TREATMENT OPPORTUNITIES FOR COLORECTAL LIVER METASTASES

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ABSTRACT

Colorectal liver metastases (CLM) are the most common hepatic malignancy and are caused by disseminated tumour cells (DTCs) seeded early in the tumourigenesis of colorectal cancer. Despite optimal treatment, CLM are associated with high mortality rates. This review provides an overview of three promising strategies to extend survival in CLM: treatment of DTCs, immunotherapy, and new surgical resection techniques.

Keywords: Colorectal liver metastases (CLM), circulating tumour cells, immunotherapy, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), resection and partial liver segment II/III transplantation with delayed total hepatectomy (RAPID).

INTRODUCTION

Colorectal cancer (CRC) is the second most common cancer in Europe, with an estimated 447,000 new cases and 215,000 deaths in 2012.[1] Distant metastasis (advanced CRC) is the major cause of CRC-related death, and ultimately develops in 50% of patients with CRC.[2] The liver is the most common metastatic site and is involved in approximately 70% of cases.[3] Colorectal liver metastases (CLM) are thereby the most common hepatic malignancy.[4] When CLM are left untreated, median survival rarely exceeds 9 months.[5] Improvements in treatment over recent decades have increased survival, but so far disease-specific 10-year survival in advanced CRC remains ~5%.[6]

CRC spreads to the liver via the blood as disseminated tumour cells (DTCs), and the metastases that arise are difficult to treat, especially in the liver. This review provides an overview of three strategies that can potentially prolong survival in patients with CLM: treating the DTCs, immunotherapy, and new techniques allowing larger liver resections.

DISSEMINATED TUMOUR CELLS

Shedding of cells to the circulation does not exclusively occur in malignancy; circulating epithelial cells are found in various benign conditions.[7] However, in contrast to their benign counterparts, some DTCs can extravasate and colonise distant sites. The low concentration of DTCs (approximately 2 cells per mL are usually expected) makes them difficult to detect in peripheral blood, and even with current technologies the median DTC detection rate in advanced CRC is ~35%.[8] Preclinical studies suggest that the concentration of DTCs is high, even in early phases of cancer development.[9] More than a decade ago, Flatmark and colleagues[10] sampled bone marrow at the time of CRC resection and detected DTCs in 10% of the patients with Stage I CRC. Similarly, by sampling mesenteric venous blood during CRC resection, Seng et al.[11] found DTCs in almost all patients with Stage I CRC. However, DTCs were also detected in the bone marrow of a small number of patients with colorectal adenomas.[10] In a breast cancer study of similar design, DTCs were also detected in the bone marrow of patients with premalignant lesions.[12] Consequently, the mere presence of DTCs in bone marrow may have limited prognostic value; in breast

cancer, a pooled analysis revealed that two-thirds of these patients have a good prognosis.[13] Taken together, this suggests that DTCs from CRC are probably a heterogeneous population in which only a subset correspond to an adverse prognosis.

By comparing the genetic footprint of primary CRCs and their liver metastases, many studies have found a remarkable degree of similarity.[14-16] It is therefore probable that the DTCs responsible for metastasis share the mutational pattern of their parental tumour. However, despite extensive research, no metastasis-specific mutation(s) has been found; the same genetic alterations that cause malignant transformation also confer metastasis potential.[17] Two mutations in CRC, however, are particularly associated with a distinct metastasis pattern: mutations in the *KRAS* oncogene (occurring in 30–50% of patients with advanced CRC) have a higher probability of metastasising to the lungs, and are associated with increased recurrence rates following resection; while mutations in the *BRAF* oncogene (occurring in 5–15% of patients with advanced CRC) are associated with increased peritoneal and distant lymph node metastasis.[18]

CRCs can be categorised based on the type of genomic instability they display. Within this, approximately 60–70% display chromosomal instability (CIN), whereas 10–15% display microsatellite instability (MSI).[19,20] CIN cancers have a higher recurrence rate compared with diploid cancers (a recent study reported rates of 43% versus 22% in Stage II CRC),[19] while MSI cancers harbour a significantly higher number of mutations, are more immunogenic, and have lower recurrence rates.[21]

Taken together, certain mutation patterns and types of genomic instability may increase risk of metastasis, but they cannot predict or explain metastasis in a meaningful way. Other mechanisms must be responsible.

It is known that a large percentage of DTCs die before landing at distant organs, but a subset of DTCs have been found to have stem-cell-like properties. These properties include an epithelial to mesenchymal phenotype, increased capacity for migration and invasion, and resistance to apoptosis.[22] CD133 may be a promising marker for these properties in CRC as higher percentages of CD133$^+$ cells are detected in liver metastatic tissues than in primary tumours.[23] High levels of CD133 in the primary tumour is associated with shorter overall survival,[24] and a high number of CD133$^+$ DTCs in mesenteric blood at the time of CRC resection is associated with relapse.[11]

The ability of different organs to support CRC DTC growth is variable, as is evident from the relatively high incidence of DTCs in the bone marrow, but incidence of actual bone metastasis in advanced CRC is low. This suggests that CRC DTCs survive in the bone marrow in a non-proliferative state.[25] The liver, on the other hand, may be a more 'permissive' environment for DTC growth.[25] A recent, preclinical study has demonstrated that DTC growth in the liver is facilitated by certain tumour-derived vesicles called exosomes.[26] Exosomes encoding the integrin αvβ5 were found to specifically bind Kupffer cells in the liver, upregulating pro-inflammatory signalling pathways and increasing liver metastasis. Consequently, by deploying integrin-blocking decoy peptides, the liver metastasis was successfully ablated. Finally, the authors showed that αv expression in patient-derived exosomes could predict metastasis to the liver.[26]

The process of colonisation by CRC DTCs also elicits inflammation in the liver.[27] An example is the activation of the bone morphogenetic protein (BMP) signalling pathway,[28] which is usually considered tumour suppressive.[29] However, certain DTC mutations (including, but not limited to, *SMAD4*) change the effects of this pathway, allowing or even stimulating growth of DTCs in the liver when BMP activity is increased.[29,30] A similar pattern is observed for transforming growth factor-β signalling;[31] certain mutations thus enable DTCs to thrive in otherwise unfavourable conditions.

An increasing body of evidence suggests that the DTCs, when arriving in the liver, lodge in an area around the capillaries named the perivascular niche.[32] This niche is highly similar to, and possibly the same as the niche wherein tissue-resident stem cells reside.[32] In patient-derived CLM specimens, CD133$^+$ cells were found to be concentrated around the microvasculature.[33] It is proposed that the endothelial cells mediate DTC survival here as CD133$^+$ CRC cells were found to be highly resistant to the chemotherapeutics fluorouracil and oxaliplatin *ex vivo* when the culture medium was conditioned by liver endothelial cells. However, without the conditioning, the cells died.[33]

There are several conceivable strategies for dealing with the DTCs in the perivascular niche, for

example, given their reliance on angiogenesis for growth, it is conceivable that a continuous administration of anti-angiogenic drugs could maintain the dormancy of the DTCs here.[34,35] The efficacy of an mTOR inhibitor for this purpose has been demonstrated pre-clinically.[36] Alternatively, blocking endothelial-derived resistance factors, such as the Notch ligand,[33] possibly in combination with chemotherapeutics, are another option. For instance, a recent Phase I study of a Notch inhibitor demonstrated tolerability of the drug in advanced cancer.[37] Lastly, interfering with pathways that are affected by DTC mutations may also be necessary. For *SMAD4* mutated cancers, the Rho-kinase inhibitor fasudil may be a candidate drug.[30]

IMMUNOTHERAPY

Tumour cells are constantly identified and eradicated by the immune system. Patients with a suppressed immune system, for example those taking immunosuppressive drugs following an organ transplant, or HIV-positive patients, have an increased incidence of malignancies.[38] Consequently, it is conceivable that stimulating the immune system in a specific fashion can lead to tumour rejection and elimination. More than 30 years ago, Rosenberg and colleagues[39] showed that administration of interleukin-2 (a T cell growth factor) can cause durable regression of metastatic disease, one of the first demonstrations of this principle.

T cells have been a major focus in immunotherapy due to their capacity for selective recognition of peptides (antigens), their capacity to directly kill antigen-expressing cells, and their ability to orchestrate diverse immune responses involving both the adaptive and innate immune system.[40] The process of T cell activation first involves the T cell receptor binding to an antigen presented on a major histocompatibility complex molecule on the surface of an antigen-presenting cell. Following this, co-receptors and co-ligands, as well as adhesion molecules, assemble to form what is known as an immunological synapse.[41] In this synapse, co-stimulatory and co-inhibitory signals (immune checkpoints) ultimately define the T cell response.[41] Some of the important receptors and ligands in this synapse are schematically illustrated in Figure 1.

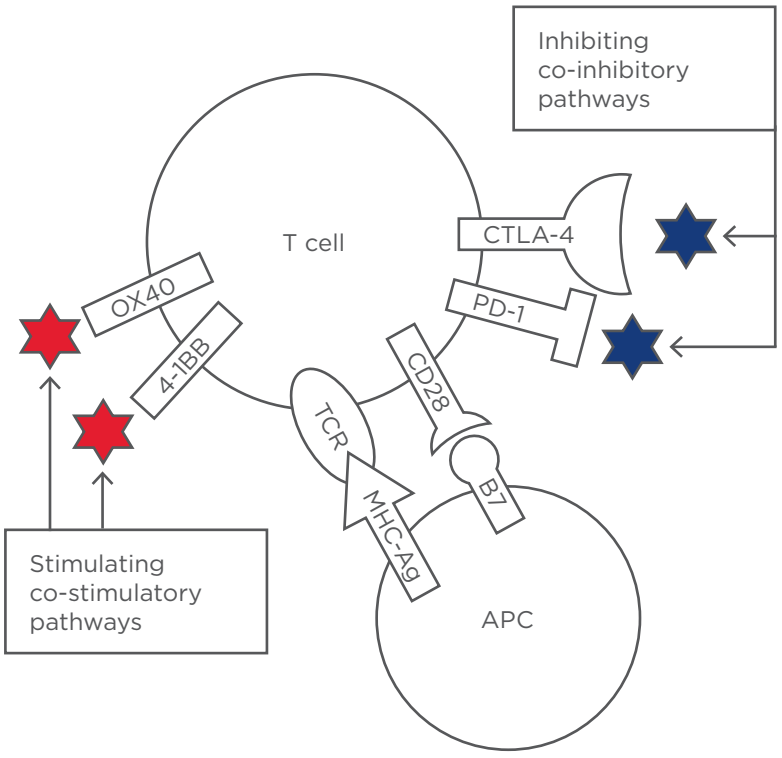

Figure 1: Druggable co-signalling receptors in the immunological synapse. The goal is to evoke an endogenous anti-tumour response.

MHC-Ag: major histocompatibility complex - antigen; APC: antigen presenting cell; TCR: T cell receptor; CTLA-4: cytotoxic T lymphocyte-associated protein-4; PD-1: programmed cell death protein-1.

Essentially, genetic and epigenetic alterations in tumours provide antigens that distinguish the tumours from normal cells. In order to evade immune destruction, tumours evolve several mechanisms to reduce their immunogenicity. The overexpression of inhibitory ligands and receptors that regulate T cell effector functions is one such mechanism.[41]

Monoclonal antibodies (mABs) targetting cytotoxic T-lymphocyte associated protein (CTLA)-4 became the first approved drug targeting a T cell checkpoint. CTLA-4 is expressed exclusively on T cells, where it surfaces upon T cell activation and competes for B7, the ligand of CD28, thereby inhibiting co-stimulation and dampening T cell effector function. Blocking CTLA-4 has shown promising results in treating advanced malignant melanoma, but has no meaningful response as a single agent in advanced CRC.[42] In contrast to CTLA-4, programmed death (PD)-1 is not solely expressed on T cells, but also on B cells and natural killer cells. PD-1's main function is to limit the activity of T cells in peripheral tissues. One of its ligands, programmed cell death-ligand 1 (PD-L1), is expressed on many tumour cells. mABs that neutralise PD-L1 have been developed, but their efficacy is primarily restricted to cancers that express, or have immune-infiltrating cells expressing PD-L1.[43] Recently, an objective response was achieved in 4 out of 10 patients with in MSI-advanced CRC receiving anti-PD-L1.[44]

In addition to blocking co-inhibitory receptors, enhancing anti-tumour immunity by targeting co-stimulatory molecules is also a possibility. Upon ligation, the tumour necrosis factor (TNF) superfamily member 4-1BB receptor evokes robust effector T cell responses.[45] A 4-1BB agonist is currently being evaluated in combination with an epidermal growth factor receptor (EGFR) antagonist in advanced CRC.[46] Another promising receptor in the TNF superfamily is OX40. High OX40 expression has been linked to a favourable outcome in CRC.[47] Clinically, in a mixed population of metastatic solid tumours, response was achieved in at least 1 tumour nodule in 12 out of 30 patients given an OX40 agonist.[48] A clinical trial using mABs stimulating OX40, in combination with liver resection in advanced CRC, is currently recruiting patients.[49]

The mutational load of a tumour seems to predict response to immunotherapy.[50] This translates to MSI tumours for patients with advanced CRC. Furthermore, it is likely that the key to success lies in combinations of checkpoint drugs: a combination of anti-PD-L1 and anti-CTLA-4 is currently being evaluated in MSI CRC patients,[51] however, immunotherapy is only effective if an immune response is present prior to therapy.[40] It may be necessary to combine these strategies with therapies that induce *de novo* immune responses, such as vaccines.[40] Alternatively, a readily available therapy is the combination of conventional radiotherapy or chemotherapy with immunotherapy. Preliminary results from a study combining anti-PDL1 with conventional chemotherapy and anti-VEGF in patients with advanced CRC achieved objective responses in 12 out of 30 patients.[52] Finally, recent studies have also suggested that cells of the innate immune system can be modulated to play a role in combatting cancer.[53] It is likely that, in order to make immunotherapy widely applicable, not only must T cells be stimulated, but antigen-presenting cells also.[53]

SURGERY

The liver is the sole organ with metastases in approximately a third of patients with advanced CRC, but traditionally only ~20% present with resectable disease.[54,55] The rationale for trying to increase resectability lies in the dramatic survival benefit as 5-year overall survival approaches 50% after surgery, while for chemotherapy the 5-year survival rate is approximately 10%.[56,57]

The liver has eight segments, each with a portal vein, hepatic artery, bile duct, and hepatic vein branches. This means that they can be resected individually, leaving the other segments uncompromised.[58] The liver is a solid parenchymal organ, which makes its division somewhat challenging. The traditional finger fracture technique for transecting the parenchyma has been replaced by newer surgical instruments, such as the ultrasound aspirator, water jet dissector, or LigaSure™. Combined with surgical staplers for the larger vessels, good cooperation with the anaesthetist to ensure a low central venous pressure and, if needed, clamping of the blood flow into the liver (Pringle manoeuvre) means that liver surgery can currently be performed with very little blood loss.

Traditionally, CRC is resected, followed by chemotherapy and hepatic resection of CLM. In the time period between the colorectal and hepatic resection(s), the CLM may continue to grow, in some

cases rendering the CLM unresectable. This is a pressing concern when there are complications following CRC surgery that delay chemotherapy and any subsequent hepatic resection(s). A decade ago, a 'liver-first' strategy was proposed as a possible solution.[59] In this approach, systemic chemotherapy is followed by resection of synchronous CLM, and following this, resection of the primary colorectal tumour. Advantages include possible downstaging of both primary tumour and liver metastases prior to surgery and avoidance of time loss. The liver-first approach is feasible, but an effect on patient survival has not yet been demonstrated.[60,61]

The liver has a unique ability to regenerate, but approximately 20–25% of the total functional liver volume should remain as a minimal remnant (and potentially more if there is chemotherapy-induced liver injury)[62] to avoid post-hepatectomy liver failure. Recently, a new technique for hepatic resection has been described, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS; Figure 2).[63]

The ALPPS technique relies on the fact that ligation of the portal vein to a lobe of the liver results in atrophy of the corresponding lobe, and hypertrophy of the other. The hypertrophy may partly be due to increased portal blood flow, but the procedure also increases levels of proinflammatory mediators and potentially levels of growth factors, which may also be a contributing factor.[64] ALPPS is most commonly performed to allow a safe resection of a large part of the right liver. In the first part of the procedure, the right liver is mobilised by dividing accessory hepatic veins. Next, the common bile duct, left and right hepatic artery, right branch of the portal vein, portal vein branch for segment IV, and the right hepatic vein are identified. The right portal branch and the branch for segment IV are subsequently isolated and ligated. Following this, the liver parenchyma is transected all the way to the anterior surface of the inferior vena cava (Figure 2A).[65,66] Finally, the arterial supply and the venous drainage of the right liver are kept intact, the right liver is left *in situ*, and the wound closed.

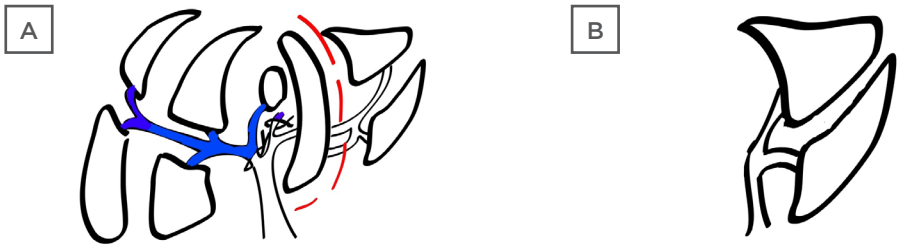

Figure 2: The ALPPS procedure.
A: The right portal vein and the portal branch to segment IV is ligated. The blue areas indicate stasis of blood. The liver parenchyma is transected along the dotted red line. B: Status post-hepatectomy (only the portal vein is depicted).
ALPPS: associating liver partition and portal vein ligation for staged hepatectomy.

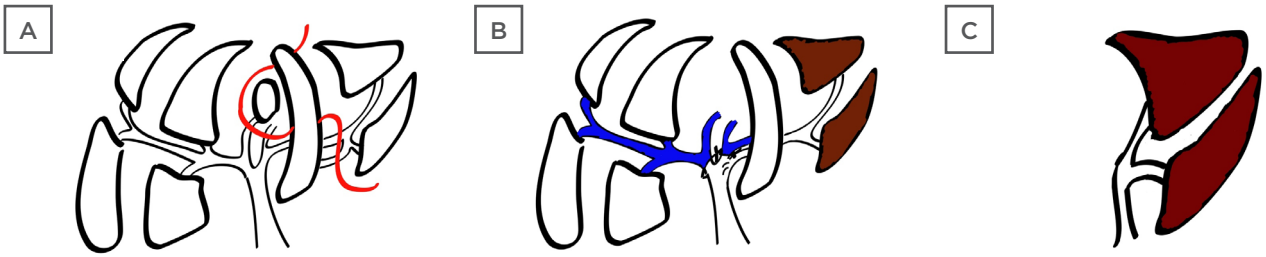

Figure 3: The RAPID procedure.
A: Liver segments I–III are resected along the red line. B: A segment II/III donor graft, coloured brown, is transplanted. Subsequently, the portal vein branches to the remaining liver are divided, the blue areas indicate stasis of blood. C: Status post-hepatectomy (only the portal vein is depicted).
RAPID: resection and partial liver segment II/III transplantation with delayed total hepatectomy.

A computed tomography scan is performed 5–7 days later; if the remnant liver (the left liver) has increased to ~30% of the total liver volume, the second step of the procedure is performed. In the second step, the right hepatic vein, right hepatic artery, right branch of the bile duct, and arterial branch to segment IV are identified, ligated, and divided. The right liver is then removed (Figure 2B).[65,66] Commonly an increase of ~70% is achieved in the remnant liver after 1 week.[66] The procedure can be performed with very low morbidity and mortality.[66] ALPPS enables large liver resections; and large resections, e.g. leaving just one segment of the liver, have been reported in the literature.[67]

Motivated by the large differences in survival between surgical resection and chemotherapy for CLM, Hagness et al.[68] evaluated liver transplant in 21 patients with unresectable CLM. The 5-year estimated overall survival was 56% in these patients (median follow-up, 65 months).[68,56] In a similar cohort of patients receiving first-line chemotherapy, the corresponding 5-year overall estimated survival was 9% (median follow-up, 60 months).[56]

A shortage of organ donors will limit the application of whole-organ liver transplantation for CLM; however, a strategy with the potential to circumvent the shortage of organ donors has recently been proposed (Figure 3): the Resection And Partial liver segment II/III transplantation with Delayed total hepatectomy (RAPID) procedure.[69] In this case, liver segments I–III are resected in the first part of the procedure, avoiding tumour-affected parts (Figure 3A). Thereafter, segment II/III of a donor graft is transplanted, anastomosing the graft liver vein to the vena cava, the portal vein, and the hepatic artery of the graft in an end-to-side fashion to the main portal trunk and the common hepatic artery, respectively. Then, similarly to ALPPS, the portal branches to the remaining liver are ligated (Figure 3B). Care is taken to avoid portal hypertension (>20 mmHg) in the graft; if needed, the right portal branch may be banded (instead of ligated) to achieve subtotal stenosis as opposed to complete occlusion. After a period of approximately 2 weeks, the second-stage hepatectomy may be performed (Figure 3C).[69]

With the RAPID approach, the prospect of living donation segment II/III grafts to patients with liver-only advanced CRC is conceivable.

CONCLUDING REMARKS

Although the mean age upon diagnosis of CRC is approximately 70 years[10] and CRC is generally considered a disease of the old, a significant proportion of the afflicted are considerably younger. Half of CRC patients develop metastases, and in this population 50% die within 2 years,[56] a testament to the considerable potential for improvement. Many hoped that whole cancer genome sequencing would identify key mutations responsible for tumour aggressiveness, but the key feature that emerged may be the immense heterogeneity of cancer.[17] Currently, the only application of cancer genomics in CRC is ruling out those with mutations in *RAS* and *BRAF* for anti-EGFR therapy, but perhaps accumulating data from transcriptomics and proteomics will change this over time. Pragmatically, mutational analysis of a limited number of genes seems sensible, perhaps even in the entire CRC population. Additionally, identifying patterns of genomic instability, as well as the expression levels of some key markers, such as PD-L1, CD133, and as recently suggested, CDX2,[70] may help identify patients with risk of relapse or those that might benefit from adjuvant chemo or immunotherapy.

Indeed, immunotherapy offers great hope, but presently it remains to be seen whether this will improve long-term survival, and whether its use will be limited to particular patient groups.[71] Stimulating T cells inherently carries risk of auto-immunity; the potential for fatal pneumonitis or even hepatitis is a concern.[71]

Removing the tumour load in the liver in advanced-CRC patients has a dramatic effect on survival, as recently demonstrated by the pilot trial of liver transplantation for unresectable CLM.[56] Interestingly, of the 21 patients transplanted, not a single patient had the liver as the first site of recurrence.[56] This is in contrast to the situation after liver resection, where approximately 40% of patients have the liver as the first site of recurrence.[56] These observations may, at least in part, be attributed to microscopic, undetectable tumour residue in the remaining liver, perhaps in the form of dormant DTCs. One may further speculate whether the mTOR-based immunosuppression in transplanted patients is suppressing the growth of DTCs. Taken together, one may argue that maximum surgical debulking in advanced CRC, even without a tumour-free result, may be the way forward.

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