MANAGEMENT OF METASTATIC COLORECTAL CANCER
Current Treatments and New Therapies

http://www.lebanesemedicaljournal.org/articles/63-4/review2.pdf

Anna-Maria HENAINE[1,2], Georges CHAHINE[4], Pascale SALAMEH[5], Edouard ELIAS[4], Marcel MASSOUD[4] Daniel HARTMANN[1,2], Gilles AULAGNER[1,2,3], Xavier ARMOIRY[2,6]

ABSTRACT • Colorectal cancer represents 8% of metastatic cancers. For decades, the gold standard therapy has been infusional chemotherapy with 5-Fluorouracil (5-FU) associated to folinic acid. The discovery of irinotecan, oxaliplatin and oral forms of 5-FU in the nineties is considered a milestone in the treatment of this disease. Since 2004, targeted therapy with monoclonal antibodies including anti-EGFR and angiogenesis inhibitors showed superiority in terms of mortality compared to conventional therapy. Metastatic colorectal cancer, however, remains an incurable disease. We present the current treatments of metastatic colorectal cancer, the clinical development of these emerging treatments, and their position in the Lebanese health care system.

Keywords: metastatic colorectal cancer, chemotherapy, monoclonal antibodies

RÉSUMÉ • Le cancer colorectal représente 8% des cas de cancers métastatiques. Pendant des décennies, la chimiothérapie associant le 5-Fluorouracil (5-FU) et l'acide folinique a constitué le traitement conventionnel de référence. L'arrivée de l'irinotécan, de l'oxaliplatine et des formes orales de 5-FU au cours des années 90 a été considérée comme une grande avancée thérapeutique. À partir de 2004, la thérapie ciblée, avec les anticorps monoclonaux visant les récepteurs EGF et l'angiogenèse (VEGF), a permis un bénéfice en termes de survie même si le cancer colorectal métastatique reste, à ce jour, incurable. Nous présentons la prise en charge actuelle du cancer colorectal métastatique, le développement clinique de ces traitements émergents et leur positionnement dans le système de santé libanais.

LIST OF ABBREVIATIONS

5-FU : 5-Fluorouracil
5-FULV : 5-Fluorouracil/Leucovorin
APC : Protein coding
ASPECCT : A Study of Panitumumab Efficacy and safety Compared to Cetuximab Trial
BRAF : V-Raf murine sarcoma viral oncogene homolog B1
CORRECT : Regorafenib monotherapy for previously treated metastatic colorectal cancer
CRC : ColoRectal Cancer
CRYSTAL : Cetuximab Combined With Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer
CYP3A4 : Cytochrome P
DNA : DeoxyriboNucleic Acid
EGF : Epidermal Growth Factor
EGFR : Epidermal Growth Factor Receptor
FGFR : Fibroblast Growth Factor Receptor
FIRE : Trial comparing Cetuximab and bevacizumab
FOLFIRI : 5-FULV + Irinotecan
FOLFOX : 5-FULV + Oxaliplatin
FOLFOXIRI : 5-FULV + Oxaliplatin + Irinotecan
GSK3B : Glycogen Synthase Kinase 3B
HER : Human Epithelial Growth Factor Receptor
Ig : Immunoglobulin
KRAS : Kirsten RAt Sarcoma 2 viral oncogene homolog
MAPK : Mitogen Activated Protein Kinase

mCRC : metastatic colorectal cancer
NRAS : Neuroblastoma RAS viral oncogene homolog
OPUS : Oxaliplatin and Cetuximab in First-Line Treatment of Metastatic Colorectal Cancer
OR : Overall response
ORR : Overall response rate
OS : Overall survival
PDGF : Platelet Derived Growth Factor
PEAK : A Randomized, Multicenter Phase II Study of Panitumumab + Modified Fluorouracil, Leucovorin, and Oxaliplatin (mFOLFOX6) or Bevacizumab + mFOLFOX6 in Patients With Previously Untreated, Unresectable, Wild-Type KRAS Exon 2 Metastatic Colorectal Cancer
PFD : Progression free disease
PFS : Progression free survival
PI3K/Akt : Phosphoinositide 3-kinase
PIGF : Placental growth factor
PRIME : Panitumumab Randomized trial In combination with chemotherapy for Metastatic colorectal cancer to determine Efficacy
RR : Response rate
UGT1A : UDP-glucuronosyltransferase 1A
VEGF : Vascular Endothelial Growth Factor
VEGFR : Vascular Endothelial Growth Factor Receptor
VELOUR : Phase III study of aflibercept and FOLFIRI in patients with metastatic colorectal cancer after failure of an oxaliplatin regimen.
WT : Wild

[1]Université de Lyon, Claude Bernard Lyon 1, Lyon, France.   [2]UMR CNRS 5510 MATEIS, Lyon, France
[3]Hospices Civils de Lyon, Groupement Hospitalier Est, Service pharmaceutique, Bron, France.
[4]Hôtel-Dieu de France (Beyrouth) & Hôpital Notre-Dame de Secours (Byblos), Liban.
[5]Université libanaise, Laboratoire clinique et épidémiologique, Faculté de Pharmacie, Hadath, Liban.
[6]Hospices civils de Lyon, Cellule Innovation/Délégation à la Recherche Clinique et à l'Innovation, Av. du Doyen Lépine, France.
Correspondance : *Anna Maria HENAINE, PharmD. UMR CNRS 5510 MATEIS. 8 Avenue Rockefeller. 69373 Lyon. France.*
e-mail : annamaria.henaine@gmail.com

BACKGROUND

Metastatic colorectal cancer (mCRC) is a major cause of morbidity and mortality throughout the world. It is the fourth most common cancer diagnosed in the U.S. and the second leading cause of cancer death in 2014. In Lebanon, colorectal cancer is the third leading cause of death from cancer and the most common cancer both sex combined [1-2]. In 2012, according to national cancer statistics, the number of eligible new cases diagnosed in the hospital system was 2035, 979 men and 1056 women [3].

About 75% of patients with CRC have sporadic disease with no apparent evidence of having inherited the disorder. The remaining 25% of patients have a family history of CRC suggesting a hereditary contribution, common exposures among family members, or a combination of both.

Numerous factors are found important in the development of CRC including high risk luminal environment, inflammation, as well as lifestyle factors such as diet, tobacco and alcohol consumption. In recent years, focus has turned towards the genetics and molecular biology of CRC and several interesting and promising correlations and pathways have been discovered. The major genetic pathways of CRC are the chromosome instability pathway representing the pathway of sporadic CRC through the KRAS, APC and p53 mutations, and the microsatellite instability pathway of hereditary non-polyposis through mutations in mismatch repair genes [4].

Metastases are observed in 40-60% of cases of CRC of which 25% are synchronous. Among patients without metastases at diagnosis, the risk of metachronous is 30-40% [5]. Without any treatment, patients with mCRC have a median survival of seven months.

In some cases, the metastases reveal the cancer (precessive metastases) whose primary site is not known and should be identified. Approximately 30-50% of CRC patients develop liver metastases, responsible alone for two thirds of the deaths. Being able to consider their resection is a major challenge since the resection significantly improves the survival of patients. The extra-hepatic locations are rare and of poor prognosis.

Currently, a multitude of clinical evidences have shed a new light on the issues such as the selection and duration of therapy, the associations with targeted agents and treatments tailored towards the clinic and molecular factors. Moreover, knowledge of prognostic and predictive biomarkers such as mutation in KRAS, NRAS, and BRAF, is of growing interest to allow a better selection of drugs and treatment strategies.

The aim of this paper is to review the updates to the management of mCRC.

THERAPEUTIC STRATEGIES in the TREATMENT of mCRC

The objectives of the management of patients with mCRC are generally palliative rather than curative, with the exception of a small proportion of patients with resectable liver metastases and controllable by radiofrequency (or cryotherapy) or locoregional treatment by direct injection of radioactive spheres [6].

Recent advances in chemotherapy regimens have increased the median overall survival (OS) from 6 months to over 30 months through the targeted therapy and double or triple combination protocols [7].

The three active agents for mCRC, known as conventional chemotherapy, are fluoropyrimidines (5-FU or its oral prodrug), irinotecan and oxaliplatin. The associations FOLFOX/XELOX and FOLFIRI are commonly used in the Lebanese hospitals [8] with no evidence from literature of differences between both regimens in terms of OS [9]. Triple associations such as FOLFOXIRI induce a median OS without disease progression with greater response rate than FOLFIRI regimen, but are associated with a poor safety profile [10]. In fact, the FOLFOXIRI has been compared to FOLFIRI in two randomized clinical trials in unresectable patients: in both studies, FOLFOXIRI led to an increased in R0 secondary resection rates: 6% *vs* 15% ($p = 0.033$) in the Gruppo Oncologico Nord Ovest (GONO) trial and 4% *vs* 10% ($p = 0.08$) in the Gastrointestinal Committee of the Hellenic Oncology Research Group (HORG) trial. In a follow-up study of the GONO trial, the 5-year survival rate was higher in the group receiving FOLFOXIRI (15% *vs* 8%) with a median OS of 23.4% *vs* 16.7% month ($p = 0.026$) [11-13].

The addition of targeted therapies to these four cytotoxic regimens has shown better outcomes in the majority of cases. However, the best strategy sequence and the respective roles of each combination are still debated.

PRINCIPAL AGENTS USED in the TREATMENT of mCRC

Table I summarizes the principal drugs used to treat mCRC.

Cytotoxic chemotherapy drugs
Cytotoxic drugs induce acute cell death (necrosis), programmed cell death (apoptosis), growth arrest or differentiation. Many antineoplasic drugs have multiple actions on the cell:

5-FU is activated to 5-fluorodeoxyuridylate, which, in the presence of a reduced folate co-factor, inhibits the enzyme thymidylate synthase. This blocks the production of thymidine phosphate which is required for DNA synthesis. The 5-fluorodeoxyuridylate may also be fraudulently incorporated into DNA causing a form of DNA damage [14].

Capecitabine is an orally bioavailable 5-FU prodrug that undergoes sequential hydrolysis and deamination reactions in the liver to produce 5'-deoxy-5-fluorouridine. It is converted to 5-FU by thymidine phosphorylase (also known as platelet-derived growth factor). As this enzyme is abundant in tumor tissue there is some tumor specificity in the patient's exposure to 5-FU.

The adverse effects, such as hand and foot syndrome, of capecitabine resemble those of 5-FU when given by protracted infusion.

TABLE I
SUMMARY of APPROVED THERAPIES for METASTATIC COLORECTAL CANCER in LEBANON

Drug	Class	Mode of administration	Pathway
CHEMOTHERAPY			
5-Fluorouracil (5-FU®)	Antimetabolite (pyrimidine antagonist)	IV	DNA/RNA damage
Capecitabine (Xeloda®)	Antimetabolite (pyrimidine antagonist)	PO	DNA/RNA damage
Oxaliplatine (Eloxatine®)	Alkylating agent (Platinum compound)	IV	DNA/RNA damage (nonspecific cell cycle)
Irinotecan (Campto®)	Topoisomerase inhibitor	IV	DNA damage
TARGETED AGENTS			
Cetuximab (Erbitux®)	Monoclonal antibody (IgG1 chimeric)	IV	PI3K/Akt, MAPK
Panitumumab (Vectibix®)	Monoclonal antibody (fully human)	IV	PI3K/Akt, MAPK
Bevacizumab (Avastin®)	Monoclonal antibody (recombinant humanized)	IV	Angiogenesis
Aflibercept (Zaltrap®)	Recombinant fusion protein	IV	Angiogenesis
Regorafenib (Stivarga®)	Tyrosine kinase inhibitor	PO	Angiogenesis, oncogenesis tumor microenvironment

Ig: immunoglobulin   **MAPK**: mitogen-activated protein kinase   **PI3K/Akt**: phosphoinositide 3-kinase   **IV**: intravenous   **PO**: per os

Other oral 5-FU prodrugs (e.g. S-1, UFT) have been the subject of extensive clinical trials [15]. These are mostly combinations of 5-FU prodrugs with uracil, which is an inhibitor of dihydropyrimidine dehydrogenase, a ubiquitous enzyme that rapidly degrades 5-FU.

Oxaliplatin is a diaminocyclohexane platinum derivative and a bifunctional alkylator capable of reacting with adjacent guanine residues in DNA. It provides either intra- or inter-strand DNA cross-links, which interfere with DNA processing.

Oxaliplatin was evaluated as the first line metastatic combination with 5-FULV (FOLFOX4). Three trials showed a significant improvement in the objective response and a progression-free survival, but no significant difference in term of OS [16]. The two limiting toxicities of FOLFOX4 were neutropenia and the specific reversible sensory neuropathy of oxaliplatin. The results of the OPTIMOX 1 study [17] showed that a "stop and go" approach using oxaliplatin free-interval resulted in a decrease in neurotoxicity without affecting the OS. Therefore, the panel recommends adjusting the schedule/timing of the administration of this drug as a means of limiting this adverse effect. The current clinical data are insufficient to support the routine use of calcium/magnesium infusions to prevent the oxaliplatin related neurotoxicity.

Irinotecan is a topoisomerase I inhibitor used to treat several solid tumor types, especially in combination with other chemotherapeutic agents in the treatment of mCRC. Inhibition of topoisomerase I by irinotecan and its active metabolite, SN-38, prevents re-ligation of single stranded DNA breaks induced during DNA synthesis phase of cellular replication. Cell death ultimately occurs. Adverse effects include severe diarrhea, myelosuppression and neutropenia, likely induced by inefficient metabolism and

excretion of SN-38, which undergoes glucuronidation primarily in the liver by UGT1A prior to excretion through the kidneys. UGT1A locus is alternatively spliced to produce nine isoenzymes and the UGT1A1 isoform is solely responsible for the phase II metabolism of bilirubin, numerous endogenous hormones and pharmacologic compounds, including irinotecan. Thus, genetic variation in UGT1A correlates with adverse events caused by irinotecan toxicity [18].

Evidence indicates that, at relatively high irinotecan level (> 250 mg/m^2), patients who are homozygous for the UGT1A1*28 variant experience a greater risk of clinically important neutropenia, especially with particular chemotherapy agents/oxaliplatin [19]. That's why it is reasonable to determine the UGT1A genetic background to assist in toxicity management. Unfortunately, this molecular testing is not present in our Lebanese hospitals to date.

Irinotecan is also an inhibitor of acetyl cholinesterase and patients may experience an acute onset of cholinergic-like symptoms including lacrimation, sweating, abdominal cramping and diarrhea.

Irinotecan, in combination with fluorouracil (FOLFIRI), in the second-line, is considered a good standard in the palliative treatment of mCRC. Several studies [20-21] have shown a significant increase in the response rate, PFS and OS with FOLFIRI compared to 5-FULV alone.

MODULATORS of the TARGETED BIOLOGICAL RESPONSE

As observed in a retrospective cohort study conducted in Lebanese hospitals [8], approximately 75% of patients with mCRC receive a biological agent during their treatment course targeting one of two main pathways: the EGFR cascade and the VEGF signaling.

TABLE II — SUMMARY of the ANTIANGIOGENIC DRUGS CURRENTLY APPROVED for mCRC TREATMENT: BEVACIZUMAB, AFLIBERCEPT & REGORAFENIB

	Bevacizumab	Aflibercept	Regorafenib
Approval date	2004	August 2012	September 2012
Registration in Lebanon	2006	2014	2014
Origin	Genentech Inc., South San Francisco, CA, USA	Sanofi-Aventis, Bridgewater, NJ, USA	Bayer AG, Leverkusen, Germany
Mechanism of action	Targets VEGF-A (only)	VEGFR decoy binding VEGF-A, VEGF-B & PlGF	Multikinase inhibitor against VEGFR1, VEGFR2, VEGFR3, PDGFR-β, FGFR1, TIE2, KIT, RET as well as a signal transduction inhibitor of the RAF/MEK/ERK pathway
Extracellular/Intracellular	Extracellular	Extracellular	Intracellular
Indication	1st/2nd line mCRC + combination chemotherapy	2nd line in mCRC + FOLFIRI	3rd line mCRC as single agent, in refractory/ progressive disease
Dosing	5-10 mg/kg IV every 2 weeks in combination with FOLFOX or FOLFIRI	4 mg/kg IV every 2 weeks in combination with FOLFIRI	160 mg daily for 21 days of an every 28d cycle
Common & classically side effects	Hypertension, proteinuria, thromboembolism, gastrointestinal perforation (rare), hemorrhage, delay wound healing, RPLS	Hypertension, proteinuria, diarrhea, bone marrow suppression (neutropenia), delay wound healing, fistula, RPLS, thromboembolism	Hypertension, asthenia, gastrointestinal perforation (rare), hemorrhage, hand-foot syndrome, rash, hepatotoxicity, RPLS
Black box warnings	Arterial & venous thromboembolism, hypertension, wound healing impairment.	Wound healing impairment, hemorrhage, gastrointestinal perforation (rare)	Hepatotoxicity (rare), RPLS, dehydration, cardiac disease
Monitoring parameters	Renal function, proteinuria, blood pressure, bleeding	Renal function, CBC, infection, proteinuria, blood pressure, bleeding, dehydration	Blood pressure, liver functions, electrolytes (if diarrhea), INR (if the patient is taking warfarin), callous in the hands or feet

FGFR: fibroblast growth factor receptor   PDGFR: platelet-derived growth factor receptor   PIGF: placental growth factor   VEGF: vascular endothelial growth factor   VEGFR: vascular endothelial growth factor receptor
FOLFIRI: 5-FU/LV/Irinotecan   mCRC: metastatic colorectal cancer   RPLS: reversible posterior leukoencephalopathy syndrome   CBC: complete blood count

Triggering cell death via cancer-directed immunotherapy or immunomodulation with the aim to overcome major mechanisms of immune resistance is a newly arising field [22-23]. We will discuss the major approaches and current therapies in the treatment of mCRC.

Inhibitors of angiogenesis

Angiogenesis is a central mechanism in human CRC development and growth [24]. In particular, VEGF is closely associated with the induction of neovascularization in human colon cancer and is one of the most important endogenous ligands of receptors present on the endothelial cell plasma membrane; it's binding leads to intracellular signaling and, ultimately, gene transcription that promotes endothelial proliferation, migration, and tube formation of endothelial cells resulting in tumor growth and metastasis.

Given the crucial importance of neoangiogenesis in mCRC, anti-VEGF pathway therapies have been intensively investigated. The monoclonal antibody bevacizumab is the first antiangiogenic agent to be approved in mCRC treatment, because of response rate and survival benefit [25-26].

The three antiangiogenic agents available within Lebanese hospitals are presented in Table II.

Bevacizumab is a recombinant humanized IgG1 monoclonal antibody (7% murine) directed against VEGF-A.

After showing its efficacy in several murine xenograft models of human tumors, bevacizumab alone or in combination with other cytotoxic agents, was shown to be well tolerated in phase I trials apart from some side effects such as thromboembolic events, bleeding, hypertension and proteinuria.

The association of bevacizumab with 5-FU increased the OS (12.9 months for FOLFOX4/bevacizumab *vs* 10.8 months for FOLFOX4 alone) and the disease free progression (DFP) significantly (7.3 months *vs* 4.7 months respectively). In November 2012, bevacizumab was approved in combination with chemotherapy based on fluoropyrimidine, irinotecan or oxaliplatin for the treatment of mCRC disease. This approval allows the patients, who received first-line treatment with bevacizumab plus irinotecan-based chemotherapy or with oxaliplatin, to continue receiving bevacizumab plus other chemotherapy based on oxaliplatin or irinotecan as second-line treatment after progression of the disease. In the Lebanese hospitals, the practice is to preferably use the protocol FOLFOX-Bevacizumab as first-line [8].

Aflibercept also known as VEGF Trap, is a fully human soluble recombinant protein composed of extracellular domains from both VEGFR-1 and VEGFR-2 fused to the Fc (a) region of human IgG1 [27]. It was designed to act as a decoy VEGF receptor, binding VEGF-A, VEGF-B, and PlGF, subsequently preventing their interactions with VEGFR-1 and VEGFR-2 [28-29].

Aflibercept was studied in combination with FOLFIRI in a prospective, randomized, double-blind, multicenter, study on patients with mCRC previously treated with an oxaliplatin-based chemotherapy regimen. Patients were randomized either to FOLFIRI plus aflibercept (4 mg/kg intravenously every 2 weeks) or FOLFIRI plus placebo. The aflibercept arm showed significant improvement in the primary endpoint of OS (13.50 months *vs* 12.06 months). PFS and ORR were also increased (6.90 months *vs* 4.67 months and 19.8% *vs* 11.1% respectively). In subgroup analyses of patients with or without prior-bevacizumab regimen, PFS was significantly improved in both. OS was significantly improved in the bevacizumab-naïve subset, but did not achieve significance in the prior-bevacizumab subset.

Based on these results, the FDA approved, on August 2012, the use of aflibercept in combination with FOLFIRI for the treatment of patients with mCRC that is resistant to or that has progressed after an oxaliplatin-containing regimen. Both antiangiogenic-associated and chemotherapy-related adverse effects were seen with greater incidence in the aflibercept arm (83.5% of grade 3 or 4 adverse events). The antiangiogenic toxicities described were hypertension, hemorrhage, and arterial and venous thromboembolism, diarrhea, and asthenia.

Regorafenib is an oral small-molecule multikinase inhibitor, inhibiting multiple oncogenic kinases, PDGF, FGFR and VEGFR-1. Its mechanism is not clearly understood, but studies have shown that it induces expression of PUMA, p53 target and critical mediator of apoptosis in colorectal cells after inhibition of ERK and activation of GSK3B through the cascade of NF-kB [30-31].

Regorafenib was approved for the treatment of mCRC patients previously treated with fluoropyrimidine, oxaliplatin, or irinotecan-based chemotherapy regimens, with prior anti-VEGF and/or anti-EGFR (KRAS wild) therapies. Regorafenib was evaluated in the Phase III CORRECT trial [32] on patients with mCRC who had progressed during or within three months after the above therapies. Patients on the study were randomized to receive either regorafenib (160 mg/day/21 days) or placebo. Treatment with regorafenib was associated with a modest but significant increase in survival (6.4 months *vs* 5.0 months). The CORRECT trial showed a survival benefit with regorafenib in an extensively treated patient population, also about 40% of patients achieved disease stabilization. Adverse events associated with regorafenib are hand-foot skin reaction, fatigue, diarrhea, mucositis, dysphonia, weight loss, infection, hypertension (impaired angiogenesis). Hepatotoxicity (sometimes fatal), increased incidence of hemorrhage, myocardial ischemia, infarction, reversible post leukoencephalopathy syndrome, gastrointestinal perforation and fistula are rare and should be closely monitored.

The bioavailability of regorafenib is 69% after oral administration in tablet form. Absorption is dependent on the fat content of a meal (better with low-fat diet). It is metabolized by CYP3A4 and UGT1A9 to two active metabolites (M-2 & M-5) and is excreted primarily by the feces [33].

Regorafenib may interact strongly with CYP3A4 inducers (rifampin, phenytoin, and carbamazepine), CYP3A4 inhibitors (ketoconazole, voriconazole, grapefruit juice, clarithromycin, itraconazole) and substrates of UGT1A1 (irinotecan) [34].

EGFR (epidermal growth factor receptor) inhibitors
EGFR is a transmembrane cell surface glycoprotein and a member of the human epithelial growth factor receptor (HER) family of tyrosine kinases. Binding of ligands to the EGFR leads to homodimerization or heterodimerization of HER family members and results in autophosphorylation of intracellular tyrosine residues, initiating several key cell-signaling pathways including RAS/RAF/MAPK axis, involved in cell proliferation and PI3K/AKT pathway, involved in cell survival and motility. Binding to EGFR enhances processes critical to tumor growth and progression such as angiogenesis, apoptosis inhibition, tumor invasiveness and metastatic spread [35]. RAS is one of the most important molecules in the signaling pathway downstream of the EGFR. Three human RAS genes have been identified: HRAS, KRAS and NRAS. Since the activation of the EGFR leads to activation of KRAS, it was hypothesized that mutations in signaling pathway downstream of the EGFR may result in receptor independent pathway activation that renders the tumors unresponsive to EGFR blockage at the cell surface. Mutations in KRAS codons 12 and 13 (exon 2) are present in 35-45% of CRC with high concordance between primary and metastatic lesions and have been established as predictors of lack of response to anti-EGFR treatment. Consequently, KRAS exon 2 mutation testing is widely implemented for selection of patients with wild-type mCRC for anti-EGFR treatment. A moderate response rate of 40-60%, also in KRAS exon wild-type tumors, has motivated assessment of the predictive role of additional alterations, primarily within the RAS-RAF-MAPK and PI3K-AKT-mTOR pathways. Additional mutations in KRAS as well as NRAS (effector binding domains identical to KRAS and present in 10% of mutations) have been suggested to infer resistance to anti-EGFR therapies and the European label for panitumumab treatment was recently modified to require testing also for KRAS mutations outside of exon 2 and for NRAS mutations. The added treatment predictive value of mutation in BRAF (principal effector of KRAS and present in 8% of mutations), PIK3CA and PTEN is more uncertain [36]. According to the most current data, the BRAF biomarker is deemed to be a negative predictive biomarker as well as a prognostic biomarker.

The BRAF and KRAS status must be determined to be wild-type to produce a positive response to anti-EGFR therapies.

Figure 1 shows the important role of the BRAF & KRAS status in the response of anti-EGFR drugs.

Testing is based on lesions with greater than 50% tumor. This is a tumor percentage that can be easily obtained from an untreated primary lesion or a well-defined metastasis without treatment or extensive inflammatory infiltrate. It is estimated that 20% of patients present with metastatic disease, so mutation testing based on a primary lesion is not always feasible. Although most studies have found that metastases maintain the mutation profile of the primary lesion in more than 90% of cases, 54-60% discordance may arise for technical reasons or, rarely, because of metastases from separate primaries. For this reason, it is preferable to test the metastases rather than the primary tumor, if possible, because it's the former that is being treated by the EGFR targeted therapy.

Two monoclonal antibodies that block EGFR signaling and prevent endogenous receptor activating ligands are currently approved for the treatment of mCRC: cetuximab and panitumumab. The efficacy of these drugs is limited to patients with wild-type (non-mutated) KRAS (KRAS WT) (cetuximab) and non-mutated RAS (panitumumab) tumors.

A multitude of recent clinical trials have indicated that expanded RAS testing is more effective than traditional KRAS testing for patients with CRC. As a result of these findings, the number of patients with CRC who are eligible for EGFR inhibitors has been reduced to approximately 40% [37].

Cetuximab is a recombinant human/mouse chimeric IgG1 monoclonal antibody that binds specifically to the extracellular domain of the EGFR on both normal and tumor cells, and competitively inhibits the binding of EGF and other ligands, such as transforming growth factor-α. The recommended dose of cetuximab, in combination with irinotecan or as monotherapy, is 400 mg/m^2 as an initial loading dose administered as a 120-minute IV infusion. The recommended weekly maintenance dose is 250 mg/m^2 infused over 60 minutes. WT KRAS status is required for initiation of treatment with cetuximab but further studies also show that WT RAS status (mutations at exons 2, 3 and 4 of KRAS, NRAS and BRAF) is mandatory prior to initiation of treatment with cetuximab using a validated method of analysis, newly applied in Lebanon.

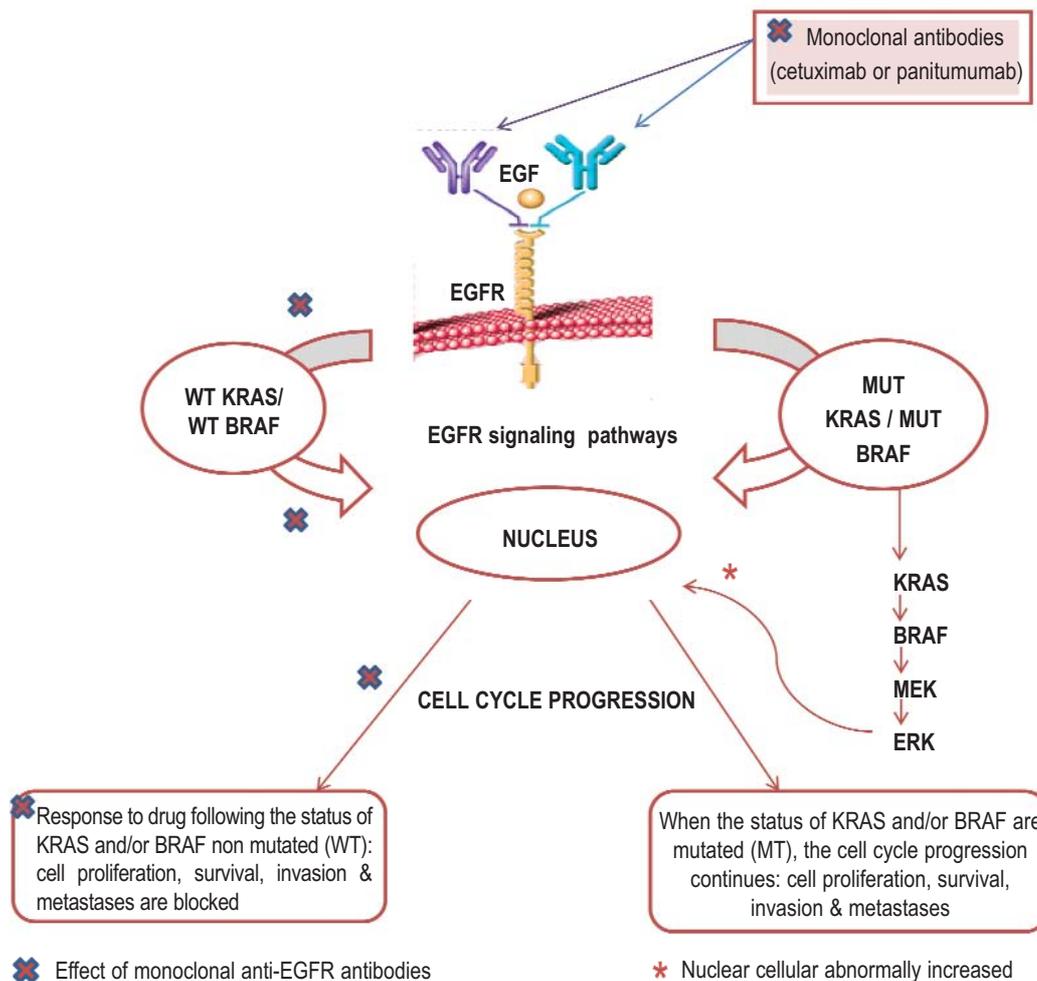The addition of cetuximab improved the resection rate of initially unresectable, liver-limited, KRAS WT mCRC

FIGURE 1

KRAS (Kirsten rat sarcoma viral oncogene homolog) / BRAF (V-Raf murine sarcoma viral oncogene homolog B1): mutations and anti-EGFR therapy in mCRC. Anti-EGFR drugs block receptor signals thus preventing downstream events such as KRAS or BRAF mediated effects.

when compared with the corresponding chemotherapy regimen alone. The efficacy of cetuximab was derived from two randomized controlled trials. The CRYSTAL study is a phase III, multicenter, open-label randomized controlled trial, which compared cetuximab in combination with FOLFIRI with FOLFIRI alone. The OPUS study trial is a phase II, multicenter, open-label randomized controlled trial, which compared cetuximab in combination with FOLFOX4 with FOLFOX4 alone [38-39]. The results of the CRYSTAL trial for the KRAS WT subgroup showed a statistically significant increase in PFS with cetuximab in combination with FOLFIRI (9.9 months vs 8.7 months). The OPUS trial also showed a statistically significant increase in PFS with a median PFS of 7.7 months for cetuximab in combination with FOLFOX compared with 7.2 months for FOLFOX alone. The RR was also improved: 60.7% in the cetuximab + FOLFOX vs 37.0% FOLFOX alone. The OPUS trial included 30.5% patients with additional RAS mutations; those in the cetuximab + FOLFOX arm had inferior survival, PFS and objective RR than those assigned to receive FOLFOX alone. Safety evaluations showed no new findings attributable to cetuximab when comparing WT and mutated RAS populations: the inferior outcome was due to lack of efficacy in combination with the known toxicity profile. As a result, some evidence of WT RAS status and of KRAS (and NRAS) is required before initiating treatment with cetuximab and that this drug is contraindicated in case of mCRC with mutant RAS or unknown RAS status. The most serious adverse reactions observed in clinical trials of cetuximab, alone or in combination with irinotecan, were infusion reactions, dermatologic toxicity (acneiform rash), interstitial lung disease, fever, sepsis, renal dysfunction, pulmonary embolism, dehydration and diarrhea.

Panitumumab is a fully humanized IgG2 monoclonal antibody that binds to the extracellular domain of the EGFR of both tumor and normal cells with high affinity. By competing with endogenous ligand binding, panitumumab inhibits receptor phosphorylation and activation of EGFR associated cell signaling [40]. This also results in down regulation of EGF receptors through receptor internalization, induction of apoptosis, autophagy and inhibition of angiogenesis. Panitumumab was approved for use by the FDA in September 2006 but registered in Lebanon since 2014. It is administered intravenously at a recommended dosing schedule of 6 mg/kg once every 2 weeks. The elimination half-life of panitumumab is approximately 7 days, longer than cetuximab where the half-life is 4 days, providing the rationale for the weekly schedule of cetuximab versus the alternate weekly administration schedule for panitumumab. It also has dual clearance mechanisms. Specific clearance of panitumumab involves binding to EGFR, internalization into the cell and degradation in lysosomes.

The evidence to support the use of panitumumab in the first-line treatment setting comes from the PRIME study (phase III trial) and two phase II trials [41-44]. The PRIME trial randomized untreated patients with mCRC to FOLFOX chemotherapy and panitumumab versus FOLFOX chemotherapy. The results of the study are shown in table III. Patients with WT KRAS mCRC who reported grades 2 to 4 skin toxicities (rash, acneiform dermatitis, pruritus, dry skin, skin fissures or erythema), diarrhea and electrolytes deficiencies (hypokalemia) had longer PFS and OS as well as ORR. The development of skin toxicity is an early clinical indicator of the panitumumab regimen's efficacy. This updated analysis confirms the value of adding panitumumab to FOLFOX in the first-line treatment of patients whose tumors are determined to be WT KRAS. Because crossover to an EGFR-targeted monoclonal antibody after progression on FOLFOX was permitted, potential survival differences may be attenuated related to panitumumab. PRIME also confirms that patients whose tumors are KRAS-mutated should not be treated with panitumumab. The recently completed PEAK study (also panitumumab in first-line treatment but phase II trial) is the first head-to-head analysis of FOLFOX combined with either panitumumab or bevacizumab in KRAS WT mCRC (Table III).

The use of panitumumab in second-line treatment of KRAS WT mCRC comes from two large phase III randomized trials, and two single-arm phase II trials (Table III).

To summarize, the addition of a biological agent to a chemotherapy backbone containing oxaliplatin or irinotecan may increase the number of patients potentially eligible for resection and improve outcomes.

COMPARATIVE DATA
BETWEEN mCRC TARGETED THERAPIES

The ASPECCT trial is an open-labeled, randomized, phase III trial designed to compare the effects of panitumumab and cetuximab for monotherapy of chemorefractory patients with KRAS WT tumors. The ORR was 22% for the panitumumab arm compared to 19.8% for the cetuximab arm. The PFS was 4.1 months in patients treated with panitumumab versus 4.4 months in patients treated with cetuximab. The study met its primary endpoint, demonstrating that panitumumab was non-inferior to cetuximab for OS (10.4 months vs 10 months). Therefore, based on these results and those from other previous trials, there is no therapeutic preference in clinical practice for using cetuximab versus panitumumab either as monotherapy or in combination with chemotherapy in naïve or chemorefractory mCRC.

Treatment selection is an art more than an algorithm. The first step is to take into account the goal of therapy and the toxicity profile that the patient is willing to live with.

The use of anti-EGFR antibody treatment in combination with chemotherapy in earlier lines of treatment is a less common and more complex issue. For RAS WT patients the optimal targeted agent in first-line therapy remains undefined with the addition of either bevacizumab or an anti-EGFR antibody supported by multiple phase III

TABLE III

PROGRESSION-FREE SURVIVAL and OVERALL SURVIVAL in FIRST & SECOND TRIALS of PANITUMUMAB [46]

TRIALS	KRAS status	Treatment regimen (control)	PFS (months)	Hazard ratio	Median OS (month)	Hazard ratio	ORR
First line							
PRIME							
(N = 1183)	KRAS WT	FOLFOX4 + P	9.6	0.8*	23.9	0.83	55%
		(FOLFOX4)	8.0		19.7		48%
	KRAS MT	FOLFOX4 + P	7.3	1.29*	15.5	1.24	40%
		(FOLFOX4)	8.8		19.3		40%
PEAK							
(N = 285)	KRAS WT	FOLFOX + P	10.9	0.87	Not reached	0.72	58%
		FOLFOX + Bev	10.1		25.4		54%
Second line							
(N = 1186)	KRAS WT	FOLFIRI + P	5.9	0.73*	14.0	0.85	35%
Peeters *et al.*		FOLFIRI	3.9		12.5	0.94	10%
	KRAS MT	FOLFIRI + P	5.0	0.85	11.8		13%
		FOLFIRI	4.9		11.1		14%
PICCOLO							
(N = 460)	KRAS WT	IrPan	NR	0.78*	10.4	1.01	34%*
		Irinotecan	NR		10.9		12%
SPIRITT	KRAS WT	FOLFIRI + P	7.7	1.01	18.0	1.06	32%
(N = 182)		FOLFIRI + Bev	9.2		21.4		19%

CI: 95% confidence interval　　**PFS**: progression-free survival　　**OS**: overall survival　　**ORR**: objective response rate　　**N**: number of patients
KRAS: Kirsten rat sarcoma gene　　**FOLFOX**: infusional 5-Fluorouracil/folinic acid/oxaliplatin　　**P**: panitumumab　　**MT**: mutated
WT: wild-type (nonmutated)　　**Bev**: bevacizumab　　**FOLFIRI**: infusional 5-Fluorouracil/folinic acid/irinotecan　　**IrPan**: irinotecan/panitumumab
NR: not reported　　* $p < 0.05$

trials. Several factors may lead the clinician to prefer an anti-EGFR antibody to bevacizumab. In patients with a contraindication to bevacizumab, the addition of anti-EGFR antibody to chemotherapy improves PFS and ORR compared with chemotherapy alone.

The consistent effects on response and survival from RAS mutations outside of KRAS exon 2 clearly suggests that mutation testing for ant-EGFR prediction should move from selective KRAS testing to broader RAS testing. Recently, data from the FIRE-3 study [45-46], which is the first head-to-head comparison between two biological agents for the treatment of mCRC, have been designed to compare FOLFIRI + cetuximab with FOLFIRI + bevacizumab in the first-line setting for patients with KRAS exon 2 WT mCRC. The primary endpoint of this study, the ORR, was not met (62% for FOLFIRI-cetuximab *vs* 58% for FOLFIRI-bevacizumab). No difference in PFS was observed (10.0 months *vs* 10.3 respectively), however, a significant difference in OS was observed in favor of FOLFIRI-cetuximab (28.7 months *vs* 25.0 respectively). In a preliminary analysis, the effects of mutations in KRAS and BRAF on ORR, PFS, and OS were evaluated. Patients with WT RAS had a median OS of 33.1 months in the FOLFIRI-cetuximab group *vs* 25.6 months with FOLFIRI-bevacizumab. However, in patients with mutant RAS, there was no difference between the two regimens. Therefore, a complete determination of RAS status may be necessary

before prescribing EGFR inhibitors in first-line treatment of mCRC, and the prescription of cetuximab should be restricted to patients with WT RAS (and WT KRAS). Thus far, the factors that contribute to the difference in OS are unclear.

Figure 2 summarizes the therapeutic strategies in the treatment of metastatic colorectal cancer.

CONCLUSION and PERSPECTIVES

The outlook for the adjuvant treatment of CRC results out of the progress in the treatment of mCRC for the last five years. Such progress has been marked by the advent of combination therapies and the very recent emergence of biotherapy improving the effectiveness of chemotherapy. With the discovery of selective targeted drugs, a new era for the treatment of mCRC has been opened. Adding these drugs, such as bevacizumab and/or cetuximab, to standard chemotherapy resulted in improved PFS and OS in patients with mCRC, either as first or second-line of therapy.

Considering the substantial cost of targeted therapies for mCRC, their cost-effectiveness has also to be measured within the Lebanese society's perspective. This type of evaluation is currently ongoing based on data from two Lebanese university hospitals [8].
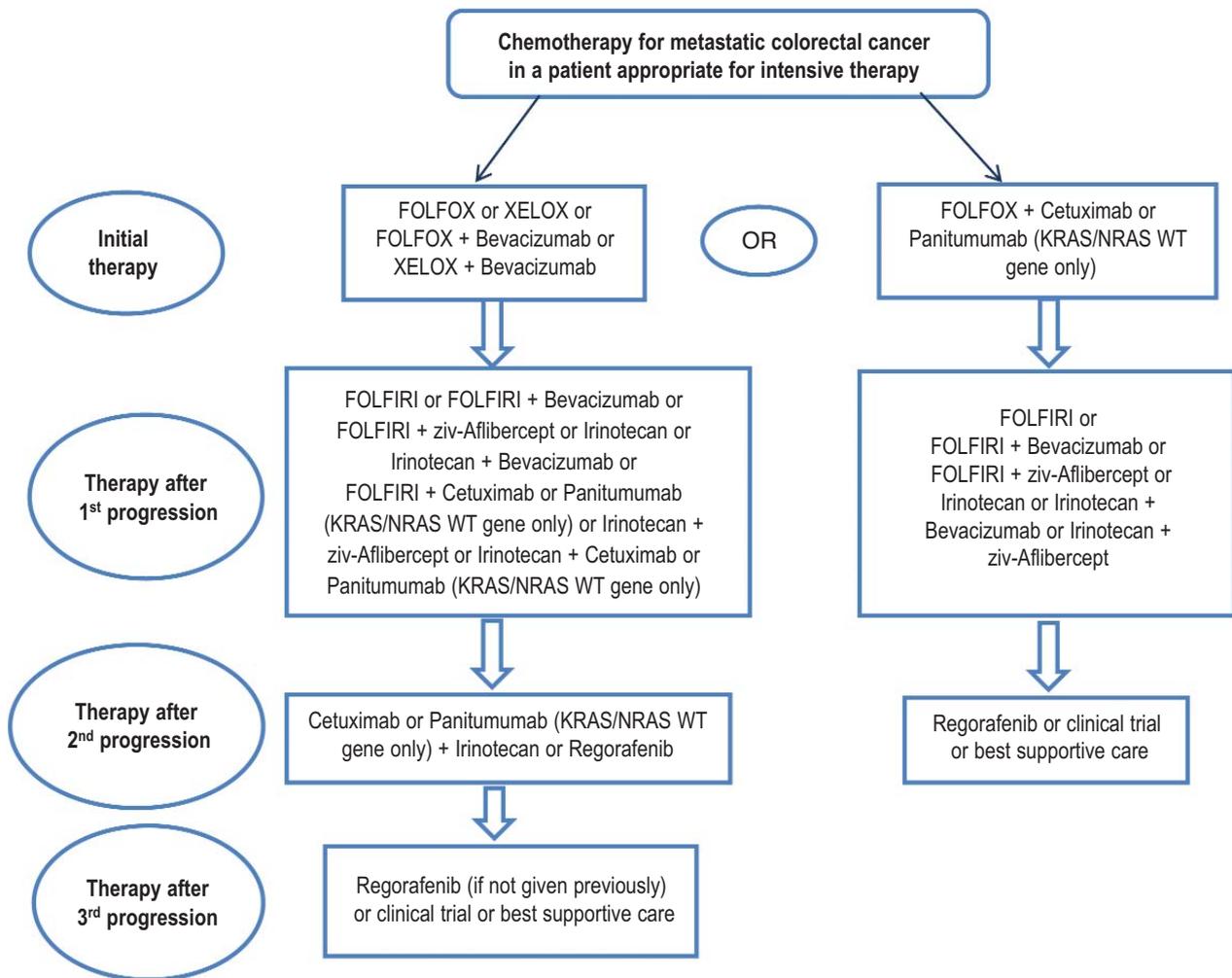
FIGURE 2

Chemotherapy for advanced or metastatic disease applied in the Lebanese hospitals [8].

FOLFIRI: infusional 5-Fluorouracil/folinic acid/irinotecan   **FOLFOX**: infusional 5-Fluorouracil/folinic acid/oxaliplatin   **KRAS**: Kirsten rat sarcoma gene
NRAS: RAS oncogene   **WT**: wild type or non-mutated

REFERENCES

1. Siegel R, Ma J, Zhaohui Z, Jennel A. Cancer Statistics, 2014. CA Cancer J Clin 2014; 64 (2): 104-17.
2. American Cancer Society. Cancer facts and figures, 2014.
3. www.moph.gov.lb/Publications/cancer statistics 2012.
4. Raskov H, Pommergard HC, Burtcharth J et al. Colorectal carcinogenesis, updates and perspectives. World J Gastroenterol 2014 Dec 28; 20 (48): 18151-64.
5. Venook A, Curley S. Management of potentially resectable colorectal cancer liver metastases. Jan 2015 Uptodate.
6. Bismuth H, Adam R, Vibert E. L'apport de la chimiothérapie dans le traitement des métastases hépatiques colorectales. Académie nationale de Chirurgie 2008; 7 (2) : 20-5.
7. Temraz S, Mukherji D, Shamseddine A. Sequencing of treatment in metastatic colorectal cancer: Where to fit the target? World J Gastroenterol 2014 Feb 28; 20 (8): 1993-2004.
8. Henaine AM, Chahine G, Salame P et al. Management of mCRC in Lebanese hospitals and estimation of direct cost: a retrospective cohort study. Oral communication presented at the Lebanese Order of Pharmacists 22nd Pharmaceutical Congress 24-26 October 2014, Hilton Habtoor Grand Hotel, Sin El Fil, Beirut, Lebanon.
9. Tourmigand C, Andre T, Achille E et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced CRC: a randomized GERGOR study. J Clin Oncol 2004; 22: 229-37.
10. Masi G, Vasile E, Loupakis F et al. Randomized trial of two induction chemotherapy regimens in metastatic colorectal cancer: An updated analysis. J Natl Cancer Inst 2011; 103 (1): 21-30.
11. Falcone A, Ricci S, Brunetti I et al. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatine & irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin & irinotecan (FOLFIRI) as 1st line treatment for mCRC: the Gruppo Oncologico Nord Ovest (GONO). J Clin Onc 2007; 25 (13): 1670-6.
12. Falcone A, Cremolini C, Masi G et al. FOLFOXIRI/bevacizumab vs FOLFIRI/bevacizumab as 1st line treatment for mCRC: results of the phase III TRIBE trial by GONO group. 2013 ASCO Annual meeting.
13. Gruenberger T, Bridgewater JA, Chau I et al. Randomized phase II study of bevacizumab with mFOLFOX6 or FOLFOXIRI in patients with initially unresectable liver

metastasis from CRC: resectability & safety in OLIVIA. Ann Oncol 2015; 26 (4): 702-8.

14. Miura K, Kinouchi M, Ishida K et al. 5FU metabolism in cancer and orally administrable 5FU drugs. Cancers 2010; 2: 1717-30.

15. Twelve C, Gollins S, Grieve R, Samuel L. A randomized cross-over trial comparing patient preference for oral capecitabine and 5-FULV regimens in patients with advanced colorectal cancer. Ann Oncol 2006; 17: 239-45.

16. Grothey A, Deschler B, Kroening H et al. Phase III study of bolus 5-FU/LV (Mayo) vs weekly high-dose 24h 5-FU infusion/LV + Oxaliplatin (OXA) (FUFOX) in advanced colorectal cancer. Proc Am Soc Clin Oncol 2002; 21: 129.

17. Loumigand C, Cervantes A, Figer A et al. OPTIMOX 1, a randomized study of FOLFOX-4 or FOLFOX-7 with oxaliplatine in a stop & go fashion in advanced CRC. A GERGOR study. J Clin Oncol 2006; 24: 394-400.

18. Cecchin E, Innocenti F, D'Andrea M et al. Predictive role of the UGT1A1, UGT1A7, and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan. J Clin Oncol May 20 2009; 27 (15): 2457-65.

19. Innocenti F, Undevia SD, Iyer L et al. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol Apr 15 2004; 22 (8): 1382-8.

20. Douillard JY, Cunningham D, Roth AD et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicenter randomized trial. Lancet 2000; 355: 1041-7.

21. Saltz LB, Cox JV, Blanke C et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 2000; 343: 905-14.

22. Koehler BC, Jager D, Shulze BH et al. Targeting cellular death signaling in CRC: current strategies and future perspectives. World of Gastroenterol 2014; 20 (8): 1923-34.

23. Bergers G, Hanahan D. Modes of resistance to antiangiogenic therapy. Nat Rev Cancer 2008; 8: 592-603.

24. Staton CA, Chetwood ASA, Cameron LC et al. The angiogenic switch occurs at the adenoma stage of the adenoma-carcinoma sequence in colorectal cancer. Gut 2007; 56 (10): 1426-32.

25. Bendell JC, Bekaii-Saab TS, Cohn AL et al. Treatment patterns and clinical outcomes in patients with metastatic colorectal cancer initially treated with FOLFOX-Bevacizumab or FOLFIRI–Bevacizumab: Results from ARIES, a bevacizumab observational cohort study. The Oncologist 2012; 17: 1486-95.

26. Stathopoulos GP, Batziou C, Trafalis D et al. Treatment of colorectal cancer with and without Bevacizumab: a phase III study. Oncology 2010; 78: 376-81.

27. Giordano G, Febbraro A, Venditti M et al. Targeting angiogenesis and tumor microenvironment in metastatic colorectal cancer: Role of aflibercept. Gastroenterology Research and Practice, 2014.

28. Patel A, Sun W. Ziv-aflibercept in metastatic colorectal cancer. Biologics 2014; 8: 13-25.

29. Ciombor KK, Berlin J, Chan E. Aflibercept. Clin Cancer Res 2013 April 15; 19 (8): 1920-5.

30. Wilhelm SM, Dumas J, Adnane L et al. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. Int J Cancer 2011 Jul 1; 129 (1): 245-55.

31. Mross K, Frost A, Steinbild S et al. A phase I dose-escalation study of regorafenib (BAY 73-4506), an inhibitor of oncogenic, angiogenic, and stromal kinases, in patients with advanced solid tumors. Clin Cancer Res 2012 May 1; 18 (9): 2658-67.

32. Schultheis B, Folprecht G, Kuhlmann J et al. Regorafenib in combination with FOLFOX or FOLFIRI as 1rst or 2nd line treatment of CRC: results of a multicenter phase Ib study. Ann Oncol 2013; 24 (6): 1560-7.

33. Sujan R, Ashwani K Singal. Regorafenib: an evidence-based review of its potential in patients with advanced liver disease. Core Evid 2014; 9: 81-7.

34. DeWit M, Boers-Doets CB, Saettini A et al. Prevention and management of adverse events related to regorafenib. Support Care Cancer 2014; 22 (3): 837-46.

35. Jean GW, Shah SR. Epidermal growth factor receptor monoclonal antibodies for the treatment of metastatic colorectal cancer. Pharmacotherapy 2008 Jun; 28 (6): 742-54.

36. Therkildsen C, Henrichsen-Schnack T, Bergmann TK et al. The predictive value of KRAS, NRAS, BRAF, PIK3CA & PTEN for anti-EGFR treatment in mCRC: a systematic review and meta-analysis. Acta Oncol 2014; 53: 852-64.

37. Bendell J, Cannon S, Fakih M. Optimizing treatment with EGFR inhibitors in CRC. http://www.onclive.com/peer-exchange/refractory-crc/Optimizing-Treatment-With-EGFR-Inhibitors-in-CRC.

38. Tejpar S, Lenz HJ, Köhne CH et al. Effect of KRAS and NRAS mutations on treatment outcomes in patients with metastatic colorectal cancer (mCRC) treated first-line with cetuximab plus FOLFOX4: New results from the OPUS study. J Clin Oncol 2014; 32 (Suppl 3).

39. Van Custem E, Lenz HJ, Köhne CH et al. Fluorouracil, Leucovorin & Irinotecan + Cetuximab treatment & RAS mutations in CRC. J Clin Oncol 2015; 33 (7): 692-700.

40. Hocking CM, Price TJ. Panitumumab in the management of patients with KRAS wild-type metastatic colorectal cancer. Therap Adv Gastroenterol 2014 Jan; 7 (1): 20-37.

41. Douillard JY, Oliner KS, Siena S et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013; 369: 1023-34.

42. Patterson S, Peeters M, Siena S et al. Comprehensive analysis of KRAS and NRAS mutations as predictive biomarkers for single agent panitumumab response in a randomized, phase III metastatic colorectal cancer study J Clin Oncol 2013; 31 (Suppl.): Abstract 36/7.

43. Seymour MT, Brown SR, Middleton G et al. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. Lancet Oncol 2013 Jul; 14 (8): 749-59.

44. Van Cutsem E, Peeters M, Siena S et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007 May 1; 25 (13): 1658-64.

45. Heinemann V, von Wekersthal L, Decker T et al. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. The Lancet Oncology 2014 Sep; 15 (10): 1065-75.

46. Hocking CM, Price TJ. Panitumab in the management of patients with KRAS wild type mCRC. Therap Adv Gastroenterol 2014; 7 (1): 20-37.