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COLORECTAL LIVER METASTASIS: TOWARDS THE INTEGRATION OF CONVENTIONAL AND MOLECULARLY TARGETED THERAPEUTIC APPROACHES

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1. ABSTRACT

Radical surgery currently represents the only treatment with curative potential for patients with colorectal cancer (CRC) liver metastases. Unfortunately, only a minority of cases is eligible for hepatic resection and many patients still develop recurrent disease, which underscores the need for more effective adjuvant treatments. In case of unresectable disease, locoregional therapeutic strategies can obtain significant tumor regression/local disease control rates, but there is no definitive evidence of their effect on patients' survival. In regards to systemic chemotherapy, the conduction of randomized controlled trials has led to a substantial progress in terms of both tumor response and survival rates. Despite these results, most patients ultimately die of their disease due to hepatic and/or extra-hepatic cancer progression. Therefore, novel therapeutic strategies are urgently needed to improve the prognosis of patients with metastatic CRC. The elucidation of CRC biology is paving the way to the development of molecularly targeted strategies, and results from controlled clinical trials have already demonstrated that some agents targeting tumor-specific molecules can significantly improve the therapeutic efficacy of conventional antineoplastic drugs. The dissection of the molecular mechanisms of CRC metastatization and tumor/host interactions will not only accelerate the development of more effective and less toxic anticancer strategies but also will allow for the personalization of the therapeutic regimen according to the molecular features of individual patients and their tumors. Only the broader clinical implementation of these novel molecular oncology findings and the optimal integration of conventional and molecularly targeted therapeutic approaches will enable clinicians to provide patients with a better chance of cure.

2. INTRODUCTION

Colorectal cancer (CRC) is the fourth most commonly diagnosed cancer and the second leading cause of cancer death in Western countries (1). Nearly 50% of CRC patients will develop liver metastases during the course of their disease, with half having hepatic metastases at the time of primary diagnosis and another half developing metachronous disease. Furthermore, over 50% of patients who die of CRC have liver metastases at autopsy, and the majority of these patients die as a result of their metastatic liver disease. Until the early 1980s, metastatic CRC to the liver was often left untreated, the median survival being 5-10 months (2, 3). By contrast, current therapeutic options allow to state that, unlike most other types of cancer, the presence of distant metastases from CRC does not preclude curative treatment (4).

Here, we review the results of conventional treatments (surgery, systemic chemotherapy and locoregional approaches) for CRC liver metastases and summarize the most promising findings regarding the clinical implementation of molecularly targeted therapeutic strategies. To this aim, PubMed searches of the National Library of Medicine were performed with appropriate key-words, with the only restriction being English language. For ongoing clinical trials, the National Cancer Institute dedicated website (http://cancer.gov/clinicaltrials) was also searched.

3. RESECTABLE DISEASE

3.1. Surgery

Radical resection is the gold standard for the treatment of CRC hepatic metastasis, and should be considered in all patients when the disease is confined to the liver and can be removed adequately, while leaving enough functional liver reserve (4-8). Several studies have demonstrated that 5-year overall survival (OS) rates following resection of isolated CRC liver metastases vary from 30 to 50% (5-8). Data on longer follow-up are still rare: two studies report 10-year OS rates of 20 and 23%, suggesting/indicating that liver resection can cure patients with CRC liver metastases (9). Unfortunately, only 30% of patients with CRC liver metastases have no other sites of disease and, among them, only 35-50% are candidates for surgical resection according to respectability/operability criteria (5-8, 10).

Though in the absence of randomized controlled trials (RCT), the analysis of published data (5-8, 11) suggests that patients are candidates for surgical resection if: 1) no extrahepatic disease is present; 2) all liver metastases can be resected with tumor-free margin; and 3) adequate (≥30%) residual liver parenchyma can be spared. As regards the first point, it must be remembered that the presence of metastatic lymph nodes of the hepatic hilum worsens the prognosis, although it cannot be considered an absolute contraindication to resection provided that a complete regional lymphadenectomy is performed (12). Also the width of disease-free margin (microscopic vs <1 cm vs >1 cm) has been uniformly demonstrated to affect the clinical outcome, while the impact of number/size of metastatic lesions, metastasis onset (synchronous/metachronous) and the stage of primary tumor are not universally accepted prognostic factors. The assessment and preservation of the liver functional reserve still represent a limiting step in the decision making process for hepatic metastasectomy. Currently, standard biochemical tests and calculation of liver parenchyma percentage of replacement based on radiological imaging constitute the basis for judging liver operability (13). Portal vein embolization targeting the diseased lobe is followed by contralateral lobe compensatory hypertrophy and can allow for extended hepatic resections otherwise life-threatening (14). Interestingly, some authors have reported that unresectable multiple bilobar liver metastases can be safely treated by combining this technique with two-stage hepatectomy (15).

The operative mortality for major hepatic resections has declined to <5% with improved operative techniques and postoperative care, but morbidity (*e.g.* hemorrhage, biliary leak, hepatic failure, peri-hepatic abscess, wound infection, pneumonia, and myocardial infarction) remains significant (22-39%) (5-9).

Despite careful selection, most patients who undergo resection of CRC liver metastasis will have recurrence of their cancer, the most common sites of recurrence being liver and lungs. Repeat liver resections for hepatic metastases have been reported by several groups (3, 16, 17): remarkably, the 5-year OS rates (30-35%) are not strikingly different from those achieved in patients undergoing first hepatic resection, which strengthens the recommendation to submit all patients with potentially resectable second liver recurrence to surgery as a first-line treatment option. Finally, some investigators have reported on the surgical resection of initially unresectable CRC liver metastases following tumor regression from chemotherapy administered/given either through the systemic route (18, 19) or hepatic arterial infusion (HAI) (20, 21). In the largest series so far reported, systemic chemotherapy allowed for the surgical rescue of 12.5% of such patients, with a 5-year OS of 33% (19). Larger trials and longer follow-ups are warranted to demonstrate the benefit of this strategy in terms of patients' OS.

3.2. Adjuvant treatments

Whether the use of adjuvant chemotherapy after resection of liver CRC metastases can decrease the rate of disease recurrence is still a matter of debate. Results from RCT are controversial. A first study failed to show any survival benefit following adjuvant 5-fluorouracil (5-FU)-based HAI chemotherapy (22), which is supported by subsequent non-randomized studies (23). Another RCT comparing adjuvant HAI/systemic chemotherapy to systemic chemotherapy alone showed a trend towards improvement in 2-year progression-free survival (PFS) (57% *vs* 42%, P=0.07) and an improved OS rate in the combined chemotherapy arm (86% *vs* 72%, P=0.03) (24). When the same research group compared surgery plus systemic (5-FU) and HAI (fluorodeoxyuridine, FUDR) chemotherapy with surgery alone, the 4-year recurrence-free survival was better in the chemotherapy arm (67% *vs*

43%; P=0.03), but no difference in OS was observed (median survival: 49 and 63 months for the control and the chemotherapy arm respectively, P=0.6), although the study was underpowered for OS analysis (25). In a recent meta-analysis (n=592), HAI delays recurrence in the remaining liver but does not improve OS, which led the authors to state that this added procedure cannot be recommended as a routine clinical practice (26).

The implementation of newer antineoplastic agents (*e.g.* irinotecan, pirarubicin) and schedules (pre- and post-resection administration) for adjuvant HAI/systemic chemotherapy is in its infancy (27, 28). An ongoing RCT is comparing surgery alone with surgery plus pre- and post-resection systemic chemotherapy using 5-FU and oxaliplatin.

4. UNRESECTABLE DISEASE

4.1. Systemic chemotherapy

Systemic chemotherapy is the mainstay of treatment for patients with unresectable metastatic CRC (29, 30). Most studies do not differentiate between patients with liver metastases only and those with hepatic and extra-hepatic disease. However, considering the trials in which the results are discussed separately, the overall response rates well correlate with those reported for the liver only: this justifies the use of the former percentage as a reliable indicator of chemotherapy activity in patients with hepatic disease only.

4.1.1. 5-Fluorouracil

5-FU, a fluorinated pyrimidine, has been and remains the most widely used chemotherapy employed as either a single agent or as a component of combination therapy for the treatment of CRC, both in the adjuvant and metastatic setting. Following metabolic activation, 5-FU binds to methylene-tetrahydrofolate and inhibits thymidylate-synthase, a key enzyme in DNA synthesis.

After the pivotal trial demonstrating that 5-FU plus leucovorin (LV) was associated with better survival when compared to 5-FU alone (31), this regimen became the standard first-line treatment for metastatic CRC. A recent meta-analysis including 2,751 patients with advanced CRC who were randomized to 5-FU/LV or 5-FU alone demonstrated that the addition of LV led to a doubling in response rates (23% *vs* 12%; P<0.0001) with a modest but significant improvement in 1-year OS (48% *vs* 43%; P=0.003) (32).

Schedules of 5-FU continuous infusion may result in better clinical outcome compared with bolus 5-FU schedules (33), which should be balanced against the extra costs and morbidity of central venous access devices.

4.1.2. Capecitabine

5-FU cannot be administered orally due to its inconsistent absorption and rapid catabolic clearance. The 5-FU prodrug capecitabine is an oral fluoro-pyrimidine reliably absorbed in the gastrointestinal tract and ultimately converted to 5-FU by thymidine-phosphorylase, an enzyme that is present in higher concentrations in tumor rather than in normal tissues. There have been two randomized comparisons of capecitabine with bolus 5-FU/LV in a total combined sample size exceeding 1,200 patients. The results consistently showed equivalent survival efficacy with a more favorable toxicity profile for capecitabine (34, 35), which is now approved as a first-line treatment for metastatic CRC.

4.1.3. Irinotecan

Irinotecan (also known as CPT-11) is a semisynthetic derivative of the plant alkaloid camptothecin that inhibits the function of the enzyme topoisomerase-I, a key factor for relaxation of supercoiled DNA during cell replication. The dose-limiting toxicity of irinotecan is delayed-onset diarrhea. Two RCT evaluated single-agent irinotecan as a second-line treatment. In one study investigators compared irinotecan to continuous 5-FU and found improved OS (10.8 *vs* 8.5 months; P=0.035) and median time-to-progression (4.2 *vs* 3.9 months; P=0.03) favoring the irinotecan arm (36). The second study compared irinotecan to best supportive care alone. Patients on the irinotecan arm manifested improved OS (9.2 *vs* 6.5 months; P=0.0001) (37). Subsequently, two RCT performed in previously untreated patients receiving first-line chemotherapy established the activity and toxicity profile of the combination of irinotecan with 5-FU/LV. Among 683 patients randomized to irinotecan plus bolus 5-FU/LV (IFL), 5-FU/LV, or irinotecan alone, IFL led to an improved response rate (39% *vs* 21%; P=0.001) and overall survival (14.8 *vs* 12.6 months; P=0.04) when compared to 5-FU/LV (38). Results for irinotecan monotherapy were similar to 5-FU/LV. These findings were consistent with an earlier study indicating a superior response rate (35% *vs* 22%; P<0.001) and survival (17.4 *vs* 14.1 months; P=0.031) for patients receiving irinotecan coupled with weekly or biweekly infusions of 5-FU/LV compared to the infusion of 5-FU/LV alone (39). In subsequent trials, concerns regarding IFL toxicity have been raised (40). The majority of unexpected early deaths were associated with multiple gastrointestinal toxicities or various thromboembolic events, prompting recommendations for vigilant clinical monitoring and aggressive supportive intervention for patients experiencing toxicity after treatment with IFL.

4.1.4. Oxaliplatin

Oxaliplatin is a third-generation, platinum-based compound with a 1,2-diaminocyclohexane carrier ligand, which forms DNA adducts and results in strand breaks. Oxaliplatin has two types (acute and chronic) of distinctive sensory neurotoxicity. The chronic neuropathy exhibits either complete or partial reversibility in 75% of affected patients within 3 to 5 months of treatment discontinuation.

Oxaliplatin administered alone exhibits single-agent activity in 18-20% of chemotherapy-naive patients and in 10% of patients who have previously failed 5-FU therapy (41). When administered with 5-FU/LV, oxaliplatin produces response rates of 20-26% in 5-FU refractory disease (42). Results from a RCT comparing infusional 5-FU/LV (LV5FU2), single-agent oxaliplatin, and the

combination (FOLFOX) in 463 patients with recurrence following IFL demonstrated better response rates (0% *vs* 1.1% *vs* 9.9%; P<0.0001) and longer PFS (2.7 *vs* 1.6 *vs* 4.6 months; P=0.07) in patients assigned to FOLFOX, but no significant survival advantage (43). Promising first-line treatment results were reported in two RCT. The first one (powered for PFS) compared FOLFOX with LV5FU2: improved PFS (9.0 *vs* 6.2 months; P=0.0003) was observed, but there was no significant improvement in OS (16.2 *vs* 14.7 months; P =0.12) (44). Chronomodulated infusions of 5-FU alone or with oxaliplatin were compared in the second RCT, in which better response rates (53% *vs* 19%; P<0.001) and PFS (8.7 *vs* 6.1 months; P=0.048) were reported with the addition of oxaliplatin (45). A survival advantage for FOLFOX has been confirmed in a three-arm RCT of patients with advanced CRC randomly assigned to bolus IFL, FOLFOX or a combination of irinotecan plus oxaliplatin (IROX) (46). FOLFOX was associated with better response rates (45% *vs* 31%; P=0.002), longer time-to-progression (8.7 *vs* 6.9 months; P=0.0014), and improved median OS (19.5 *vs* 14.8 months; P=0.0001) compared to IFL; noticeably, FOLFOX was also superior to IROX. Accordingly, either oxaliplatin or irinotecan in combination with 5-FU/LV (preferably as an infusion regimen) currently represent the reasonable strategies for first-line chemotherapy in patients with unresectable metastatic CRC. As for irinotecan, combination regimens containing daily bolus 5-FU/LV and oxaliplatin can be associated with severe gastrointestinal toxicity and relatively high mortality rates (8.5%) (47).

4.1.5. Irinotecan versus oxaliplatin

A single completed RCT comparing an oxaliplatin- to an irinotecan-based regimen coupled with bolus and then infused 5-FU/LV (FOLFOX *vs* FOLFIRI) has been reported (48). In this study, patients crossed-over to the alternative treatment arm upon progression while on their first regimen. Because the trial was powered for PFS on second-line therapy as the endpoint, only 226 patients were enrolled. The overall response rate (ORR) was 56% for FOLFIRI in first-line and 4% for FOLFIRI in second-line. For FOLFOX the ORR was 54% in first-line and 15% in second-line. While these patients were initially judged to be unresectable, sufficient responses were observed in 22% of the FOLFOX- and 9% of the FOLFIRI-treated patients to permit surgical interventions that culminated in complete resections in the majority of patients. The overall PFS on first-line therapy was 8.5 months for FOLFIRI and 8.1 months for FOLFOX (P=0.24). The second-line PFS was 2.5 months for FOLFIRI and 4.2 months for FOLFOX (P=0.003). The median OS did not differ between strategies at 21.5 months with FOLFIRI/FOLFOX and 20.6 months with FOLFOX/FOLFIRI (P=0.99); moreover, higher rates of grade 3-4 febrile neutropenia, alopecia, nausea and stomatitis occurred with FOLFIRI, while neutropenia and paresthesias were more common with FOLFOX, indicating equivalent activity and moderate toxicity differences between these two strategies.

4.2. Locoregional therapeutic approaches

4.2.1. Hepatic arterial infusion

The unique differential blood supply of the liver (portal vein → healthy parenchyma; hepatic artery → metastatic disease) underlies the rationale for HAI chemotherapy for CRC liver metastatic disease (49). FUDR is the preferred agent for HAI owing to its short half-life and high-rate of hepatic extraction leading to a 100- to 400-fold ratio of hepatic-to-systemic drug exposure. Biliary sclerosis is the dose-limiting toxicity, which has been reduced with the use of dexamethasone as part of the treatment (50), whereas catheter displacement/occlusion remains the most frequently reported complication (51, 52). Although randomized trials comparing HAI with systemic chemotherapy have demonstrated higher response rates as compared to systemic 5-FU, the clinical utility of HAI remains uncertain (53). A meta-analysis including 654 patients with unresectable hepatic metastases enrolled in seven RCT comparing HAI to systemic 5-FU therapy did show greater ORR with HAI (41% *vs* 14%; P<0.001), but no OS advantage (16 *vs* 12.2 months; P=0.14) (54). More recently, investigators randomized 209 patients to systemic therapy (LV5FU2) or HAI with 5-FU/LV (55). No differences in PFS or OS (14.7 *vs* 14.8 months; P=0.79) were observed. However, because of technical challenges inherent to HAI, 37% of HAI-assigned patients did not start their treatment and an additional 29% were unable to receive more than two cycles due to catheter displacement/failure. A later study enrolled 117 patients with liver-limited unresectable metastases who were randomized to bolus 5-FU/LV or HAI with FUDR (56). ORR (51% *vs* 24%; P=0.009) and survival (22.7 *vs* 19.8 months; P=0.027) favored HAI, although time to extrahepatic progression was significantly shorter for HAI patients (7.8 *vs* 23 months; P=0.0007). Overall, with the availability of more effective systemic chemotherapy regimens the value of HAI in unresectable liver CRC is currently questioned. In particular, extra-hepatic disease recurrence is an undisputed limit of locoregional therapies/approaches. Addition of 5-FU-based systemic chemotherapy to HAI does not appear to offer any advantage for non-resectable CRC liver metastases (57, 58). Newer chemotherapeutic agents (*e.g.* irinotecan, oxaliplatin) may prove to be more efficient in reducing the extrahepatic failure rates, as suggested by the encouraging results from a recent study (HAI + systemic irinotecan) (59).

4.2.2. Isolated hepatic perfusion

Another locoregional therapeutic approach to unresectable liver metastases is hyperthermic isolated hepatic perfusion (IHP) (60). This is a surgically demanding procedure (in terms of both manpower and cost) that can only be performed in highly specialized centers. The mean operative time is 6±4 h, a few days in the intensive care unit are usually necessary and the mean hospital stay ranges between 10 and 29 days. Although other drugs have been employed (*e.g.* 5-FU, mitomycin-C) (61), melphalan is the most frequently administered agent, either alone or in combination with tumor-necrosis-factor (a cytokine with anti-tumor and anti-angiogenic properties (62)). Since hepatocellular damage is the limiting toxicity, cirrhosis, portal hypertension and tumor liver replacement ≥50% are regarded as exclusion criteria in IHP protocols. IHP-related mortality rates vary between 0% and 18%; ORR and median OS range from 20% to 83% and 9 to 28.8

months, respectively (63-68). Response rates and median survivals after IHP are not strikingly different from those obtained with HAI. However, it must be remembered that several patients responded to IHP after HAI and/or systemic chemotherapy failure (69). As already suggested (66), the duration of tumor regression might be improved by combining IHP and HAI, which would serve as induction and maintenance therapy respectively. Ongoing trials are testing the efficacy of the combination of IHP with both HAI and systemic chemotherapy. The potential use of IHP as a neoadjuvant treatment remains another open question: some authors successfully performed hepatic resections after IHP (61), although in such cases surgery can be technically challenging because of the major inflammatory response following the locoregional treatment. Overall, as no RCT has been performed, IHP remains an investigational treatment to be performed only within the frame of clinical trials.

4.2.3. Ablative techniques

Techniques for local tumor destruction such as radiofrequency and cryoablation can be used to clear the liver from metastatic tumor lesions (70). Both procedures can be used alone or in combination with liver resection (71-73). During this last approach, which is most often used, lesions surgically accessible are resected, while ablative techniques are used to treat unresectable lesions. To date, there is no evidence that local tumor ablation is as efficient as resection in terms of survival benefit: consequently, ablative techniques should be reserved for unresectable lesions (74-76).

Using the combined approach (local tumor destruction + surgery), most series using cryoablation describe 1- and 2-year OS rates of 80 and 60%, respectively (9), with median OS varying from 26 to 32 months. For radiofrequency, 1- and 2-year survival rates of 81 and 67% have been reported, respectively, with median OS ranging between 18 and 45 months. It has been claimed that these results are better than those obtained during chemotherapy alone. However, these superior results may be due to a biased patient selection: in fact, patients selected for local treatment usually have only a limited number of metastatic nodules (generally <10), while patients treated with chemotherapy often show widespread liver involvement. Because disease recurrence after local ablative tumor treatment is mainly outside the area treated by local ablative therapy, a combined treatment regimen of local tumor destruction and systemic/locoregional chemotherapy is encouraged by many centers at this stage. Results from small series of patients treated with ablative techniques and HAI are conflicting (77).

Overall, although some studies show significant treatment responses after cryoablation or radiofrequency, the precise impact of local tumor ablative therapy on OS of patients with CRC liver metastasis is still unclear, and a RCT (radiofrequency plus chemotherapy *vs* chemotherapy alone) is still ongoing.

5. MOLECULARLY TARGETED THERAPY

The expression “molecularly targeted therapy” has been defined in different ways (78). The Food and Drug Administration has considered targeted therapy as a drug with an approved label in which there is a specific reference to a simultaneously or previously approved diagnostic test that must be performed before the patient can be considered eligible to receive the drug. Examples of such definition are the co-approvals of trastuzumab along with the eligibility diagnostic test for the selection of patients featuring HER-2/neu protein overexpression or gene amplification (breast carcinoma), cetuximab along with the eligibility test for EGFR overexpression (CRC), and imatinib along with the eligibility test for the expression of the translocation fusion gene Bcr-Abl (chronic myelogenous leukemia, CML) or the tyrosine-kinase receptor c-Kit (gastro-intestinal stromal tumors, GIST). A more general definition of targeted therapy is that of a drug/therapeutic strategy with a focused mechanism specifically acting on a well-defined target or biologic pathway that - when inactivated - causes regression or destruction of cancer (Figure 1). These anticancer therapies can be classified into three main categories: a) those interfering with cancer-related signaling pathways at protein level (i.e., development of molecularly targeted drugs); b) gene therapy, which aims at correcting gene imbalances underlying tumor survival/aggressiveness; c) active specific immunotherapy (vaccination), which exploits the potential of an entire cell network (the immune system) to selectively recognize and kill malignant cells.

The following section is not meant to be a comprehensive description of all targeted drugs/strategies recently developed for the treatment of CRC, but is aimed at briefly describing the mechanism of action and the clinical results of some of the most promising classes of such antineoplastic approaches.

5.1. Molecularly targeted drugs

5.1.1. Anti-angiogenic agents

Angiogenesis is a complex multistep process that plays an essential role in cancer progression and has become an attractive therapeutic target with the potential to be effective for a variety of malignancies (79). Vascular endothelial growth factor (VEGF) is a key promoter of cancer angiogenesis and is overexpressed in several tumor types, including CRC (80). Bevacizumab is a recombinant humanized monoclonal antibody against VEGF that serves as a “trap” neutralizing free VEGF. This antiangiogenic agent has demonstrated clinically significant synergistic activity against a variety of solid tumors (81-83). As regards CRC, in a phase II RCT the addition of bevacizumab to 5-FU/LV resulted in both higher ORR (40% vs 17%; P=0.029) and longer median PFS (9 vs 5.2 months; P=0.005) (84). In a subsequent phase III RCT of fist-line treatment for metastatic CRC, 815 patients were randomized to IFL

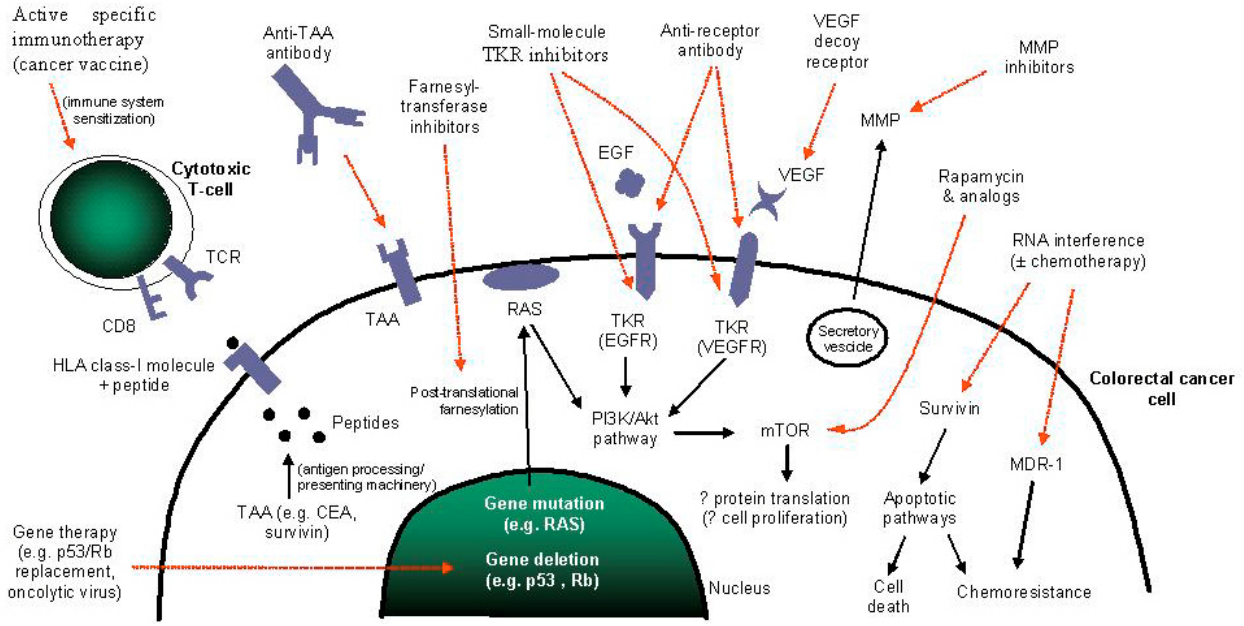

Figure 1. Examples of molecular targets for colorectal cancer-selective therapeutic strategies. TKR: tyrosine-kinase receptor; EGF: epidermal growth factor; EGFR: EGF receptor; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptor; MMP: matrix metallo-proteinase; mTOR: mammalian target of rapamycin; PI3K: phosphatidylinositol-3-kinase; MDR-1: multi-drug resistance protein-1; TAA: tumor-associated antigen; TCR: T-cell receptor.

versus IFL plus bevacizumab (85): the combination regimen was associated with improved ORR (45% *vs* 35%; P=0.0029), PFS (10.6 *vs* 6.2 months; P<0.00001) and median OS (20.3 *vs* 15.6 months; P=0.00003). An unusual but easily treated toxicity (hypertension) was higher with bevacizumab (10.9% *vs* 2.3%), but no increase in bleeding or thrombotic events occurred.

Another antiangiogenic strategy is to block the tyrosine-kinase activity of the VEGF receptor catalytic domain. To this aim, a number of small molecule inhibitors have been developed: one of them (vatalanib, PTK787/ZK222584) (86), is under investigation in phase III RCT for the treatment of metastatic CRC.

5.1.2. Growth factor signaling pathway inhibitors

One of the most active and promising areas of investigation in the field of molecular oncology is the development of drugs inhibiting growth-factor signaling pathways (87), such as the ErbB receptor family, a group of tyrosine-kinase receptors including ErbB1 (also known as epidermal growth-factor receptor, EGFR, or HER1), ErbB2 (HER2 or HER2/neu), ErbB3 (or HER3) and ErbB4 (or HER4).

Cetuximab is chimeric monoclonal antibody that neutralizes the activity of EGFR and synergistically enhances the antitumor activity of both chemotherapy (88) and radiotherapy (89). In a phase II trial of cetuximab given as monotherapy in 57 patients with EGFR-positive CRC refractory to both 5-FU and irinotecan, 9% of patients achieved a partial response (90). In another phase II trial (n=121) of irinotecan plus cetuximab, the ORR in patients who had prior exposure to irinotecan was 19% (91). In a larger trial employing a 2:1 randomization scheme, 218 patients who were known to express EGFR and had progression after treatment with irinotecan were assigned to cetuximab plus irinotecan and 111 patients were assigned to cetuximab alone (92). ORR was 23% to cetuximab/irinotecan and 11% to cetuximab alone, indicating that antibody therapies are active even in a treatment-refractory population.

Another way to oppose the tyrosine-kinase activity of EGFR is to target its catalytic domain by means of small-molecule inhibitors (*e.g.* erlotinib, gefitinib), which have shown significant anticancer activity in patients with advanced/metastatic non-small cell lung carcinoma (93) and are being evaluated in CRC patients in association with both conventional chemotherapy and other targeted drugs.

5.2. Gene therapy

Although safety concerns regarding the clinical implementation of viral vectors (94) have tempered the enthusiasm surrounding this approach, advances in gene-delivery (*e.g.* development of third-generation lenti-viral vectors, adenoviral vectors, and encapsulated methods of delivering naked DNA (95)) and gene knock-down (i.e. RNA interference (96)) technology are nourishing the hope of investigators to fight cancer through this approach.

One strategy consists of replacing tumor-suppressor genes (*e.g.* p53, Rb) lost by malignant cells during the carcinogenesis/progression process. Preclinical findings show that exposure of p53-deficient CRC cells to

vectors encoding wild-type p53 has definite antiproliferative effect and sensitizes malignant cells to conventional cytotoxic agents such as 5-FU (97). In a phase I study of adenoviral mediated p53 gene (wild-type) delivery through the hepatic artery, the treatment was well tolerated and - of 12 patients who went on to receive HAI of FUDR - 11 had a significant (>50%) tumor shrinkage (98). The safety of systemic p53 gene therapy using the canarypox virus has been recently reported as well (99).

In another approach (called suicide gene therapy), cancer cells are transduced with a gene encoding an enzyme (*e.g.* thymidine kinase, cytosine deaminase) that converts an inactive pro-drug (gancyclovir, 5-fluorocytosine, respectively) into an active cytotoxic agent (phosphogancyclovir, 5-FU, respectively). In phase I trials the safety of this type of gene therapy administered by intratumoral injection has been proven, but no significant tumor regressions have been reported (100-102).

Increasing understanding of the virus-host interactions has led to the improvement in the design of genetically engineered oncolytic viruses (103). For instance, onyx-015 is an adenovirus lacking the E1B gene product for p53 degradation, which makes the virus to selectively replicate in p53-defective malignant cells. Although this allows for the intravenous administration of the virus, no tumor regression has been observed using such gene therapy alone (104). By contrast, when onyx-015 is administered through HAI in combination with systemic 5-FU/LV, the ORR was 25% in patients previously resistant to chemotherapy (105).
Although the clinical implementation of cancer gene therapy can be considered in its infancy, these and other results support further investigation in this field to fully explore its therapeutic potential.

5.3. Cancer vaccines

Active specific immunotherapy embodies the ideal tumor-killing system for three main reasons: 1) unlike chemotherapy, which follows a log-kill kinetics, immune system cell mediators can hunt-down the minimal residual disease on a single cell basis; 2) its potentially extreme tumor specificity, which has so far no equals among anticancer agents/strategies, guarantees minimal toxicity; 3) once appropriately trained, the immune system can mount a "cytotoxic memory" against the targeted tumor, ensuring further protection against disease recurrence. Despite these premises and several successes in animal models, the results of such cancer biotherapy in the clinical setting have not met the expectations (106). The molecular identification of tumor-associated antigens (TAA) coupled with other insights/advances in tumor immunology have recently renewed the enthusiasm for the development of anticancer vaccines (107, 108).

Several trials of vaccination for the treatment of metastatic CRC patients have been carried out (109, 110). Results demonstrate that different vaccination regimens/strategies (*e.g.* autologous/allogeneic tumor cells, heat-shock-proteins, viral/plasmid vectors coding for CEA/p53, anti-idiotypes mimicking TAA) can induce the immune system to recognize and destroy CRC cells in humans, with no significant toxicity. Although significant (partial/complete) CRC regression have been rarely observed (110), patients showing an immunological response to vaccination have been repeatedly reported to have a better clinical outcome as compared to non-responders (111-114). Similar findings have been reported in the adjuvant setting (after primary CRC resection or liver metastasectomy) (115, 116). Since - under particular circumstances - vaccination appears to effectively circumvent the phenomenon of tumor immune escape/resistance, the major challenge of tumor immunologists is to manipulate the immune response so to reproduce these conducive conditions in a larger set of patients. Several strategies have been validated in preclinical models to break immune tolerance towards malignant cells, some of them being tested in clinical trials (108). As regards CRC, the implementation of vaccine regimens based on dendritic cells – the most powerful antigen-presenting cells – has yielded encouraging results (117, 118) that justify further investigation. Peptides - the 8-10 amino-acid long TAA segments recognized by T-lymphocytes on the surface of antigen-presenting and malignant cells - have been largely experimented in patients with a variety of tumor types. Pilot studies in subjects with metastatic CRC have been recently published (114, 117, 119, 120) and others are underway, some using peptides derived from non-vital TAA (*e.g.* CEA), others from TAA playing a crucial role in tumor cell survival (*e.g.* survivin, an anti-apoptotic protein).

Overall, preliminary results from small/non-randomized studies do not allow to judge the efficacy of these novel vaccination strategies in patients with CRC. Only the conduction of larger/randomized trials and the clinical implementation of recent tumor immunology insights will allow investigators to define the role of cancer vaccines (alone or combined with other/conventional treatments) in the therapeutic management of metastatic CRC (107).

6. TREATMENT PERSONALIZATION

Current treatment strategies for CRC are far from optimal, due in part to the inability to accurately distinguish subgroups of patients that differ in their prognosis (likelihood of experiencing disease relapse and thus of dying from CRC) and their probability of responding to a given treatment (4). Currently, ~80% of CRC patients receiving 5-FU-based chemotherapy do not benefit from this treatment, either because they have been already cured by surgery, or because their tumor is refractory to the administered antineoplastic agents, or because they do not receive the optimal drug dosage according to their own capability of metabolizing the therapeutic drugs. Therefore, the identification of biomarkers capable of distinguishing between these patient subsets would be of paramount clinical value for several reasons. First, patients unlikely to respond to a given therapeutic regimen could be spared the toxicity and the expenses associated with the treatment itself. Secondly, these subjects could be placed on alternate therapies. Third, many chemotherapeutic agents may

promote the acquisition of multidrug resistance that – if not promptly recognized at molecular level – allows the tumor to progress while the patient is still on treatment.

6.1. Pharmacogenetics/genomics

Genetically determined variability of the function of certain key enzymes has been shown to influence chemotherapy toxicity/response and ultimately CRC patients' survival (121). The study of the influence of genotype on drug activity and efficacy (pharmacogenetics) and the genome-wide approach to drug discovery and interpretation of complex pharmacological responses (pharmacogenomics) are gaining momentum in current molecular medicine as the pharmaco-dynamics/-kinetics of antineoplastic agents is elucidated and the phenomenon of drug resistance is dissected (122, 123).

Most studies have examined the predictive value of the expression levels of thymidylate synthetase (TS) and other related enzymes (*e.g.* thymidine phosphorylase, TP) in affecting 5-FU metabolism (124-126) in CRC patients undergoing chemotherapy. Unfortunately, while several reports have linked low TS expression with improved response to 5-FU *in vivo*, others have shown no relationship between these parameters. The predictive efficacy of TP is also unclear, with both high and low levels of TP linked to 5-FU response depending on whether the studies were performed *in vitro* or *in vivo*, respectively. Other studies suggest that factors involved in regulating cell growth and apoptosis, (*e.g.* p53, c-Myc, Bcl-2 family members, DCC, p21/WAF1/cip1, p27/kip1), can predict response to 5-FU-based therapy, although some conflicting data have been reported (127). Furthermore, various allelic deletions and mismatch repair status may identify tumor subsets with differential 5-FU sensitivity. For example, tumors that are mismatch repair-deficient have been reported to show improved response to 5-FU, although studies reporting no difference, and the converse, have also been published (128).

More recently, factors correlated with tumor sensitivity to newer chemotherapeutic agents (*e.g.* irinotecan, oxaliplatin) (129) as well as targeted agents (*e.g.* EGFR-targeted agents) (130, 131) have been described, although the experience is obviously limited and the clinical value still to be determined.

6.2. Identification of patients at risk of recurrence

Following radical surgery or chemotherapy-induced complete tumor remission, the identification of patients with minimal residual disease would allow clinicians to treat only patients who need further therapy. As the molecular mechanisms underlying CRC aggressiveness/metastatic potential are elucidated, putative molecular prognostic factors expressed by the primary tumor are proposed to select patients at higher risk of disease relapse (Table 1) (132-135). Unfortunately, none of these factors has been so far demonstrated of routine clinical value 4, due to their insufficient prognostic power in individual patients.

Another approach to the issue of defining the risk of disease recurrence is to directly detect the minimal residual disease in the peripheral blood (circulating tumor cells) by means of highly sensitive molecular biology techniques (*e.g.* quantitative real-time PCR (136)) as already validated in hematological malignancies and proposed for other solid tumors such as melanoma (137) and breast carcinoma (138). As regards CRC, preliminary results are encouraging (139-141); however, the experience is still limited, some findings are conflicting (142, 143), and lager studies are warranted to prove the clinical usefulness of these strategies.

Molecularly based imaging technologies (*e.g.* positron emission tomography based on molecularly targeted probes) for the detection of the minimal residual disease on a cellular basis are being investigated (144, 145), but no data are yet available concerning patients with metastatic CRC.

6.3. Implementation of high-throughput technologies

As above outlined, the up- or down-regulated expression of several genes/proteins in primary/metastatic CRC has been found to correlate with different survival rates of patients, often allowing to subdivide a given TNM stage into prognostic subcategories. However, none of these biomarkers is utilized in the routine clinical practice, due to their insufficient prognostic power in individual patients. Moreover, some of these biomarkers (*e.g.* apoptosis-related and DNA damage repair molecules, enzymes involved in antineoplastic drug metabolism) have been linked to CRC sensitivity to treatment, but – again - their predictive value remains insufficient to permit their implementation in the therapeutic decision-making process of single patients. A further limitation of these factors is that they are often designed to predict response to a specific agent (most often 5-FU), and thus generally fail to identify alternative treatment options: a robust assay, capable of predicting the probability of response of a given tumor to the multiple therapeutic regimens that are increasingly becoming available, would therefore have significant clinical utility. Finally, most studies have so far relied on the expression of single prognostic/predictive factors despite the knowledge that cancer development/progression as well as treatment sensitivity/resistance are multifactorial phenomena. The complexity of the gene/protein abnormalities defining a given CRC argues that an assay capable of collectively considering all these variables may be more informative for the classification and determination of prognosis and response to therapy. The sequencing of the human genome, combined with the development of high-throughput screening technologies such as gene microarray (146), tissue microarray (147) and - more recently - proteomics (148) platforms now make such an approach possible (149). *In vitro*, it has been demonstrated that measurement of multiple rather than single biomarkers results in more accurate prediction of drug sensitivity when compared to traditional determinants of 5-FU and oxaliplatin response (129, 150). In patients with CRC, preliminary results on the utilization of high-throughput technologies for predictive and prognostic purposes are encouraging (151-156): nonetheless, only the broad implementation of such data in the protocols of large clinical trials will assess the ability of molecularly based treatment personalization to positively impact on the management of patients with CRC metastasis.

Table 1. Examples of biomarkers correlated with prognosis and/or treatment response in patients with colorectal carcinoma

Tumor suppressor genes and oncogenes	• K-ras • c-Myc • p53 • DCC (deleted in colon cancer) • smad4 • nm23
Apoptosis and survival-related factors	• Bcl-2 • Bax • Survivin • Telomerase
Growth-factorsand growth-factor receptors	• TGF-α (transforming growth-factor alpha) • TGF-β • CTGF (connective tissue growth-factor) • HER-2/neu • EGFR (epidermal growth-factor receptor) • c-Met (hepatocyte growth-factor receptor)
Mismatch repair genes	• MSH2 • MLH1
Angiogenesis-related molecules	• VEGF (vascular endothelial growth factor) • Endoglin (CD105) • HIF (hypoxia inducible factor)
Cyclin-dependent kinase inhibitors	• p27/kip1 • p21/waf1/cip1 • p16
Adhesion molecules	• CD44 • E-cadherin • ICAM-1
Markers of invasiveness	• MMP (matrix metallo-proteinases) • TIMP (tissue inhibitor of metallo-proteinase) • uPA (urokinase-type plasminogen activator)
Markers of proliferation	• Ki-67 • Mib-1 • PCNA (proliferation cell nuclear antigen) • β-catenin (Wnt pathway)
Drug metabolism enzymes	• TS (thymidylate synthetase) • TP (thymidine phosphorylase) • DPD (dihydropyrimidine dehydrogenase) • ERCC-1 (excision repair cross-complementing gene) • XPD (xeroderma pigmentosum group-D gene) • XRCC1 (X-ray cross-complementing group-1 gene) • PARP (poly(ADP)-ribose polymerase)

7. CONCLUDING REMARKS

Radical surgery currently provides the best chance of cure for patients with CRC liver metastases, with acceptable mortality/morbidity rates. Unfortunately, only a minority of them is eligible for hepatic resection owing to either insufficient liver functional reserve, or extra-hepatic disease or poor general conditions. Moreover, several patients who underwent radical surgery develop hepatic and/or extra-hepatic recurrence, which underscores the need for more effective adjuvant treatments.

Locoregional strategies (HAI, IHP, ablative techniques) can obtain significant tumor regression/local disease control rates, but there is no definitive evidence of their impact on patients' OS. Likely, only the implementation of more active antineoplastic agents (HAI, IHP) and/or the combination with systemic treatments will maximize the advantages (*e.g.* favorable pharmacokinetics) proper of these approaches. In regards to systemic chemotherapy, the conduction of RCT over the past two decades has led to tangible progresses in the optimization of the drug regimen with significant improvements in terms of both tumor response and OS rates.

Despite these advances, most patients ultimately die of their disease due to hepatic and/or extra-hepatic cancer progression. Therefore, novel therapeutic strategies are urgently needed to improve the prognosis of patients with metastatic CRC. Thanks also to the implementation of novel high-throughput technologies that allow for a

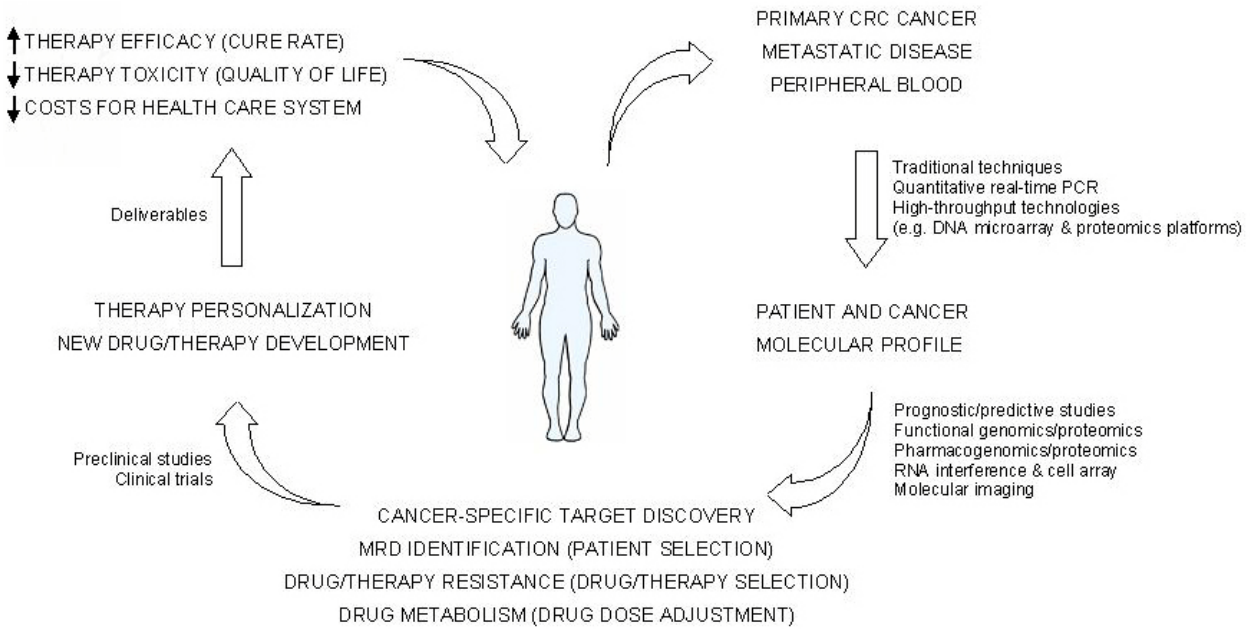

Figure 2. Bench-to-bedside translational approach to the therapeutic management of patients with colorectal cancer (CRC). MRD: minimal residual disease.

comprehensive evaluation of the molecular signature underlying malignant cell behavior, molecularly targeted therapeutic strategies are being developed. Controlled clinical trials have already demonstrated that some agents targeting tumor-specific molecular derangements can significantly improve the therapeutic efficacy of conventional antineoplastic drugs against CRC as well as other solid malignancies in the advanced/metastatic setting (78), which has inaugurated a new era in the field of oncology. On the basis of the results yielded in the treatment of advanced/metastatic CRC, it is reasonable to believe that these novel therapeutic regimens will provide similar survival advantages in the adjuvant setting (*e.g.* after primary/metastatic CRC resection), as investigators are verifying in ongoing trials. The dissection of the molecular mechanisms underlying cancer development/progression and tumor/host interactions will not only facilitate the discovery of novel tumor "Achilles' heels" potentially targetable by novel cancer-selective strategies, but also will allow for the personalization of the therapeutic regimen according to the molecular features of individual patients/tumors. Current criteria for the formulation of patient prognosis and prediction of treatment responsiveness rely upon traditional clinico-pathological factors (*e.g.* primary tumor TNM stage, liver metastases number/size, lymph-node involvement, margin width, expression of single molecular markers), which are likely inadequate to accurately identify metastatic disease with greater intrinsic aggressiveness/treatment resistance. The better understanding of the cascade of molecular events underlying CRC aggressiveness and treatment sensitivity is providing investigators with pathogenesis-based information, which is essential both for the identification of patients requiring adjuvant therapy after hepatic resection and the selection of the therapeutic approach most likely to be effective in each given patient (Figure 2). Hopefully, in the near future the broader implementation of these molecular oncology findings and concepts in clinical protocols for the multidisciplinary approach to CRC liver metastasis will translate into a better chance of cure for patients (4).

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