring tumor cell viability through increased oxygenation. Numerous angiogenic factors contribute to metastasis formation in CRC including VEGF/VEGFR, angiopoietins, and thrombospondin amongst others. VEGF is a direct acting endothelial cell mitogen that induces cell migration, proliferation, invasion, and increased vascular permeability [64] and functions by binding one of three endothelial cell tyrosine kinase receptors: VEGFR-1, 2, and 3. Increased VEGF in primary tumor is associated with poor prognosis, with VEGF being up-regulated in primary CRC cells and down-regulated in the corresponding liver metastasis [65, 66]. Angiopoietins are a family of four (Ang-1 to Ang-4) angiogenic factors that are ligands for the endothelium specific tyrosine kinase Tie receptors [67]. Ang-1 and Ang-2 have been found to have an imbalance in their expression levels in CR with Ang-2 frequently expressed in primary and metastatic CRC and Ang-1 infrequently expressed, leading authors to believe that Ang-2 is an initiating factor for tumor angiogenesis and may aid in liver metastasis [68]. Ang-2 has also been found to be a significant predictor of poor prognosis [69] (Table 2).

Survival and apoptosis

While p53 plays a pivotal role in CRC [70], it's role in metastasis is controversial [71–74]. Survivin, a member of the inhibitor of apoptosis protein family, functions to inhibit the intrinsic pathway of apoptosis by localizing to the mitochondria as well as regulating cell division [75]. Higher expression of survivin has been shown to be a significant predictor of lower survival [76]. Continued research into the complex biologic growth and signaling factors and further understanding of potential inhibitors may lead to deeper understanding of the metastatic process involved in colorectal cancer progression and lead to development of more effective targeted therapy.

Table 3 Results lymph node biopsy technique

1 slice H&F	The standard 1st slice H&E	The new OSNA	The reference MLS + H&E/IHC
Macromets patients	7	7	7
Micromets patients	0	1	2
ITC patients	0	3	9
pN0 patients	15	11	4
Total patients	22	22	22

MLS multi-level sectioning, *ITC* isolated tumor cells

Molecular staging of lymph nodes in colon cancer patients using one-step nucleic acid amplification (OSNA) is equivalent to routine use of histopathology

Markus Zuber, MD et al.

Extended Abstract

Objective: Small nodal tumor infiltrates are identified by applying multilevel sectioning and immunohistochemistry (IHC) in addition to H&E (hematoxylin and eosin) stains of resected lymph nodes [77]. However, the use of multilevel sectioning and IHC is very time- consuming and costly. The current standard analysis of lymph nodes in colon cancer patients is based on one slide per lymph node stained by H&E. A new molecular diagnostic system called "One Step Nucleic Acid Amplification" (OSNA) was designed for a more accurate detection of lymph node metastases [78–81]. The objective of the present investigation was to compare the performance of OSNA to current standard histology (H&E) [82, 83], We hypothesize that OSNA provides a better staging than the routine use of one slide H&E per lymph node [84] (Table 3).

Methods: From 22 colon cancer patients 307 frozen lymph nodes were used to compare OSNA with H&E. The lymph nodes were cut into halves. One half of the lymph node was analyzed by OSNA. The semi-automated OSNA uses amplification of reverse-transcribed cytokeratin19 (CK19) mRNA directly from the homogenate. The remaining tissue was dedicated to histology (the reference method), with 5 levels of H&E and IHC staining (CK19).

Results: On routine evaluation of one H&E slide 7 patients were nodal positive (macro-metastases). All these patients were recognized by OSNA analysis as being positive (sensitivity 100 %). Two of the remaining 15 patients had lymph node micro-metastases and 9 isolated tumor cells. The two patients with micro-metastases in the reference method only one was positive in OSNA but negative in H&E. For patients with isolated tumor cells, H&E was positive in 0/9 cases whereas OSNA was positive in 3/9 patients.

There was only one OSNA positive case (IHC negative) which could not be confirmed by RT-PCR (data not shown). On the basis of single lymph nodes the sensitivity of OSNA and the 5 levels of H&E and IHC was 94.5 % (data not shown).

Conclusion: OSNA is a novel molecular tool for the detection of lymph node metastases in colon cancer patients which provides better staging compared to the current standard evaluation of one slide H&E stain. Since the use of OSNA allows the analysis of the whole lymph node, sampling bias and undetected tumor deposits due to uninvestigated material will be overcome. OSNA improves staging in colon cancer patients and may replace the current standard of H&E staining in the future.

Keywords One-step nucleic acid amplification, Colon cancer, Staging, Lymph node, Histopathology

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Colorectal hepatic metastases—selecting the optimal surgical approach

Michael I. D'Angelica, MD

The clinical issue of patients with limited and resectable hepatic metastases from colorectal cancer (CLM) is a relatively common problem. Of the 150,000 patients that present with primary colorectal cancer in the United States, 15–25 % will have synchronous hepatic metastases and an additional 25–50 % will ultimately develop hepatic metastases [85]. If you then consider that up to 25 % of these patients are estimated to have resectable CLM then there may be as many as 12,500 cases a year in the United States.

The rationale for hepatic resection for CLM was originally based on retrospective data that documented that patients who underwent partial hepatectomy for resectable CLM had 5-year survivals of approximately 25–40 % [85]. Unresectable patients did poorly and rarely survived 5 years. Some studies were able to document a group of patients who had resectable disease but didn't undergo resection for various reasons. These patients survived longer than those with unresectable disease but 5 year survival was <5 % (Scheele BJS 90) [86]. From these data it was concluded that hepatic resection was associated with long-term survival that was not possible with chemotherapy or supportive care. Through the 1980s, 1990s and 2000s there were then many reported series of hepatic resection that documented 5-year survival rates ranging from 25 % to as high as 58 % in the modern era [87].

These data that were used to justify hepatic resection for CLM however, had significant limitations. The studies were uncontrolled retrospective case series that utilized predictive statistics. The denominator from which these patients were chosen was unknown. It was possible that the survival data were more a reflection of extreme selection bias than the operation itself. Early on, prominent surgeons voiced their doubt about the value of liver resection for these patients [88].

While a prospective randomized controlled trial would have addressed this issue, there are certain observations that do not require such trials. It has now been demonstrated that hepatic resection for CLM can result in long-term disease free cure. Since this is not possible in patients treated with chemotherapy this potential justifies the operation in properly selected patients. We published a study that analyzed 612 patients that had undergone complete resection of CLM with 10-year actual survival data [89]. The median survival for these patients was 44 months. There were 102 actual 10-year survivors and only 1 documented disease-specific death after 10 years. Of the 102 10 year survivors, 99 were free of disease. Eighty-six of them remained free of disease after a single liver resection. Given that there was an additional group of long-term disease-free survivors that almost survived 10 years but could not be documented to be alive beyond this mark, the potential 10 year cure rate ranged from 17 to 25 %. Approximately one-third of the 5-year survivors went on to die of their disease and therefore patients

require follow up to 10 years after resection [89]. Patients with limited and resectable metastatic CLM are an example of a remarkably interesting biologic phenomena; that patients are cured of "metastatic disease" by a complete resection.

The alternative treatment option for patients with CLM is systemic chemotherapy. Chemotherapy for metastatic colorectal cancer significantly improved during the 1990s. Modern systemic chemotherapy now has response rates in excess of 50 percent, and in those that don't undergo resection the median survival approaches 2 years [90]. However, the median time to progression is approximately 1 year, and once you progress, second and third line therapies have very limited efficacy. Long-term survival with chemotherapy is uncommon and cure probably does not occur. Therefore, although chemotherapy has improved, it is not a potentially curative treatment.

One of the biggest challenges in this field has been predicting outcome in patients who undergo resection of CLM. There are a number of risk scoring systems that combine clinical and pathologic factors to help predict outcome. Most risk scoring schemes are based on multivariate analyses of large clinical databases and combine these factors (weighted or not) to optimally predict outcome. One well known example of this is a study by Fong et al. that identified 5 preoperative factors independently associated with worse survival [91]. The predictive factors included a node positive primary tumor, a disease-free interval <12 months, >1 hepatic tumor, size of >5 cm and a serum CEA > 200 ng/dl. A point is assigned for each factor yielding a sum ranging from 0 to 5. This score correlates very well with 5-year survival and has been a consistently good predictor of outcome in our subsequent studies [91]. Interestingly, when you take these scores and you stratify people with actual long-term follow-up you can predict differences in outcome but the worst scores do not preclude cure [89]. Therefore even in the highest risk patients a chance of long-term survival and cure with resection exists limiting the clinical utility of these scores. Furthermore, the only factor that we find that precludes 10-year survival is a positive margin. There is therefore a great need for effective and clinically useful markers in this disease.

To add further complexity to this field it has become apparent that the context within which these resections are performed is changing. Hepatic resection has become safer in experienced hands with less blood loss, shorter hospital stays and a mortality of 1 percent or less for parenchymal resections in patients without cirrhosis [92]. Furthermore, we and others have developed the technical ability to resect extensive bilobar disease with parenchymal sparing resections, intraoperative thermal ablation, two-stage resections and the use of portal vein embolization [93, 94]. We also now have effective systemic and regional chemotherapy options for our patients. These changes over time have likely contributed to improved survival. We and others have published data demonstrating improved long-term survival in the modern era [92].

Given the improving safety of hepatic resection and better survival one question is whether the indications for hepatectomy for CLM should expand. Before addressing this question we should first address the classical indications for this operation. Based on case series evaluating survival from the 1970s through the early 1990s, the following contraindications were typically proposed: the presence of extrahepatic disease (EHD), four or more liver metastases, and anticipated margins less than 1 cm [95]. These contraindications were not always consistently supported in studies, they were based on small numbers of patients and they were from an era of poor imaging and staging. Furthermore, there was no effective chemotherapy for metastatic colorectal cancer at that time. These 3 contraindications will be individually reviewed and their modern day context analyzed.

Liver resection for CLM has a sound biologic rationale, which reflects the concept of regionally confined metastases and is supported by rigorous clinical data demonstrating potential for long-term cure. The results of hepatic resection for regionally confined CLM do not naturally extend to patients with concurrent EHD. The relevant question is therefore whether resection of CLM combined with resection of EHD is curative or a cytoreductive noncurative operation. Combined resections of CLM and EHD have been performed more frequently and a number of institutional case series have demonstrated the possibility of long term survival with this approach. In general, patients without EHD have better survival than those with it, but 5 year predicted survival for those with EHD are approximately 20–30 % [96, 97]. Five-year survival rates like this appear better than outcomes for chemotherapy alone and many feel that patients with resectable CLM and limited and resectable EHD should proceed with resection. However, these survival data are likely as much a reflection of selection bias and the underlying indolent tumor biology as they are of the operation.

Predictive survival statistics alone do not tell us whether resection of CLM and concurrent EHD is a potentially curative operation. The question is how frequently patients recur after such operations. We have analyzed this question. Excluding R2 resections, 116 patients underwent a complete resection of CLM and EHD at our institution from 1992 to 2007. Of these 116 patients, 110 (95 %) have recurred with a median time to recurrence of 9 months. There were 5 patients free of recurrence: 2 have less than 2 year follow up, 2 had histologic invasion of the diaphragm which could be considered locally invasive rather

truly EHD, and 1 patients with a lung metastasis is free of recurrence at 40 months from the operation [97]. These data support the idea that nearly everyone undergoing this operation ultimately will recur. The likely reason that these patients are surviving a long time is our selection bias. They have single sites of EHD with limited liver disease. They are relatively young and otherwise healthy patients and the majority are well selected with a prolonged test of time on preoperative chemotherapy.

In summary, resection of CLM and concurrent EHD is associated with better long-term survival than would be expected with chemotherapy alone but in general, these operations cannot be considered potentially curative. Whether the survival is a result of surgery or selection bias is impossible to know but the 2 probably both contribute. It is critical for both surgeon, oncologist and patient to understand that most, if not all of these patients spend a substantial part of their lives getting treated for recurrent disease.

It is a somewhat similar story when we look at the outcome in patients, with four or more CLM. Predicted 5-year survival rates range from 30 to 50 % but recurrence rates are high. Most case series report recurrence rates of 80–85 % with a median time to recurrence of 10–12 months. Due to incomplete follow up it is likely that these recurrence rates will be higher and be over 90 % [98–100]. Unlike the patients with EHD, there does appear to be some chance of cure in these patients. Our actual 10 year data suggest that approximately 5–10 % are probably cured [89]. The excellent predicted 5-year survival rates likely reflect a combination of selection, tumor biology and resection. Therefore, patients with 4 or more CLM that undergo complete resection have an excellent associated 5-year survival but high recurrence rates. While we likely cure 5–10 % of patients with resection, the reality for the great majority of these patients is chronicity of care with treatment of recurrent disease.

An anticipated close or positive margin has also been a historical contraindication to resection for CLM, Margins are a difficult issue since we cannot always predict them preoperatively and it is often unclear whether they are reflective of technical failure or underlying tumor biology. In general, positive margins have been associated with dismal outcomes with universal recurrence and death from disease. In our study of actual 10 year survivors after resection of CLM, a positive margin was the only factor that precluded cure [89]. Interestingly, in most studies the width of a negative margin does not independently correlate with outcome [101]. In our own studies, patients with positive margins do poorly and the width of the margin closely correlates with survival [102]. Close margins, however, should not preclude resection since they are still associated with good long-term survival and cure. On multivariable analysis the width of the margin did hold up as an independent predictor of outcome in our dataset [102]. The association between margin and outcome is clearly a complex interrelationship between tumor biology and surgical technique that is difficult if not impossible to. In summary, margins probably do matter. Positive margins do poorly and the width of the margin may be important. Unfortunately, predicting the margin pre or intra-operatively is difficult and therefore excluding patients based on radiologically close margins is not recommended.

It is therefore apparent that the contraindications to liver resection have never been well defined. Historically accepted contraindications (EHD, 4 or more CLM, close margins) to hepatic resection are no longer strictly applicable because of the associated long-term survival that does not appear to be possible with chemotherapy alone. Unfortunately, in patients with these historical contraindications cure is very uncommon. While hepatic resection in these situations is reasonable and justified in well selected patients, it is critical that we understand the reality of resecting extensive disease. The great majority of these patients will receive chemotherapy for long durations of time. If they are lucky and they have an indolent and favorable disease recurrence pattern they will have repeat surgical ablative procedures and live for years. Many patients will have both chemotherapy and repeat ablative/surgical procedures. It is equally important to understand the benefits of surgery. Resection accomplishes a 'complete response' and can provide a significant amount of time off of chemotherapy for some patients. Resection may also alter the disease pattern decreasing and perhaps eliminating bulky hepatic metastases that may be more tolerable and may afford long-term survival despite recurrent disease. Most importantly, we need to discuss these issues openly and honestly with patients and describe realistic outcome expectations.

Although the historically described contraindications to hepatic resection for CLM are not strictly applicable any more, they are still relevant. If we consider patients with less than 4 CLM, no EHD and apparently wide margins, hepatic resection is potentially curative. Outcomes and cure rates in this situation are similar to and better than some primary non-metastatic tumors that are treated with surgery. It should be quite clear that resection is indicated in these patients. However, when resections are performed for extensive CLM, limited EHD or with positive margins it is a very different situation. While there is associated long-term survival, resection is rarely curative. It is beyond the scope of this writing but these patients are a good group of patients to consider neoadjuvant chemotherapy with a significant test of time in which one could consider operations for the well selected patients who have done well.

In summary, hepatic resection for limited liver metastases is potentially curative and our ability to predict outcome in a clinically relevant manner is poor. There is clearly a need for better biomarkers and effective predictors of outcome. An expansion of indications for hepatic resection for CLM is warranted due to the associated survival figures but as we expand these indications, recurrence rates rise and become nearly universal in some situations. Therefore, based on historical contraindications we can divide resections into potentially curative and non-curative (or very low chance of cure) operations and treatment strategies can be devised based on these distinctions.

Regional therapy of liver metastases

Nancy Kemeny, MD

Lymph node metastases are an important prognostic predictor for survival in patients with colorectal metastases [103]. Disease from colorectal cancer can spread to the lymph nodes, but it can also spread directly to the liver and at times bypass the lymph nodes [104]. In patients with metastatic disease, 50 % of patients will have liver metastases which are the major cause of morbidity and mortality. In an autopsy series of patients who died from colorectal cancer, 46 % had only liver metastases. Metastases can travel up the portal vein into the liver, since the venous drainage of the colon and rectum is via the portal vein; therefore, some patients with liver metastases do not have lymph node metastases [105]. The concept of stepwise pattern of metastatic progression has been described by Weiss et al. and is based on observations of the patterns of metastasis in >1500 autopsies of CRC patients. He describes a "cascade" model whereby CRC metastasis progresses stepwise first into the liver and then into the lungs and finally into other organs [106]. One implication of this model may be that the liver acts as a gatekeeper for further metastatic spread. Thus, an important aspect of treatment of hepatic metastasis is removing them or directly treating them and thus cutting off the cascade of metastasis.

The rationale for hepatic arterial chemotherapy

The rationale for hepatic arterial chemotherapy has an anatomic and pharmacologic basis.

1. Liver metastases greater than 2–3 months are perfused almost exclusively by the hepatic artery while normal hepatocytes derive their blood supply from both the portal vein and hepatic artery [107, 108]. By injection of labeled H3 FUDR (5-fluoro-2′-deoxyuridine) into either the hepatic artery or portal vein of patients, mean tumor FUDR levels are significantly increased (15-fold) when the drug is injected via the hepatic artery [109] while mean liver concentrations of drug do not differ depending on the route of injection.
2. Drugs that are largely extracted by the liver during the first pass results in high local concentrations with minimal systemic toxicity. Ensminger et al. [110] demonstrated that 94–99 % of FUDR is extracted by the liver during the first pass, compared to 19–55 % of fluorouracil (FU). This makes FUDR an ideal drug for hepatic arterial chemotherapy [111].
3. Drugs with a steep dose–response curve are more useful for hepatic infusion since small increases in the concentration of drug can be given and will result in a large improvement in response [112].
4. Drugs with a high total body clearance are also more useful for hepatic infusion. The area under the concentration versus time curve (AUC) is a function not only of drug clearance, but also of hepatic arterial flow. Since hepatic arterial blood flow has a high regional exchange rate (100–1,500 ml/min), drugs with a high clearance rate are needed [113]. If a drug is not rapidly cleared, recirculation through the systemic circulation mitigates the advantage of intra-arterial therapy over systemic therapy [113].

Collins established that increased local concentrations of HAI are dependent on the ratio of the total body clearance of a particular drug (CL_{TB}) to the regional exchange (Q) for a particular body compartment: CL_{TB}/Q.

Multiple agents have been studied but 5-fluoro-2-deoxyuridine (FUDR) demonstrates superior properties for HAI, such as a very short half-life, and extensive first pass extraction by the liver, which results in an up to 400 fold difference between the systemic concentration and the hepatic intratumoral concentration. Recently oxaliplatin has been studied for use in HAI study by Dzodic et al. [114] did observe significant advantage in tissue concentrations when comparing HAI and IV administration of oxaliplatin, with HAI offering an advantage over IV administration. A recent study by Ducreux et al. [115] evaluated a 2-h HAI oxaliplatin administration with concurrent intravenous 5FU and leucovorin in 28 patients with inoperable metastatic liver lesions from colon cancer and showed an objective response rate of 64 %. Although this study did not directly compare to intravenous administration of oxaliplatin, the HAI seems to have a longer time to the emergence of neurotoxicity, which may be due to decreased systemic availability by this route, but did also seem to cause an increase in abdominal pain over IV administration. Irinotecan has also been recently studied for HAI. A Phase I study by Van Riel et al. [116] showed

increased systemic levels of the active metabolite SN38 during HAI as compared to intravenous infusion of irinotecan, however it did not exhibit increased activity when delivered by HAI in a Phase II Study.

Initial trials of hepatic arterial infusion

The initial trials of hepatic arterial infusion (HAI) utilized external pumps and percutaneous placed catheters which produced problems such as catheter dis-lodgment and bleeding [117]. A totally implantable infusion device provided a new stimulus for this type of treatment and these pumps offer several advantages over external pumps including a reduction in catheter related complications such as thrombosis and precise drug administration and patient acceptance [118].

The early randomized studies compared HAI therapy alone to 5FU. In total, 10 prospective randomized phase III trials comparing HAI chemotherapy to systemic treatment have been published to date [119]. These trials have consistently shown higher response rates for HAI treatment, with response rates ranging from 42–62 versus 9–21 % in patients treated with systemic chemotherapy. Conclusions regarding overall survival were difficult to assess in many of these studies since crossover to hepatic therapy after failure of systemic therapy was performed. Some of the trials included patients with extrahepatic disease and in some the hepatic arterial therapy was never administered. One of the most recent of these trials is the Cancer and Leukemia Group B (CALGB) 9,481 study published in 2006, which compared patients treated with HAI FUDR and dexamethasone to systemic 5-FU and Leucovorin. This study of 135 patients demonstrated a significant increase in survival in the HAI arm (24.4 vs. 20 months $P = 0.0034$) (Fig. 1) [120]. In this study the design did not permit crossover. Toxicity showed increased biliary toxicity with HAI and increased systemic to toxicity with systemic therapy. Additionally, the HAI group showed a longer time to progression and higher quality of life assessment scores (Slide 4).

A recent meta-analysis evaluating the aggregated data from these 10 prior studies demonstrated significantly increased response rate, 42.9 versus 18.4 %, for the HAI versus systemic treatment ($P = 0.0001$) [121]. The difference in median overall survival was 15.0 months (HAI) versus 12.4 months (systemic) but did not reach statistical significance ($P = 0.24$). Since the meta-analysis relied on the above studies, the shortcomings of the individual studies go into the meta-analysis.

Pump complications

The surgical challenges associated with HAI pump placement may be minimized by an appropriate preoperative work-up including a CT scan with angiography to identify any aberrant arterial anatomy that may require alterative surgical approaches. Increased surgical experience, refinements of surgical techniques, and improvements in pump design have decreased complication rates over time. In an institutional review of pump complications seen in 544 patients treated at MSKCC between 1986 and 2001, complications during the earlier half of the study period (1986–1993) were significantly higher (25 %) than the later half of the study time (1994–2001, 18 %) [122].

Mechanical complications of HAI pump placement include hepatic artery thrombosis, catheter thrombosis, extrahepatic pump perfusion, and incomplete hepatic perfusion. A 2.4 % risk of early thrombosis was observed in a series of 544 patients. Of these cases, approximately one-third were salvaged with anti-coagulation or lytic therapy. Catheter thrombosis was observed as a late complication as well (2 %), and was associated with technical errors such as inadequate filling of the pump or back-bleeding into the catheter during port manipulation. Complications related to inappropriate pump perfusion may be related to thrombosis, aberrant anatomy, or surgical complications.

Chemical complications observed early in the development of HAI chemotherapy included a chemical cholecystitis and biliary sclerosis. The complication of cholecystitis is now preemptively addressed by a routine cholecystectomy performed at the time of HAI pump placement. The susceptibility of bile ducts to the effects of HAI chemotherapy results from their primary perfusion by the hepatic artery [123]. This toxicity can be moderated by the addition of dexamethasone to the HAI pump infusion. A randomized trial comparing patients treated with FUDR alone versus FUDR with dexamethasone demonstrated that the steroid treated group benefited with a trend towards lower bilirubin levels, and high response and survival. Reviewing several studies performed at MSKCC, greater than two-fold elevations in alkaline phosphatase were seen in 27–43 % of patients, bilirubin increases greater than 3 mg/dl was seen in 6–19 % of patients, and transaminitis was seen in 37–59 %. An algorithm for dose reduction has been developed where elevations in AST, alkaline phosphatase, or total bilirubin dictate reduced dosages (or held doses) of chemotherapy.

Liver resection followed by adjuvant therapy

HAI chemotherapy has also been evaluated as an adjuvant treatment for CRC patients with hepatic metastases who have undergone surgical resection of the liver. Relapse occurs in 65–80 % of patients after surgical resection of liver metastases, with the liver being the most common site of recurrence [124]. The two phase III trials addressing this question of systemic therapy after liver resection showed a trend in favor

of adjuvant treatment, but failed to demonstrate statistical significance [125]. Since that recurrent metastatic disease after hepatic resection is most likely to occur in the liver (slide 5). HAI chemotherapy in the adjuvant setting has also been investigated in several studies [126]. A benefit in hepatic free survival has been demonstrated in three studies: an MSKCC trial [127, 128], an ECOG/SWOG trial [129], and a Greek trial [130]. No advantages seen in German study [131]. Among these, the largest study with the longest follow-up is the MSKCC trial, (slide 6) which accrued 156 patients [132]. The endpoint was 2 year survival which was significantly increased in HAI group (Fig. 2). This study compared patients treated with HAI chemotherapy combined with systemic 5-FU/LV to those treated with systemic chemotherapy alone (Fig. 2). The 10-year survival rate for patients receiving HAI treatment was 41 % compared with a 27.2 % survival for systemic treatment alone (Fig. 3). The hepatic DFS and overall DFS were significantly increased in the HAI group in this study as well as other studies (Fig. 4). Additional studies are beginning to explore the role of combination HAI/systemic chemotherapy in the adjuvant setting. The combination of HAI chemotherapy with systemic 5-FU, LV, and oxaliplatin in surgically resected patients has shown 5-year survival rates of up to 88 % [133]. A new trial adding Bev to HAI + SYS did not improve results over HAI + SYS alone but both groups had excellent 4 year survival of 85 and 81 % for no Bev versus Bev groups, respectively (slide 11) [134]. A retrospective trial by House et al. looked at the survival of patients after liver resection who received modern systemic chemotherapy and compared them to those who had HAI plus systemic chemotherapy. There was a significant increase in RFS and overall survival in the HAI plus systemic group versus modern systemic therapy alone (slide 12).

Unresectable disease

Historically, patients with unresectable colorectal cancer with hepatic metastasis treated with systemic chemotherapy (5-FU/LV) might expect a response rate in the range of 20–30 %. Newer systemic chemotherapy regimens utilizing agents such as oxaliplatin and irinotecan resulted in high response rates in the range of 40 %. When HAI chemotherapy is combined with systemic chemotherapy. Several recent trials have demonstrated response rates of 70–90 %. This strategy offers the benefit of focused control of hepatic disease along with prevention and control of extrahepatic metastasis. There also appears to be an additive effect with regard to disease response in the liver. The most recent of these studies examined the response of 49 patients with unresectable liver metastasis treated with a combination of HAI with FUDR and dexamethasone plus systemic chemotherapy with oxaliplatin and irinotecan (Fig. 5). In this series, a total of 92 % of patients had a response, 84 % demonstrating a partial response and 8 % achieving complete response. Moreover, 47 % of patients had a response that was significant enough to permit surgical resection of their liver metastases, and among treatment-naïve patients, 57 % converted to surgical resection. The toxicities associated with combinations of HAI and systemic chemotherapy was similar to the toxicities reported for individual agents with myelosuppression and diarrhea from the systemic chemotherapy and LFT abnormalities from the HAI chemotherapy. The volume of reductions shown in Fig. 6 are very great thus allowing a large number of patients to get to resection even if not resectable at initiation of therapy.

Conclusion

Liver metastases are a significant cause of morbidity and mortality in patients diagnosed with colorectal carcinoma. Surgical resection of hepatic metastases offers an opportunity for cure, but relapse occurs frequently, especially within

CALGB Trial - Metastatic Colorectal Cancer Randomized Study HAI-FUDR vs SYS-FU/LV

Survival and Response

	HAI n=68	SYS n=67	p-value
Survival (mos)	24.0	20.0	0.0034
Response (CR/PR)	47%	24%	0.012
Hepatic Disease Free Survival (mos)	9.8	7.3	0.034

Fig. 1 CALGB study comparing treatment with HAI, FUDR, dexamethasone to Systemic 5FU and Leucovorin

Adjuvant Therapy After Liver Resection HAI + SYS versus SYS

Endpoint: increase 2 year survival

Statistical Method: 156 pts were required to detect increase in 2-year survival with 80% power at a 5% significance level

Results:

	HAI + SYS (n=74)	SYS (n=82)	p Value
2-year Survival	85%	69%	0.02

Fig. 2 MSKCC study comparing 2 year survival rate of adjuvant therapy after liver resection of Hepatic Metastases: HAI (FUDR/Dexamethasone) + FU/LV vs. Systemic therapy only

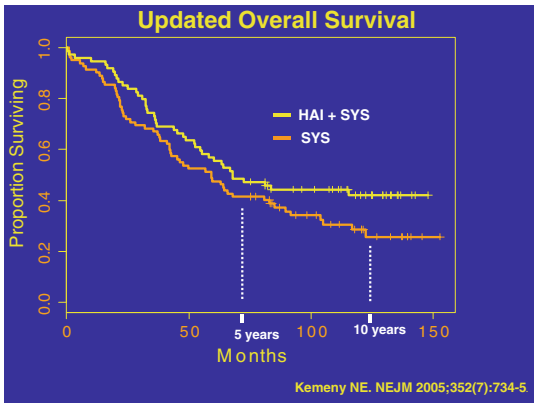

Fig. 3 Updated 10 yr survival post adjuvant therapy HAI and Systemic vs. Systemic therapy only

Randomized Studies After Liver Resection
HAI vs SYS or Control

Disease-Free Survival

Studies	#pts.	% 2-year HAI	% 2-year SYS	% 5-year HAI	% 5-year SYS	*P* value
MSKCC	156	55	45	40	30	.02
ECOG	75	60	40	40	20*	.03
Lorenz	186	median		20/12.6*		NS
Lygidakis	122	66	48	60	35	.0002
Tono	19	75	20	60	20	.045

*No treatment in control arm

Fig. 4 Randomized studies after liver resection HAI vs Systemic therapy or control

HAI FUDR and Dex plus Systemic Oxal/CPT-11
n=47

	Previous Sys. chemo (n=26)	No prior therapy (n=21)
Complete Response	2	2
Partial Response	21	19
Stable Disease	1	1
Response Rate	23/26 (88%)	21/21 (100%)
Median Survival	35 months	50 months
Patients resected	10 (45%)	12 (57%)

Fig. 5 Outcome following HAI, FUDR and Dexamethasone and Systemic Oxaliplatin and Irinotecan in unresectable colorectal metastases

the liver. For patients with unresectable disease, advances in systemic chemotherapy, surgical approaches, as well as RFA and cryoablation have improved the odds. HAI

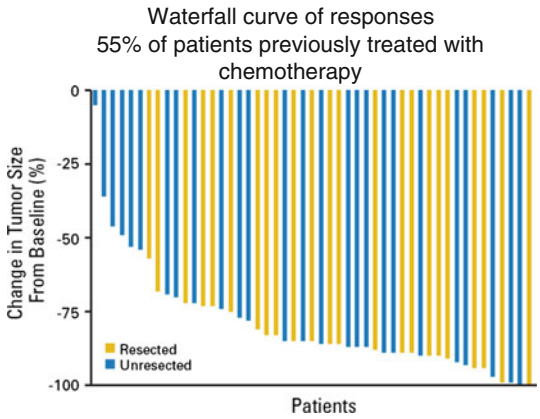

Fig. 6 Volume of response to HAI therapy in patients with hepatic colorectal metastases (55%) previously treated with chemotherapy

chemotherapy, by targeting liver metastatic disease offers a potent treatment for liver confined metastases, and when combined with systemic chemotherapy, may offer very good chance for conversion to surgical resection and the possibility of a cure. Larger, randomized, controlled trials comparing new systemic chemotherapy agents to HAI chemotherapy in combination with systemic treatments are needed to assess the relative benefits of each of these treatments. For patients after resection, recurrence is a significant likelihood and additional studies are needed to further elucidate the role for HAI and systemic chemotherapy in reducing the risk of relapse (Figs. 1, 2, 3, 4, 5, 6).

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