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New Strategies to Enhance the Efficacy of Surgical Treatment for Colorectal Liver Metastasis

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1. Introduction

Colorectal cancer (CRC) is the second most common cause of cancer death worldwide [1]. The liver is the most frequent site of metastasis in CRC, both at the time of diagnosis (15-20% of cases) and after apparently radical surgery on the primary tumour (nearly 40% of cases). If patients with colorectal liver metastasis (CRC-LM) are not treated, prognosis is very poor, with a near zero five-year survival rate. At present, liver resection is the only treatment modality that has the potential to achieve long-term survival and to offer the possibility of a cure. Patients who undergo complete (R0) resection of liver metastases have a five-year survival rate of approximately 40-50% [2]. Unfortunately, however, 50% to 70% of patients develop secondary metastatic disease after R0-resection of CRC-LM [3].

In order to obtain the best results in a systemic disease such as metastatic CRC (mCRC), the optimal integration of medical treatment and surgery is essential. The introduction of several effective cytotoxic and targeting agents, in combination with surgical treatment, has extended survival [4]. In addition, promising emerging therapies –cancer stem cell (CSC)-targeted therapies, pathway inhibitors for CRC, induction of tumour cell differentiation, improving liver regeneration, and nanoparticle (NP)-guided tumour ablation, among others– may be found to be effective in achieving better control and even complete eradication of CRC-LM. If confirmed, these strategies will bring significant benefits to patients, particularly in terms of long-term survival. Further, in this era of multimodality treatment of CRC, it is critically important to identify effective biomarkers for prognosis and prediction of individual treatment responses, and these are expected to become useful tools for improving therapeutic approaches.

The present chapter aims i, to present the state of the art related to criteria for appropriate decision making for CRC-LM treatment; ii, to review the current data on the role of biomarkers used for the prediction of response to CRC-LM therapies; and iii, to outline emerging targeting agents and new therapeutic techniques to improve life expectancy and quality of life in CRC-LM patients.

2. Therapeutic approaches to colorectal liver metastases: The state of the art

More than half of patients with CRC will develop liver metastases, and nearly 80% of them are initially unresectable. Hence, optimal management of hepatic metastases often requires a multidisciplinary approach. The availability of new medical therapies, including neoadjuvant chemotherapy and targeted therapies, render a considerable percentage (up to 40%) of initially unresectable patients potentially resectable, improving the overall outcomes of patients with mCRC.

Nowadays, to optimise the integration between surgery and medical approaches in the treatment of mCRC it is necessary to consider different groups, based on current guidelines for stratification of patients according to clinical goals and treatment. In this section, we summarise the recommended therapeutic approaches for CRC-LM according to the aforementioned patient stratification.

2.1. Selection criteria for resection of colorectal liver metastases: Definition of resectability

Patients diagnosed with mCRC should undergo an upfront evaluation by a multidisciplinary team, including medical oncologists, surgeons, radiotherapists and radiologists, in order to assess resectability status and to achieve the best therapeutic results [5]. The target end point for assessing resectability is the potential of surgery to cure the disease when achieving RO resection of all evident disease. Incomplete resection (macroscopic or microscopic), so-called debulking surgery, has not been found to help achieve this end point [6].

Classically, surgical criteria have determined resectability [7], but over recent years several authors have questioned the relevance of many of them. Nowadays, the only surgical criteria that continue to be widely used are complete tumour resection with the preservation of two contiguous liver segments (with adequate vascular inflow and outflow) and an adequate liver remnant (at least 25% of the total liver volume considering the healthy organ) [8]. If, however, we seek a more comprehensive definition of resectability, we should also take into account prognostic evaluation and predicted response to different treatments, by including multiple clinical and molecular factors, which influence patient outcome. Some validated clinical scores are already available, while molecular factors are still under investigation (discussed in more detail below in the section entitled *Predictive biomarkers for response to treatment in colorectal cancer*). In relation to this, the study conducted by Fong *et al.* [9] at the Memorial-Sloan Kettering Cancer Center has been one of the most useful attempts to define prognosis after surgical management of CRC-LM. The score proposed integrates a range of risk factors which influence the risk of death after surgery: preoperative carcinoembryonic antigen (CEA) level > 200

ng/ml, synchronous metastases or metachronous metastases with a disease-free interval of less than twelve months, more than one metastasis, extrahepatic disease, a tumour > 5 cm in diameter and lymph node involvement associated with the primary tumour.

Over recent years, several studies, well summarised in the systematic review of Quan *et al.* [10], have investigated the validity of these various criteria. Notably, the value of the following indicators have been questioned: the number of lesions, maximum lesion dimensions, timing of metastases, absence of metastatic spread outside the liver, and margin of healthy liver tissue, as has the definition of an adequate liver remnant after resection [11].

In summary, the criteria listed in this section can be regarded as an invaluable tool for patient stratification before liver resection, but failure to meet them should not constitute an absolute contraindication to surgery.

2.2. Optimal chemotherapy timing and regimes

In the case of resectable CRC-LM, current guidelines recommend the administration of a course of an active systemic chemotherapy regimen for a total perioperative treatment time of approximately six months [12]. The preferred regimens are combination chemotherapy based on fluoropyrimidines XELOX (capecitabine plus oxaliplatin), FOLFOX (5-fluorouracil, leucovorin and oxaliplatin) and FOLFIRI (5-fluorouracil, leucovorin and irinotecan), optionally together with antiangiogenic biological agents (bevacizumab, cetuximab or panitumumab). In order to improve the selection of the regimen to be used, KRAS mutation status should be determined in all patients at the time of diagnosis of metastatic disease. If no KRAS mutations are identified, BRAF testing should be considered [13]. New targeting biological agents are emerging and they can be expected to lead to improvements in clinical effectiveness. Their effects, pathways and theoretical and practical applications will be discussed in more detail below in the section entitled *Emerging targeting agents for colorectal liver metastases*.

In patients with few metastases that are easy to surgically resect and no poor prognostic indicators, postoperative chemotherapy is usually preferred [14]. On the other hand, in other clinical situations, perioperative (neoadjuvant plus postoperative) chemotherapy can be used [15]. The optimal sequencing of chemotherapy is not clear, however, and recent studies have assessed the pros and cons of different possible timings of administration [16-17]. Potential advantages of administering chemotherapy preoperatively include earlier treatment of micrometastatic disease, evaluation of responsiveness to chemotherapy (which can be prognostic and help decision making) and avoidance of surgery in patients who progress early. On the other hand, several disadvantages have also been highlighted: the risk of missing the window of opportunity for resection, which may be due to disease progression or due to a complete response making it difficult to identify areas for resection; radiological complete response does not always mean pathological response, as viable cancer cells can remain at the original sites of metastases [18]; hepatotoxicity develops with some regimens with serious clinical implications both before and after surgery [19]; and finally, frequent radiological examinations must be undertaken to determine the appropriate timing for surgery [20]. There is now a general trend towards the use of perioperative chemotherapy for patients with

resectable CRC-LM, but more studies are necessary to provide stronger evidence regarding the benefit of this approach.

It is important to note that patients diagnosed with rectal cancer and resectable synchronous liver metastases usually need a specific approach due to the risk of locoregional failure. Preoperative chemoradiotherapy in locally advanced rectal tumours (cT3/4, cN+) decreases the risk of pelvic recurrence after surgery, and postoperative chemotherapy is also mandatory [21]. Some studies suggest that pelvic radiotherapy diminishes tolerance to biological agents, but there is not enough data to guide decisions on when this approach may be suitable. After surgical resection of metastases and rectal lesions, pathological rectal disease determines adjuvant therapy. Specifically, postoperative chemoradiotherapy is advised for patients who have not received prior chemoradiation and have a higher risk of pelvic recurrence (pT3/4 or pN1/2) [22]. Patients with pT1-2pN0 tumours should receive six months of adjuvant chemotherapy without pelvic radiation.

In brief, the choices of type of chemotherapy/chemoradiotherapy regimen and its timing depend on a number of factors, namely a patient's disease history and pathological status, tumour gene expression, and previous treatment (including chemotherapy and associated drug toxicity/safety), as well as institutional preferences.

2.3. Conversion or downsizing chemotherapy

When patients present initially unresectable disease, owing to technical difficulties and/or the presence of poor prognostic factors, treatment decisions are difficult. In this clinical situation, preoperative chemotherapy is being considered in highly selected cases in an attempt to downsize CRC-LM and convert them to a resectable status. In these cases, any active metastatic chemotherapy regimen can be used, the goal being to reduce the size of the visible metastases as much as possible. Several trials have been conducted using different combinations of chemotherapy and biological agents, but they have not provided compelling evidence to favour one regimen over another [23-24].

Further, there are other factors we must keep in mind when considering this kind of treatment. Some chemotherapy regimens may cause hepatotoxicity (steatohepatitis and sinusoidal liver injury, among others) [25] with clinical implications for liver surgery, and we must measure responsiveness, so radiological and clinical reassessment should be scheduled approximately every two months after the initiation of chemotherapy. If the disease becomes resectable, surgery should be performed as soon as possible, in order to limit toxicity.

In addition, we must optimise imaging of CRC-LM choosing the most accurate methods. Radiological imaging techniques, namely computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography-PET/CT in selected cases, are the essential tools to measure patient tumour response and resectability, while intraoperative ultrasonography (IOUS) remains mandatory in all patients undergoing surgical resection of CRC-LM. Indeed, several authors have demonstrated that IOUS can change the surgical management in up to 35% of patients [26-27].

Furthermore, it must be taken into account that sometimes a radiological complete response can be achieved in patients with one or more metastases. However, a systematic review of the literature suggests that between 17 and 51% of such patients will have residual microscopic disease [28-29]. Accordingly, all liver metastases, including those with radiological complete response after chemotherapy, should be resected when technically feasible. In addition, almost all authors recommend postoperative chemotherapy in these cases.

Overall, recent studies have reported that resectability is achieved in 10 to 40% of selected patients after chemotherapy. It remains difficult, however, to draw firm conclusions as the definition of unresectable disease, patient selection criteria, drug regimens, and outcome measures vary, and there is insufficient data from randomised controlled trials [10].

2.4. Surgical strategy

Liver resection remains the only treatment modality that can achieve long-term survival and offers a possibility of a cure [2]. Selection criteria for resection of CRC-LM are continually being refined (see the aforementioned *Definition of resectability*). In this field, advances in surgical strategy (timing, techniques, etc.) have improved results, but patient management is complex and tends to require a combination of different approaches (colorectal resection, liver resection, chemotherapy, and radiotherapy, among others).

At present, when oncology committees evaluate CRC-LM for surgical treatment, several clinical scenarios are considered: resectable synchronous metastases, resectable metachronous metastases and unresectable disease amenable to conversion chemotherapy.

2.4.1. Resectable synchronous metastases

Close to a third (15-34%) of patients have liver metastases at the time of diagnosis (synchronous metastases). Evidence-based protocols for the management of synchronous metastases are, however, poor, and few prospective studies have been published recently, such results as are available being difficult to generalise [30]. In any case, some suggestions can be made.

One of the open questions is the timing of colorectal and liver surgery (simultaneous versus staged). The traditional approach has been to perform surgery on the primary lesions (colorectal resection), followed by chemotherapy and subsequent liver surgery. Nowadays, simultaneous colorectal and liver resections are preferred when feasible [31]. This combined surgery can be safely performed whenever minor hepatectomies are planned, but there is no consensus on the best approach in cases requiring major hepatectomies. Considering the largest series, some show similar rates in simultaneous and staged resections [32], but a multicentre database analysis in the USA found increased morbidity and mortality after simultaneous major hepatectomy and colorectal resection [33]. Thus, in the absence of clear evidence, the decision to undertake more complex procedures must be made on a case-by-case basis. Even though surgery is the key treatment in patients with CRC-LM, chemotherapy (and sometimes radiotherapy) must also be administered. The sequencing of chemotherapy has been discussed above, and often influences surgical timing.

On the other hand, we should not forget that patients with synchronous metastases have a poorer prognosis [34]. Various strategies have been proposed in an attempt to improve results. One possibility, suggested by Mentha *et al.* [35], is a change in the treatment sequence, the so-called reverse approach, which consists of treatment with systemic chemotherapy followed by liver resection and subsequent primary tumour treatment (with the option of radiotherapy of the rectal tumour prior to resection). More recently, Bruquet *et al.* [36], have reported their experience at the MD Anderson Cancer Center comparing the traditional approach, combined or simultaneous resection, and a “reverse” strategy. These authors found similar oncological outcomes, morbidity and mortality with the three options. This lack of strong evidence makes us cautious, but we believe that the reverse strategy can be considered a reasonable option in patients with asymptomatic primary CRC but advanced CRC-LM.

2.4.2. Resectable metachronous metastases

Most patients with CRC-LM (2/3 of cases) develop liver metastases after initial treatment, during the course of the disease (metachronous metastases). The management of resectable metachronous disease is distinct from that of synchronous disease, though it should also include diagnostic imaging of CRC-LM, as well as evaluation of the chemotherapy and surgical history.

In this group of patients, PET/CT should be considered preoperatively to characterise the extent of metastatic disease and to identify possible sites of extrahepatic disease that could preclude surgery [37]. In addition, it is important to evaluate previous chemotherapy to guide the choice of regimen. In general, six months of perioperative (pre- and/or postoperative) chemotherapy is recommended, but in selected cases observation is also considered appropriate. In addition, previous hepatotoxicity and other side effects should be taken into account [12].

In relation to the type of surgery to be performed, previous surgery can preclude surgical treatment of metachronous metastases, especially if upfront liver resection was performed, it being necessary to assess the remnant liver and technical difficulties. Finally, recent data suggest that it is safe to adopt a surgical approach to the treatment of recurrent hepatic disease isolated to the liver, but that survival decreases with each subsequent curative surgical approach [38].

2.4.3. Unresectable disease amenable to conversion chemotherapy

In this clinical situation, preoperative chemotherapy is considered in highly selected cases, with the aim of downsizing CRC-LM [39]. After disease becomes resectable, surgery should be performed as soon as possible and all liver metastases, including those with radiographic complete response after chemotherapy, should be resected when technically feasible [10]. The treatment of the primary tumour can be postponed until the completion of adjuvant therapy [36].

2.4.4. New surgical techniques

A feasible approach has emerged for patients who would be left with an inadequate future liver remnant (FLR) if complete disease clearance were to be attempted with a single hepatec-

tomy: this is a two-stage hepatectomy for bilobular liver metastases in combination with selected use of portal venous embolization. It offers the best chance of achieving an adequate degree of liver remnant hypertrophy, being more effective than portal venous embolization before a single hepatectomy [40].

Although the standard of care for resectable liver metastases is surgical resection, in selected patients other liver-directed therapies can be used in addition to or instead of surgical resection. These include intrahepatic arterial chemotherapy [41], radiofrequency tumour ablation [42], radioembolization [43] and stereotactic external beam radiation [44]. Some of these innovative techniques will be discussed in more depth below in the section entitled *New therapeutic techniques for colorectal liver metastases*.

3. Predictive biomarkers for response to treatment in colorectal cancer

Over the past decade, developments in CRC-LM therapy have improved the prognosis of patients. Combination chemotherapy, such as FOLFOX or FOLFIRI, has become the standard regimen for unresectable advanced or recurrent CRC, and high response rates have been reported. On the other hand, not all patients respond well to these therapies, and it has been suggested that differential responses are due to the specific molecular profile of each patient and/or tumour. To facilitate the design of personalised therapeutic strategies for CRC patients, it is therefore important to identify biomarkers which are able to accurately predict the sensitivity of patients to the potential therapies and to estimate the likely course of the illness.

Recent advances in the fields of genomics and proteomics have contributed to our understanding of CRC at the molecular level by evaluating the expression profiles of genes and proteins in tissues and body fluids. To date, some of the candidate biomarkers have yielded somewhat contradictory results. On the other hand, several studies have identified candidate molecular biomarkers that may help to predict the response to cytotoxic chemotherapy and guide treatment selection.

3.1. Molecular predictors of response to chemotherapy

5-Fluorouracil (5-FU) is the main stay of all current standard CRC chemotherapy regimens, despite the fact that it causes serious side effects (grade 3 or 4) in up to 30% of patients. Several enzymes involved in 5-FU metabolism have been proposed as predictors of response to fluoropyrimidine treatment: 5-FU exerts its activity by inhibiting thymidylate synthase (TS), a key enzyme of nucleotide pyrimidine metabolism that is essential for DNA synthesis and cellular proliferation. Thymidine phosphorylase (TP) is another enzyme involved in thymidine metabolism, which regulates the conversion of thymine to thymidine; this is why it is thought to limit the toxicity of high levels of thymidine and prevent replication errors during DNA synthesis. In this role, TP degrades 5-FU, limiting the activity of this chemotherapeutic agent. Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting hepatic enzyme involved in the catabolism of 5-FU [45]. It was found that low levels of expression of TP, DPD and TS were independently associated with improved overall survival [46]. Specific-

ly, deficiency in DPD activity caused by mutations in the gene encoding DPD (the *DYPD* gene) can lead to severe 5-FU-related toxicities, which can be fatal. However, as mutations in the *DPYD* gene are responsible for only some of the adverse reactions to 5-FU and the association between genotype and phenotype is not clear [47], further assay development and prospective trials are needed to evaluate the clinical usefulness of these enzymes in predicting which patients are likely to develop serious, life-threatening toxicity to 5-FU [48]. *Irinotecan* (*CPT-11*), a topoisomerase inhibitor, shows efficacy in the treatment of mCRC when used either as a single agent or in combination with radiotherapy and/or other chemotherapeutic drugs. Irinotecan acts as an inhibitor of DNA topoisomerase I (Topo I) and exerts a cytotoxic effect in replicating cells by inducing DNA strand breaks [49]. The active metabolite of irinotecan is SN-38, which is metabolised *in vivo* through conjugation by the liver enzyme uridine diphosphate glucuronosyltransferase (UGT1A1). A variant of the gene encoding this enzyme (UGT1A1*28) has come to be considered the main pharmacogenetic marker for severe haematological toxicity (neutropaenia) of the drug. Nevertheless, UGT1A1*28 testing as a predictive marker of adverse effects needs to be further investigated before translation to clinical practice and the available data are not conclusive in defining a precise genotype-based dosage [50-51]. In addition, tumour expression of Topo-I has been explored as a biomarker of the efficacy of irinotecan-based therapies [52]. Although further studies are needed, it has been shown that high levels of Topo-I in tumour tissue are associated with a good response to irinotecan [53-54]; these data are consistent with the hypothesis that a larger amount of Topo I would facilitate the activity of a Topo I inhibitor [55]. *Oxaliplatin* is a platinum analogue that improves response rate and survival in patients with advanced CRC. DNA kinking is the major feature of platinum-DNA adducts that block DNA replication and lead to cancer cell death. These DNA strand breaks are recognised and repaired by the nucleotide excision repair (NER) pathway, whose major components are excision repair cross-complementation group (ERCC1 and ERCC2) proteins, acting as the rate-limiting enzymes of oxaliplatin efficacy. Several studies have demonstrated that low levels of ERCC1 and/or ERCC2 gene expression correlate well with better response rates following oxaliplatin-based therapy in advanced CRC patients, leading to improved survival [56-58]. Despite these promising findings, most of the studies have been retrospective and differed significantly in design, and results have not been consistent. Further prospective trials are needed to assess the correlation between decreased expression of *ERCC1* and *ERCC2* and platinum toxicities.

3.2. Molecular predictors of response to anti-EGFR therapy

We should also consider other markers which it has been suggested may be useful for predicting patient responses to biological agents, in particular anti-epidermal growth factor receptor (EGFR) monoclonal therapy. EGFR is a member of the transmembrane tyrosine kinase receptor family ErbB, involved in tumour cell proliferation, inhibition of apoptosis, invasion, migration and angiogenesis [59-60]. When a ligand binds the EGFR homo or hetero-dimers are formed with other ErbB family members, initiating two main intracellular cascades, which are important for cell survival, proliferation and migration. On the one hand, membrane localization of the lipid kinase PIK3CA counteracts PTEN and promotes AKT1 phosphoryla-

tion and, on the other, KRAS activates BRAF, which in turn triggers the mitogen-activated protein kinases [61]. Abnormal expression of EGFR has been demonstrated in many advanced tumours, including in breast cancers, gliomas and lung cancer. In the case of mCRC, EGFR overexpression has been detected in 60-80% of cases [59] and a correlation has been reported with early tumour recurrence and extra-hepatic metastasis [62]. For these reasons, researchers started to explore therapeutic strategies to disrupt EGFR function.

Cetuximab, a chimeric IgG1 monoclonal antibody (mAb), and panitumumab, a humanised IgG2 mAb, target the EGFR and have small, but nonetheless clinically important response rates of around 10% in unselected patients with chemotherapy-refractory metastatic CRCs. However, its exact role in the CRC metastatic cascade has not yet been characterised due to controversial results obtained with anti-EGFR antibody therapy. In fact, it has been shown that the response to this therapy is independent of EGFR expression in tumour tissue [63]. In relation to this, some studies suggest that EGFR expression in the primary tumour does not necessarily correspond with the level of expression in metastatic tissue, while other studies have reported 78-100% concordance in EGFR expression in the two tissue compartments [64]. These findings prompted an effort to identify alternative predictive molecular biomarkers that could help to select patients more likely to benefit from anti-EGFR agents. Candidates that have been investigated so far include not only molecular alterations affecting the EGFR, but also molecular events downstream in the pathway, such as aberrations in the interlinked RAS-RAF-mitogen-activated protein kinase and PI3K-AKT-mTOR intracellular signalling transducers.

KRAS encodes for a cytoplasmic GTP-binding protein with low inherent GTPase activity. When the KRAS protein is bound to GTP, it relays signals of cellular proliferation and inhibition of apoptosis, acting as a typical oncogene. Activating mutations in KRAS lead to a gain in function of this gene, and hence over-expression of RAS/RAF-dependent proteins. Specifically, mutations in codons 12 and 13 of exon 2 have been demonstrated to predict low response rate to EGFR monoclonal antibodies-targeted therapy [65]. A lack of efficacy and also a possible detrimental effect on anti-EGFR-based chemotherapy in KRAS-mutated patients have been suggested by some trials using cetuximab in first-line therapy for mCRC, such as OPUS (oxaliplatin plus cetuximab) [66] and CRYSTAL (irinotecan plus cetuximab) [67]. These data indicate that KRAS mutations can be considered a highly specific biomarker to predict poor response to treatment with anti-EGFR mAbs. In addition, due to the fact that anti-EGFR agents fail to achieve either objective responses or disease stabilization in a substantial proportion of patients with wild-type KRAS tumours also, it seems necessary to investigate mutations in other genes involved in signalling pathways downstream of EGFR, including NRAS, BRAF, PIK3CA and PTEN.

Activating mutations in other members of the RAS family are less common than those found in KRAS. For instance, the reported frequency of NRAS mutations is 2.2 to 2.6%. Patients with tumours with these mutations had a significantly poorer response rate to anti-EGFR therapy [68], although no significant differences were seen in overall survival between patients with wild-type and mutant NRAS.

Mutations in BRAF, the major effector of KRAS, have also been associated with reduced sensitivity to EGFR-directed therapy. In a retrospective study, Di Nicolantino *et al.*

[69]examined tumours from patients who had received anti-EGFR therapy. They found that none of the patients carrying the BRAF V600E mutation responded and that none of the responders had a BRAF mutation. Moreover, De Roock *et al.* [68]recently conducted a large trial in which they analysed tumour specimens from CRC patients treated with the anti-EGFR agent cetuximab. They found that KRAS and BRAF mutations were mutually exclusive; the BRAF mutation was identified in 4.7% of cases and those carrying the mutation had a significantly lower response to anti-EGFR therapy, than those with BRAF wild-type tumours.

The phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin, *PI3K/AKT/mTOR*, pathway is another major intracellular signalling effector pathway activated by EGFR stimulation. Mutations in this pathway are present in as many as 30-40% of CRC patients. In particular, mutations in *PIK3CA* have been described in 15% of colon carcinomas, 20% of these being found in exon 20. Patients carrying these mutations and treated with anti-EGFR therapy have poorer clinical outcomes than wild-type *PIK3CA* carriers. However, because the number of patients with these mutations is very low in most studies, there is a need for controlled trials to assess whether the use of BRAF and *PIK3CA* mutation analysis as predictors of anti-EGFR therapy efficacy improves clinical outcomes [70].

PTEN is the only tumour suppressor gene involved in the *PI3K/AKT-mTOR* pathway. Loss of *PTEN* function, due to mutations, deletions or epigenetic silencing, leads to activation of this pathway. On the other hand, intact *PTEN* expression in metastatic tissue was found to be predictive of response to cetuximab, while this was not observed in patients with intact *PTEN* expression in primary tumour tissue [71]. These data are, however, limited and the findings need to be explored in larger confirmatory studies.

3.3. Molecular predictors of response to anti-VEGFR therapy

VEGF is overexpressed in CRC and the level of expression is directly correlated with the development of metastasis [72]. The VEGF family is made up of six growth factors (GFs) which exert their effects via binding to one of the three VEGFRs which belong to the tyrosine kinase receptor (TKR) family, mostly found in endothelial cells and angioblasts [64]. Bevacizumab is a humanised mAb which binds to VEGFA blocking the binding of this GF to VEGFR, thereby avoiding the corresponding intracellular signal transduction. Although several groups have focused their research efforts on finding a biomarker to accurately predict the clinical benefit of adding bevacizumab to therapy, no predictive molecules have yet been identified.

Several candidate predictive biomarkers similar to the KRAS mutation for cetuximab, have been proposed for bevacizumab, but they have remained elusive [73]. Specifically, it has been shown that the efficacy of bevacizumab therapy is independent of KRAS, BRAF and p53 status [74-75].

Another candidate is Ang-2, a regulator of angiogenesis that exerts context-dependent effects on endothelial cells. Although this ligand binds the endothelial-specific receptor tyrosine kinase 2 (TIE2) and acts as a negative regulator of angiogenesis, recent data from analysis of tumours indicate that, under certain conditions, Ang-2 can stimulate endothelial cells, acting as an anti-apoptotic agent in these cells [76]. In this context, serum Ang-2 has been proposed as a candidate biomarker due to the fact that patients having low pre-therapeutic Ang-2 serum

levels was significantly associated with response rate after receiving bevacizumab-containing treatment, though results should be further validated [77].

Recently, the development of quantitative predictive biomarkers has led to the increased use of imaging in the evaluation of tumour angiogenesis. Dynamic contrast enhanced-MRI (DCE-MRI) is a technique that can assess tumour perfusion and microvascular vessel wall permeability. Although it is difficult to evaluate the subtle changes occurring during bevacizumab treatment, correlations between tumour grade, microvessel density and VEGF expression in clinical trials of angiogenesis inhibitors have led to DCE-MRI parameters being proposed as biomarkers of drug efficacy [73]. Lastly, some authors have investigated changes in plasma cytokines and angiogenic factors during treatment as potential markers of therapeutic response and resistance [78].

3.4. DNA microarray-based gene expression profiling

Due to the genetic heterogeneity of CRC, many authors agree that it is likely to be necessary to assemble a panel of biomarkers to obtain high enough sensitivity to use these types of biomarkers as a screening test in clinical practice [48, 52]. To date, however, a limited number of markers have been identified in CRC, and their individual use has led to conflicting results.

In this context, advances in genomic techniques, such as DNA microarrays (allowing high-throughput analysis of genes), are very important as they provide large volumes of data which increases the probability of uncovering potential biomarkers. Recently, a total of 66 genes associated with benefit from adjuvant 5-FU/leucovorin treatment were identified. Six of the so-called “chemotherapy benefit genes” were selected to create treatment score algorithms. If validated, these signatures will quantify the likelihood of differential treatment benefit from 5-FU-based therapy [79]. Further, DNA microarray-based gene expression profiling provides a strategy to search systematically for molecular markers of colon cancer. Gene expression analysis studies have already resulted in many new insights into cancer biology and mRNA expression analysis is turning out to be a very useful tool for disease outcome prediction [80-81].

4. Emerging targeting agents for colorectal liver metastasis

The current treatment recommendations for mCRC indicate that therapeutic approach should be multidisciplinary [82], as surgery plus perioperative treatment offers better survival than surgery alone in patients with resectable or potentially resectable disease. Thus, whereas primary surgery is the gold standard for individuals with a single metastasis, it seems that for multinodular disease, neoadjuvant chemotherapy followed by surgery may be more appropriate [83]. Though in cases of unresectable aggressive disease, treatment should be decided on a case-by-case basis (adapting the strategy to the characteristics of the patient), a multidisciplinary approach should be taken to planning treatment from the outset. In fact, in select unresectable patients chemotherapy allows subsequent rescue surgery and achieves a significant increase in five-year survival rates [39]. An essential aspect of the treatment strategy for advanced CRC is the consideration of treatment as a continuum. Thus, sequential admin-

istration of conventional drug combinations based on fluoropyrimidines plus oxaliplatin or irinotecan, results in longer survival. While XELOX, FOLFOX and FOLFIRI are the schemes most commonly used, the trend has been for the standard of care chemotherapy for first-line mCRC to change from FOLFIRI to FOLFOX [84]. In order to improve the poor prognosis of patients with mCRC, treatment intensification has been also tested using the combination of the three active agents 5-FU/leucovorin, oxaliplatin and irinotecan (FOLFOXIRI), and this has achieved increase in R0 secondary resection rate and in overall survival [85]. Other schemes are currently being evaluated in randomised phase II trials as first-line chemotherapy for advanced CRC; these include TOMOX (oxaliplatin plus raltitrexed) which has been found to have a similar efficacy to FOLFOX [86].

The development of new drugs that selectively target specific molecular pathways involved in tumour progression (targeted therapy) has resulted in one of the most important advances in mCRC in the last decade, with biological agents today being a commonly used weapon in the armamentarium against mCRC, particularly in chemorefractory patients and those who are not initially suitable liver resection candidates [87]. In recent years, intense efforts have been focused on developing new molecules to inhibit targets that are critical for CRC, including new anti-angiogenesis agents, novel tyrosine kinase inhibitors (TKIs), agents to act on the PI3K/Akt signalling pathway, modulators of autophagy, and proteasome inhibitors, as well as targeted therapies against cancer stem cells, among others.

4.1. Targeting angiogenesis

GFs have been identified as important targets [88] and the development of targeting biological agents, directed to block effects of GFs on tumour cells, and their integration with cytotoxic chemotherapy regimens has resulted in significant improvements in efficacy outcomes.

One of the most important effects of some GFs is the promotion of angiogenesis, an essential mechanism for both primary tumour growth and metastasis. Due to this, novel therapeutic approaches have focused on the role of angiogenesis-targeting inhibitors. So far, three antiangiogenic biological agents have been approved for the treatment of patients with mCRC: bevacizumab, cetuximab and panitumab. The first successful targeting agent was bevacizumab; today, there is clear evidence to recommend addition of this anti-VEGF antibody to cytotoxic therapy (irrespective of the selected chemotherapy regimen) in both the first- and second-line treatment, this significantly increasing overall survival [75]. Moreover, it has been shown that bevacizumab, combined with FOLFOX or FOLFIRI, may also be active in chemorefractory and selected mCRC patients [89]. Currently, new trials (CHARTA and PERIMAX) are being conducted with bevacizumab plus FOLFIRI, designed to assess the benefits and limitations of a highly active four-drug regimen in mCRC [4]. In addition, it is also important to note that in patients with mCRC on a bevacizumab-containing regimen who show disease progression and hence need a change in the chemotherapy regimen, maintenance therapy with bevacizumab appears to be associated with significantly longer overall survival than the same regimen without bevacizumab [90]; this fact highlights the importance of bevacizumab therapy beyond disease progression in patients with mCRC, although this use is not currently recommended outside clinical trials.

The other two antiangiogenic biological agents, cetuximab and panitumumab target the ligand-binding domain of EGFR. Signalling of this receptor appears to modulate angiogenesis via the upregulation of VEGF and other angiogenic factors [60]. The use of these EGFR inhibitors was approved for mCRC in patients with wild-type KRAS, whose tumours express EGFR. In fact, as described previously, BRAF and codon 12 KRAS mutations are predictive of adverse outcome in CRC patients receiving cetuximab, being associated with a shorter time to progression and poor survival [91]. In contrast, it has been demonstrated that the combination of chemotherapy, such as irinotecan or FOLFIRI, and cetuximab as a first line in patients with wild-type KRAS significantly improves survival [67, 92]. Cetuximab is also indicated as a monotherapy in such patients following failure of both irinotecan- and oxaliplatin-based chemotherapy [93], while panitumumab is also a valid second-line option for wild-type KRAS patients, as a monotherapy or combined with FOLFIRI [94], though the addition of this EGFR inhibitor to oxaliplatin-based chemotherapy in first-line treatment of mCRC did not improve survival or response rate [95]. In an attempt to increase anti-tumour activity by simultaneously blocking both VEGF and EGFR pathways, some randomised studies have explored the combination of cetuximab or panitumumab with bevacizumab plus chemotherapy, but no benefits were observed, and in some cases the outcome was actually poorer with a greater toxicity, so this type of combination is not recommended for mCRC [64].

In relation to toxicity, treatment with these biological agents is associated with a wide range of adverse events that sometimes require discontinuation of treatment; these include severe hypersensitivity and skin toxicities, in the case of EGFR mAbs, and hypertension, thromboembolic events, bleeding and proteinuria, with bevacizumab treatment [93].

Despite the aforementioned advances, the targeted biological agents currently available are only effective in a small subset of patients (for example, less than half of the KRAS-wild type patient population benefits from anti-EGFR strategies) [96] and their overall impact on the treatment of mCRC has been relatively modest (beneficial effects only lasting on the order of weeks to a few months). These limited results, coupled with the undesirable effects, has led to intensification of the search for novel antiangiogenic therapies to increase the anti-tumour activity in advanced CRC. There are currently several molecules in phase II and III trials for treatment of mCRC that target various members of the VEGF family (aflibercept), signalling by VEGFRs (ramucirumab and IMC-18F1) or the tyrosine kinase components of these receptors (regorafenib, brivanib alaninate, cediranib and linifanib) [93, 97].

Aflibercept is a multiple angiogenic factor trap designed to block the angiogenesis network by binding VEGF-A, VEGF-B and placental growth factor (PLGF) [98]. The recent results of a multinational phase III study (VELOUR trial: aflibercept/FOLFIRI *vs.* placebo/FOLFIRI) demonstrated significant improvements in median overall survival, supporting the use of this VEGF Trap as a second-line option for patients with prior oxaliplatin treatment [99]. Ramucirumab is a fully humanised mAb directed against the extracellular domain of VEGFR-2, which binds VEGF-A and is believed to be the key VEGFR involved in tumour angiogenesis. Like aflibercept, ramucirumab is currently being evaluated in combination with FOLFIRI in a phase III trial for the second-line treatment of mCRC patients for whom prior oxaliplatin- and bevacizumab-containing initial therapy has failed [100]. In addition, a phase II study of

ramucirumab in the first-line setting in combination with FOLFOX6 therapy is also in progress [101]. Another anti-VEGFR mAb, IMC-18F1, which targets VEGFR-1 has been developed recently [102], and is also being studied in a phase II trial in mCRC.

Other candidate molecules represent new approaches to intracellular signal blockade of the VEGF and fibroblast growth factor (FGF) signalling pathways, via TKIs. In a recent phase III study (CORRECT trial), regorafenib has been found to improve survival in mCRC patients who progressed after all standard therapies, making it the first small-molecule multikinase inhibitor to have demonstrated survival benefits in such patients [103]. Brivanib alaninate is an oral TKI that specifically inhibits the VEGFR-1 and FGFR. This FGF signalling blockade may represent an important advantage, since it has been suggested that resistance to bevacizumab is associated with increased expression of FGF [78], and hence brivanib could have antiangiogenic activity in bevacizumab-resistant patients. In addition, a phase III trial combining brivanib and cetuximab in second/third line therapy in patients with advanced wild-type *KRAS* mCRC found improved progression free survival with no impact in overall survival [97]. Cediranib is an inhibitor of VEGFRs, platelet-derived growth factor-(PDGF) receptor beta and FGF receptor, whose activity has been compared with that of bevacizumab as a first-line treatment in combination with FOLFOX (HORIZON phase III trial) for mCRC patients; although cediranib activity was comparable to that of bevacizumab, the patient-reported outcomes were significantly less favourable [104]. Similarly, linifanib (a TKI that targets both VEGFRs and PDGFRs), in combination with FOLFOX, did not offer any advantages (over bevacizumab) in a randomised phase II trial as a second-line treatment for mCRC [105].

4.2. Targeting PI3K/Akt/mTOR pathway

The PI3K/Akt/mTOR pathway, an essential regulator of protein translation and cell proliferation, is another important target being investigated for mCRC in phase II and III trials. The PI3K/AKT/mTOR signalling cascade is constitutively active in many types of cancer and, in particular, it plays a critical role in the growth and progression of CRC. In addition, it has been demonstrated that this pathway may be upregulated after blockade of both VEGF- and EGFR-mediated signalling [101].

These aforementioned data provide the rationale for targeting this pathway therapeutically in CRC patients. Perifosine is an oral alkylphospholipid that targets both AKT and nuclear transcription factor-kappa B (NF- κ B) pathways. This novel molecule appears to enhance the cytotoxic effects of 5-FU: it has produced promising results in a phase II randomised trial of capecitabine \pm perifosine in previously treated patients with mCRC and, hence, is currently in phase III clinical development in combination with this 5-FU prodrug [106].

Activation of the PI3K/AKT cascade promotes mTOR, a serine-threonine kinase whose activation results in cell cycle progression and protein synthesis, and is involved in the CRC metastatic process. The mTOR inhibitors are analogues of rapamycin, including everolimus and temsirolimus, which are being investigated in clinical trials in combination with irinotecan, cetuximab, FOLFOX, bevacizumab or panatimumab in patients with mCRC progressing on prior chemotherapy [101]. Current expert opinion suggests that mTOR inhibitors may represent an attractive anti-tumour target in combination with strategies to target other

pathways that may overcome resistance [107]. In relation to this, it has been demonstrated that addition of the multikinase inhibitor, sorafenib, enhances the therapeutic effect of rapamycin on induction of apoptosis and inhibition of cell-cycle progression, migration and invasion of CRCs [108]. In addition, it has been suggested that mTOR inhibition by metformin (an antidiabetic drug), via activation of the AMP-activated protein kinase (AMPK) pathway, which functions as a sensor for cellular nutrient and energy levels, could be a new option for CRC [109].

4.3. Autophagy modulators and proteasome inhibitors

Autophagy is a multistep process of sequestration and subsequent elimination of cytosolic proteins, damaged organelles and protein aggregates in autophagosomes [110]. This self-degradation, via the lysosome, is responsible for the maintenance of intracellular homeostasis and enables cell survival under stress conditions. In the cancer cell, autophagy can be used as a strategy of self-adaption to generate nutrients and energy during tumour progression and in periods of hypoxia and stress, such as induced by chemotherapy, leading to development of drug resistance. The role of autophagy after chemotherapy remains controversial, it having been suggested that autophagy induction may increase efficacy of other anti-tumour agents, while most evidence suggests that the inhibition of autophagy is what can increase the effectiveness of these agents. As autophagy inhibitor, an analogue of chloroquine, hydroxychloroquine (HCQ), is currently involved in two different phase II studies for advanced CRC in combination with FOLFOX/bevacizumab or capecitabine/oxaliplatin/bevacizumab [111]. Given that HCQ induces ocular toxicities, such as retinopathy, novel autophagy inducers, such as Lys05, are currently being investigated in CRC [112].

In relation to autophagy inducers, since the PI3K/Akt/mTOR pathway is a key regulator of autophagy [113], mTOR inhibitors have been also used to modulate this mechanism, proving to be effective in many models for CRCs, but their clinical use has been less successful [114]. Proteasome inhibitors have also been described as autophagy inducers. It has been shown that proteasome inhibition generates a stress response through alteration of the protein milieu, which, in turn, induces endoplasmic reticulum stress; this causes an accumulation of misfolded proteins in the endoplasmic reticulum lumen and, consequently, induction of cellular stress responses, such as the unfolded protein response and autophagy to maintain endoplasmic reticulum homeostasis [110]. Bortezomib, the main proteasome inhibitor, was shown to induce autophagy in CRC cells [115]. However, in a randomised phase II study in relapsed or refractory CRC, bortezomib alone or in combination with irinotecan was not effective [116]. There are current trials examining combinations of bortezomib with other chemotherapies, such as oxaliplatin, 5-FU and leucovorin, in patients with advanced CRC [117]. In view of the limited results with autophagy inducers, some authors have suggested that optimal anti-tumour efficacy might be achieved by the combination of proteasome inhibitors and autophagy inhibitors [118].

4.4. Targeting cancer stem cells, Wnt pathway inhibitors, and tumour cell differentiation inducers

Cancer stem cells (CSCs) are a subpopulation of tumour cells that possess the capacity to self-renew and to give rise to the heterogeneous lineages of cancer cells that comprise the tumour,

and it is believed that they could be crucial in controlling and curing cancer [119]. Specifically, there is increasing evidence that CSCs play an important role in the occurrence, growth, and progression of tumours, as well as possibly in the initiation of distant metastases. In addition, CSCs are also involved in resistance to conventional chemotherapeutic drugs, novel tumour-targeted drugs, and radiation therapy [120].

CSCs have been identified not only in leukaemias, but also in solid tumours, including CRC. In fact, it has been suggested that CRC stem cells are responsible for tumour relapse, because conventional drugs fail to eliminate the CSC reservoir [121]. Due to this important clinical feature, CRC stem cells have recently been identified as a rational therapeutic target. Several CSC-targeted therapies have been proposed, including microbial- and plant-derived biomolecules; therapies directed at CSC-specific surface markers; some classical drugs, such as tranilast, curcumin and thioridazine; and reversal of their resistance to anti-tumour agents; so far, however, the toxicity of some of these approaches in normal stem cells and treatment resistance remain important limitations [122].

Various signalling pathways, such as Wingless/Int (Wnt), Hedgehog and Notch, are involved in maintaining the stemness of CSCs. Among these, Wnt, stands out for being particularly active in the majority of CRCs, and hence is the first being investigated for therapeutic targeting in CRC. A primary consequence of Wnt signalling activation is the stabilization of β -catenin in the cytoplasm, resulting in an increased translocation of β -catenin to the nucleus and, in turn, activation of Wnt target gene expression. Misregulation of the canonical Wnt/ β -catenin pathway and aberrant activation of Wnt signalling target genes are common in CRC and contribute to cancer progression [123]. Despite the importance of this pathway, few compounds have progressed beyond preclinical development. Efforts have been made to investigate the inhibition of a number Wnt genes, including the matrix metalloproteinases (MMPs), which play an important role in the degradation of extracellular matrix component, crucial for invasion and metastasis. Some studies have shown that increased expression of various MMPs (MMP-1, MMP-2 and MMP-9) favours CRC progression and could predict liver metastasis. Further, several therapeutic MMP inhibitors have been developed, but so far they have failed to produce a survival benefit and, in addition, they have been associated with adverse effects, such as musculoskeletal syndrome. The development of more selective MMP inhibitors is seen as a possible way forward [124].

Another novel compound is salinomycin, a polyether ionophore antibiotic that has been shown to kill CSCs in various types of human cancer, including CRC cells, mostly by interfering with ABC drug transporters and the Wnt/ β -catenin signalling pathway. Salinomycin inhibits the migratory and invasive capacity, and reduces the proportion of CD133 CSCs in HT29 and SW480 CRC cells [125]. The results from preclinical trials and its ability to kill therapy-resistant cancer cells make salinomycin a promising anticancer drug [126].

In recent years, other agents have been shown to suppress the self-renewal of CSCs *in vitro* and *in vivo*; these include metformin, DECA-14, rapamycin, oncostatin M, some natural compounds, oncolytic viruses, microRNAs, TNF-related apoptosis inducing ligand, telomerase inhibitors, mAbs and all-*trans* retinoic acid (ATRA). It has been suggested that combina-

tions of these agents and conventional therapy could significantly reduce tumour growth, metastasis and recurrence [127].

CSCs are characterised by two main properties of normal stem cells, self-renewal and differentiation. Given this, the induction of differentiation using retinoids would be a plausible therapeutic strategy. ATRA, a potent differentiating agent, has been demonstrated to induce CSC growth inhibition, and this has been associated with down-regulation of Wnt/ β -catenin signalling [128]. In a CRC tumour model of liver metastasis, we have demonstrated the anti-tumour effects of ATRA. This pro-differentiating agent hindered or completely abolished the pro-tumour stimulus produced by serum obtained from hepatectomised rats, and by a wide variety of GFs (HGF, VEGF, PDGF, EGF, and bFGF). In addition, in combination with 5-FU, an additive effect was observed in *in vitro* studies [129]. In *in vivo* experiments, ATRA also reduced tumour progression, though it failed to increase survival, both alone and in combination with 5-FU (unpublished data).

5. New therapeutic techniques for colorectal liver metastasis

Although surgical excision of tumour tissue remains the only potentially curative treatment for CRC-LM, several other techniques are now being developed to be used when surgery is not feasible or to improve surgical results.

The list of possible new therapeutic techniques for CRC-LM seems likely to increase over the next five years, including:

- Surgical approaches focused on increasing the size of the liver lobes (staged surgery or portal branch ligatures), as the percentage of remnant liver after hepatectomy is a limiting factor in many patients
- Techniques for percutaneous tumour ablation, which can reduce CRC-LM volume and allow surgery or at least delay the progression of the illness
- Nanoparticles (NPs) to selectively deliver drugs to tumour cells or induce local hyperthermia.

First, let us consider patients who could benefit from surgical excision of their liver metastases, but in whom the FLR would be less than 25%, which is currently considered the threshold of what can be tolerated. Initially, strategies for such cases were focused on selectively increasing the liver mass of liver lobes free of tumour. Some clinical trials have found that portal vein embolization (PVE) of the lobes bearing metastases induces regeneration of the other lobes, and this has been found to result in a 20-45% increase in their relative volume in two to eight weeks [130]. However, the clinical benefit of this procedure is not clear and, as there were also reports of tumour progression due to hepatectomy, it has not been widely adopted.

In patients who have inadequate FLR to undergo disease clearance with a single hepatectomy, two-stage hepatectomy for bilobar liver metastases in combination with selected use of portal venous embolization is feasible. It offers the best chance of achieving adequate FLR hypertro-

phy, better than a strategy involving PVE before a single hepatectomy. In addition, rates of macroscopic surgical clearance greater than 65% have been reported [131].

Surgical strategies must be individualised after careful assessment of disease distribution and its relationship to key underlying vascular and biliary structures. Thus, the majority of authors perform the first surgical stage focused on the minor hepatectomy and concurrent procedures as required. When necessary, ligation or embolization of the portal vein is carried out, to enhance the hepatic regeneration response induced by hepatectomy [132]. Nowadays, embolization is preferred to avoid surgical manipulation of the porta hepatis prior to major hepatectomies, and to achieve segment IV total portal inflow occlusion if a right hepatectomy is planned. Hypertrophy after PVE is maximal in the first three weeks, and tends to plateau after this period [133]. The time interval between hepatectomies must be long enough for adequate recovery from the first hepatectomy and liver hypertrophy, but not so long as to enable disease progression, since tumour volume may still increase within the occluded liver [134]. This interval is not well defined but is believed to be around eight to sixteen weeks. In the second-stage operation, the liver surgery usually is complex, often involving other procedures such as radiofrequency ablation. Consequently, postoperative morbidity is significant (50-60%) after this second surgery, particularly due to transient or permanent liver insufficiency. Nevertheless, when performed in referral centres for hepatic surgery the mortality rate is low (2.6-5%). Further, reported three-year survival rates after two-staged hepatectomy range from 30 to 58% [135], and in all series were significantly higher than in those patients treated with best palliative chemotherapy. Given this survival benefit and the feasibility of the surgery, this two-stage approach can be justified in suitably selected patients.

More recently, *in situ* liver transection with portal vein ligation has been proposed as a useful alternative for patients who have some segments of the left liver free of tumour, but an FLR that is too small [136]. In a first surgical intervention, arterial vessels and veins draining the lobes containing metastases are dissected and marked with vessel loops; then, the portal branches to those lobes are severed (most commonly, all right portal branches and segment I and IV branches). Some clinical trials have found that a 40-80% increase in FLR is achieved after three to eight days, and the patient can be re-operated on to remove the previously prepared lobes [137]. The results so far reported (daily increases of FRL up to 22%) are promising, but further clinical trials need to be carried out before this procedure can be generally recommended.

A quite different approach is percutaneous tumour ablation, an old design that is continuously being refined and improved with new technical developments. Initially, ablation of liver metastases was achieved by alcoholisation (ethanol injection), this being used as a downstaging procedure prior to surgery, then came radiofrequency thermoablation [42], and this was soon followed by microwave thermoablation. These procedures proved to be useful tools to reduce tumour volume, but only provided a transient effect when applied to CRC-LM. More recently, laser tumour ablation and cryoablation have gone through experimental trials in animals and are now being tested in patients, but still limited to primary liver tumours. As with previous treatments, if and when they prove to be useful in hepatocarcinomas, they will be tried in CRC-LM [138]

A different, perhaps more subtle, approach is embolization of the arterial vessels supplying the tumour. Transarterial embolisation with 300- to 500- μ m microspheres has been widely used either as a downstaging procedure or as a palliative treatment. This classic technique was improved by adding selective transarterial chemotherapy prior to embolisation, which allowed higher doses of chemotherapy with fewer side effects [139].

One of the problems in treating CRC-LM is the low tolerance of the liver parenchyma to radiation [140]. An elegant solution to overcome this limitation is known as *selective internal radiation therapy* and consists in the administration of ^{90}Y -resin microspheres through the arterial branches supplying the tumour. First applied to non-resectable hepatocarcinoma patients, achieving a reduction in tumour burden, relief of symptoms and increase in survival, it is now being tested in CLR-LM with promising results. However, the type of radiolabelled microspheres, indications and dosing schedules have to be better defined [141].

A novel approach has been the use of precharged particles to chemoembolise liver tumours. Smaller (50- to 100- μ m) electrically-activated microspheres are exposed to a chemotherapeutic agent which binds to them by electrostatic forces. These spheres are delivered to the vascular tumour bed where they are widely and uniformly seeded; then the drug is released and exerts its effect specifically on the tumour tissue, while the spheres block further blood supply. Preliminary reports have been quite promising, and may lead to the procedure being applied in CRC-LM [142].

All these techniques can, however, only be applied to selected macroscopic liver metastases, leaving untreated residual microfoci responsible for tumour recurrence. We need therapeutic tools to attack individual cancer cells seeded throughout the whole liver parenchyma from the primary colorectal tumour. Currently, one of the most promising avenues is radioimmunotherapy, with ongoing preclinical and clinical studies in CRC. This type of therapy involves the administration of radiolabeled mAbs that are directed specifically against tumour-associated antigens or against the tumour microenvironment. Some phase II trials have suggested that radioiodinated antibodies against CEA, as an adjuvant treatment after R0-resection of CRC-LM, improve overall survival [143]. More recently, new studies are being undertaken to assess the safety and efficacy of combining anti-CEA-RIT and kinase inhibitors, such as imatinib, to increase antibody distribution in CRC tumours [144].

On the other hand, the new field of nanosystems for cancer diagnostics and treatment is highly promising [145]. NPs, which easily escape detection and destruction by our immune system, are being used to deliver drugs directly to the tumour bed and selectively destroy cancer cells. It has been suggested that this strategy may be able to overcome tumour resistance and reduce toxicity in healthy organs. Tumour tissue tends to retain NPs, probably due to its particular characteristics (abnormally leaky endothelium and underdeveloped lymphatic drainage) [146], and this could explain the tendency of NPs to accumulate in liver metastases more than in normal liver parenchyma when administered through the hepatic artery, as we have recently shown [147]. Further, in order to decrease the severe dose-limiting toxicity of 5-FU and to enhance the concentration of this agent in the tumour mass, some researchers are investigating the use of 5-FU-loaded biodegradable NPs, and have already shown a significant improvement in the anticancer activity of the drug in an *in vitro* CRC model [148]. Finally, magnetic NPs are

being investigated in combination with high-frequency magnetic fields to induce local hyperthermia in the tumour, promising results having been obtained in experimental settings (CRC-LM in rats) [149].

6. Conclusion

Liver metastases are a common undesirable development in CRC and represent the leading cause of death in this high-prevalence disease. The management of CRC-LM has significantly changed over the past two decades, with dramatic improvements in patient outcomes. This has been made possible by the application of several key concepts when implementing different therapeutic approaches for subsets of patients with mCRC. Firstly, there is a clear consensus that the best management is achieved with a multimodality approach, including surgery, perioperative chemotherapy, biological agents and/or radiotherapy. Secondly, the therapeutic option with the best potential for cure in patients with CRC-LM remains complete resection of the metastases. Strategies to facilitate liver resection are allowing significantly increases in overall survival in this complex disease. In relation to this, the use of optimal first-line chemotherapy doublet (FOLFOX, FOLFIRI, XELOX) or triplet regimens (FOLFOXIRI) in combination with targeted therapy is now recognised as a good therapeutic approach in potentially resectable patients. In particular, the development of new biological molecules for targeted therapy (bevacizumab, cetuximab, and panatimumab) has been a key factor in the most important advances in mCRC treatment.

Nevertheless, much remains to be done. The fact that these current targeted biological agents are only effective in small subsets of patients with mCRC, and that their overall impact in the management of the disease is still relatively modest, has encouraged researchers to search for novel molecules that selectively target specific molecular pathways. This has resulted in a plethora of new antiangiogenic agents (afibercept, ramucirumab, regorafenib, etc.) and novel molecules directed against new biological targets (such as, autophagy or CSCs) or various different signalling pathways (Wnt and PI3K/AKT/mTOR, among others); these are currently being tested in preclinical studies or in phase II and III trials.

Additionally, new therapeutic techniques, such as surgical approaches focused on increasing the size of the liver lobes, SIRT, radiofrequency ablations and, more recently, NPs to selectively deliver drugs or to induce local hyperthermia in the tumour bed, promise to increase overall outcome in patients with advanced mCRC, in particular, in those with special characteristics that complicate treatment of their disease (inadequate FLR and others).

Finally, it is important not to forget the need to continue the search for new biomarkers to enable better patient stratification for each treatment option. Based on a better understanding of the process involved in the development and progression of CRC, biomarker panels will be developed and this will greatly facilitate the design of personalized medicine for CRC patients.

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