TARGETED THERAPY IN COLORECTAL CANCER

Christos Karapetis and Ganessan Kichenadasse

Department of Medical Oncology, Flinders Medical Centre, Bedford Park, South Australia.

Email: C.Karapetis@flinders.edu.au

Abstract

Our understanding of the molecular pathways that mediate cancer cell proliferation has increased significantly and with this comes the rapid development of molecular targeted therapies. The epidermal growth factor receptor and the vascular endothelial growth factor are two such targets that have proven to be important in the treatment of advanced colorectal cancer. Successful inhibition of these targets, utilising monoclonal antibodies bevacizumab, cetuximab and panitumumab, has led to improved patient outcomes. Prolongation of patient survival and improvement in quality of life has been associated with the use of these antibodies. Such therapies are now becoming part of standard management of advanced colorectal malignancy. Predictive biomarkers that allow for a more rational and effective utilisation of these new molecular targeted therapies are being discovered. The number of potential molecular targets seems infinite as new drugs are rapidly processed through new accelerated clinical trial designs and drug development programs. Many challenges remain in the successful development of molecular targeted therapies, including overcoming mechanisms of resistance, optimal drug delivery, the issues of the financial cost of these new drugs and equitable access to the new therapies.

Colorectal carcinoma is the third most common malignancy of both sexes in developed countries.[1] The spread of colorectal cancer to distant sites (Duke's stage D) represents essentially incurable disease, except for selected cases where complete surgical resection can be applied. Chemotherapy for advanced colorectal cancer can prolong survival and provide symptomatic benefit and quality of life improvement.[2-8] Over the last decade new cytotoxics, including irinotecan and oxaliplatin, have produced further survival benefit.[9-13] Therapeutic options for patients with metastatic colorectal cancer who have failed these treatments are limited.

The epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) represent the two molecular structures and associated pathways that have proven to be successful targets in the development of new drugs. Other targets are being evaluated in ongoing clinical trials. We will outline the results obtained in trials that have evaluated molecular targeted strategies and briefly outline the challenges of successful implementation of such treatment, in the context of advanced colorectal cancer.

Targeted therapy directed against epidermal growth factor and associated pathways

Cetuximab is a chimeric immunoglobulin G1 monoclonal antibody that binds to the EGFR with high affinity.[14] Panitumumab is a humanised monoclonal antibody that has the same effect on EGFR.[15] This association of antibody and receptor competitively inhibits ligand binding and leads to inhibition of phosphorylation and subsequent activation of downstream signalling pathways. These antibodies also stimulate EGFR internalisation, effectively removing the receptor from the cell surface.[14] Blocking EGFR can lead to cell cycle arrest in the G_1 phase,[16] and cell death via apoptosis.[17]

Single-agent therapy with cetuximab or panitumumab has demonstrated activity in patients with refractory, metastatic EGFR-positive colorectal carcinoma. The objective response rates observed in these studies vary between 8 to 12% for single-agent EGFR directed therapy.[18-21] The response rate appears similar (10%) when cetuximab is used as a single agent as first line therapy for previously untreated patients.[22] Two Phase III randomised control trials have confirmed a benefit of EGFR directed therapy when compared to best supportive care. Cetuximab demonstrated an overall survival advantage, and a quality of life benefit.[20] Panitumumab was associated with a progression free survival benefit, but not an overall survival advantage, although the trial design allowed for cross-over from best supportive care to panitumumab on disease progression.[19] An acneiform skin rash is the principal side-effect of such a treatment approach. An increased severity of skin rash has been associated with a greater response rate.[20,23] The problem with using rash as predictive markers is that the drug must first be administered to observe the rash. It is therefore not a predictive factor that can be used prior to therapy, and cannot be used to select patients for initiation of therapy.

Efficacy has also been demonstrated with the combination of either panitumumab or cetuximab and irinotecan in patients with irinotecan-refractory EGFR-positive metastatic colorectal cancer.[23,24] Response rates of 19 to 23% with median duration of response of four to six months have been observed, but the median survival of approximately six to eight months remains similar to that observed in the single agent EGFR antibodies.[20,23,25] The combination of FOLFIRI and cetuximab was associated with a modest prolongation of progression-free survival when used as a first line treatment for advanced colorectal cancer. A trial comparing irinotecan plus cetuximab versus irinotecan alone as second line treatment, showed no overall survival difference between the two arms.[26] The

combination of oxaliplatin, fluoropyrimidines and EGFR directed antibody has also been evaluated, with evidence of higher response rates with the antibody-chemotherapy combination approach.

Targeting the EGFR pathway via the associated tyrosine kinase has been tried, with initial studies suggesting possible efficacy. Single agent tyrosine kinase inhibitor (TKI) therapy was associated with stable disease in almost 40% of patients, but no objective responses.[27] Phase I and II trials combining TKI with chemotherapy initially suggested safety and possible efficacy, but subsequent studies have been disappointing.[28,29] The addition of gefitinib does not overcome fluoropyrimidine

Table 1: *Chemotherapy naive*

	Chemotherapy alone median survival (months)	Bevacizumab with chemotherapy median survival (months)	Absolute benefit (months)	HR	P value
Bevacizumab with Fluoropyrimidine monotherapy					
Kabbinavar et al (Combined analysis)[36]					
PFS	5.6	8.8	+ 3.2	0.63	0.0001
OS	14.6	17.9	+ 3.3	0.74	0.0081
Bevacizumab with Irinotecan based chemotherapy					
Hurwitz et al (AVF2107g)[35]					
PFS	6.2	10.6	+ 4.4	0.54	< 0.001
OS	15.6	20.3	+ 4.7	0.66	< 0.001
Bevacizumab with Oxalipaltin based chemotherapy					
Saltz et al (NO 16966)[37]					
PFS	8.0	9.4	+1.4	0.83	0.0023
OS	19.9	21.3	+1.4	0.89	0.07
Hochster et al (TREE)[38]					
PFS					
mFOLFOX6	8.7	9.9	+ 1.2		
bFLOX	6.9	8.3	+ 1.4		
CapOx	5.9	10.3	+ 4.4		
OS	18.2	23.7	+ 5.5		
mFOLFOX6	19.2	26.1	+ 5.9		
bFLOX	17.9	20.4	+ 2.5		
CapOx	17.2	24.6	+ 4.4		

Table 2: *Previously treated with chemotherapy, bevacizumab naive*

	Chemotherapy alone median survival (months)	Bevacizumab with chemotherapy median survival (months)	Absolute benefit (months)	HR (95% CI)	P value
Bevacizumab with Oxalipaltin based chemotherapy (Irinotecan Refractoy)					
Giantonio et al (E3200)[40]					
PFS	4.8	7.2	+ 2.4	0.64	<0.0001
OS	10.8	12.9	+ 2.1	0.76	0.0018
Bevacizumab with Fluoropyrimidine monotherapy (Irinotecan/Oxaliplatin refractory)					
Chen et al (TRC-0301)[41]					
PFS	–	3.5	–	–	–

OS (Overall survival); PFS (Progression free survival); HR (hazard ratio); FOLFOX6 (bolus and infusion fluorouracil [FU] and leucovorin [LV] with oxaliplatin); bFLOX (bolus FU and low-dose LV with oxaliplatin); CapOx (capecitabine with oxaliplatin).

resistance.[30] In a randomised Phase II trial the addition of gefitinib to FOLFIRI did not provide benefit,[31] and in another Phase II study the combination of irinotecan and gefitinib was associated with increased toxicity.[32]

Targeted therapy directed against the vascular endothelial growth factor and associated pathways

Angiogenesis plays an important role in the growth and progression of cancers like colorectal cancers. VEGF pathways play a key role in this process. The two major players are VEGF receptors (VEGFR) and their ligands, VEGF glycoproteins. There are five major ligands, VEGF-A through E, while the receptors include VEGFR-1, 2 and 3. The binding of VEGF ligands to the receptors triggers a series of events involving endothelial cell proliferation, migration and survival, in addition to altering vascular permeability, thereby controlling the physiological and tumour angiogenesis.

Angiogenesis plays an important role in cancer survival and progression.[33] There are two major anti-angiogenic approaches – monoclonal antibodies or small molecules directed against the VEGF pathways. Other approaches like antisense oligonucleotides and aptamers are still in early research phase. Worldwide, several such drugs like bevacizumb, sunitinib and sorafenib, are approved for routine clinical use to treat patients with colorectal cancer, renal cancers, lung and breast cancers, based on efficacy data from well conducted Phase III trials. In this review, we present the evidence to show how targeting angiogenesis has changed the way we manage colorectal cancer.

Bevacizumab, a recombinant humanised monoclonal antibody against VEGF-A ligand, was the first anti-angiogenic drug to show impressive survival benefit in clinical trials. Most studies indicate that bevacizumab in combination with chemotherapy is better than chemotherapy alone in terms of survival for metastatic colorectal cancer. Bevacizumab acts as a chemo-sensitiser by reducing new blood vessel formation and inducing apoptosis in addition to normalising the tumour vasculature, improving delivery of chemotherapy.[34]

Hurwitz et al compared the benefit of adding bevacizumab to irinotecan and 5-fluorouracil in patients with previously untreated metastatic colorectal cancer.[35] There was a significant difference in response rates, overall survival and progression free survival in favour of the bevacizumab arm.

The consistent survival benefit of adding bevacizumab to other chemotherapy regimens like bolus 5-FU/LV,[36] FOLFOX[37] and capecitabine/oxaliplatin[38] confirmed that the approach of combining anti-angiogenic drugs and chemotherapy is beneficial. The survival advantage ranged from 1.4 to 4.7 months. Interestingly, the NO16966 study showed a progression-free survival benefit, but no significant differences in overall survival and response rates.[37] Early cessation of bevacizumab in this trial has been postulated as a reason for the smaller observed benefit, and has led to recommendations that bevacizumab should be continued beyond the completion of first-line chemotherapy.[39] Results from trials in which bevacizumab has been added to chemotherapy in the first line and second line setting are summarised in tables 1 and 2.

Chen et al studied the efficacy of bevacizumab and 5-FU (bolus or infusion) in heavily pre-treated patients who were refractory to irinotecan and oxaliplatin in a single arm Phase II trial.[41] The progression-free survival was 3.5 months and response rate was just 1%. The Eastern Co-operative Oncology Group trial (E3200) was a Phase III trial comparing FOLFOX with or without bevacizumab in irinotecan refractory patients. A survival benefit was demonstrated in the bevacizumab treated patients.[40]

There are several ongoing studies which evaluate the role of bevacizumab to the standard adjuvant chemotherapy with FOLFOX for Duke's C or high risk Duke's B colorectal cancer. Since bevacizumab is a potent radio-sensitiser through its normalisation of tumour vasculature, it is being evaluated in combination with radiotherapy for rectal cancers. The results of these trials are eagerly awaited before bevacizumab can be recommended in this setting.

Targeting angiogenesis with small molecule TKIs has not met with such success as the antibody-based approach. Vatalinib is one such TKI of multiple targets including VEGFR, platelet derived growth factor and C-KIT. The advantage of this drug is oral administration in addition to multi-targeting activity. Vatalinib has been studied extensively in very large Phase III trials (Colorectal Oral Novel Therapy for the Inhibition of Angiogenesis and Retarding of Metastases – CONFIRM 1 and 2) in combination with chemotherapy in the first line and second line setting.[42,43] There was no benefit in survival or response rates. A subsequent meta-analysis showed that the addition of vatalinib improved the progression-free survival in patients with elevated lactate dehydrogenase.[44] The valuable lessons learnt from these studies indicate the need for better patient selection using validated predictive factors.

There are several ongoing studies with other anti-angiogenic drugs including sunitinib, sorafenib and cediranib in combination with chemotherapy drugs. The results of these trials are still awaited.

There is a well recognised toxicity profile related to anti-angiogenic drugs, including hypertension, proteinuria and bleeding. The trials of bevacizumab have identified proteinuria (28%), hypertension (25%), haemorrhage (2 to 9%), arterial thromboembolism (0 to 3.8%), wound healing complications (2%) and gastrointestinal perforation (1.5 %).[45] Several Phase IV studies of bevacizumab in combination with various chemotherapy agents in community practice have highlighted similar incidence of adverse events.[46-48] Early identification and prompt therapy of these complications cannot be overemphasised.

Combination of bevacizumab and EGFR-directed monoclonal antibodies

Preliminary results from Phase II and III trials have failed to demonstrate a benefit in combining bevacizumab with EGFR-directed monoclonal antibodies.[49-51] These studies raise concerns about increased toxicity and reduced treatment efficacy when combining these

molecular targeted therapies. We have much to learn about the interaction of these drugs and our understanding of 'multi-targeting' remains rudimentary.

Biomarker predictors of benefit to molecular targeted therapy

Colorectal cancer is a multi-step process characterised by a sequence of genetic alterations in cell growth regulator genes, such as K-RAS, p53 and DCC genes.[52] EGFR is a logical potential biomarker, but as measured by immunohistochemistry, is not a useful predictive factor. There is no significant relationship between EGFR expression as determined by immuno-histochemistry and the likelihood of response to cetuximab.[23,25,53,54] K-RAS gene mutations occur early in the stages of carcinogenesis, as the colorectal adenoma progresses to develop into a carcinoma. The RAS/RAF/MAP kinase and the PTEN/PI3K/AKT signalling pathways are activated by ligand binding and activation of EGFR, and these pathways form a network that plays a central role in cancer progression and survival.[55] Mutations in K-RAS can lead to constitutive activation of the pathway, and this may render inhibitors of components of the cascade upstream of EGFR ineffective. K-RAS mutations are found in 30-50% of colorectal cancers with most mutations found in exon 2 of the K-RAS gene.[56,57-64]

Previous studies have compared the efficacy of EGFR-directed monoclonal antibodies across wild-type K-RAS and mutant K-RAS tumours. Median survival was longer and responses were seen almost exclusively in the wild-type K-RAS subsets.[58-60,65] In a randomised control trial, panitumumab benefit when compared to best supportive care was confined to patients with wild-type K-RAS tumours.[56] For patients with K-RAS mutant tumours, there was no difference in progression-free survival between the panitumumab and best supportive care groups, but median progression-free survival for patients with wild-type K-RAS tumours was 12.3 weeks with panitumumab and 7.3 weeks with best supportive care. All of the objective responses occurred in patients with panitumumab treated wild-type K-RAS tumours.[56]

In the first line setting, K-RAS mutation status has a similar predictive significance. Results of the CRYSTAL study demonstrated that benefit through the addition of cetuximab to FOLFIRI chemotherapy is restricted to patients with wild-type K-RAS tumours. Patients with colorectal tumours that contain K-RAS mutations did not obtain a benefit with cetuximab.[66] Similar results were observed in patients receiving the FOLFOX chemotherapy combination as first line therapy, and the progression free survival was trending lower in those patients with K-RAS mutations when treatment included cetuximab versus chemotherapy alone.

Other gene mutations, such as mutations involving the PTEN, BRAF or PI3KCA genes, can also lead to unrestricted cancer cell growth and may also be useful predictive biomarkers. Loss of PTEN activity, for example, has been associated with lack of efficacy as measured by radiological response, with none of 11 patients responding to cetuximab in a recently reported series.[60] Moving upstream in the signalling pathway, high EGFR ligand expression, particularly amphiregulin and epiregulin, has been observed in tumours responding to cetuximab and these ligands represent attractive targets for future biomarker research.[67] So far, there are no reliable biomarkers to predict benefit from bevacizumab.

Real progress but significant challenges

Over the last decade there has been a paradigm shift in the way we manage patients with advanced colorectal cancer. Multi-agent chemotherapy and multiple lines of therapy are now part of optimal treatment strategies. Anti-angiogenic therapy has contributed to improving outcomes, particularly prolonging disease control with an associated prolongation of overall survival. The benefit has been observed when bevacizumab is used as part of either first or second line therapy. EGFR directed therapy, particularly utilising cetuximab or panitumumab, also prolongs survival, but this appears to be restricted to patients with tumours that are have wild-type K-RAS. These new targeted therapies are relatively expensive. In the UK National Health Service review, bevacizumab was not considered to be cost effective in combination with chemotherapy,[68] and in 2008 bevacizumab and the EGFR-directed antibodies are not funded on the Pharmaceutical Benefits Scheme in Australia. The cost effectiveness improves when these treatments can be delivered to patients with a higher chance of benefiting. Avoiding therapy in patients that have little chance of responding can help to eliminate toxicity of ineffective therapy and allow other treatment approaches to be pursued. Accurate and reliable biomarkers that allow selection of patients with advanced colorectal cancer, who will benefit from new therapies, would represent a significant advance in the clinical management of this disease. The K-RAS correlative analyses have identified a biomarker that can effectively exclude a significant proportion of patients, 40% with tumours that have K-RAS mutations, from EGFR monoclonal antibody therapy. Other prognostic and predictive variables, preferably ones that are reliably and easily measured, need to be identified.

Conflict of interest statement: Christos Karapetis has served on advisory boards for Merck Serono, Astra Zeneca, AMGEN and Roche.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55(2):74-108.
2. Nordic Gastrointestinal Tumor Adjuvant Therapy Group. Expectancy or primary chemotherapy in patients with advanced asymptomatic colorectal cancer: a randomised trial. J Clin Oncol. 1992;10(6):904-11.
3. Rougier P, Laplanche A, Huguier M, Hay JM, Ollivier JM, Escat J, et al. Hepatic arterial infusion of floxuridine in patients with liver metastases from colorectal carcinoma: long-term results of a prospective randomized trial. J Clin Oncol. 1992;10(7):1112-8.
4. Allen-Mersh TG, Earlam S, Fordy C, Abrams K, Houghton J. Quality of life and survival with continuous hepatic-artery floxuridine infusion for colorectal liver metastases. Lancet. 1994;344(8932):1255-60.
5. Scheithauer W, Rosen H, Kornek GV, Sebesta C, Depisch D. Randomised comparison of combination chemotherapy plus supportive care with supportive care alone in patients with metastatic colorectal cancer. BMJ. 1993;306(6880):752-5.
6. Cunningham D, Glimelius B. A phase III study of irinotecan (CPT-11) versus best supportive care in patients with metastatic colorectal cancer who have failed 5-fluorouracil therapy. V301 Study Group. Seminars in oncology. 1999;26(1 Suppl 5):6-12.
7. Glimelius B, Hoffman K, Graf W, Pahlman L, Sjoden PO. Quality of life during chemotherapy in patients with symptomatic advanced colorectal cancer. The Nordic Gastrointestinal Tumor Adjuvant Therapy Group. Cancer. 1994;73(3):556-62.
8. Graf W, Pahlman L, Bergstrom R, Glimelius B. The relationship between an objective response to chemotherapy and survival in advanced colorectal cancer. Br J Cancer. 1994;70(3):559-63.

9. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet. 2000;355(9209):1041-7.
10. Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, et al. A randomised controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol. 2004;22(1):23-30.
11. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. New Engl J Med. 2000;343(13):905-14.
12. Grothey A, Sargent D, Goldberg RM, Schmoll HJ. Survival of patients with advanced colorectal cancer improves with the availability of fluorouracil-leucovorin, irinotecan, and oxaliplatin in the course of treatment. J Clin Oncol. 2004;22(7):1209-14.
13. Mayer RJ. Moving beyond fluorouracil for colorectal cancer. New Engl J Med. 2000;343(13):963-4.
14. Baselga J, Norton L, Masui H, Mendelsohn J. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. J Nat Cancer Inst. 1993;85(16):1327-33.
15. Wainberg ZA, Hecht JR. Panitumumab in colorectal cancer. Expert review of anticancer therapy. 2007;7(7):967-73.
16. Chou JL, Fan Z, DeBlasio T, Koff A, Rosen N, Mendelsohn J. Constitutive overexpression of cyclin D1 in human breast epithelial cells does not prevent G1 arrest induced by deprivation of epidermal growth factor. Breast Cancer Res Treat. 1999;55(3):267-83.
17. Wu X, Fan Z, Masui H, Rosen N, Mendelsohn J. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. J Clin Invest. 1995;95(4):1897-905.
18. Lenz HJ, Van Cutsem E, Khambata-Ford S, Mayer RJ, Gold P, Stella P, et al. Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. J Clin Oncol. 2006;24(30):4914-21.
19. Van Cutsem E, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, 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):1658-64.
20. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, et al. Cetuximab for the treatment of colorectal cancer. New Engl J Med. 2007;357(20):2040-8.
21. Hecht JR, Patnaik A, Berlin J, Venook A, Malik I, Tchekmedyian S, et al. Panitumumab monotherapy in patients with previously treated metastatic colorectal cancer. Cancer. 2007;110(5):980-8.
22. Pessino A, Artale S, Sciallero S, Guglielmi A, Fornarini G, Andreotti IC, et al. First-line single-agent cetuximab in patients with advanced colorectal cancer. Ann Oncol. 2008;19(4):711-6.
23. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. New Engl J Med. 2004;351(4):337-45.
24. Berlin J, Posey J, Tchekmedyian S, et al. Panitumumab with irinotecan/leucovorin/5-fluorouracil for first-line treatment of metastatic colorectal cancer. Clinical colorectal cancer. 2007;6(6):427-32.
25. Saltz LB, Meropol NJ, Loehrer PJ, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol. 2004;22(7):1201-8.
26. Sobrero AF, Maurel J, Fehrenbacher L, Scheithauer W, Abubakr YA, Lutz MP, et al. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26(14):2311-9.
27. Townsley CA, Major P, Siu LL, Dancey J, Chen E, Pond GR, et al. Phase II study of erlotinib (OSI-774) in patients with metastatic colorectal cancer. Brit J Cancer. 2006;94(8):1136-43.
28. Van Cutsem E, Verslype C, Beale P, Clarke S, Bugat R, Rakhit A, et al. A phase Ib dose-escalation study of erlotinib, capecitabine and oxaliplatin in metastatic colorectal cancer patients. Ann Oncol. 2008;19(2):332-9.
29. Meyerhardt JA, Zhu AX, Enzinger PC, Ryan DP, Clark JW, Kulke MH, et al. Phase II study of capecitabine, oxaliplatin, and erlotinib in previously treated patients with metastastic colorectal cancer. J Clin Oncol. 2006;24(12):1892-7.
30. Stebbing J, Harrison M, Glynne-Jones R, Bridgewater J, Propper D. A phase II study to determine the ability of gefitinib to reverse fluoropyrimidine resistance in metastatic colorectal cancer (the INFORM study). Brit J Cancer. 2008;98(4):716-9.
31. Santoro A, Comandone A, Rimassa L, Granetti C, Lorusso V, Oliva C, et al. A phase II randomized multicenter trial of gefitinib plus FOLFIRI and FOLFIRI alone in patients with metastatic colorectal cancer. Ann Oncol. 2008.
32. Chau I, Cunningham D, Hickish T, Massey A, Higgins L, Osborne R, et al. Gefitinib and irinotecan in patients with fluoropyrimidine-refractory, irinotecan-naive advanced colorectal cancer: a phase I-II study. Ann Oncol. 2007;18(4):730-7.
33. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.
34. Jain RK. Normalisation of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. (New York, NY 2005;307(5706):58-62.
35. Hurwitz H, Kabbinavar F. Bevacizumab combined with standard fluoropyrimidine-based chemotherapy regimens to treat colorectal cancer. Oncology. 2005;69 Suppl 3:17-24.
36. Kabbinavar FF, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR, et al. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. J Clin Oncol. 2005;23(16):3697-705.
37. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol. 2008;26(12):2013-9.
38. Hochster HS, Hart LL, Ramanathan RK, Childs BH, Hainsworth JD, Cohn AL, et al. Safety and efficacy of oxaliplatin and fluoropyrimidine regimens with or without bevacizumab as first-line treatment of metastatic colorectal cancer: results of the TREE Study. J Clin Oncol. 2008;26(21):3523-9.
39. Cilley JC, Barfi K, Benson AB, 3rd, Mulcahy MF. Bevacizumab in the treatment of colorectal cancer. Expert Opin Biol Ther. 2007;7(5):739-49.
40. Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol. 2007 Apr 20;25(12):1539-44.
41. Chen HX, Mooney M, Boron M, Vena D, Mosby K, Grochow L, et al. Phase II Multicenter Trial of Bevacizumab Plus Fluorouracil and Leucovorin in Patients With Advanced Refractory Colorectal Cancer: An NCI Treatment Referral Center Trial TRC-0301. J Clin Oncol. 2006 Jul 20;24(21):3354-3360.
42. Hecht JR, Trarbach T, Jaeger E, Hainsworth Jea. A randomized, double-blind, placebo-controlled, phase III study in patients (Pts) with metastatic adenocarcinoma of the colon or rectum receiving first-line chemotherapy with oxaliplatin/5- fluorouracil/leucovorin and PTK787/ZK 222584 or placebo (CONFIRM-1). J Clin Oncol. 2005;23 (June 1 suppl; abstr 3a).
43. Koehne C, Bajetta E, Lin E, Valle Jea. Final results of CONFIRM 2: A multinational, randomized, double-blind, phase III study in 2nd line patients (pts) with metastatic colorectal cancer (mCRC) receiving FOLFOX4 and PTK787/ZK 222584 (PTK/ZK) or placebo. J Clin Oncol. 2007;25(June 20 suppl; abstr 4033a).
44. Major P, Trarbach T, Lenz H, Kerr Dea. A meta-analysis of two randomized, double-blind, placebo-controlled, phase III studies in patients (pts) with metastatic colorectal cancer (mCRC) receiving FOLFOX4 and PTK/ZK to determine clinical benefit on progression-free survival (PFS) in high LDH pts. J Clin Oncol. 2006;24 (June 20 suppl; abstr 3529a).
45. Saif MW, Mehra R. Incidence and management of bevacizumab-related toxicities in colorectal cancer. Expert opinion on drug safety. 2006;5(4):553-66.
46. Ackland SP, Clarke S, Perez-Carrión R, Chiara Sea. Updated efficacy data from AVIRI: A large phase IV trial of first-line bevacizumab plus FOLFIRI in patients with mCRC. J Clin Oncol 2008;26 (May 20 suppl; abstr 463a).
47. Berry SR, Van Cutsem E, Kretzschmar A, Michael Mea. Final efficacy results for bevacizumab plus standard first-line chemotherapies in patients with metastatic colorectal cancer: First BEAT. J Clin Oncol 2008;26 (May 20 suppl; abstr 4025a).
48. Purdie DM, Berlin JD, Flynn PJ, Grothey Aea. The safety of long-term bevacizumab use: Results from the BRiTE observational cohort study (OCS). 2008 (May 20 suppl; abstr 4103a).
49. Bokemeyer C, Bondarenko I, Hartmann JT, De Braud FGea. KRAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: The OPUS experience. J Clin Oncol 2008;26 (June 20 suppl; abstr 4000).
50. Punt CJ, Tol J, Rodenburg CJ, Cats Aea. Ranndomized phase III study of capecitabine, oxaliplatin, and bevacizumab with or without cetuximab in advanced colorectal cancer (ACC), the CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG). J Clin Oncol. 2008;26(June 20 suppl; abstr LBA4011).
51. Meyerhardt JA, Stuart K, Fuchs CS, Zhu AX, Earle CC, Bhargava P, et al. Phase II study of FOLFOX, bevacizumab and erlotinib as first-line therapy for patients with metastatic colorectal cancer. Ann Oncol. 2007;18(7):1185-9.
52. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. New Engl J Med. 1988;319(9):525-32.
53. Zhang W, Gordon M, Press OA, Rhodes K, Vallböhmer D, Yang DY, et al. Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with Cetuximab. Pharmacogenet Genomics. 2006;16(7):475-83.
54. Wierzbicki RD, Jonker DJ, Moore MJ, et al. A phase II multicenter study of cetuximab monotherapy in patients with EGFR-undetectable refractory metastatic colorectal carcinoma (mCRC). J Clin Oncol. 2008;26 (May 20 suppl; abstr 4065).
55. Mendelsohn J, Baselga J. Epidermal growth factor receptor targeting in cancer. Seminars in oncology. 2006;33(4):369-85.
56. Amado RG, Wolf M, Peeters M, Cutsem EV, Siena S, Freeman DJ, et al. Wild-Type KRAS Is Required for Panitumumab Efficacy in Patients With Metastatic Colorectal Cancer. J Clin Oncol. 2008.
57. Fransen K, Klintenas M, Osterstrom A, Dimberg J, Monstein HJ, Soderkvist P. Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas. Carcinogenesis. 2004;25(4):527-33.

58. De Roock W, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, 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):508-15.
59. Di Fiore F, Blanchard F, Charbonnier F, Pessot FE, Lamy A, Galais MP, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. Brit J Cancer. 2007;96(8):1166-9.
60. Frattini M, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Brit J Cancer. 2007;97(8):1139-45.
61. Andreyev HJ, Ross PJ, Cunningham D, Clarke PA. Antisense treatment directed against mutated Ki-ras in human colorectal adenocarcinoma. Gut. 2001;48(2):230-7.
62. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, Zanon C, Moroni M, Veronese S, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res. 2007;67(6):2643-8.
63. Esteller M, Gonzalez S, Risques RA, Marcuello E, Mangues R, Germa, JR, et al. K-ras and p16 aberrations confer poor prognosis in human colorectal cancer. J Clin Oncol. 2001;19(2):299-304.
64. Bazan V, Agnese V, Corsale S, Calò V, Valerio MR, Latteri MA, et al. Specific TP53 and/or Ki-ras mutations as independent predictors of clinical outcome in sporadic colorectal adenocarcinomas: results of a 5-year Gruppo Oncologico dell'Italia Meridionale (GOIM) prospective study. Ann Oncol. 2005;16 Suppl 4:iv50-5.
65. Lievre A, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol. 2008;26(3):374-9.
66. Van Cutsem E, Lang I, D'haens G, Moiseyenko Jea. KRAS status and efficacy in the first-line treatment of patients with metastatic colorectal cancer (mCRC) treate with FOLFIRI with or without cetuximab: The CRYSTAL experience. J Clin Oncol. 2008;26 (June 20 suppl; abstr 2).
67. Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol. 2007;25(22):3230-7.
68. Tappenden P, Jones R, Paisley S, Carroll C. Systematic review and economic evaluation of bevacizumab and cetuximab for the treatment of metastatic colorectal cancer. Health technology assessment (Winchester, England) 2007;11(12):1-128, iii-iv.