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Combining chemotherapy and targeted therapies in metastatic colorectal cancer

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Abstract

Colorectal cancer remains one of the major causes of cancer death worldwide. During the past years, the development of new effective treatment options has led to a considerable improvement in the outcome of this disease. The advent of agents such as capecitabine, irinotecan, oxaliplatin, cetuximab and bevacizumab has translated into median survival times in the range of 2 years. Intense efforts have focused on identifying novel agents targeting specific growth factor receptors, critical signal transduction pathways or mediators of angiogenesis. In addition, several clinical trials have suggested that some of these molecularly targeted drugs can be safely and effectively used in combination with conventional chemotherapy. In this article we review various treatment options combining cytotoxic and targeted therapies currently available for patients with metastatic colorectal cancer.

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Key words: Targeted therapy; Chemotherapy; Combinations; Clinical trials; Colorectal cancer

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INTRODUCTION

Chemotherapy remains the cornerstone of treatment

of metastatic colorectal cancer (mCRC) and, with the exception of a minority of patients (pts) who are candidates for salvage surgery, the goal of chemotherapy is palliation. Remarkable and clinically relevant advances have been made in the last 5 years in the treatment of this disease, essentially owing to the introduction of combination chemotherapy regimens containing oxaliplatin and irinotecan (CPT-11)^[1]. The addition of either drug to 5-fluorouracil/leucovorin (5-FU/LV) proved to significantly increase overall response rates and survival times. Indeed, median overall survival is highly correlated with the percentage of patients who receive the three cytotoxic agents in the course of their disease. Results from a Phase III study by Falcone *et al*^[2] suggested that the up-front use of a triplet combination of irinotecan, oxaliplatin and 5-FU/LV significantly improved the outcome in terms of response rate (RR) and survival times compared to a standard doublet of irinotecan and 5-FU/LV.

Interestingly, with the more recent incorporation of bevacizumab and cetuximab into the treatment armamentarium, the median overall survival (OS) has doubled from 12 mo to approximately 2 years in Phase III trials. In fact, most recent trials that attempt to expose patients to all five drug classes (fluoropyrimidines, irinotecan, oxaliplatin, bevacizumab and anti-EGFR antibody) target an OS well over 2 years. In this review we will summarize some of the available therapeutic repertoire based on targeted therapies in combination with chemotherapy for patients with mCRC.

COMBINING CHEMOTHERAPY AND EGFR-TARGETED THERAPIES

The epidermal growth factor receptor (EGFR), a transmembrane tyrosine kinase, is one of four members of the HER receptor family. This receptor is overexpressed in a number of solid tumors of ectodermal origin, including colon adenocarcinoma^[3]. EGFR over expression has been correlated with disease progression, poor prognosis and reduced sensitivity to chemotherapy^[4]. Therefore, several strategies have been developed to target EGFR, including small molecule tyrosine kinase inhibitors and monoclonal antibodies^[5].

Cetuximab-based combination therapy

Cetuximab is the most advanced monoclonal antibody against EGFR in clinical development. Since preclinical

and early clinical studies suggested that Cetuximab might revert irinotecan resistance in CRC both *in vitro* and *in vivo*, a phase II trial of cetuximab with irinotecan was performed in patients with EGFR positive colorectal cancer that was refractory to both 5-fluorouracil (5-FU) and Irinotecan. Among the 120 patients treated with this regimen, overall response rate was 22.5%^[6].

To confirm these clinical findings, 329 EGFR-positive, irinotecan-refractory mCRC patients were randomized in a 2:1 ratio to receive cetuximab plus irinotecan (arm A; $n = 218$) or cetuximab alone (arm B; $n = 111$) with the option to switch to the combination of cetuximab with irinotecan after failure of cetuximab as a single agent. Both the response rate (22.9% *vs* 10.8%, $P = 0.007$) and the median time to progression (4.1 *vs* 1.5, $P < 0.001$) favored the combination arm. Although no survival benefit was observed for arm A, cetuximab was demonstrated to have clinically significant activity when given alone or in combination with irinotecan and consequently received FDA approval^[7].

More recently, MABEL trial^[8] investigated the combination of cetuximab and CPT-11 at a dose and schedule as pre-study in an uncontrolled, multicenter study including 1123 mCRC pts with detectable EGFR. 64% of the patients had received ≥ 2 lines of chemotherapy. 76% had also been pretreated with cetuximab. The estimated median survival was 9.2 mo at an expense of an acceptable toxicity profile, including grade 3-4 diarrhea (20%) acne-like rash (19%), neutropenia (9%) and asthenia (8%). MABEL clearly confirmed in a wider setting the efficacy and safety of C225 plus CPT-11 seen in previous studies. Similarly, EPIC trial is a randomized phase III trial comparing cetuximab plus irinotecan to irinotecan as second line therapy in patients with EGFR-expressing mCRC who have failed first line oxaliplatin in combination with a fluoropyrimidine. Accrual is currently ongoing^[9].

Cetuximab-based combinations as salvage therapy:

Several trials have addressed the potential of cetuximab-based combinations in heavily pretreated patients. Vincenzi *et al*^[10] evaluated the efficacy of cetuximab plus oxaliplatin in patients previously failed on an oxaliplatin-based regimen in first line, irinotecan-based regimen in second line, and cetuximab plus irinotecan in third line. No objective clinical response was identified after the interim analysis planned according to the two-staged Simon accrual design. The same group^[11] evaluated the activity of cetuximab and weekly irinotecan (90 mg/m²) in patients refractory to one oxaliplatin-based chemotherapy regimen (Capecitabine + Oxaliplatin or FOLFOX IV regimen, as first line) and one Irinotecan-based chemotherapy (FOLFIRI regimen, as second-line chemotherapy) for at least 2 mo. Overall response rate was 25.4% (95% CI: 21.7%-39.6%); 38.2% (95% CI: 18.6%-39.8%) of patients showed a disease stability as the best response. The median time to progression was 4.7 mo (95% CI: 2.5-7.1 mo) and the median survival time was 9.8 mo (95% CI: 3.9-10.1 mo). The most common G3-4 noncutaneous side toxicities were diarrhoea (16.4%), fatigue (12.7%), stomatitis (7.3%) and skin toxicity (32.6%). A statistically significant ($P = 0.006$) association between the cutaneous toxicity

and both tumour response and time to progression was observed. The authors also identified a borderline significant difference in terms of overall survival.

The combination of Cetuximab plus FOLFIRI has been prospectively evaluated in 41 EGFR expressing mCRC pts refractory to prior FOLFIRI for metastatic disease^[12]. Most of the patients were treated in third line. A 20% overall response rate was recorded, with a median PFS of 4.3 mo and a median overall survival of 5 mo.

Cetuximab-based combinations in front-line therapy:

Cetuximab established activity in the salvage setting prompted its incorporation to first-line combination therapy. Available preliminary data from Phase II trials combining cetuximab with either irinotecan or oxaliplatin-based chemotherapy have shown very encouraging activity. CALGB 80203^[13] randomized untreated mCRC patients to FOLFOX or FOLFIRI with or without C225 independent of EGFR status. ORR was similar in the FOLFIRI or FOLFOX arms, while C225 containing arms had a higher ORR (49% *vs* 33%, $P = 0.014$) when compared to non cetuximab containing arms. No significant differences in grade 3 diarrhea or any grade 4 toxicity were seen with the addition of C225. Preliminary results of the combination of C225, capecitabine (800 g/m² bid po on d 1 to 14) and Irinotecan (200 g/m² i.v on d 1) *vs* C225 combined with capecitabine (1000 mg/m² bid in d 1-14) and oxaliplatin (130 g/m² on d 1) reported an overall response rate of 41% (95%; 22% to 61%) and 71% (95%; 48% to 89%) respectively, with both arms showing a manageable toxicity profile^[14].

Promising results have also been reported^[15] combining cetuximab with (AIO) infusional 5-FU/FA plus irinotecan regimen in EGFR-expressing mCRC. Grade 3 or 4 toxicities were acne-like rash (38%), diarrhea (29%), cardiovascular events (20%) and nausea/vomiting (5%). Objective responses were observed in 67% of the patients. The median time to progression was 9.9 mo and the median survival time was 33 mo.

The combination of cetuximab with modified FOLFOX 6 in 83 chemo-naïve mCRC pts with positive or undetectable EGFR expression show a preliminary ORR of 53%^[16]. Main grade 3-4 toxicities included neutropenia (38%), diarrhea (10%), rash (10%) and neurotoxicity (7%). The combination of FOLFOX-4 plus C225^[17] has also been evaluated in 47 EGFR-expressing mCRC, with a reported ORR of 68%. Grade 3-4 adverse events included acne-like rash (18%) diarrhea (7%), nausea and vomiting (4%) and anemia (4%).

Preliminary results of the OPUS trial^[18], a randomized phase II study in the first line treatment of mCRC, confirmed the superiority of FOLFOX plus cetuximab *vs* FOLFOX in terms of overall response rate (45.6% *vs* 36.8%).

These small trials supported the conduct of a multicenter Phase III clinical trial that compared FOLFIRI plus Cetuximab with FOLFIRI alone in 1217 EGFR-expressing chemotherapy-naïve patients. Cetuximab plus FOLFIRI significantly increased response rate and progression-free survival, reducing the relative risk of progression by approximately 15%^[19].

Panitumumab-based combination therapy

Panitumumab is a fully human IgG2 monoclonal antibody directed against the epidermal growth factor receptor. Its use in combination with IFL and FOLFIRI in first line treatment of metastatic CRC has been evaluated in a multicenter, single arm, phase 2 trial^[20]. Panitumumab was given at a weekly dose of 2.5 mg/kg i.v. over 60-90 min followed by chemotherapy. The combination with IFL was considered too toxic, with grade 3-4 diarrhea in 47% of the patients. The FOLFIRI plus panitumumab combination was associated with a more manageable side effect profile with grade 3-4 diarrhea in 25% of the pts and grade 3-4 hypomagnesemia in 8%. Skin and nail toxicities occurred in at least 20% of patients but were rarely severe (grade 3 in 2 out of 24 pts). The objective response rate with FOLFIRI plus panitumumab was 66%, with a disease control rate of 79%. Median progression free survival was 10.9 mo. Further investigation of FOLFIRI with an every two weeks schedule of panitumumab is ongoing in randomized phase 3 trials.

Cetuximab-induced papulopustular skin rash is thought to be mechanism- and dose-related, and may be a surrogate indicator of an adequate degree of receptor saturation by cetuximab. The possibility of increasing Cetuximab efficacy by inducing skin rash has been recently confirmed. Cetuximab dose escalation up to 500 mg/m² improves response rate in patients with absent or slight skin reaction on standard dose treatment^[21].

Future directions

Large studies validating molecular predictive markers are needed in order to identify the subset of patients more likely to respond to EGFR-targeted therapies. Candidate markers include total and phosphorylated EGFR, total and phosphorylated forms of AKT, mitogen-activated protein kinase (MAPK), mitogen-activated protein/ERK (MEK), ERK, signal transducers and activators of transcription (STAT), PTEN and mTOR^[22]. Although EGFR gene copy number has also been proposed^[23], EGFR amplification, measured by FISH is a rare event (4%) in colorectal cancer^[24]. Other potential predictive markers are k-ras^[25] cyclin D1 A870G polymorphisms^[26], HER-2 expression^[27] or higher gene expression levels of VEGF^[28]. More recently, a combination of various predictive biomarkers has retrospectively been able to identify subsets of patients more likely to benefit from cetuximab therapy^[29]. In addition, several polymorphisms in genes involved in the EGFR and angiogenesis pathway have been associated with clinical outcome^[30]. Prospective studies are clearly needed to confirm these preliminary findings.

EGFR tyrosine kinase inhibitor (TKI)-based combination therapy

Gefitinib: Gefitinib (ZD1839) selectively inhibits the EGFR tyrosine kinase and has approximately 100-fold greater potency against EGFR compared with other tyrosine or serine/threonine kinases. Unlike cetuximab, gefitinib does not induce EGFR internalization or degradation in CRC cells, nor does it reduce EGF binding sites or EGFR protein content. Both *in vitro* and *in vivo* studies indicated that gefitinib monotherapy

had antitumour activity in some CRC cell lines^[31]. However, phase I / II clinical studies in patients with mCRC indicated that gefitinib had negligible activity^[32,33]. Preclinical suggestions of a supra-additive, growth-inhibitory effect of gefitinib and a wide variety of cytotoxic drugs with different mechanism(s) of action^[34] prompted several trials of gefitinib in combination with chemotherapy in mCRC patients.

Gefitinib plus fluoropyrimidines: In preclinical models a strong synergistic interaction between gefitinib and 5'-deoxy-fluorouridine (5'-DFUR) was demonstrated when ZD1839 was applied before or concurrently with 5'-DFUR^[35]. Subsequently, the combination of intermittent gefitinib (250-500 mg/d on d 1-14) plus 5-FU/LV administered as a bolus in a dose-reduced Mayo Clinic regimen (370/20 mg/m²) on d 8-12 with 5-FU and leucovorin as first-line therapy in mCRC was tested, with no evidence of cumulative toxicity or major drug-drug pharmacokinetic interactions^[36]. In the second part of the study, gefitinib was administered continuously at 500 mg/d, and 5-FU/LV was added to the schedule on d 8-12 and 36-40. Overall response rate was 23%, with the most common toxicities being rash and diarrhea.

Preliminary results from a small phase I / II trial combining gefitinib 250-mg daily with capecitabine 1000-1250 mg twice daily after failure of first-line therapy^[37] also suggest some evidence of activity .

Gefitinib plus irinotecan-based therapy: A dose-finding trial of irinotecan plus gefitinib in mCRC patients pretreated with fluoropyrimidine-based chemotherapy defined irinotecan given at a dose of 225 mg/m² every 3 wk plus gefitinib at a dose of 250 mg/d as the maximum tolerated dose (MTD) of this regimen^[38]. Dose-limiting toxicities (DLTs), such as neutropenia and diarrhea, occurred at unexpectedly low doses of irinotecan. Disease stabilization was achieved in 21% of the patients.

The combination of gefitinib plus FOLFIRI in both chemotherapy-naïve mCRC patients^[39] and as salvage therapy^[40] was considered too toxic despite reduced weekly doses of 5-FU, LV, and irinotecan.

Gefitinib plus oxaliplatin-based therapy: Gefitinib plus FOLFOX has been tested in both the first line and the salvage setting. Kuo *et al*^[41] reported data on a phase II study of one cycle of FOLFOX-4, and then additional cycles of FOLFOX-4 with 500 mg/d of gefitinib in 27 patients with documented progressive colorectal cancer after at least one chemotherapeutic regimen (usually irinotecan based). 33% of the patients achieved objective responses, whereas 48% had stable disease for a prolonged period. Response rates did not differ depending on number of prior regimens. Median event-free survival was 5.4 mo, and overall survival was 12 mo. Another feasibility study assessed the combination of gefitinib (250 mg/d) plus capecitabine (2000 mg/m² per day, d 1-15) plus oxaliplatin (120 mg/m² every 3 wk for six courses) as first-line treatment in patients with mCRC^[42]. The most common grade 3 adverse events were diarrhea and neutropenia. A clinical benefit rate of 58% has been noted.

Overall, toxicity rates with the addition of gefitinib to an oxaliplatin-fluoropyrimidine combination are markedly more favorable than with the irinotecan-based regimens, although higher incidences of grade III or IV diarrhea, nausea, and vomiting than with FOLFOX alone are noted. Further studies of TKI-based therapy for CRC are planned or recruiting.

Erlotinib: Erlotinib, an orally reversible TKI reduces intratumoral EGFR autophosphorylation^[43] with no effect on EGFR expression or surface receptor density. Evidence of single agent erlotinib activity in mCRC patients derived from disease-specific phase II studies^[44] led to the design of several trials in combination with chemotherapy.

Tarceva plus fluoropyrimidines: Additive activity of capecitabine and erlotinib in tumor models^[45] supported a phase 2 trial evaluating the combination of erlotinib 150 mg daily with capecitabine 1000 mg/m² bid. for 14 d every 3 wk in chemotherapy-naïve mCRC patients. Grade 3 diarrhea (30%) grade 3 renal insufficiency (10%) and grade 3 hiperbilirubinemia (10%) were the most troublesome toxicities. Regarding efficacy, no complete responses were achieved whereas disease control rate was 34%^[46].

Tarceva plus oxaliplatin: Meyerhardt *et al*^[47] reported on the results of a triplet regimen of erlotinib, 100 mg/d, capecitabine, 1650 mg/m² per day (d 1-14), and oxaliplatin, 130 mg/m² every 3 wk in 32 patients mostly pretreated with an irinotecan-containing regimen. By intent-to-treat analysis, 25% of the patients experienced a partial response and 44% had stable disease for at least 12 wk. 29% of the patients discontinued study therapy due to toxicity.

Other TKIs-based combinations

EKB-569, an irreversible dual inhibitor of the EGFR and HER-2 tyrosine kinases, inhibits the growth of tumor cells that overexpress EGFR or HER-2 *in vitro* and *in vivo*^[48]. Dose-limiting toxicities with EKB-569 plus FOLFIRI in 47 chemotherapy-naïve mCRC patients^[49] were grade 3 diarrhea and grade 3 fatigue. The MTD was selected as 25 mg EKB-569. The response rate was 38% and the clinical benefit rate was 85%. EKB-569 treatment resulted in complete inhibition of pEGFR and significant inhibition of pMAPK in both skin samples (11 patients) and tumor samples (three patients) with no change in pAkt activity.

In a dose-escalation study^[50] with FOLFOX-4 plus EKB-569, 25-75 mg/d, starting from d 3, DLTs were observed with EKB-569 at a dose of 35 mg/d (grade III diarrhea and febrile neutropenia), leaving an MTD of 25 mg/d. The most common grade III or IV adverse events were neutropenia (32%; 9 of 29 patients) and diarrhea (8%; 2 of 29 patients).

COMBINING CHEMOTHERAPY AND VEGF-TARGETED THERAPIES

Bevacizumab

Clinical development of Bevacizumab (BV) has rapidly progressed to Phase III trials after a preliminary randomized

Phase II trial in which 104 previously untreated mCRC patients were randomized to two doses of BV (5 and 10 mg/kg) in addition to bolus 5-FU/LV (high dose, Rosewell-Park regimen) or to 5-FU/LV alone^[51]. The combination of 5-FU/LV with low-dose BV (5 mg/kg every 2 wk) demonstrated superiority compared with the control monotherapy arm and to the BV-containing arm at a higher dose. These results provided the rationale for the key front-line Phase III study by Hurwitz *et al*^[52] which demonstrated superiority of IFL plus BV over IFL plus placebo in terms of RR (45% *vs* 35%), PFS(10.6 mo *vs* 6.2 mo) and OS(20.3 mo *vs* 15.6 mo). A subanalysis of this trial has recently established the benefit of Bevacizumab in mCCR patients with poor conditions^[53].

The second trial (E3200) was a second-line Phase III study, designed for patients who already failed an irinotecan-containing therapy and did not receive BV in first-line treatment^[54]. Initially, the study included three randomization arms: FOLFOX4 plus BV 10 mg/kg, FOLFOX4 alone or BV 10 mg/kg alone. The BV single-agent arm was closed ahead of time since it was clearly inferior to both other arms (RR 3% and PFS 2.7 mo). The results again largely favored the BV-containing arm, especially in terms of RR (21.8% *vs* 9.2%, $P < 0.0001$) and PFS (7.2 mo *vs* 4.8 mo, $P < 0.0001$). The primary end point of the study was reached, since a statistically significant increase in median survival was obtained in the experimental arm (12.5 mo *vs* 10.7 mo, $P < 0.0024$).

Finally, updated results of N016966, a randomized phase III trial evaluating the addition of bevacizumab to oxaliplatin-based first line chemotherapy have been reported. Bevacizumab-containing arms demonstrated a significant benefit in terms of progression-free survival, although overall response rate did not significantly differ^[55].

More recently, several phase II trials have addressed the feasibility and activity of bevacizumab when combined with various cytotoxic regimens. The First BEATrial^[56] enrolled 1927 chemotherapy-naïve patients treated with a combination of bevacizumab and several first-line chemotherapies, including FOLFOX, FOLFIRI and XELOX. Median PFS was 10.4 mo. Combinations of XELOX or XELIRI plus bevacizumab have yielded tumor control rates in the range of 80% as front-line therapy for mCRC^[57].

In contrast to its efficacy when used in combination with first- and second-line chemotherapy, activity of bevacizumab in chemoresistant disease has been disappointing. Chen *et al* developed a treatment referral center (TRC) protocol (TRC-0301) for patients with mCRC in the third-line setting with the aim of evaluating the safety and activity of BV plus FU/LV in patients progressed after treatment with both irinotecan-based and oxaliplatin-based chemotherapy regimens^[58]. Independent review confirmed one PR (1%; 95% CI, 0% to 5.5%). Median PFS in this cohort was 3.5 mo (95% CI, 2.1 mo to 4.7 mo) and median OS was 9.0 mo (95% CI, 7.2 mo to 10.2 mo). The authors conclude that BV, alone or in combination with an ineffective chemotherapy in the third-line setting, is likely to be of minimal, if any, clinical benefit.

An important question that remains unresolved is

whether to continue bevacizumab with second-line therapy following failure of a bevacizumab-containing first-line regimen. Although retrospective data from the BRiTE trial suggest that the use of bevacizumab beyond first progression correlate with an improved survival, more mature data are required to draw any firm conclusion^[59].

VEGF Tyrosine kinase inhibitors (TKI)-based combination therapy

Tyrosine kinase inhibitors of vascular endothelial growth factor receptors (VEGFRs) are low molecular weight, ATP-mimetic proteins that bind to the ATP-binding catalytic site of the tyrosine kinase domain of VEGFRs, resulting in a blockade of intracellular signaling. Several of these molecules have entered clinical evaluation.

Semaxanib: Semaxanib is a small, lipophilic, synthetic molecule that inhibits VEGFR-1, and -2 tyrosine kinases^[60]. A promising response of 31.6% was observed with semaxanib at two different dose levels, 85 and 145 mg/m² twice weekly in combination with fluorouracil plus leucovorin as first-line therapy for 28 patients with mCRC^[61]. However, a randomized, multicenter, phase III trial failed to show any improvement in clinical outcome with semaxanib in combination with fluorouracil and leucovorin (Roswell Park regimen) *versus* fluorouracil and leucovorin alone as first-line therapy for 737 mCRC patients; moreover, worse toxicity in the semaxanib arm (in terms of diarrhea, cardiovascular events, vomiting, dehydration, and sepsis) was observed^[62].

Vatalanib: Vatalanib is a synthetic, low molecular weight, orally bio-available agent that inhibits all known VEGFR tyrosine kinases, platelet-derived growth factor receptor beta (PDGFR- β) and c-Kit tyrosine kinase^[63].

Vatalanib was evaluated in two phase I / II studies as a single daily dose in combination with FOLFOX-4 or FOLFIRI. In the first study, the pharmacokinetics and toxicity profiles of both vatalanib and FOLFOX-4 were unaffected by co-administration^[64]. The reported response rate was 54%, with a median PFS of 11 mo and an estimated median OS time of 16.6 mo. In the second study^[65], co-administration of vatalanib at 1250 mg/d with FOLFIRI had minor effects on irinotecan exposure but lowered by 40% the AUC of SN-38 in patients' serum. The response rate was 41%, with a median PFS duration of 7.1 mo and a median OS time of 24.3 mo. Two large, randomized, double-blinded, placebo-controlled, phase III trials compared the efficacy of oral vatalanib in combination with FOLFOX-4 with FOLFOX-4 alone in patients with mCRC, and none of them met the primary end points. In the CONFIRM-2 trial, the addition of PTK/ZK to FOLFOX-4 in previously treated mCRC did not meet the primary end points of the study. OS was 12.1 mo in the PTK/ZK arm and 11.8 mo in the placebo arm. The overall response rate was, respectively, 18.5 and 17.5%. PFS was significantly longer in the PSK/ZK arm (5.5 mo *vs* 3.8, $P = 0.026$) As in confirm 1 trial, patients with pretreatment high LDH showed a strong improvement in PFS^[66]. Adverse events were similar to those of the CONFIRM-1 trial. Thrombotic and embolic events of all

grades occurred in 6% of the patients treated with PTK/ZK *vs* 1% in the placebo arm. Trying to further analyze the relation between LDH levels and clinical outcome with PTK/ZK, Fixed paraffin embedded tumor samples from 36 mCRC not included in the CONFIRM trials were analyzed and tumor gene expression correlated with serum levels of LDH in the same group of patients. Intratumoral levels of LAMA, hypoxia inducible factor 1 (HIF-1), Glut-1 and VEGFA were significantly correlated. Moreover, patients with high serum LDH showed increased intratumoral gene expression of VEGFA, supporting the hypothesis of serum LDH levels as a surrogate maker for activation of the hypoxia inducible factor related genes in the tumor^[66].

AZD2171: Preliminary data of a phase I evaluation of AZD2171, a highly potent and selective inhibitor of VEGFR signaling, in combination with several chemotherapy regimens including FOLFOX-6 and CPT-11, has shown some evidence of activity^[67].

Vandetalib: Vandetalib, a once-daily oral inhibitor of VEGFR-dependent tumor angiogenesis, EGFR- and RET-dependent tumor proliferation, in combination with FOLFOX6^[68] or FOLFIRI^[69] has also shown some evidence of activity in mCRC, with diarrhea and neutropenia being the most frequent grade 3 toxicities.

Future directions

So far, clinical, biochemical, and molecular markers have failed to discriminate which patients are more likely to benefit from bevacizumab-containing regimens. An analysis of predictive markers showed indeed that bevacizumab increased the activity of irinotecan plus FU/LV regardless of the level of VEGF expression, thrombospondin expression, and microvessel density^[70]. Mutations of *k-ras*, *b-raf*, and *p53* could not predict for a prolonged survival on bevacizumab plus irinotecan plus bolus FU/LV^[71]. Recently, Shaye *et al* evaluated functionally significant polymorphisms of genes involved in the angiogenesis pathway in mCRC patients who receive bevacizumab as part of their front-line therapy. There were statistically significant associations between genomic polymorphisms of KDR, CXCR2, MMP7, leptin and both progression-free survival and response rate. Hopefully, prospectively collected samples from patients enrolled onto cooperative group studies and the development of selective micro arrays to define the angiogenesis-related genes in individual tumors, and at different stages of therapy and tumor progression may allow improved therapeutic efficacy.

COMBINATION OF TARGETED THERAPIES

The assumption that most advanced solid tumors derive their growth advantage from more than a signaling pathway and the significant level of compensatory cross talk among receptors within a signaling network as well as with heterologous receptor systems has provided the basis of a combined molecular targeting approach, in which more than one class of inhibitor is applied simultaneously.

A phase II study with the combination of FOLFOX,

bevacizumab (5 mg/kg) and erlotinib (150 mg/d) every two weeks in 31 chemotherapy naive mCRC patients has been recently conducted. Grade 3-4 adverse events included diarrhea (29%) neutropenia (29%) rash (18%), fatigue (14%) and neuropathy (11%) 78% of the patients had at least one grade 3-4 toxicity. Remarkably, as much as 42% of the patients came off for toxicity. Similar results have been reported in the DREAM-OPTIMO3 study, with a 70% incidence of grade 3-4 toxicity when adding erlotinib to a combination of bevacizumab and XELOX^[73].

A phase II trial of FOLFOX plus bevacizumab and cetuximab in 67 chemotherapy-naïve mCRC patients yielded a 55% response rate, with a median PFS of 9.6 mo and 71% of the patients progression-free for at least 8 mo^[74].

The combination of FOLFOX or FOLFIRI with panitumumab and AMG706, an oral multikinase inhibitor targeting VEGF, PDGF and Kit receptors has been tested in 45 mCRC patients, with no apparent PK/PD interactions and an overall response rate in the range of 50%^[75].

Based on these results, combinations of monoclonal antibodies are currently being actively tested in first-line therapy of mCRC. The Cancer and Leukemia Group B (CALGB)/South West Oncology Group (SWOG) Intergroup 80405 Phase III trial randomizes patients to either cetuximab or bevacizumab, or both antibodies in combination, with the oncologist's choice of FOLFOX or FOLFIRI. In addition, the Panitumumab Advanced Colorectal Cancer Evaluation (PACCE) trial is currently evaluating the efficacy of FOLFOX or FOLFIRI (depending on the investigator choice) plus BV, versus the same combination plus panitumumab.

OTHER TARGETED THERAPIES-BASED COMBINATIONS

Cell cycle inhibitors

Kortmanský *et al*^[76] reported the results of the combination of 5-FU and UCN-01, a selective inhibitor of a number of serine-threonine kinases, including calcium and phospholipid-dependent protein kinase C and cell cycle specific kinases, among 35 patients with advanced solid tumors, the majority of them with a diagnosis of mCRC. No objective responses were observed, although eight patients had stable disease. Most of the patients with stable disease had previously received and progressed on 5-fluorouracil. There was minimal toxicity attributed to the combination, although expected toxicities associated with UCN-01 were observed.

Apoptosis modifiers

Bcl-2 plays a pivotal role in the regulation of caspase activation and apoptosis. Its overexpression is found in 30%-94% of clinicopathological colorectal carcinoma specimens and confers a multidrug resistant phenotype in several cell lines. In support of this data, antisense oligonucleotide therapy directed against bcl-2 was shown to significantly enhance the chemosensitivity in several cancer cell lines compared with controls *in vitro*.

A recently published phase I trial assessed the feasibility and pharmacokinetic behaviour of the combination of oblimersen sodium, a phosphorothioate antisense oligonucleotide that hybridizes to the first six codons of the bcl-2 open reading frame mRNA, with CPT-11 in 20 pts with mCRC. Among them, 1 pt experienced a PR while 10 additional patients had stable disease lasting 2.5-10 mo. The authors recommend oblimersen at 7 g/kg/d, d 1-8 with CPT-11 280 mg/m² on d 6 once every 3 wk was the RD for further development in phase II trials^[77].

Proteasome inhibitors

The proteasome inhibitor Bortezomib (PS-341), at a dose of 1.3 mg/m² administered twice weekly every 21 d in pretreated patients with mCRC did not prove to have clinical activity^[78].

The main nonhematologic toxicities were elevation of alkaline phosphatase, constipation, fatigue, nausea, and sensory neuropathy. A pharmacokinetic and pharmacodynamic analysis of topotecan plus PS-341 in 22 patients with advanced solid malignancies found that, with the addition of PS-341, peripheral blood mononuclear cells (PBMC) topoisomerase I levels got stabilised or increased. These findings suggest that PS-341 may overcome resistance to topoisomerase I inhibitors, since *in vitro* exposure to camptothecin results in down-regulation of the target enzyme. Preliminary data of the combination of FOLFOX4 plus bortezomib in mCRC patients^[79] show evidence of clinical activity, with bortezomib at a dose of 1 mg/m² being the RD for phase II trials.

COX inhibitors

Numerous clinical trials are ongoing to test the efficacy of nonsteroidal anti-inflammatory COX-2 inhibitors in combination regimens for therapy of advanced solid tumors^[80]. Preliminary data on the combination of rofecoxib (50 mg/d) with weekly irinotecan and infusional fluorouracil demonstrated a good tolerability up to the irinotecan dose of 125 mg/m²/wk. The phase II study showed a 36.7% objective response rate, a clinical benefit of 76.7% and a median TTP and overall survival of 4 and 9 mo, respectively. The combination was feasible and safe, with a reduced rate of mucositis and diarrhea^[81].

However, in the BICC-C trial^[82], addition of celecoxib to several Irinotecan/fluoropyrimidine combinations did not impact safety or efficacy. Results of larger studies seem warranted.

Histone deacetylase inhibitors

Histone acetylation by histone acetyltransferases is important for promoting the action of several transcription factors. Acetylation facilitates binding of transcription factors to specific target DNA sequences by destabilizing nucleosomes bound to the promoter region of the target genes^[83].

Vorinostat, a novel histone deacetylase inhibitor that potentiates 5-FU through a decrease in thymidilate synthase (TS) expression has been tested in combination with FOLFOX, in a phase I study that enrolled mCRC patients who had failed prior FOLFOX, irinotecan and cetuximab

therapy. Tolerance was acceptable, and some evidence of both, clinical activity (SD in some patients) and biological activity (down regulation of TS) are suggested^[84].

mTOR inhibitors

Rapamycin displays potent antimicrobial and immunosuppressant effects as well as antitumor properties. Rapamycin's antiproliferative actions are due to its ability to modulate key signal transduction pathways that link mitogenic stimuli to the synthesis of proteins necessary for the cell cycle to progress from the G1 to S phase^[85].

Rapamycin clinical development has been hampered due to the poor aqueous solubility and chemical stability of the macrolide. CCI-779, a rapamycin ester derived from 2, 2-bis (hydroxymethyl) propionic acid, is one analog that was selected for further development due to its promising pharmacological, toxicological and antitumor profiles^[86].

A phase I study of escalating doses of CCI-779 in combination with 5-FU/leucovorin in patients with advanced solid tumors, including mCRC reported preliminary evidence of activity including 1 complete response in a patient with mCRC receiving the 15 mg/m² dose and several patients with stable disease of a maximum duration of 12 mo. Further studies are required to determine appropriate regimens with this combination treatment^[87].

CONCLUSION

In conclusion, the biological agents have clearly increased the therapeutic armamentarium of patients with metastatic CRC and offer also prospects for an increased chance of a longer survival. Eventually, the availability of more predictive biological factors may allow oncologists to tailor individualized targeted combination therapy to a specific patient with a specific tumor. However, the cost of novel therapies for mCRC is particularly high. Such a heavy economical burden may be counterbalanced either by a very significant breakthrough in treatment efficacy or by selection of patients with a higher chance of responding to a specific treatment.

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