symposium article

Annals of Oncology 21 (Supplement 6): vi1–vi10, 2010
doi:10.1093/annonc/mdq273

Molecular markers and biological targeted therapies in metastatic colorectal cancer: expert opinion and recommendations derived from the 11th ESMO/World Congress on Gastrointestinal Cancer, Barcelona, 2009

E. Van Cutsem[1,*], M. Dicato[2], N. Arber[3], J. Berlin[4], A. Cervantes[5], F. Ciardiello[6], A. De Gramont[7], E. Diaz-Rubio[8], M. Ducreux[9], R. Geva[1,3], B. Glimelius[10], R. Glynne Jones[11], A. Grothey[12], T. Gruenberger[13], D. Haller[14], K. Haustermans[1], R. Labianca[15], H. J. Lenz[16], B. Minsky[17], B. Nordlinger[18], A. Ohtsu[19], N. Pavlidis[20], P. Rougier[18], W. Schmiegel[21], C. Van de Velde[22], H. J. Schmoll[23], A. Sobrero[24] & J. Tabernero[25]

[1]University Hospital Gasthuisberg, Leuven, Belgium; [2]Centre Hospitalier de Luxembourg, Luxemburg; [3]Tel Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv, Israel; [4]Vanderbilt University Medical Center, Nashville, USA; [5]Hospital Clínico, Incliva, University of Valencia, Valencia, Spain; [6]Second University of Naples, Naples, Italy; [7]Hôpital Saint-Antoine, Paris, France; [8]San Carlos Hospital Clinic, Madrid, Spain; [9]Institut Gustave Roussy, Villejuif, France; [10]University of Uppsala, Uppsala and Karolinska Institutet, Stockholm, Sweden; [11]Centre for Cancer Treatment, Mount Vernon Hospital, Northwood, Middlesex, UK; [12]Mayo Clinic, Rochester, MN, USA; [13]Medical University Vienna, Vienna, Austria; [14]University of Pennsylvania Cancer Center, Philadelphia, PA, USA; [15]Ospedale Riuniti, Bergamo, Italy; [16]University of Southern California, Norris Cancer Center, Los Angeles, CA, USA; [17]University of Chicago Medical Center, Chicago, USA; [18]Hôpital Ambroise Paré, Boulogne, France; [19]National Cancer Center Hospital East, Kashiwa, Japan; [20]University of Ioannina, Ioannina, Greece; [21]Ruhr-University Bochum, Bochum, Germany; [22]Leiden University Medical Center, Leiden, The Netherlands; [23]Martin-Luther-Universität, Halle, Germany; [24]San Martino Hospital, Genova, Italy and [25]Vall d'Hebron University Hospital, Barcelona, Spain

The article summarizes the expert discussion and recommendations on the use of molecular markers and of biological targeted therapies in metastatic colorectal cancer (mCRC), as well as a proposed treatment decision strategy for mCRC treatment. The meeting was conducted during the 11th ESMO/World Gastrointestinal Cancer Congress (WGICC) in Barcelona in June 2009. The manuscript describes the outcome of an expert discussion leading to an expert recommendation. The increasing knowledge on clinical and molecular markers and the availability of biological targeted therapies have major implications in the optimal management in mCRC.

introduction

Colorectal cancer is one of the leading causes of death from cancer worldwide [1]. In Europe alone 412 900 new cases were diagnosed in 2006 comprising 12.9% of all new cancer cases [2]. The decrease in mortality spanning across the last decade is attributed to early detection and improved therapy. The median survival of patients with metastatic colorectal cancer (mCRC) participating in clinical trials has improved from approximately 6 months to 2 years. This increase in survival can be attributed mainly to two treatment advances. The first treatment advance is the introduction of new cytotoxics and biologicals, as well as the better selection of patients; the second, the establishment of a multidisciplinary approach to metastatic colorectal cancer, improved techniques to resect metastatic disease particular in liver metastases and the development of new techniques such as two-staged metastasectomy, incorporation of portal vein embolization for increasing liver reserve and the combination of surgery with localized therapy such as radiofrequency ablation [3].

Current standard treatment for mCRC patients includes a fluoropyrimidine backbone either intravenously or orally, with oxaliplatin and/or irinotecan with the incorporation of the biological targeted therapies bevacizumab, cetuximab, or panitumumab. The choice of a treatment regimen is highly directed by the planned strategy: resectable or potentially resectable if tumor shrinkage/control exists versus non-resectable metastases and by the need for an aggressive treatment or not. Currently implemented treatment strategies follow the conventional treatment paradigm proved across the years of 'more is better'.

One of the important questions is whether to combine or to 'pile up' as many different agents as possible (e.g. two cytotoxics and two biologicals) in the first-line treatment of mCRC or rather administer the different cytotoxics in a more sequential approach, as has been studied in the FOCUS [4] and

Correspondence to: Prof. Dr E. Van Cutsem, Digestive Oncology Unit, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. Tel: +32-16–344225; Fax: +32-16–344419; E-mail: eric.vancutsem@uz.kuleuven.ac.be

CAIRO-1 [5] studies. This sequential approach was, however, associated with a relatively short median survival. Recently the attempt to combine different biologicals with two cytotoxics was evaluated in the PACCE [6] and in the CAIRO-2 [7] studies, testing the addition of cetuximab or panitumumab to a cytotoxic doublet plus bevacizumab. Both trials failed to show an advantage of the four-drug combination and a concern for a possible detrimental effect was raised, in particular in patients with KRAS mutated tumors. It is shown that patients exposed to all three cytotoxics, regardless of the sequence, during their disease, have a longer overall survival [8]. In this regard an Italian study showed that the combination of the three cytotoxics led to an improved efficacy of the combination of 5-fluorouracil (5-FU), oxaliplatin and irinotecan compared to the combination of 5-FU/irinotecan (FOLFIRI) [8], while a Greek study did not show a superiority of the three cytotoxics compared to the FOLFIRI regimen [9]. Phase II studies are ongoing evaluating the combination of three cytotoxics plus a biological targeted agent.

The development of the targeted agents has clearly improved the therapeutic outcome of patients with mCRC [3] and has changed the treatment options for these patients. With the introduction of the targeted agents, the need for better patient selection became obvious.

Our knowledge of the molecular development of CRC has greatly increased in recent years, as well as the unraveling of the different signal transduction pathways and their variance amongst different individuals. The introduction of single nucleotide polymorphisms (SNPs) has added to our awareness of how individually profiled each patient could be. Molecular markers clearly have the potential to help the prediction of the activity or the lack of activity of a biological agent. The best example is the introduction of the *KRAS* mutation status for the prediction of resistance to anti-epidermal growth factor receptor (EGFR) antibodies. Other emerging markers are under evaluation. The classical efficacy evaluation by RECIST is challenged by the fact that some compounds, in particular the angiogenesis inhibitors, may not shrink the size of the metastatic disease but change the nature of the metastases tissue composition to a more avascular/necrotic area [10]. Functional imaging with positron emission tomography (PET) scan, dynamic contrast enhanced ultrasonography and magnetic resonance imaging (MRI) are coming into the research arena and are likely to be implemented in clinical practice in the future. Validation of the clinical utility of functional imaging techniques is, however, still needed, as well as the optimization of the endpoints in clinical trials in mCRC.

Molecular markers can be prognostic, predictive, or both. Prognostic markers identify patients with different risks of a specific outcome of the disease, either in the absence of a systemic therapy and/or associated with a differential outcome regardless of treatment. A prognostic marker usually does not determine the specific choice of a therapy. A predictive marker can predict differential efficacy of a particular therapy according to the marker status and therefore helps to guide the therapeutic choice [11].

The expert panel discussed the current status of the molecular markers and provides recommendations for clinical practice and future research for the treatment of patients with mCRC.

methods

A panel of experts from different countries and different disciplines involved in the treatment of metastatic colorectal cancer convened in the Barcelona ESMO/World Congress on Gastrointestinal Cancer, held in June 2009. Experts were selected on scientific merits and recognition as international opinion leaders. Specific questions on the issue were sent around to all experts and returned before the meeting. Answers were analyzed and presented in the meeting and re-discussed in the expert forum. The experts' opinions are mainly based on scientific studies and on evidence coming from well-performed clinical trials, although attention was given to personal experience. Conclusions from the discussions are thereby published aiming to help clinicians in daily decision making. These are not official guidelines or consensus statements, but are really expert recommendations.

The panel discussed the relevant data and publications on prognostic and predictive markers for metastatic colorectal cancer. The data on the potential prognostic and predictive markers were discussed in view of their clinical relevance for the management of patients with mCRC. The significance of the markers in relation to the use of biological agents in metastatic disease has also been discussed in detail. Clinical recommendations are proposed, as well as suggestions for future trial designs.

markers in metastatic colorectal cancer

prognostic markers

patient-related prognostic markers. Patient-related markers correlating with disease prognosis have been extensively investigated, are well documented, and are readily incorporated into the stratification process of modern clinical trials.

Performance status (PS) has consistently emerged as a prognostic marker, from early trials with 5-FU alone to trials incorporating biological targeted agents [12–18]. Most recent clinical trials do not incorporate patients with ECOG PS ≥2, making a true comparison difficult. A recently published pooled analysis by Sargent et al. [19] incorporated 6286 mCRC patients from nine controlled trials, of whom 509 had a PS = 2. PS was found to be a prognostic marker for OS, progression free survival (PFS) and response rate (RR), despite a similar relative benefit from treatment. Higher rates of toxicity in patients with a PS = 2 vs PS = 0/1 were also demonstrated.

The importance of age as a prognostic marker has been controversial. Elderly patients are often an underrepresented group with little incorporation in clinical trials and 'ageism' may be a common practice, depicted by less frequent use of combined treatment and less invasive interventions in the elderly [20].

More than 70% of deaths from CRC in the Western world occur in patients ≥65 years old. The fact that the outcome in this group of patients is often worse may be attributed to more advanced stage at diagnosis and often, less aggressive treatment and decreased use of second- and third-line therapies [20–21]. Elderly patients fit enough to be eligible for clinical trials appear

to derive a similar benefit from the treatment. In general, fit elderly patients tolerate the chemotherapy quite well, but some studies reported that elderly patients may have more specific toxicity from cytotoxics: e.g. increased neutropenia in patients ≥75 years when treated with irinotecan [22] and an increase in bevacizumab-related arterial thrombosis in patients ≥70 year [23]. Co-morbidity and socio-economic status further impact treatment availability and prognosis. Several additional patient-related factors that have been shown to produce improved prognosis include female sex [12, 24] and limited weight loss at diagnosis.

tumor-related prognostic markers. Tumor-related biomarkers and factors remain the most important prognostic variables. The stage of disease currently described by TNM, as published by the American Joint Committee on Cancer (AJCC) and American Cancer Society (ACS) [25] is one of the strongest prognostic factors [24].

A colonic primary tumor is a positive prognostic marker compared to a rectal tumor [13–14]. A mucinous histology is usually accepted as being related to a worse outcome. Metachronous metastases have a better outcome compared to synchronous metastases. Metastatic spread confined to the liver shows improved OS rates as opposed to spread to multiple organ sites [12]. In contrast, patients with liver metastases show a shorter survival compared to lung metastases [14, 17]. Peritoneal metastases are related to a worse outcome. The higher the number of metastatic sites, the worse the prognosis is [14]. In patients with mCRC, a prior adjuvant treatment is considered as a negative prognostic factor, especially when treated with oxaliplatin-based adjuvant chemotherapy [12, 24]. The number of previous lines of chemotherapy is also accepted as a negative prognostic factor [16].

center-related factors. The center in which treatment is directed influences the prognosis, with experience and volume [26–27] of patients playing a role, as well as possible deviation from standard clinical practice [28] for a variety of reasons. The treatment center is therefore a common stratification factor in multi-centric and especially in international trials.

biochemical prognostic markers. Biochemical markers should optimally be combined together and not be seen as single factors. Many of these factors have a prognostic as well as a predictive significance. Pretreatment blood count values with low hemoglobin levels [13, 15] and high white blood cell (WBC) counts [13, 17] depict poor prognosis. Increased alkaline phosphatase baseline levels are considered one of the strongest poor prognostic factors for survival in mCRC [12–14, 18]. Lactate dehydrogenase (LDH) participates in anaerobic glycolytic metabolism in tumor cells and is postulated as a biomarker for high angiogenic tumors [29]. High base line LDH measurements predict poor prognosis [12, 17]. High levels of serum bilirubin and low levels of serum albumin also represent poor prognosis. Preoperative CEA has been advocated as prognostic for disease free survival as well as persistent disease after surgery [30]. Baseline levels of CEA prior to treatment in the metastatic setting also seem to have some prognostic relevance [12, 17]. A multivariate analysis [14], on a total of 3825 patients from 19 prospective trials treated with 5-FU-based therapy for mCRC distinguished three risk groups based on the main prognostic markers; a low risk group described as ECOG 0/1 and only one tumor site; an intermediate risk group defined as patients with ECOG 0/1 and more than one tumor site and alkaline phosphatase <300 U/l or patients with ECOG >1, WBC count $<10 \times 10^9$/l and only one tumor site; and a high risk group of patients with ECOG 0/1 and more than one tumor site and alkaline phosphatase of ≥300 U/l or patients with ECOG >1 and more than one tumor site or WBC count $>10 \times 10^9$/l. The median survivals were 15 months, 10.7 months and 6.1 months, respectively. A recommendation to stratify future trials based on these prognostic factors is sometimes made.

molecular prognostic markers. Real evidence as to the validity of the many proposed prognostic markers published in the literature is scarce. Two molecular markers seem to have a consistent prognostic value.

microsatellite instability: The development of CRC through microsatellite instability (MSI) is attributed to the hereditary HNPCC syndrome patients and to about 15% of sporadic CRC patients [31]. MSI-H patients have a phenotype characterized by right-sided location and a relatively early stage at diagnosis. In a study [32] in 607 patients at ages ≤50 diagnosed with CRC, 17% were found to have high MSI that was associated with a significant survival advantage regardless of standard prognostic factors, including stage. An Italian study in 1263 CRC patients detected 20% of the tumors as MSI-H showing a favorable prognosis [33]. A subset analysis according to stage, though, showed this advantage was not maintained in stage IV patients. A meta-analysis by Popat et al. [34] spanning across 32 studies with 7642 cases of whom 1277 (16.7%) were MSI-H reported the hazard ratio estimate for overall survival associated with MSI as 0.65 (95% CI, 0.59 to 0.7) maintained through stages II–IV.

BRAF mutations: The V600E mutation in *BRAF* has been implicated in several trials as a poor prognostic marker in CRC. Ogino et al. [35] analyzed 649 colon cancers of all stages. Analysis adjusted for other prognostic markers revealed that *BRAF* mutation was associated with a significantly high cancer-specific mortality (multivariate HR 1.97; 95% CI 1.13 to 3.42). Roth et al. [36] reported also that *BRAF* is a negative prognostic factor in stage II/III colon in the framework of the PETACC-3 study and Van Cutsem et al. [37] reported the negative prognostic significance of *BRAF* mutations in metastatic CRC treated in the Crystal trial [37]. The relationship between *BRAF* mutation and MSI was assessed by Somawitz et al. [38] in 911 CRC patients. The V600E mutation was found in 9.5% of the overall population, in 5% (40/803) of microsatellite-stable (MSS) cancers and in 52% (43/83) of MSI tumors. The *BRAF V600E* mutation was associated with a significantly worse survival in stages II to IV MSS colon cancers. No effect on prognosis in MSI tumors was detected.

Several conflicting data have been published on the prognostic significance of *KRAS* mutations. Two recent large studies, however, showed no prognostic role of *KRAS* mutations in stage III and II/III colon cancer [36, 39].

predictive markers

Most if not all proclaimed predictive markers have not been validated in prospectively designed trials and must therefore be used with caution. Some of these markers are, however, sometimes used in every day clinical practice to guide the clinicians.

A distinction must also be made between markers that can be assessed prior to the start of treatment as opposed to those revealed only once treatment has started. The first might help determine if treatment should be started while the other might help determine if treatment should be stopped.

patient-related predictive markers for outcome. Markers predicting outcome have been investigated for specific treatment regimens and as such can only be considered when these protocols are implemented.

general clinical and biochemical factors: Patient and tumor variables are strong prognostic factors and as such often predict efficacy of the chemotherapeutic treatment mCRC. Several trials using regimens based on 5FU, irinotecan and/or oxaliplatin reported that baseline hemoglobin and WBC levels, LDH levels, time since diagnosis, low number of organs involved, performance status, and age are predictive of response and survival [40–45]. The data in these studies usually do not clearly distinguish between the prognostic and predictive value of these markers.

Extensive and symptomatic peritoneal carcinomatosis is often related to a lower chance of response to an antitumoral therapy, on top of its prognostic significance. However, peritoneal carcinomatosis is often difficult to evaluate properly.

The ability of carcinoembryonic antigen (CEA) kinetics to predict response to chemotherapy had been debated. Clearly absolute correlation does not occur as patients presenting with decreased markers may still show progression on imaging. Strimpakos et al. [46] retrospectively examined 670 patients receiving first-line chemotherapy and having baseline CEA measurements plus at least two more measurements during treatment. CEA levels were described as flare in 78 (12%) patients (flare defined as a ≥15% rise from baseline with a minimum 4 μg/l rise followed by subsequent ≥15% decrease from baseline). Those were compared to patients with increasing CEA and were found to have a significantly better ORR (73% versus 11%; respectively, $P < 0.001$), similar significant differences were seen with PFS and OS. Other series support this observation [47]. According to a few small trials, a CEA response after treatment, at least in patients who had pretreatment elevated levels, correlated with RR [48–50]. The use of CEA is, however, not recommended to routinely replace imaging in assessing response.

skin rash: One of the more consistent clinical parameters is the association found between the development of skin toxicity, mainly rash, and response on EGFR-targeting agents: the more severe the rash (usually acneiform) is, the better the RR, PFS and/or survival is for patients treated with EGFR inhibitors.

This correlation has been shown in early phase II trials, as well as in phase III trials [51, 52]. It is reported for cetuximab and panitumumab in colorectal cancer as well as in other disease sites and for the small molecule tyrosine kinase inhibitors: e.g. erlotonib in pancreatic cancer [53]. Starting from this information, the Everest study has explored whether dose increase of cetuximab in patients with no rash or a slight rash can increase the activity of cetuximab [54]. Although the RR was slightly higher in patients receiving a dose increase of cetuximab, with the aim to induce a more pronounced rash and a higher activity, this strategy cannot be recommended outside of clinical trials before further validation studies have been reported.

hypertension: Preliminary data of a small phase II study in 39 patients treated with bevacizumab/ irinotecan/5-FU has suggested the correlation of activity and early hypertension [55]. This correlation has also been suggested in pancreatic cancer patients treated with axitinib, a multitarget tyrosine kinase inhibitor [56]. The data on the correlation of hypertension and benefit from an angiogenesis inhibitor are at this moment weak without clear therapeutic implications in clinical practice [57].

clinical and biochemical predictive markers for toxicity.

renal function and capecitabine: Pharmacokinetic studies of capecitabine showed that moderate impairment of creatinine clearance causes an increase in the AUC of the key metabolite 5′-DFUR (the immediate precursor to 5-FU) and that this correlates with more adverse events [58]. A combined safety analysis on two phase III trials [59] with capecitabine revealed an increase in grade 3–4 toxicity in patients with moderate renal impairment at baseline (defined as creatinine clearance 30–50 ml/min calculated by the Cockcroft and Gault formula [60]). More dose reductions were needed during the treatment and a 75% starting dose was therefore recommended in patients with a moderately impaired renal function without compromising activity. An increase in toxicity was also observed in patients with impaired renal function treated with 5-FU/LV.

bilirubin levels and UGT polymorphism and irinotecan: Irinotecan's active metabolite SN-38 is inactivated through glucuronidation by uridine diphosphate glucuronosyltransferases (UGTs) mainly in the liver and is excreted through the bile ducts. Bilirubin being a substrate of UGT1A1 is a possible indirect marker of its activity. An analysis of four phase II trials with irinotecan revealed that increased bilirubin levels correlate with a high risk for grade 3–4 neutropenia [45]. A meta-analysis demonstrated that *UGT1A1*28* genotype is moderately predictive of severe irinotecan induced hematologic toxicity at moderate doses of irinotecan and strongly predictive at high doses, but at low doses these patients have a comparable incidence of toxicity to other patients [61–62]. In 2005 the FDA added a warning to the irinotecan packaging label indicating that patients with the *UGT1A1*28* genotype were at increased risk for neutropenia and that a reduced initial irinotecan dosage should be considered. However, the precise implications of *UGT1A1* genotype on the safety, efficacy, and development of individualized patient dosing of irinotecan are not yet clear. Significant unexplained variability exists within the UGT1A1 subgroups, and other characteristics of the patients have been understudied. For example, Miya et al. [63] identified many

additional clinical characteristics that affected SN38 distribution and levels such as sex, BSA, and age. Thus possible confounders to the *UGT1A1*28* allele-irinotecan adverse event relationship include: other enzymes and transporters that play a role in irinotecan disposition as well as other genetic variation in the UGT1A1 gene; age, gender, and ethnicity; comorbidity and performance status; pretreatment serum bilirubin concentrations; use of other drugs that share the irinotecan metabolizing pathway. Important questions still remain before implementing UGT1A1 testing in clinical practice such as how accurately does genotyping of UGT1A1 predict total irinotecan metabolic capacity.

cardiovascular disease as risk for toxicity on bevacizumab: Patients treated with bevacizumab are more likely to develop an arterial thrombosis [23, 64]. Patients with a history of arterial thrombotic disorders such as angina pectoris, myocardial infarction, or stroke and elderly patients above 65 years are especially at risk. In these patients the potential benefit of an antiangiogenic treatment should always be put into balance with the potential adverse events [65].

molecular predictive markers for efficacy

predictive markers for anti-EGFR antibodies. KRAS mutations (most frequently analyzed those in codon 12 and 13) are strongly associated with resistance to the anti-EGFR antibodies cetuximab and panitumumab. This has been consistently shown in many small retrospective data sets, retrospective analyses of larger phase III studies, and also in a few prospective studies [52, 66–71]. Approximately 40% of patients with CRC harbor a *KRAS* mutation and will derive no benefit from cetuximab or panitumumab. Of the remaining patients with a *KRAS* wild-type tumor, approximately 15–20%, will have an objective response when treated with an antibody alone and approximately 35–40% when treated with cetuximab plus irinotecan in irinotecan refractory CRC, leading to the statement that *KRAS* mutations predict resistance to cetuximab and panitumumab as single agent and in combination with chemotherapy in pretreated as well as in early lines of treatment in combination with a cytotoxic partner. It has even been reported that a *KRAS* mutation is associated with a deleterious effect when patients are treated with an oxaliplatin based backbone in combination with an anti-EGFR antibody. Several small studies have reported that there is a more than 95% concordance between the presence of a *KRAS* mutation in the primary tumor and in the metastases [72]. This consistent association has led the major regulatory bodies to limit the use of cetuximab and panitumumab to *KRAS* wild-type tumors with mandatory evaluation of mutations prior to the administration.

emerging potential predictive markers for anti-EGFRs. BRAF and *KRAS* mutations are considered mutually exclusive and apparently mutated at a similar phase of the tumorigenesis [73]. The *BRAF* mutation (mainly V600E) is detected in 6–10% of patients with mCRC [37, 75]. Several studies have reported consistently that the presence of the *BRAF* mutation V600E correlates with resistance to cetuximab and panitumumab in chemorefractory mCRC [74–77]. In the updated analysis of the Crystal trial [37] 9% of the *K-ras* wild-type patients had a *BRAF* mutation (6% of all patients with CRC). This analysis showed that the presence of *BRAF* mutations had a strong negative prognostic significance. Whether *BRAF* mutations have also a predictive significance for cetuximab in combination with a standard cytotoxic combination in the first line treatment of mCRC cannot be shown, but cannot be excluded either in view of the important prognostic significance of *BRAF* mutations, the potential interaction with chemotherapy, and the relatively low frequency of *BRAF* mutations. Similar findings were reported in the Opus study [78]. Similar to *KRAS*, a retrospective analysis showed a high concordance between the presence of B-raf mutations in the primary tumor and in metastases [72, 79].

Amphiregulin and epiregulin are ligands of the EGFR. Preliminary evidence from small series showed correlation between ligand expression levels and efficacy under anti-EGFR treatment. Recently larger retrospective studies have confirmed the potential predictive role of these ligands in patients with a *KRAS* wild-type tumor. High epiregulin gene expression was found to be associated with improved median PFS (5.4 versus 1.9 months; $P < 0.0001$, respectively), and median OS (9.8 versus 5.1 months; $P < 0.001$, respectively) in *KRAS* wild-type patients treated with cetuximab in chemorefractory CRC in the NCIC CTG CO.17 phase III trial [80]. A correlation between epiregulin and amphiregulin expression was also reported in 200 *KRAS* wild-type chemorefractory CRC by Jacobs et al. [81]. Further validation of the ligand data is however needed in prospective trials, as well as in earlier lines of treatment in combination with chemotherapy. Moreover the linear correlation between the level of amphi- and epiregulin may render clinical decisions more difficult.

Additional effectors participating in the EGFR downstream signal transduction are *PI3K*, *PTEN* and *NRAS*. It is biologically reasonable to assume that further mutations might preclude the efficacy of anti-EGFR antibodies. Preliminary evidence to this effect is emerging [76, 82–84]. According to some recent analysis *NRAS* mutations may also play an important role in determining resistance to anti-EGFR antibodies. However, the data on *PI3K* mutations are less clear. According to a European Consortium analysis *PI3K* mutations are not clearly associated with resistance to cetuximab [77]. The actual data on the role of *PTEN* immunohistochemistry as a predictive marker are not so convincing [85]. *PTEN* was only positive in the metastatic site making it a difficult marker to use in the clinic.

other potential markers for chemotherapy efficacy and toxicity.

ERCC1 for oxaliplatin efficacy: The mechanism of action of platinum agents is via creating cross-links in the DNA strands. The nucleotide excision repair mechanism removes these cross-links and results in resistance to the platinums. One of the key proteins in this process is the ERCC1. Several retrospective analyses in patients with mCRC treated with oxaliplatin based regimens, showed an association between ERCC1-118 T/T polymorphism and treatment efficacy [86–88]. A similar trend was found with ERCC1 expression assessed by IHC [89] or mRNA [90].

potential markers for 5FU efficacy and toxicity: Thymidylate synthase (TS) is the key enzyme targeted by 5-FU. Numerous studies were published discussing the role of TS levels and/or the role of TS polymorphisms, either by IHC or genetic analysis in predicting efficacy. Most of the accumulated evidence was performed on small population sizes and evidence is conflicting [91–93]. This precludes any official recommendation.

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme in the catabolism of 5-FU. A correlation between the DPD expression levels and outcome was reported in several studies [94], but the data are not strong and consistent enough to determine DPD routinely in clinical practice. More important are the data of several retrospective series that demonstrated the importance of DPD activity level in developing toxicity from 5-FU. Low activity levels of DPD appear to cause more rapid development of severe toxicity and even higher mortality rates than normal DPD levels. The most frequent mutation leading to decreased activity is IVS14+1G>A [95–97]. The current recommendation is to look for DPD deficiency in patients with severe fluoropyrimidine related toxicity in order to avoid further exposure to 5-FU that may lead to major toxicity.

expert panel recommendations for molecular predictive markers testing.

KRAS mutation status – *for anti-EGFR treatment*: Analysis for *KRAS* mutation should be routinely done before chemotherapy for metastatic CRC is given. Analysis can be done on paraffin-embedded tumor block of the primary or metastatic site.

BRAF mutation status – for anti-EGFR treatment: BRAF mutations have a strong prognostic significance. In chemorefractory mCRC, the evidence is accumulating for a predictive role of *BRAF* mutations as a marker for resistance and testing can be considered before an anti-EGFR antibody is considered. However, in earlier lines of treatment routine testing of *BRAF* mutations cannot currently be recommended.

MSI: In the adjuvant setting, an emerging role of MSI in stage II colon cancer has been shown. In young patients and in families with clinical features of Lynch syndrome, diagnosed with mCRC, MSI testing is part of the genetic evaluation in the exploration of genetic predisposition for Lynch syndrome and is mandatory for genetic counseling. There is however, not sufficient evidence to guide treatment choice in patients with mCRC based on MSI or MSS status.

treatment strategy for metastatic colorectal cancer

Patients suspected to have metastatic spread should be thoroughly examined with available imaging techniques. An assessment of the tumor burden as well as the relevant prognostic and predictive markers should be done in order to clearly define the treatment goal and strategy.

The optimal treatment strategy of patients with mCRC should be discussed in a multidisciplinary expert team, at least for those patients with metastases limited to the liver and/or the lungs. The experience of the team has a clear impact on the outcome of the patient.

The majority of patients with metastatic disease have lesions that initially are not suitable for resection. It is important to select patients in whom the metastases are suitable for resection and those with initially unresectable disease in whom the metastases can become suitable for resection after a major response has been achieved with combination chemotherapy. The aim of the treatment in the last group of patients may therefore be to reverse initially unresectable metastatic CRC to resectable CRC, i.e. conversion therapy. Some patients may initially have a 'technically' resectable disease, but it is biologically not sound to do a resection. A response to therapy in these patients may tell that it later is biologically sound to do even extensive surgery. The determination of the goal of therapy (palliative versus potentially curative) will therefore determine the selected treatment option (Table 1) [98].

unresectable metastatic colorectal cancer

The first line strategy is shown in Figure 1.

patients needing or desiring an aggressive approach. The primary decision directing strategic planning is the goal of the treatment. An aggressive approach with combination

Strategy according to treatment aim

Clinical situation	What is needed?	Treatment intensity
• liver (lung) metastases ✓ potentially resectable • multiple metastases with ✓rapid progression ✓tumour related symptoms ✓risk for rapid deterioration	Maximal tumour-shrinkage required Control of progressive disease	Upfront combination treatment: multidrug regimens
• unresectable metastases ✓ no option for resection ✓ no symptoms ✓ risk for rapid deterioration ✓ comorbidity	Tumour shrinkage less relevant Control of further progression Prevention from toxicity	Start with single agent (sequential approach) or with doublets

Table 1. Treatment strategy according to clinical situation.

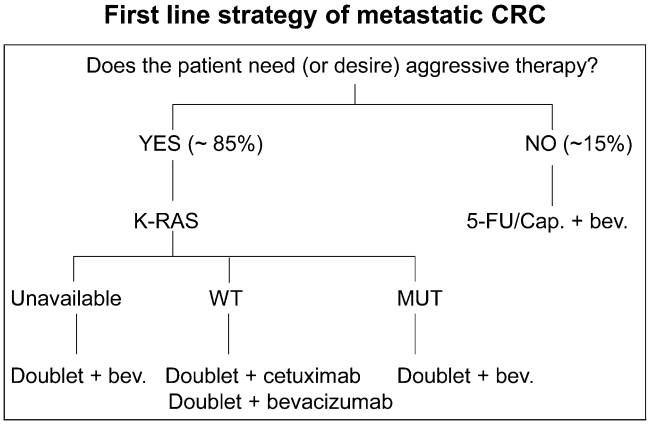

Figure 1. Clinical strategy in first-line chemotherapy directed by need for aggressive therapy and *KRAS* status. WT, wild type; MUT, mutant; Cap., capecitabine; bev., bevacizumab; doublet, doublet of cytotoxics.

chemotherapy is indicated when the patient can withstand such treatment and falls under one of the following groups, since a maximal tumor shrinkage or maximal control of tumor progression may be waranted:

- patients with potentially resectable metastases;
- patients with clearly symptomatic disease in whom tumor regression is needed;
- patients with the risk of rapid deterioration due to an aggressive tumor biology and/or extensive disease.

In these patients a cytotoxic doublet in combination with a biological is proposed. As options for cytotoxic combination FOLFOX, FOLFIRI and capecitabine/oxaliplatin are usually proposed. The combination of three cytotoxics is usually not proposed as backbone, but remains an alternative for the experts when a high shrinkage of the tumor is needed and there is contraindication to biologic targeted therapies. In this situation the determination of *KRAS* mutation status is important. In patients with a *KRAS* mutant tumor the obvious choice is a bevacizumab-containing regimen. In patients with a *KRAS* wild-type tumor bevacizumab and cetuximab in combination with a cytotoxic doublet are valid options. The data for panitumumab is less mature, still not published, but the preliminary data show efficacy results in the same direction of cetuximab and this may become an option in patients with *KRAS* wild-type tumors. There are no data available of randomized studies comparing the activity of a doublet of cytotoxics plus bevacizumab with a doublet plus cetuximab/panitumumab in the first line treatment. Toxicity considerations related to the targeted agents are important, as well as considerations that the evidence for the activity of anti-EGFR antibodies – even administered as single agents – is stronger in later lines of treatment than for bevacizumab. It may be very relevant for a patient in good condition to use the available agents in the continuum of care of his disease in order to maximize the chances for a prolongation of survival.

The combination of a doublet of cytotoxics plus cetuximab has led to higher resection rates (although still low in absolute numbers) in patients with *KRAS* wild-type, liver-limited unresectable mCRC. The combination of FOLFOX/cetuximab and FOLFIRI/cetuximab has led to similar response rates and resection rates in *KRAS* wild-type tumors. The combination of a fluoropyrimidine/oxaliplatin/bevacizumab has led to a non-significant trend in an increased resection rate compared to the cytotoxic backbone alone, although no increase in response rate was shown.

patients not needing or desiring an aggressive approach. In patients with clearly unresectable disease that is unlikely to become resectable, without relevant tumor-related symptoms, without an immediate risk of rapid progression and with important comorbidity, a sequential therapy starting with a fluoropyrimidine monotherapy in combination with bevacizumab (if no cardiovascular contraindications are present) remains a valid option. It is, however, not easy to select this group of patients, as there is no data regarding biomarkers for predicting the optimal strategy. It is estimated that up to 15%–20% of patients with mCRC belong to this group. If this less aggressive approach is considered as the suitable option for this population of patients, a close follow-up with frequent evaluations should be mandated, as if the selected treatment is not active the patients may render to a situation of rapid deterioration due to tumor burden and no further treatment options may be then considered. This effect has been suggested as one of the potential explanations why some sequential treatment strategies starting with a non-doublet fluoropyrimidine may have a worse outcome in OS as less patients have been treated at the end with all the available drugs in the continuum of care concept.

later lines of therapy. In patients for whom first-line treatment has failed, the cytotoxic backbone should be changed. In the second-line treatment the systematic use of biologic targeted agents is less established. If bevacizumab was not used in the first line, it can be recommended to add it at this stage, as there is one study showing OS advantage. The data with the anti-EGFR antibodies suggest a similar relative benefit in the second- and third-line treatment. In the third line the combination of cetuximab plus irinotecan is clearly more active than cetuximab single agent and is therefore the preferred option in fit patients who can tolerate it. The combination of panitumumab/FOLFIRI has also been shown to be more active than FOLFIRI alone in second line.

additional issues. how long to treat?: It is recommended to continue first line combination with biologic targeted therapies until disease has progressed, treatment is intolerable by the patient, or metastases have become resectable. In the event that one of the cytotoxic partners (oxaliplatin, irinotecan) is stopped for safety reasons, it is usually recommended to continue with the biological in combination with a fluoropyrimidine until disease progression or non-acceptable toxicity appears.

biological agents beyond progression: To date, there is no clear evidence as to the advantage of continuing a biological agent after disease progression while exposed to this agent.

the role of rash: Although the severity of rash correlates with the efficacy of anti-EGFR antibodies, this has no immediate implications in clinical practice. A lack of rash is not currently recommended as reason for discontinuing anti-EGFR therapy.

choice of first-line chemotherapy after adjuvant: If an oxaliplatin-based combination was administered in the adjuvant setting, the choice of an irinotecan-based combination is recommended in first-line treatment. This recommendation is stronger if the recurrence of disease occurred <12 months after stopping the adjuvant treatment.

treatment refractory disease: In patients with chemorefractory disease and a good performance status, adequate organ function and a clear wish for further treatment, combination of a fluoropyrimidine with mitomycin can be considered, although the level of evidence for activity is low as well as the level of agreement between

experts. The experts prefer in this situation to propose to patients a well-designed clinical trial.

treatment of resectable metastatic colorectal patients

resectable liver or lung metastases. The perioperative or postoperative administration of a cytotoxic doublet is the recommended approach in patients with resectable metastases. There is no evidence yet that adding a biological to a cytotoxic doublet improves the outcome in resectable metastases compared to a cytotoxic doublet alone in combination with resection of the metastases.

disclosures

The authors have not indicated financial relationships with companies whose products are mentioned in this article.

references

1. Jemal A, Siegel R, Ward E et al. Cancer statistics, 2008. CA Cancer J Clin 2008; 58(2): 71–96.
2. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 2007; 18(3): 581–592.
3. Kopetz S, Chang GJ, Overman MJ et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol 2009; 27(22): 3677–3683.
4. Seymour MT, Maughan TS, Ledermann JA et al. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. Lancet 2007; 370(9582): 143–152.
5. Koopman M, Antonini NF, Douma J et al. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. Lancet 2007; 370(9582): 135–142.
6. Hecht JR, Mitchell E, Chidiac T et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol 2009; 27(5): 672–680.
7. Tol J, Koopman M, Cats A et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med 2009; 360(6): 563–572.
8. Falcone A, Ricci S, Brunetti I et al. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. J Clin Oncol 2007; 25(13): 1670–1676.
9. Souglakos J, Androulakis N, Syrigos K et al. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). Br J Cancer 2006; 94(6): 798–805.
10. Chun YS, Vauthey JN, Boonsirikamchai P et al. Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastases. JAMA 2009; 302(21): 2338–2344.
11. Sargent DJ, Conley BA, Allegra C, Collette L. Clinical trial designs for predictive marker validation in cancer treatment trials. J Clin Oncol 2005; 23(9): 2020–2027.
12. Tournigand C, Andre T, Achille E et al. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol 2004; 22(2): 229–237.
13. Rougier P, Van Cutsem E, Bajetta E et al. Randomised trial of irinotecan versus fluorouracil by continuous infusion after fluorouracil failure in patients with metastatic colorectal cancer. Lancet 1998; 352(9138): 1407–1412.
14. Kohne CH, Cunningham D, Di CF et al. Clinical determinants of survival in patients with 5-fluorouracil-based treatment for metastatic colorectal cancer: results of a multivariate analysis of 3825 patients. Ann Oncol 2002; 13(2): 308–317.
15. Graf W, Glimelius B, Pahlman L, Bergstrom R. Determinants of prognosis in advanced colorectal cancer. Eur J Cancer 1991; 27(9): 1119–1123.
16. Lavin P, Mittelman A, Douglass H Jr, Engstrom P, Klaassen D. Survival and response to chemotherapy for advanced colorectal adenocarcinoma: an Eastern Cooperative Oncology Group report. Cancer 1980; 46(7): 1536–1543.
17. Kemeny N, Braun DW Jr. Prognostic factors in advanced colorectal carcinoma. Importance of lactic dehydrogenase level, performance status, and white blood cell count. Am J Med 1983; 74(5): 786–794.
18. Mitry E, Douillard JY, Van Cutsem E et al. Predictive factors of survival in patients with advanced colorectal cancer: an individual data analysis of 602 patients included in irinotecan phase III trials. Ann Oncol 2004; 15(7): 1013–1017.
19. Sargent DJ, Kohne CH, Sanoff HK et al. Pooled safety and efficacy analysis examining the effect of performance status on outcomes in nine first-line treatment trials using individual data from patients with metastatic colorectal cancer. J Clin Oncol 2009; 27(12): 1948–1955.
20. Papamichael D, Audisio R, Horiot JC et al. Treatment of the elderly colorectal cancer patient: SIOG expert recommendations. Ann Oncol 2009; 20(1): 5–16.
21. Pallis AG, Papamichael D, Audisio R et al. EORTC Elderly Task Force experts' opinion for the treatment of colon cancer in older patients. Cancer Treat Rev 2010; 36(1): 83–90.
22. Folprecht G, Seymour MT, Saltz L et al. Irinotecan/fluorouracil combination in first-line therapy of older and younger patients with metastatic colorectal cancer: combined analysis of 2,691 patients in randomized controlled trials. J Clin Oncol 2008; 26(9): 1443–1451.
23. Scappaticci FA, Skillings JR, Holden SN et al. Arterial thromboembolic events in patients with metastatic carcinoma treated with chemotherapy and bevacizumab. J Natl Cancer Inst 2007; 99(16): 1232–1239.
24. Edler D, Glimelius B, Hallstrom M et al. Thymidylate synthase expression in colorectal cancer: a prognostic and predictive marker of benefit from adjuvant fluorouracil-based chemotherapy. J Clin Oncol 2002; 20(7): 1721–1728.
25. AJCC Cancer Staging Manual. 7th edn. Springer 2010.
26. Smith TJ, Hillner BE, Bear HD. Taking action on the volume-quality relationship: how long can we hide our heads in the colostomy bag? J Natl Cancer Inst 2003; 95(10): 695–697.
27. Onega T, Duell EJ, Shi X, Demidenko E, Gottlieb D, Goodman DC. Influence of NCI cancer center attendance on mortality in lung, breast, colorectal, and prostate cancer patients. Med Care Res Rev 2009; 66(5): 542–560.
28. Smith TJ, Hillner BE. Ensuring quality cancer care by the use of clinical practice guidelines and critical pathways. J Clin Oncol 2001; 19(11): 2886–2897.
29. Koukourakis MI, Giatromanolaki A, Simopoulos C, Polychronidis A, Sivridis E. Lactate dehydrogenase 5 (LDH5) relates to up-regulated hypoxia inducible factor pathway and metastasis in colorectal cancer. Clin Exp Metastasis 2005; 22(1): 25–30.
30. Grem J. The prognostic importance of tumor markers in adenocarcinomas of the gastrointestinal tract. Curr Opin Oncol 1997; 9(4): 380–387.
31. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer – the stable evidence. Nat Rev Clin Oncol 2010; 7(3): 153–162.
32. Gryfe R, Kim H, Hsieh ET et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000; 342(2): 69–77.
33. Benatti P, Gafa R, Barana D et al. Microsatellite instability and colorectal cancer prognosis. Clin Cancer Res 2005; 11(23): 8332–8340.
34. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005; 23(3): 609–618.
35. Ogino S, Nosho K, Kirkner GJ et al. CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut 2009; 58(1): 90–96.
36. Roth AD, Tejpar S, Delorenzi M et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol 2010; 28(3): 466–474.

37. Van Cutsem E, Lang I, Folprecht G et al. Cetuximab plus FOLFIRI in the treatment of metastatic colorectal cancer (mCRC): The influence of KRAS and BRAF biomarkers on outcome: Updated data from the CRYSTAL trial. Gastrointestinal Cancers Symposium Orlando, 2010: 281.
38. Samowitz WS, Sweeney C, Herrick J et al. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res 2005; 65(14): 6063–6069.
39. Ogino S, Meyerhardt JA, Irahara N et al. KRAS mutation in stage III colon cancer and clinical outcome following intergroup trial CALGB 89803. Clin Cancer Res 2009; 15(23): 7322–7329.
40. Rougier P, Bugat R, Douillard JY et al. Phase II study of irinotecan in the treatment of advanced colorectal cancer in chemotherapy-naive patients and patients pretreated with fluorouracil-based chemotherapy. J Clin Oncol 1997; 15(1): 251–260.
41. Saltz LB, Douillard JY, Pirotta N et al. Irinotecan plus fluorouracil/leucovorin for metastatic colorectal cancer: a new survival standard. Oncologist 2001; 6(1): 81–91.
42. Steinberg J, Erlichman C, Gadalla T, Fine S, Wong A. Prognostic factors in patients with metastatic colorectal cancer receiving 5-fluorouracil and folinic acid. Eur J Cancer 1992; 28A(11): 1817–1820.
43. Giacchetti S, Perpoint B, Zidani R et al. Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. J Clin Oncol 2000; 18(1): 136–147.
44. 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(13): 905–914.
45. Freyer G, Rougier P, Bugat R et al. Prognostic factors for tumour response, progression-free survival and toxicity in metastatic colorectal cancer patients given irinotecan (CPT-11) as second-line chemotherapy after 5FU failure. CPT-11 F205, F220, F221 and V222 study groups. Br J Cancer 2000; 83(4): 431–437.
46. Strimpakos AS, Cunningham D, Mikropoulos C, Petkar I, Barbachano Y, Chau I. The impact of carcinoembryonic antigen flare in patients with advanced colorectal cancer receiving first-line chemotherapy. Ann Oncol 2010; 21(5): 1013–1019.
47. Sunga A, Rajput A, Yang GY et al. Carcinoembryonic antigen (CEA) surge in patients with metastatic colorectal cancer receiving systemic chemotherapy. J Clin Oncol 2005; 23: 3615.
48. Ward U, Primrose JN, Finan PJ et al. The use of tumour markers CEA, CA-195 and CA-242 in evaluating the response to chemotherapy in patients with advanced colorectal cancer. Br J Cancer 1993; 67(5): 1132–1135.
49. Wang WS, Lin JK, Lin TC et al. Carcinoembryonic antigen in monitoring of response to systemic chemotherapy in patients with metastatic colorectal cancer. Int J Colorectal Dis 2001; 16(2): 96–101.
50. Iwanicki-Caron I, Di Fiore F, Roque I et al. Usefulness of the serum carcinoembryonic antigen kinetic for chemotherapy monitoring in patients with unresectable metastasis of colorectal cancer. J Clin Oncol 2008; 26(22): 3681–3686.
51. 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; 25(13): 1658–1664.
52. Van Cutsem E, Kohne CH, Hitre E et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009; 360(14): 1408–1417.
53. Moore MJ, Goldstein D, Hamm J et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007; 25(15): 1960–1966.
54. Van Cutsem E, Humblet Y, Gelderblom H et al. Cetuximab dose-escalation study in patients with metastatic colorectal cancer (mCRC) with no or slight skin reactions on cetuximab standard dose treatment (EVEREST): Pharmacokinetic and efficacy data of a randomized study. Gastrointestinal Cancers Symposium Orlando, 2007: 237.
55. Scartozzi M, Galizia E, Chiorrini S et al. Arterial hypertension correlates with clinical outcome in colorectal cancer patients treated with first-line bevacizumab. Ann Oncol 2009; 20(2): 227–230.
56. Spano JP, Chodkiewicz C, Maurel J et al. Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. Lancet 2008; 371(9630): 2101–2108.
57. Mir O, Ropert S, Alexandre J, Goldwasser F. Hypertension as a surrogate marker for the activity of anti-VEGF agents. Ann Oncol 2009; 20(5): 967–970.
58. Poole C, Gardiner J, Twelves C et al. Effect of renal impairment on the pharmacokinetics and tolerability of capecitabine (Xeloda) in cancer patients. Cancer Chemother Pharmacol 2002; 49(3): 225–234.
59. Cassidy J, Twelves C, Van Cutsem E et al. First-line oral capecitabine therapy in metastatic colorectal cancer: a favorable safety profile compared with intravenous 5-fluorouracil/leucovorin. Ann Oncol 2002; 13(4): 566–575.
60. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16(1): 31–41.
61. Hoskins JM, Goldberg RM, Qu P, Ibrahim JG, McLeod HL. UGT1A1*28 genotype and irinotecan-induced neutropenia: dose matters. J Natl Cancer Inst 2007; 99(17): 1290–1295.
62. Hoskins JM, McLeod HL. UGT1A and irinotecan toxicity: keeping it in the family. J Clin Oncol 2009; 27(15): 2419–2421.
63. Miya T, Goya T, Fujii H et al. Factors affecting the pharmacokinetics of CPT-11: the body mass index, age and sex are independent predictors of pharmacokinetic parameters of CPT-11. Invest New Drugs 2001; 19(1): 61–67.
64. Nalluri SR, Chu D, Keresztes R, Zhu X, Wu S. Risk of venous thromboembolism with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis. JAMA 2008; 300(19): 2277–2285.
65. Pereg D, Lishner M. Bevacizumab treatment for cancer patients with cardiovascular disease: a double edged sword? Eur Heart J 2008; 29(19): 2325–2326.
66. Lievre A, Bachet JB, Le Corre D et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006; 66(8): 3992–3995.
67. De Roock W, Piessevaux H, De Schutter J et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol 2008; 19(3): 508–515.
68. Amado RG, Wolf M, Peeters M et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008; 26(10): 1626–1634.
69. Karapetis CS, Khambata-Ford S, Jonker DJ et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008; 359(17): 1757–1765.
70. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008; 358(11): 1160–1174.
71. Normanno N, Tejpar S, Morgillo F, De Luca A, Van Cutsem E, Ciardiello F. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. Nat Rev Clin Oncol 2009; 6(9): 519–527.
72. Artale S, Sartore-Bianchi A, Veronese SM et al. Mutations of KRAS and BRAF in primary and matched metastatic sites of colorectal cancer. J Clin Oncol 2008; 26(25): 4217–4219.
73. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature 2002; 418(6901): 934.
74. Di Nicolantonio F, Martini M, Molinari F et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 2008; 26(35): 5705–5712.
75. Loupakis F, Ruzzo A, Cremolini C et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. Br J Cancer 2009; 101(4): 715–721.
76. Lambrechts D, De Roock W, Prenen H et al. The role of KRAS, BRAF, NRAS, and PIK3CA mutations as markers of resistance to cetuximab in chemorefractory metastatic colorectal cancer. J Clin Oncol 2009; 27: 9020.
77. Tejpar S, De Roock W. PIK3CA, BRAF and KRAS mutations and outcome prediction in chemorefractory metastatic colorectal cancer (mCRC) patients treated with EGFR targeting monoclonal antibodies (MoAbs): results of a European Consortium. Eur J Cancer 2009; 7: 322.

78. Kohne C, Rougier P, Stroh C, Schlichting M, Bokemeyer C, Van Cutsem E. Cetuximab with chemotherapy (CT) as first-line treatment for metastatic colorectal cancer (mCRC): A meta-analysis of the CRYSTAL and OPUS studies according to KRAS and BRAF mutation status. Gastrointestinal Cancers Symposium Orlando, 2010: 406.
79. Santini D, Loupakis F, Canestrari E et al. Correlation of BRAF wild-type status (WT) in primary colorectal tumors with BRAF status in related metastatic sites: Implications for treatment with anti-EGFR antibodies. Gastrointestinal Cancers Symposium Orlando, 2010: 349.
80. Jonker DJ, Karapetis C, Harbison C et al. High epiregulin (EREG) gene expression plus K-ras wild-type (WT) status as predictors of cetuximab benefit in the treatment of advanced colorectal cancer (ACRC): Results from NCIC CTG CO.17—A phase III trial of cetuximab versus best supportive care (BSC). J Clin Oncol 2009; 27: 4016.
81. Jacobs B, De Roock W, Piessevaux H et al. Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol 2009; 27(30): 5068–5074.
82. Linot B, Capitain O, Metges J et al. Gamelin. Impact of PI3K, BRAF, and KRAS mutations on efficacy intensified FOLFIRI + cetuximab regimen in advanced colorectal cancer Gastrointestinal Cancers Symposium Orlando, 2010: 365.
83. Frattini M, Saletti P, Romagnani E et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 2007; 97(8): 1139–1145.
84. Perrone F, Lampis A, Orsenigo M et al. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. Ann Oncol 2009; 20(1): 84–90.
85. Loupakis F, Pollina L, Stasi I et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J Clin Oncol 2009; 27(16): 2622–2629.
86. Ruzzo A, Graziano F, Loupakis F et al. Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX-4 chemotherapy. J Clin Oncol 2007; 25(10): 1247–1254.
87. Liang J, Jiang T, Yao RY, Liu ZM, Lv HY, Qi WW. The combination of ERCC1 and XRCC1 gene polymorphisms better predicts clinical outcome to oxaliplatin-based chemotherapy in metastatic colorectal cancer. Cancer Chemother Pharmacol 2009 Dec 4 [Epub ahead of print].
88. Viguier J, Boige V, Miquel C et al. ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. Clin Cancer Res 2005; 11(17): 6212–6217.
89. Kim SH, Kwon HC, Oh SY et al. Prognostic value of ERCC1, thymidylate synthase, and glutathione S-transferase pi for 5-FU/oxaliplatin chemotherapy in advanced colorectal cancer. Am J Clin Oncol 2009; 32(1): 38–43.
90. Shirota Y, Stoehlmacher J, Brabender J et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 2001; 19(23): 4298–4304.
91. Soong R, Shah N, Salto-Tellez M et al. Prognostic significance of thymidylate synthase, dihydropyrimidine dehydrogenase and thymidine phosphorylase protein expression in colorectal cancer patients treated with or without 5-fluorouracil-based chemotherapy. Ann Oncol 2008; 19(5): 915–919.
92. Martinez-Balibrea E, Abad A, Aranda E et al. Pharmacogenetic approach for capecitabine or 5-fluorouracil selection to be combined with oxaliplatin as first-line chemotherapy in advanced colorectal cancer. Eur J Cancer 2008; 44(9): 1229–1237.
93. Findlay MP, Cunningham D, Morgan G, Clinton S, Hardcastle A, Aherne GW. Lack of correlation between thymidylate synthase levels in primary colorectal tumours and subsequent response to chemotherapy. Br J Cancer 1997; 75(6): 903–909.
94. Koopman M, Venderbosch S, van Tinteren H, Ligtenberg MJ, Nagtegaal I, Van Krieken JH et al. Predictive and prognostic markers for the outcome of chemotherapy in advanced colorectal cancer, a retrospective analysis of the phase III randomised CAIRO study. Eur J Cancer 2009; 45(11): 1999–2006.
95. van Kuilenburg AB, Haasjes J, Richel DJ et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res 2000; 6(12): 4705–4712.
96. van Kuilenburg AB, Muller EW, Haasjes J et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14+1G>A mutation causing DPD deficiency. Clin Cancer Res 2001; 7(5): 1149–1153.
97. Van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 2002; 12(7): 555–558.
98. Schmoll HJ, Sargent D. Single agent fluorouracil for first-line treatment of advanced colorectal cancer as standard? Lancet 2007; 370: 105–107.