Florida International University
FIU Digital Commons

HWCOM Faculty Publications | Herbert Wertheim College of Medicine

5-28-2014

Targeted therapy in advanced metastatic colorectal cancer: Current concepts and perspectives

Florian Hohla
Paracelsus Medical University of Salzburg

Thomas Winder
Medical University of Zurich

Richard Greil
Paracelsus Medical University of Salzburg

Ferenc g. Rick
Herbert Wertheim College of Medicine, Florida International University; Veterans Affairs Medical Center, ferenc.rick@fiu.edu

Norman L. Block
Veterans Affairs Medical Center; University of Miami

See next page for additional authors

NON-COMMERCIAL USE PERMITTED

This work is licensed under a Creative Commons Attribution-Noncommercial 4.0 License
Follow this and additional works at: http://digitalcommons.fiu.edu/com_facpub

Part of the Medicine and Health Sciences Commons

Recommended Citation
Hohla F, Winder T, Greil R, Rick FG, Block NL, Schally AV. Targeted therapy in advanced metastatic colorectal cancer: Current concepts and perspectives. World J Gastroenterol 2014; 20(20): 6102-6112 Available from: URL: http://www.wjgnet. com/1007-9327/full/v20/i20/6102.htm DOI: http://dx.doi. org/10.3748/wjg.v20.i20.6102

This work is brought to you for free and open access by the Herbert Wertheim College of Medicine at FIU Digital Commons. It has been accepted for inclusion in HWCOM Faculty Publications by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

Authors
Florian Hohla, Thomas Winder, Richard Greil, Ferenc g. Rick, Norman L. Block, and Andrew V. Schally

This article is available at FIU Digital Commons: http://digitalcommons.fiu.edu/com_facpub/66

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i20.6102

World J Gastroenterol 2014 May 28; 20(20): 6102-6112
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2014 Baishideng Publishing Group Inc. All rights reserved.

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (5): Colorectal cancer

Targeted therapy in advanced metastatic colorectal cancer: Current concepts and perspectives

Florian Hohla, Thomas Winder, Richard Greil, Ferenc G Rick, Norman L Block, Andrew V Schally

Florian Hohla, Richard Greil, III[rd] Medical Department with Hematology, Medical Oncology, Hemostasis, Rheumatology and Infectious Diseases, Oncologic Center, Center for Clinical Cancer and Immunology Trials, Laboratory of Immunological and Molecular Cancer Research, Paracelsus Medical University of Salzburg, A-5020 Salzburg, Austria
Thomas Winder, Department of Oncology, Medical University of Zurich, 8091 Zurich, Switzerland
Ferenc G Rick, Norman L Block, Andrew V Schally, Endocrine, Polypeptide and Cancer Institute, Veterans Affairs Medical Center and South Florida Veterans Affairs Foundation for Research and Education, Miami, FL 33125, United States
Ferenc G Rick, Department of Urology, Florida International University, Herbert Wertheim College of Medicine, Miami, FL 33199, United States
Norman L Block, Andrew V Schally, Divisions of Hematology/Oncology and Endocrinology, Department of Pathology and Department of Medicine, Miller School of Medicine, University of Miami, Miami, FL 33136, United States
Author contributions: Hohla F, Winder T, Greil R, Rick FG, Block NL and Schally AV wrote the paper; Block NL and Schally AV revised the paper and gave final approval of the version to be published.
Correspondence to: Florian Hohla, MD, III[rd] Medical Department with Hematology, Medical Oncology, Hemostasis, Rheumatology and Infectious Diseases, Oncologic Center, Center for Clinical Cancer and Immunology Trials, Laboratory of Immunological and Molecular Cancer Research, Paracelsus Medical University of Salzburg, Müllner Hauptstrasse 48, A-5020 Salzburg, Austria. f.hohla@salk.at
Telephone: +43-662-448258743 Fax: +43-662-44824103
Received: October 7, 2013 Revised: February 20, 2014
Accepted: March 12, 2014
Published online: May 28, 2014

Abstract

The introduction of new cytotoxic substances as well as agents that target vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) signaling has improved clinical outcome of patients with metastatic colorectal cancer (mCRC). In this review we summarize the most relevant clinical data on VEGF and EGFR targeting regimens in mCRC. The effects of available treatment strategies for mCRC are often temporary, with resistance and disease progression developing in most patients. Thus, new treatment strategies are urgently needed. Some GI peptides including gastrin and gastrin releasing peptide, certain growth factors such as insulin-like growth factor-Ⅰ and Ⅱ and neuropeptides such as growth hormone releasing hormone (GHRH) are implicated in the growth of CRC. Experimental investigations in CRC with antagonistic analogs of bombesin/gastrin-releasing peptide, GHRH, and with cytotoxic peptides that can be targeted to peptide receptors on tumors, are summarized in the second part of the review.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Colorectal cancer; Targeted treatment; Vascular endothelial growth factor; Epidermal growth factor receptor; Peptide receptors; Gastrin-releasing peptide; Growth hormone releasing hormone; Luteinizing hormone-releasing hormone; Cytotoxic analogs

Core tip: Our review evaluates the most recent clinical data on therapeutic reagents designed to target the vascular endothelial growth factor and epidermal growth factor receptor signaling pathways in colorectal cancer. As colorectal cancers express receptors for bombesin/gastrin-releasing peptide, growth hormone-releasing hormone, somatostatin as well as luteinizing hormone-releasing hormone, we review the implications of these pathways in the growth of colorectal cancers and summarize experimental data and clinical studies performed to date with regard to the antiproliferative action of antagonistic peptide analogs of these receptors as well as their cytotoxic analogs and their status as drug candidates for the treatment of metastatic colorectal cancer.

Hohla F, Winder T, Greil R, Rick FG, Block NL, Schally AV. Targeted therapy in advanced metastatic colorectal cancer: Current concepts and perspectives. *World J Gastroenterol* 2014; 20(20): 6102-6112 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i20/6102.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i20.6102

INTRODUCTION

Worldwide estimates of new cases of colorectal cancer (CRC) exceed 1.2 million, with more than 600000 deaths per year[1]. It is estimated that 20% of patients with CRC have metastatic disease at the time of diagnosis; 20%-25% of patients will experience metastases during the course of the disease thus resulting in a relatively high overall mortality rate of 40%-45%[2]. Beside standard chemotherapy (CTX) with 5-fluorouracil (5-FU) based regimens, the incorporation of monoclonal antibodies (mAbs) targeting vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) signaling pathways have further broadened the treatment options for metastatic colorectal cancer (mCRC) patients. Although the survival for all patients with mCRC has improved significantly, the 5-year survival rates still remain low at about 10%, with a median overall survival (OS) of 24 mo. Thus, new approaches to the treatment of mCRC are required. Antagonistic analogs of bombesin/gastrin releasing-hormone (BN/GRP) and growth hormone-releasing hormone (GHRH) as well as targeted cytotoxic analogs of luteinizing hormone-releasing hormone (LHRH) and somatostatin (SST), linked to chemotherapeutic substances, which have been developed in our laboratories over the last two decades, have been shown to be highly effective in suppressing the proliferation of experimental human CRC *in vivo* and *in vitro* and represent an entirely new class of antineoplastic agents for the treatment of mCRC. In the first part of the present review the most recent data on currently available biological agents that target the VEGF (bevacizumab, aflibercept and regorafenib) and EGFR pathways (cetuximab and panitumumab) are highlighted. In the second part, we summarize experimental studies performed so far regarding the antiproliferative action of antagonists of BN/GRP and GHRH as well as cytotoxic analogs of Somatostatin and LHRH against CRC *in vitro* and *in vivo*.

VEGF TARGETING MABS

Bevacizumab

Bevacizumab (Bev), developed in the early 1990s, is a recombinant, humanized IgG1 mAb effective against all isoforms of VEGF-A that disrupts their interactions with VEGFRs[3]. Preclinical studies have demonstrated that Bev exhibits a broad range of antitumor activity[4]. The most relevant clinical studies with Bev in combination with CTX are summarized in Table 1. In the pivotal clinical trial of Hurwitz *et al*[5], designated AVF2107, patients with untreated mCRC were given a combination of irinotecan, bolus 5-FU, leucovorin (LV) (IFL), and placebo or a combination of IFL and Bev (5 mg/kg biweekly). The study showed a significant benefit in overall response (45% *vs* 35%, $P = 0.004$), progression free survival (PFS) (10.6 mo *vs* 6.2 mo, $P < 0.001$), and overall survival (OS) (20.3 mo *vs* 15.5 mo, $P = 0.001$) for mCRC patients treated with Bev. In another phase 3 clinical trial, designated BICC-C, performed by Fuchs *et al*[6], patients with mCRC were randomly assigned to receive one of three different irinotecan-containing regiments (irinotecan plus infusional 5-FU and LV (FOLFIRI), irinotecan plus bolus 5-FU/LV (mIFL) and irinotecan plus oral capecitabine (CapeIRI) (designated as period 1). After a protocol amendment, an additional 117 patients were randomly assigned to FOLFIRI plus Bev or mIFL + Bev, whereas, due to toxicity concerns, further enrollment of CapeIRI was discontinued (designated as period 2). The results for both periods 1 and 2 demonstrated that FOLFIRI and FOLFIRI+Bev offered superior activity to their therapeutic alternatives. Furthermore, patients who received FOLFIRI+Bev showed a higher overall response rate (47% *vs* 54.4%), a longer PFS (11.2 mo *vs* 7.6 mo) and median OS (28 mo *vs* 23.1 mo) compared to FOLFIRI alone. The fact that infusional 5-FU showed a significant longer PFS compared to the oral 5-FU prodrug, capecitabine (7.6 *vs* 5.8, $P = 0.015$), led to the recommendation to preferentially use infused 5-FU, instead of its oral prodrug, in combination with irinotecan. However, a subsequently performed phase Ⅱ trial which assessed the efficacy and safety of Bev plus oral capecitabine and irinotecan or FOLFIRI as first line therapy for patients with mCRC found no difference between the oral and the infused 5-FU regimen regarding the PFS and OS (9 mo and 23 mo)[7]. The convincing results obtained by phase 3 combination studies with irinotecan and Bev led study designers to consider whether Bev could enhance the effect of any CTX regimen. However, subsequent trials with oxaliplatin-based regimens produced less robust differences[8-10]. In the Phase-Ⅲ trial, NO16966, by Saltz *et al*[10], the effect of capecitabine and oxaliplatin (XELOX) compared with those of infused 5-FU, LV and oxaliplatin (FOLFOX), with or without Bev, was evaluated in previously untreated patients with mCRC. Although the difference in PFS and OS (both 1.4 mo) was statistically significant for treatment with Bev and Oxaliplatin based combinations compared to CTX alone, the additional benefit in PFS and OS was smaller for the oxaliplatin based regimen than that achieved in the study of Hurwitz *et al*[5] (4.4 mo and 4.8 mo, respectively). Another Phase-Ⅲ trial performed by Hochster *et al*[9], the TREE study, investigated the tolerability of oxaliplatin in combination with 3 different 5-FU regimens (continuous infusion, bolus and oral) with (TREE-2 cohort) or without (TREE-1 cohort) Bev as a first-line therapy for mCRC. The study showed a benefit in overall response (52% *vs* 41%), PFS (9.9 mo *vs* 8.7 mo) and OS (24.6 mo *vs* 19.2 mo) in patients treated with FOLFOX6 + Bev compared to CTX

Table 1 Effect of bevacizumab in phase Ⅲ Studies in patients with metastatic colorectal cancer

Study	Treatment	Phase	Regimen	Patients (*n*)	Overall response	Median PFS (mo)	Median OS (mo)
Hurwitz *et al*[5], AVF2107 trial, 2004	First-line	3	IFL + Bev	402	45%	10.6	20.3
			vs				
			Placebo	411	35%	6.2	15.5
					$P = 0.004$	$P < 0.001$	$P < 0.001$
Fuchs *et al*[6], BICC-C trial, 2007	First-line	3	FOLFIRI (period 1)	144	47%	7.6	23.1
			FOLFIRI + Bev (period 2)	57	54.4%	11.2	28.0
Saltz *et al*[10], N016966 trial, 2008	First-line	3	FOLFOX-4 or XELOX + Placebo	701	38%	8.0	19.9
			vs				
			FOLFOX-4 or XELOX + Bev	699	38%	9.4	21.3
						$P = 0.0023$	$P = 0.077$
Hochster *et al*[9], TREE1/2 study, 2008	First-line	3	mFOLFOX-6	69	41%	8.7	19.2
			XELOX	48	27%	5.9	17.2
			mFOLFOX-6 + Bev	71	52%	9.9	26.1
			XELOX + Bev	72	36%	10.3	24.6
Giantonio *et al*[11], ECOG E3200, 2007	Second-line	3	FOLFOX + Bev	290	22.7%	7.3	12.9
			Placebo	289	8.6%	4.7	10.8
						$P < 0.0001$	$P = 0.0011$
Bev beyond progression Bennouna *et al*[12], ML18147 (TML), 2012	Second-line	3	Continued use of Bev + standard	409	5.4%	5.7	11.2
			2nd-line CTX *vs* 2nd-line CTX alone	411	3.9%	4.1	9.8
					$P = 0.3113$	$P = 0.0001$	$P = 0.0062$

IFL: Irinotecan/bolus 5-FU/leuvovorin; Bev: Bevacizumab; PFS: Progression free survival; OS: Overall survival; CTX: Chemotherapy.

alone. The addition of Bev to second-line CTX with FOLFOX4, after progression on a CTX regimen without Bev, was evaluated in the ECOG E3200 Phase Ⅲ trial[11]. The addition of Bev to FOLFOX4 improved response rates, PFS and OS in patients whose tumors had already progressed on irinotecan-containing CTX. These findings led to the approval of Bev in combination with CTX as second-line therapy for mCRC. The first randomized Phase Ⅲ trial which investigated the efficacy of Bev therapy continuation beyond progression was the ML18147 (TML) study performed by Bennouna *et al*[12]. In this trial, patients with mCRC who progressed after a Bev containing first-line CTX were randomly assigned to Bev + CTX and CTX alone. Continued use of Bev in combination with a standard 2nd line CTX showed a modest but significant benefit in PFS (5.7 mo *vs* 4.1 mo, $P = 0.0001$) and OS (11.2 mo *vs* 9.8 mo) compared to CTX alone.

Aflibercept

Aflibercept is a recently developed, multiple angiogenic factor trap that prevents not only VEGF-A, but also two additional members of the VEGF family, VEGF-B and placental growth factor (PlGF), from activating their native receptors (VEGFR-1)[13,14]. These findings suggest that upregulation of PlGF and VEGF-B with concurrent activation of VEGFR-1 could be a potential mechanism of tumor resistance to therapies such as Bev, which targets VEGF-A only[15-17]. The VELOUR trial evaluated aflibercept plus FOLFIRI *vs* FOLFIRI alone in patients with mCRC after progression on an oxaliplatin based CTX trial[18]. Addition of Bev significantly improved PFS (6.9 mo *vs* 4.7 mo, $P = 0.0007$) and OS (13.5 mo *vs* 12.06 mo) compared to CTX alone.

Regorafenib

Regorafenib is an inhibitor of PDGF receptors, c-KIT, FGF receptor and VEGF1-3[19]. In the pivotal Phase Ⅲ study, CORRECT, patients with mCRC who had progressed after all approved drugs were randomly assigned to Regorafenib or placebo[20]. Treatment with Regorafenib significantly prolonged OS (6.4 mo *vs* 5.0 mo, $P = 0.0052$) and PFS (1.9 mo *vs* 1.7 mo, HR = 0.49) compared to placebo.

EGFR TARGETING MABS

Cetuximab

Cetuximab is a recombinant, chimeric, human/murine immunglobulin (Ig)G1 mAb that binds specifically to the extracellular domain of EGFR in normal and tumor cells, promoting receptor internalization and degradation without receptor phosphorylation and activation[21]. The most relevant clinical studies with cetuximab in combination with CTX are summarized in Table 2. In the pivotal Phase Ⅱ study, BOND, Cunningham *et al*[22] randomly assigned patients with mCRC, who where refractory to irinotecan based CTX, to either irinotecan and cetuximab or cetuximab alone. The combination of irinotecan with cetuximab significantly improved overall response (22.9% *vs* 10.8%), median PFS (4.1 mo *vs* 1.5 mo) and OS (8.6 mo *vs* 1.5 mo) compared to cetuximab alone. These findings led to the approval of cetuximab for patients with irinotecan refractory CRC, in the United States and Europe, as well as patients who were refractory to other previous therapies. Several small, retrospective studies have shown an association between *KRAS* mutation status and responsiveness of a colorectal tumor to cetuximab[23-26].

Table 2 Effect of cetuximab in phase Ⅱ/Ⅲ studies in patients with metastatic colorectal cancer

Study	Treatment	Phase	Regimen	Patients (*n*)	Overall response	Median PFS (mo)	Median OS (mo)
Cunnigham *et al*[22], BOND study, 2004	Refractory to irinotecan	2	Irinotecan+ cetuximab	218	22.90%	4.1	8.6
			vs				
			cetuximab alone	211	10.80%	1.5	1.5
					P = 0.0074	*P* < 0.0001	*P* = 0.48
Van Cutsem *et al*[28], CRYSTAL trial, 2009	First-line	3	FOLFIRI + cetuximab	105	36.20%	7.6	17.5
			vs				
			placebo	87	40.20%	8.1	17.7
			(K-Ras mutant)		OR = 0.80	HR = 1.07	HR = 1.03
						P = 0.75	
			FOLFIRI + cetuximab	172	59.30%	9.9	24.9
			vs				
			placebo	176	43.20%	8.7	21.0
			(K-Ras wild-type)		OR = 1.91	HR = 0.68	HR = 0.84
					P = 0.004	*P* = 0.048	
Bokemeyer *et al*[29,30], OPUS trial, 2008	First-line	2	FOLFOX + cetuximab	52	33%	8.6	NR
			vs				
			placebo	47	49%	5.5	NR
			(K-Ras mutant)		OR = 0.507	HR = 1.83	
					P = 0.106	*P* = 0.0192	
			FOLFOX + cetuximab	61	61%	7.7	NR
			vs				
			placebo	73	37%	7.2	NR
			(K-Ras wild-type)		OR = 2.54	HR = 0.57	
					P = 0.011	*P* = 0.016	
Heinemann *et al*[31], FIRE-3, 2013	First line	3	FOLFIRI + cetuximab	297	62%	10.3	28.7
			FOLFIRI + bevacizumab	295	57%	10.4	25.0
					OR = 1.249	HR = 1.04	HR = 0.77
					P = 0.18	*P* = 0.69	*P* = 0.017

PFS: Progression free survival; OS: Overall survival; NR: Not reported.

In the study of Karapetis *et al*[27] patients with mCRC refractory to standard treatment were randomly assigned to receive Cetuximab plus best supportive care (BSC) or BSC alone, to detect activating mutations in exon 2 of the *KRAS* gene. Patients with tumors expressing mutant KRAS did not respond to cetuximab (overall response rate 1.2%), whereas patients with tumors harboring a wild-type KRAS did benefit from cetuximab compared to BSC alone in terms of overall response rate (12.8% *vs* 0%), PFS (3.7 mo *vs* 1.9 mo, HR = 0.4, $P < 0.001$) and OS (9.5 mo *vs* 4.8 mo, HR = 0.55, $P < 0.001$). In the patient cohort receiving BSC alone, the mutation status of the *KRAS* gene was not significantly associated with OS (HR = 1.01)[27]. Retrospective analysis of the KRAS status in the CRYSTAL trial has recently shown statistically significant differences between patients with wild-type KRAS and those with mutant *KRAS* in response to FOLFIRI plus cetuximab in terms of PFS (9.9 mo *vs* 7.6 mo) and overall response (59% *vs* 36%)[28]. In KRAS wild-type patients, treatment of mCRC patients with FOLFIRI plus cetuximab *vs* FOLFIRI alone significantly prolonged OS (24.9 *vs* 21.0, HR = 0.84)[28]. Data from the OPUS trial showed that the combination of cetuximab and FOLFOX4 has an overall response rate of 61% in patients with wild-type KRAS compared with 33% in those with mutant *KRAS*[29,30]. In the Phase Ⅲ study FIRE-3 by Heinemann *et al*[31], patients with mCRC were randomly assigned to FOLFIRI plus either Cetuximab or Bev. Patients in the cetuximab and Bev arms had similar times to disease progression (10 mo *vs* 10.3 mo), but those treated with cetuximab had a significant improved OS (28.7 mo *vs* 25 mo, HR = 0.77, $P = 0.01$).

Panitumumab

Panitumumab (Vectibix®) is a fully human, recombinant IgG2 mAB that binds specifically and with high affinity to the extracellular domain of EGFR in normal and tumor cells. Through competitive binding to EGFR ligands, panitumumab prevents EGFR dimerization, autophosphorylation and signaling, thereby inhibiting proliferation and promoting apoptosis[32]. The most relevant clinical studies with panitumumab in combination with CTX are summarized in Table 3. In a phase-3 trial Van Cutsem *et al*[33] randomly assigned patients refractory to standard treatment, to treatment with panitumumab and BSC *vs* BSC alone. Objective response rates favored panitumumab over BSC (10% *vs* 0%). Panitumumab significantly prolonged PFS (8 wk *vs* 7.3 wk, HR = 0.54) but did not influence OS (HR = 1.00). A Phase Ⅲ study, PRIME, evaluated the combination of panitumumab with FOLFOX4 *vs* FOLFOX4 alone as first-line treatment of metastatic CRC[34]. The combination therapy significantly improved PFS compared to CTX alone in patients with KRAS wild type (9.6 mo *vs* 8.0 mo, *P* =

Table 3 Effect of panitumumab in phase Ⅲ studies in patients with metastatic colorectal cancer

Study	Treatment	Phase	Regimen	Patients (*n*)	Overall response	Median PFS (mo)	Median OS (mo)
Van Cutsem *et al*[33], 2007	Refractory to standard CTX	3	Panitumumab + BSC	231	10%		
			vs				
			BSC	232	0%	HR = 0.54	HR = 1.0
					$P < 0.0001$		
Douillard *et al*[34], PRIME-trial, 2010	First-line	3	K-Ras WT				
			FOLFOX4 + panitumumab	325	55%	9.6	23.9
			FOLFOX4	331	48%	8.0	19.7
					OR = 1.35	HR = 0.8	HR = 0.83
					$P = 0.068$	$P = 0.02$	$P = 0.072$
			K-Ras MT				
			FOLFOX4 + panitumumab	221	40%	7.3	15.5
			FOLFOX	219	40%	8.8	19.3
						HR = 1.29	HR = 1.24
						$P = 0.02$	$P = 0.068$
Peeters *et al*[35], 2010	Second-line	3	K-Ras WT				
			FOLFIRI + panitumumab	303	35%	5.9	14.5
			FOLFIRI	294	10%	3.9	12.5
					$P = 0.001$	HR = 0.73	HR = 0.85
						$P = 0.004$	$P = 0.12$
			K-Ras MT				
			FOLFIRI + panitumumab	238	13%	5.0	11.8
			FOLFIRI	248	14%	4.9	11.1
						HR = 0.85	HR = 0.94
						$P = 0.14$	
Douillard *et al*[36], Update Prime-trial, 2013	First-line	3	K-Ras WT/MT other Ras				
			FOLFOX4 + panitumumab	51	NR	7.3	17.1
			FOLFOX4	57	NR	8.0	18.3
						HR = 1.28	HR = 1.29
						$P = 0.326$	$P = 0.305$
			K-Ras + N-Ras WT				
			FOLFOX4 + panitumumab	259	NR	10.1	26.0
			FOLFOX	253	NR	7.9	20.2
						HR = 0.72	HR = 0.78
						$P = 0.004$	$P = 0.043$
Schwartzberg *et al*[37], PEAK-trial, 2013	First-line	2	K-Ras WT/MT other RAS				Not
			mFOLFOX6 + panitumumab	142	NR	10.9	Reached
			mFOLFOX6 + bevacizumab	143	NR	10.1	25.4
						HR = 0.87	HR = 0.72
						$P = 0.35$	$P = 0.14$
			K-Ras / N-RAS WT				Not
			mFOLFOX6 + panitumumab	88	NR	13.0	Reached
			mFOLFOX6 + bevacizumab				
				82	NR	9.5	29.0
						HR = 0.65	HR = 0.61
						$P = 0.03$	$P = 0.09$

PFS: Progression free survival; OS: Overall survival; BSC: Best supportive care; CTX: Chemotherapy; WT: Wild-type; MT: Mutant; NR: Not reported.

0.02) and increased overall response rates (55% *vs* 48%). A non-significant increase in OS was also observed for panitumumab-FOLFOX4 *vs* CTX alone (23.9 mo *vs* 19.7 mo, respectively, $P = 0.072$). Peeters *et al*[35] randomly assigned patients with mCRC pretreated with one CTX, to panitumumab plus FOLFIRI *vs* FOLFIRI alone. In wild-type KRAS exon 2 mCRC patients a significant improvement in PFS (5.9 mo *vs* 3.9 mo, $P = 0.004$) and response rates (35% *vs* 10%) was observed with the addition of panitumumab compared to CTX alone. In patients with mutant *KRAS* exon 2, there was no difference in efficacy. In order to assess the efficacy and safety of panitumumab plus FOLFOX4 as compared with FOLFOX4 alone according to the *KRAS* (exon 2-4) and *NRAS* (exon 2-4) mutation status data of the PRIME study were updated[36]. In patients without any *RAS* mutation (*KRAS* 2-4/*NRAS* exon 2-4 wild-type) treatment with panitumumab significantly prolonged PFS (10.1 mo *vs* 7.9 mo, $P = 0.004$) and OS (26.0 mo *vs* 20.2 mo, $P = 0.043$) compared to CTX alone. In the trial designated PEAK, Schwartzberg *et al*[37] randomly assigned untreated patients with mCRC to FOLFOX4 plus either panitumumab or Bev. Again, RAS status was assessed. In RAS wild-type stratum combination of panitumumab with FOLFOX4 improved PFS (13.5 mo *vs* 9.5 mo, HR = 0.65, $P = 0.03$) and OS (HR = 0.61, $P = 0.09$) compared to Bev with the

same combination.

ANTIPROLIFERATIVE EFFECT OF BN/GRP ANTAGONISTS IN CRC

In addition to polypeptide growth factors, such as EGF family members, much evidence supports the autocrine involvement of specific neuropeptides, such as gastrin-releasing peptide (GRP), in the proliferation, local invasion, metastasis and angiogenesis of many tumors including CRC[38-42]. GRP is a member of the bombesin (BN)-like peptide family and normally functions as a gastrointestinal hormone and neurotransmitter[43]. From an oncologic point of view, GRP affects the growth and differentiation of a number of human tumors including CRC[3,40,41,44-46]. Four receptor subtypes associated with the BN-like peptide family have been identified and cloned[38,47]. Receptor subtype 1, termed GRP-R, binds BN and GRP with high affinity. Subtype 2 prefers neuromedin B and subtype 3 is classified as an orphan receptor because its natural ligand is not yet identified. A fourth subtype has a higher affinity for amphibian BN than for GRP. These receptors are coupled to G-protein via their intracellular domains and, thus, belong to the G-protein receptor superfamily. Studies have shown that receptors for GRP (GRP-Rs) are overexpressed in human CRC and human CRC cell lines when compared with normal colonic epithelial cells[48-55]. Approaches to inhibit the autocrine growth effect of GRP-like peptides on tumor growth in human and animal studies include receptor antagonists, monoclonal antibodies, vaccination against GRP, antisense oligonucleotides or bispecific molecules[56]. During the past decade, a large number of BN/GRP antagonists were synthesized in our laboratories. Among these compounds were RC-3095 and RC-3940-Ⅱ, both of which showed strong inhibitory effects on several experimental cancers including CRC *in vitro* and in mouse xenografts *in vivo*[40,41,54,57-60]. The tumor-inhibitory mechanism of BN/GRP antagonists appears to be more complex than a simple competitive action on the receptor and is incompletely understood[40,56]. In xenografts of HT-29 human CRC inhibition of tumor growth by BN/GRP antagonist, RC-3095, was linked with a significant down-regulation of EGF receptors[61]. In another experiment we showed that combined treatment with RC-3940-Ⅱ and a chemotherapeutic agent, such as 5-FU or irinotecan, resulted in a synergistic growth inhibition of experimental human colon cancers xenografted into nude mice[54]. Cell cycle analysis of *in vitro* material revealed that BN/GRP antagonist, RC-3940-Ⅱ, led to an increase in the number of cells blocked in S and G_2/M phase and fewer cells with G_0/G_1 DNA content[54]. A Phase I clinical trial with BN/GRP antagonist, RC-3095, in 25 heavily pretreated patients with advanced solid malignancies, including 2 patients with mCRC, showed no objective tumor response at the dosage used[62]. In conclusion, BN/GRP antagonists have shown impressive preclinical antitumor activity and should be further investigated in clinical trials.

ANTIPROLIFERATIVE EFFECT OF GHRH ANTAGONISTS IN CRC

Growth hormone-releasing hormone (GHRH) belongs to the family of related peptides that includes: vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), secretin and glucagon[63]. GHRH released by the hypothalamus, regulates the secretion of growth hormone (GH) by binding to specific receptors for GHRH (GHRH-R) in the pituitary gland[40,41,64]. In turn, GH induces the production of hepatic insulin-like growth factor (IGF-1), which is a known mitogen and has been linked with malignant transformation, tumor progression, and tumor metastasis[65]. In addition to its neuroendocrine action, GHRH functions as an autocrine/paracrine growth factor in various cancers, including CRC[40,66]. Antagonistic analogs of GHRH, developed our laboratories, strongly suppress the growth *in vitro* and *in vivo* of many experimental cancers including CRC[40,41,54,64,66-68]. The antitumor effects of GHRH antagonists were initially thought to be exerted only indirectly through the inhibition of serum IGF-I levels. However, evidence suggested, that the principal antiproliferative effects of GHRH antagonists are exerted directly through the blocking of the stimulatory loop formed by GHRH and its receptors on tumor cells. Our group demonstrated the presence of the pituitary type GHRH-receptor and four truncated splice variants (SVs) of GHRH-R in human cancer specimens and cancer cell lines including CRC[40,66,69,70]. Of the four isoforms, SV1 has the greatest structural similarity to the GHRH-R and it probably mediates, in concert with GHRH-R, the effect of GHRH and its antagonists on tumors. We also examined the protein and mRNA expression of GHRH-R and SV1 in normal human tissues and human CRC tissue by immunohistochemical staining and RT-PCR[70]. The main finding was that the expression of GHRH-R and SV1 was absent in normal colonic mucosa but significantly increased in tubulovillous adenomas and in colorectal cancers. We assume that this aberrant expression of GHRH-R and SV-1 in colorectal cancers may provide a molecular target for a therapeutic approach based on GHRH antagonists[70]. We showed that GHRH antagonist, JMR-132, significantly decreased the volume of HT-29, HCT-116, and HCT-15 experimental human colon carcinomas grown as xenografts in athymic nude mice by up to 75% and also extended tumor doubling times compared to controls[67]. In other studies, combined treatment *in vivo* with JMR-132 plus chemotherapeutic agents 5-FU, irinotecan or cisplatin resulted in an additive tumor growth suppression of HT-29, HCT-116 and HCT-15 human colon cancer xenografts[68]. Cell cycle analysis revealed that treatment of HCT-116 human colon cancer cells with GHRH antagonist, JMR-132, *in vitro* was accompanied by a cell cycle arrest in S-phase. Thus, we suggest that JMR-132 enhances antiproliferative effects of S-phase specific cytotoxic drugs by causing accumulation of tumor cells in S-phase[68]. The molecular mechanisms

involved in the antiproliferative effects of GHRH antagonists on tumor cells have not been completely elucidated. We showed in HCT-116 human colon cancer cells *in vitro*, that treatment with GHRH antagonist, JMR-132, causes significant DNA damage as measured by an increase in olive tail moment and loss of inner mitochondrial membrane potential. Western blotting demonstrated a time-dependent increase in protein levels of phosphorylated p53(Ser46), Bax, cleaved caspase-9, -3, cleavage of PARP and a decrease in Bcl-2 levels[67]. Also, an augmentation in cell cycle checkpoint protein $p21^{Waf1/Cip1}$ was accompanied by a cell cycle arrest in S-phase. DNA fragmentation visualized by the comet assay and by the number of apoptotic cells increased time dependently as determined by flow cytometric annexin-V and PI staining assays. Thus we suggest that GHRH antagonists exert their antiproliferative effects on experimental colon cancer cells through $p21^{Waf1/Cip1}$ mediated S-phase arrest along with apoptosis involving the intrinsic pathway[67]. So far GHRH antagonists have not been clinically tested. However, the impressive preclinical activity merits further investigations in clinical trials.

ANTIPROLIFERATIVE EFFECT OF CYTOTOXIC ANALOGS OF SOMATOSTATIN, BN/GRP AND LHRH IN CRC

On the basis of the presence of specific receptors for hypothalamic peptides on various human cancers including CRC, our group developed targeted cytotoxic analogs of somatostatin (SST) and LHRH linked to doxorubicin or 2-pyrrolinodoxorubicin[71,72].

Cytotoxic somatostatin analogs, AN-238 and AN-162

The hypothalamic neuropeptide SST exists in two main active forms: a 14-amino acid peptide and an amino terminally extended version consisting of 28 amino acids[41]. Both forms are present in the gastrointestinal tract inhibiting the secretion of many hormones including growth hormone, insulin, glucagon, gastrin, secretin and cholecystokinin[41]. At least five distinct SST receptor subtypes, $SSTR_{1-5}$, have been characterized[73,74]. These receptors are distributed in both normal and cancerous tissues, but found in higher density in the latter as well specifically as in human colon cancer cell lines[40,41,75,76]. While native SST shows high affinities to $SSTR_{1-5}$, synthetic octapeptides such as RC-160 and RC-121, synthesized in our laboratory, bind preferentially to $SSTR_2$ and $SSTR_5$, moderately affinity to SST_3 and with low affinity to SST_1 and SST_4[40,73,74,77]. In our endeavour to develop chemotherapy targeted to SSTR, we synthesized two cytotoxic hybrids of SST, AN-238, AN-162, containing DOX or the strongly active derivative of DOX, 2-pyrrolino-DOX, the latter conjugated to the octapeptide SST analog, RC-121[78]. Both cytotoxic analogs AN-238 (containing 2-pyrrolino-DOX) and AN-162 (containing DOX), significantly inhibited tumor growth of experimental human colon cancer xenografted into nude mice[75,76]. Cell cycle analysis showed that treatment of HCT-116 human colon cancer cells with AN-162 caused a significantly greater increase in the number of S-phase cells and apoptotic cells as compared to treatment with doxorubicin alone[75]. We hypothesize that the lesser effect of unconjugated doxorubicin compared to AN-162 could be the reduction of intracellular drug accumulation caused by increased drug efflux when Dox alone is used. Cellular resistance (multi drug resistance, MDR) to doxorubicin is often related to its rapid efflux from the intracellular environment by membrane transporters termed p-glycoproteins (Pgp), products of the multiple drug resistance gene 1 (*MDR-1*). To proof this concept, we treated the doxorubicin resistant mouse leukemic cell line P388/R84, which overexpresses the membrane transporter Pgp, with AN-162 and compared to unconjugated doxorubicin. Cell cycle analysis revealed that AN-162 compared to doxorubicin caused a progressive accumulation of P388/R84 cells in S and G2 phase with an increase in the number of apoptotic cells with $< G_0/G_1$ content[75]. Thus, treatment efficacy with targeted cytotoxic peptides may be related to overcoming chemoresistance.

Cytotoxic LH/RH analogs

The hypothalamic hormone, LHRH, also known as gonadotropin-releasing hormone is the primary regulator of gonadal function and reproduction in vertebrates[79]. Receptors for LHRH have been demonstrated in healthy sex organs, as well as in breast, ovarian, endometrial and prostate cancers and cell lines of colorectal cancer[71,72,80]. On the basis of the presence of receptors for LHRH on these tumors, we have developed a new class of targeted antitumor agents, AN-152 (AEZS-108) and AN-207, by linking cytotoxic radicals to LHRH agonists[72]. Thus Dox was coupled to LHRH to form the targeted cytotoxic analog AN-152 (AEZS-108). An even more potent hybrid molecule, AN-207, was synthesized by conjugating 2-pyrrolino-Dox to LHRH. Both cytotoxic LHRH analogs, AN-152 and AN-207, powerfully inhibited growth of experimental colon cancers xenografted into nude mice[80]. AN-152 (AEZS-108) has been successfully tested in one Phase I and two Phase II studies in patients with heavily pretreated LHRH-R positive recurrent ovarian and endometrial cancers[71]. Phase I / II studies with AEZS-108 in castration-resistant prostate cancer and refractory bladder cancer are presently in progress with promising results[71]. In our experimental studies, all 5 human CRC cell lines evaluated expressed LHRH receptors[80]. Currently, there are no clinical data on the expression of LBHRH receptors in CRC. However, a common practice in clinical trials with cytotoxic LHRH analog AN-152 on prostatic, bladder, ovarian and endometrial cancers is to first evaluate the expression of the LHRH receptor in the tumors of patients by immunohistochemistry. Cytotoxic LHRH analog, AEZS-108, may be a useful agent for the treatment of LHRH receptor positive advanced colorectal

carcinoma. On the basis of our results, patients with mCRC could be considered for the inclusion in future clinical trials with cytotoxic LHRH analog AEZS-108, after establishing the presence of LHRH receptors in biopsy samples.

CONCLUSION

The current management of mCRC involves various active drugs, either in combination or as single agents: 5-FU/LV, capecitabine, irinotecan, oxaliplatin, bevacizumab, aflibercept, regorafenib, cetuximab and panitumumab. The choice of therapy is based on consideration of the goals of therapy, the type and timing of prior therapy, the different toxicity profiles of the constituent drugs and the molecular characteristics of the tumor. Treatment regimens with Bev are independent of the RAS mutation status and show greater response rates, up to 10%, and significantly longer PFS and OS in combination with an irinotecan based CTX. Treatment of Bev with oxaliplatin based regimens seems to have a more moderate benefit in PFS and OS. Beyond progression after a Bev containing regimen, continued use of Bev in combination with a standard second-line CTX significantly improves PFS and OS. Bev or aflibercept, when given with second-line CTX, have comparable outcomes, each adding 1.4 mo of survival time. Regorafenib has been approved as a treatment option for patients with good performance status and who have received all available agents leading to a modest OS advantage of 1.4 mo. Recently published data from the FIRE-3, PRIME and the PEAK trial suggest, that cetuximab based regimens may lead to improved OS compared to Bev containing regimens. The observed survival benefit of EGFR targeting agents may be partially a result of excluding patients with mutated *RAS* metastatic colorectal cancers as performed in the PEAK trial and the updated PRIME study. Therefore, especially for patients with RAS wild-type mCRC a cetuximab-based treatment may be more beneficial and should be offered as first line therapy. However, response to treatment is usually temporary in patients with mCRC and leads to a median survival of 24 mo. Thus receptors for certain peptide hormones, which are highly expressed in CRC, may be investigated as therapeutic targets. Targeted cytotoxic LHRH analog AN-152 (AEZS-108), should be examined for treatment of patients with LHRH receptor positive CRC.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Song X**, Zhao Z, Barber B, Gregory C, Schutt D, Gao S. Characterizing medical care by disease phase in metastatic colorectal cancer. *Am J Manag Care* 2011; **17** Suppl 5 Developing: SP20-SP25 [PMID: 21711074]

3 **Kim KJ**, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* 1993; **362**: 841-844 [PMID: 7683111 DOI: 10.1038/362841a0]

4 **Gerber HP**, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. *Cancer Res* 2005; **65**: 671-680 [PMID: 15705858]

5 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435 DOI: 10.1056/NEJMoa032691]

6 **Fuchs CS**, Marshall J, Mitchell E, Wierzbicki R, Ganju V, Jeffery M, Schulz J, Richards D, Soufi-Mahjoubi R, Wang B, Barrueco J. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. *J Clin Oncol* 2007; **25**: 4779-4786 [PMID: 17947725 DOI: 10.1200/JCO.2007.11.3357]

7 **Ducreux M**, Adenis A, Pignon JP, François E, Chauffert B, Ichanté JL, Boucher E, Ychou M, Pierga JY, Montoto-Grillot C, Conroy T. Efficacy and safety of bevacizumab-based combination regimens in patients with previously untreated metastatic colorectal cancer: final results from a randomised phase II study of bevacizumab plus 5-fluorouracil, leucovorin plus irinotecan versus bevacizumab plus capecitabine plus irinotecan (FNCLCC ACCORD 13/0503 study). *Eur J Cancer* 2013; **49**: 1236-1245 [PMID: 23352604 DOI: 10.1016/j.ejca.2012.12.011]

8 **Cassidy J**, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Rittweger K, Gilberg F, Saltz L. XELOX vs FOLFOX-4 as first-line therapy for metastatic colorectal cancer: NO16966 updated results. *Br J Cancer* 2011; **105**: 58-64 [PMID: 21673685 DOI: 10.1038/bjc.2011.201]

9 **Hochster HS**, Hart LL, Ramanathan RK, Childs BH, Hainsworth JD, Cohn AL, Wong L, Fehrenbacher L, Abubakr Y, Saif MW, Schwartzberg L, Hedrick E. 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**: 3523-3529 [PMID: 18640933 DOI: 10.1200/JCO.2007.15.4138]

10 **Saltz LB**, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzén F, Cassidy J. 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**: 2013-2019 [PMID: 18421054 DOI: 10.1200/JCO.2007.14.9930]

11 **Giantonio BJ**, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, Schwartz MA, Benson AB. 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; **25**: 1539-1544 [PMID: 17442997 DOI: 10.1200/JCO.2006.09.6305]

12 **Bennouna J**, Sastre J, Arnold D, Österlund P, Greil R, Van Cutsem E, von Moos R, Viéitez JM, Bouché O, Borg C, Steffens CC, Alonso-Orduña V, Schlichting C, Reyes-Rivera I, Bendahmane B, André T, Kubicka S. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. *Lancet Oncol* 2013; **14**: 29-37 [PMID: 23168366 DOI: 10.1016/S1470-2045(12)70477-1]

13 **Chu QS**. Aflibercept (AVE0005): an alternative strategy for inhibiting tumour angiogenesis by vascular endothelial growth factors. *Expert Opin Biol Ther* 2009; **9**: 263-271 [PMID: 19236257 DOI: 10.1517/14712590802666397]

14 **Mitchell EP**. Targeted therapy for metastatic colorectal cancer: role of aflibercept. *Clin Colorectal Cancer* 2013; **12**: 73-85 [PMID: 23102896 DOI: 10.1016/j.clcc.2012.08.001]

15 **Rini BI**, Michaelson MD, Rosenberg JE, Bukowski RM, Sos-

man JA, Stadler WM, Hutson TE, Margolin K, Harmon CS, DePrimo SE, Kim ST, Chen I, George DJ. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. *J Clin Oncol* 2008; **26**: 3743-3748 [PMID: 18669461 DOI: 10.1200/JCO.2007.15.5416]

16 **Taylor AP**, Osorio L, Craig R, Raleigh JA, Ying Z, Goldenberg DM, Blumenthal RD. Tumor-specific regulation of angiogenic growth factors and their receptors during recovery from cytotoxic therapy. *Clin Cancer Res* 2002; **8**: 1213-1222 [PMID: 11948135]

17 **Cao Y**. Positive and negative modulation of angiogenesis by VEGFR1 ligands. *Sci Signal* 2009; **2**: re1 [PMID: 19244214 DOI: 10.1126/scisignal.259re1]

18 **Van Cutsem E**, Tabernero J, Lakomy R, Prenen H, Prausová J, Macarulla T, Ruff P, van Hazel GA, Moiseyenko V, Ferry D, McKendrick J, Polikoff J, Tellier A, Castan R, Allegra C. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol* 2012; **30**: 3499-3506 [PMID: 22949147 DOI: 10.1200/JCO.2012.42.8201]

19 **Wilhelm SM**, Dumas J, Adnane L, Lynch M, Carter CA, Schütz G, Thierauch KH, Zopf D. Regorafenib (BAY 73-4506): a new oral multikinase inhibitor of angiogenic, stromal and oncogenic receptor tyrosine kinases with potent preclinical antitumor activity. *Int J Cancer* 2011; **129**: 245-255 [PMID: 21170960 DOI: 10.1002/ijc.25864]

20 **Grothey A**, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 303-312 [PMID: 23177514 DOI: 10.1016/S0140-6736(12)61900-X]

21 **Tabernero J**. The role of VEGF and EGFR inhibition: implications for combining anti-VEGF and anti-EGFR agents. *Mol Cancer Res* 2007; **5**: 203-220 [PMID: 17374728 DOI: 10.1158/1541-7786.MCR-06-0404]

22 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313 DOI: 10.1056/NEJMoa033025]

23 **De Roock W**, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, Biesmans B, Van Laethem JL, Peeters M, Humblet Y, Van Cutsem E, Tejpar S. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008; **19**: 508-515 [PMID: 17998284 DOI: 10.1093/annonc/mdm496]

24 **Di Fiore F**, Blanchard F, Charbonnier F, Le Pessot F, Lamy A, Galais MP, Bastit L, Killian A, Sesboüé R, Tuech JJ, Queuniet AM, Paillot B, Sabourin JC, Michot F, Michel P, Frebourg T. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy. *Br J Cancer* 2007; **96**: 1166-1169 [PMID: 17375050 DOI: 10.1038/sj.bjc.6603685]

25 **Frattini M**, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M, Camponovo A, Etienne LL, Cavalli F, Mazzucchelli L. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer* 2007; **97**: 1139-1145 [PMID: 17940504 DOI: 10.1038/sj.bjc.6604009]

26 **Lièvre A**, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, Ychou M, Bouché O, Landi B, Louvet C, André T, Bibeau F, Diebold MD, Rougier P, Ducreux M, Tomasic G, Emile JF, Penault-Llorca F, Laurent-Puig P. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008; **26**: 374-379 [PMID: 18202412 DOI: 10.1200/JCO.2007.12.5906]

27 **Karapetis CS**, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; **359**: 1757-1765 [PMID: 18946061 DOI: 10.1056/NEJMoa0804385]

28 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]

29 **Bokemeyer C**, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, Celik I, Schlichting M, Koralewski P. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 2011; **22**: 1535-1546 [PMID: 21228335 DOI: 10.1093/annonc/mdq632]

30 **Bokemeyer C**, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donea S, Ludwig H, Schuch G, Stroh C, Loos AH, Zubel A, Koralewski P. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 663-671 [PMID: 19114683 DOI: 10.1200/JCO.2008.20.8397]

31 **Heinemann V**, Fischer von Weikersthal L, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, Heintges T, Lerchenmueller J, Kahl C, Stintzing S. Randomized comparison of FOLFIRI plus cetuximab vs FOLFIRI plus bevacizumab as first-line treatment of KRAS wild-type metastatic colorectal cancer: German AIO study KRK-0306 (FIRE-3). *J Clin Oncol* 2013; **31**: abstract LBA3506

32 **Gravalos C**, Cassinello J, García-Alfonso P, Jimeno A. Integration of panitumumab into the treatment of colorectal cancer. *Crit Rev Oncol Hematol* 2010; **74**: 16-26 [PMID: 19616446 DOI: 10.1016/j.critrevonc.2009.06.005]

33 **Van Cutsem E**, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, Canon JL, Van Laethem JL, Maurel J, Richardson G, Wolf M, Amado RG. 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**: 1658-1664 [PMID: 17470858 DOI: 10.1200/JCO.2006.08.1620]

34 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010; **28**: 4697-4705 [PMID: 20921465 DOI: 10.1200/JCO.2009.27.4860]

35 **Peeters M**, Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, André T, Chan E, Lordick F, Punt CJ, Strickland AH, Wilson G, Ciuleanu TE, Roman L, Van Cutsem E, Tzekova V, Collins S, Oliner KS, Rong A, Gansert J. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol* 2010; **28**: 4706-4713 [PMID: 20921462 DOI: 10.1200/JCO.2009.27.6055]

36 **Douillard JY**, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec

M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; **369**: 1023-1034 [PMID: 24024839 DOI: 10.1056/NEJMoa1305275]

37 **Schwartzberg LS**, Rivera F, Karthaus M, Fasola G, Canon JL, Yu H, Oliner KS, Go WY. Analysis of KRAS/NRAS mutations in PEAK: A randomized phase II study of FOLFOX6 plus panitumumab (pmab) or bevacizumab (bev) as first-line treatment (tx) for wild-type (WT) KRAS (exon 2) metastatic colorectal cancer (mCRC). *J Clin Oncol* 2013; **31**: abstr 3631

38 **Jensen RT**, Battey JF, Spindel ER, Benya RV. International Union of Pharmacology. LXVIII. Mammalian bombesin receptors: nomenclature, distribution, pharmacology, signaling, and functions in normal and disease states. *Pharmacol Rev* 2008; **60**: 1-42 [PMID: 18055507 DOI: 10.1124/pr.107.07108]

39 **Patel O**, Shulkes A, Baldwin GS. Gastrin-releasing peptide and cancer. *Biochim Biophys Acta* 2006; **1766**: 23-41 [PMID: 16490321 DOI: 10.1016/j.bbcan.2006.01.003]

40 **Schally AV**, Comaru-Schally AM, Nagy A, Kovacs M, Szepeshazi K, Plonowski A, Varga JL, Halmos G. Hypothalamic hormones and cancer. *Front Neuroendocrinol* 2001; **22**: 248-291 [PMID: 11587553 DOI: 10.1006/frne.2001.0217]

41 **Schally AV**, Szepeshazi K, Nagy A, Comaru-Schally AM, Halmos G. New approaches to therapy of cancers of the stomach, colon and pancreas based on peptide analogs. *Cell Mol Life Sci* 2004; **61**: 1042-1068 [PMID: 15112052 DOI: 10.1007/s00018-004-3434-3]

42 **Corral RS**, Iñiguez MA, Duque J, López-Pérez R, Fresno M. Bombesin induces cyclooxygenase-2 expression through the activation of the nuclear factor of activated T cells and enhances cell migration in Caco-2 colon carcinoma cells. *Oncogene* 2007; **26**: 958-969 [PMID: 16909108 DOI: 10.1038/sj.onc.1209856]

43 **Thomas RP**, Hellmich MR, Townsend CM, Evers BM. Role of gastrointestinal hormones in the proliferation of normal and neoplastic tissues. *Endocr Rev* 2003; **24**: 571-599 [PMID: 14570743]

44 **Cuttitta F**, Carney DN, Mulshine J, Moody TW, Fedorko J, Fischler A, Minna JD. Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. *Nature* 1985; **316**: 823-826 [PMID: 2993906]

45 **Glover SC**, Tretiakova MS, Carroll RE, Benya RV. Increased frequency of gastrin-releasing peptide receptor gene mutations during colon-adenocarcinoma progression. *Mol Carcinog* 2003; **37**: 5-15 [PMID: 12720295 DOI: 10.1002/mc.10117]

46 **Jensen JA**, Carroll RE, Benya RV. The case for gastrin-releasing peptide acting as a morphogen when it and its receptor are aberrantly expressed in cancer. *Peptides* 2001; **22**: 689-699 [PMID: 11311741]

47 **Spindel ER**, Giladi E, Segerson TP, Nagalla S. Bombesin-like peptides: of ligands and receptors. *Recent Prog Horm Res* 1993; **48**: 365-391 [PMID: 8382830]

48 **Carroll RE**, Matkowskyj KA, Chakrabarti S, McDonald TJ, Benya RV. Aberrant expression of gastrin-releasing peptide and its receptor by well-differentiated colon cancers in humans. *Am J Physiol* 1999; **276**: G655-G665 [PMID: 10070042]

49 **Chave HS**, Gough AC, Palmer K, Preston SR, Primrose JN. Bombesin family receptor and ligand gene expression in human colorectal cancer and normal mucosa. *Br J Cancer* 2000; **82**: 124-130 [PMID: 10638978 DOI: 10.1054/bjoc.1998.0888]

50 **Ferris HA**, Carroll RE, Lorimer DL, Benya RV. Location and characterization of the human GRP receptor expressed by gastrointestinal epithelial cells. *Peptides* 1997; **18**: 663-672 [PMID: 9213359]

51 **Patel O**, Dumesny C, Giraud AS, Baldwin GS, Shulkes A. Stimulation of proliferation and migration of a colorectal cancer cell line by amidated and glycine-extended gastrin-releasing peptide via the same receptor. *Biochem Pharmacol* 2004; **68**: 2129-2142 [PMID: 15498503 DOI: 10.1016/j.bcp.2004.08.009]

52 **Preston SR**, Woodhouse LF, Jones-Blackett S, Miller GV, Primrose JN. High-affinity binding sites for gastrin-releasing peptide on human colorectal cancer tissue but not uninvolved mucosa. *Br J Cancer* 1995; **71**: 1087-1089 [PMID: 7734305]

53 **Radulovic SS**, Milovanovic SR, Cai RZ, Schally AV. The binding of bombesin and somatostatin and their analogs to human colon cancers. *Proc Soc Exp Biol Med* 1992; **200**: 394-401 [PMID: 1352046]

54 **Rick FG**, Buchholz S, Schally AV, Szalontay L, Krishan A, Datz C, Stadlmayr A, Aigner E, Perez R, Seitz S, Block NL, Hohla F. Combination of gastrin-releasing peptide antagonist with cytotoxic agents produces synergistic inhibition of growth of human experimental colon cancers. *Cell Cycle* 2012; **11**: 2518-2525 [PMID: 22751419 DOI: 10.4161/cc.20900]

55 **Saurin JC**, Rouault JP, Abello J, Berger F, Remy L, Chayvialle JA. High gastrin releasing peptide receptor mRNA level is related to tumour dedifferentiation and lymphatic vessel invasion in human colon cancer. *Eur J Cancer* 1999; **35**: 125-132 [PMID: 10211100]

56 **Hohla F**, Schally AV. Targeting gastrin releasing peptide receptors: New options for the therapy and diagnosis of cancer. *Cell Cycle* 2010; **9**: 1738-1741 [PMID: 20473035]

57 **Casanueva FF**, Perez FR, Casabiell X, Camiña JP, Cai RZ, Schally AV. Correlation between the effects of bombesin antagonists on cell proliferation and intracellular calcium concentration in Swiss 3T3 and HT-29 cell lines. *Proc Natl Acad Sci USA* 1996; **93**: 1406-1411 [PMID: 8643644]

58 **Radulovic S**, Miller G, Schally AV. Inhibition of growth of HT-29 human colon cancer xenografts in nude mice by treatment with bombesin/gastrin releasing peptide antagonist (RC-3095). *Cancer Res* 1991; **51**: 6006-6009 [PMID: 1682040]

59 **Reile H**, Cai R, Armatis P, Schally A. New antagonists of bombesin gastrin-releasing Peptide with C-terminal leu-psi-(ch2n)tac-nh2. *Int J Oncol* 1995; **7**: 749-754 [PMID: 21552898]

60 **Rick FG**, Schally AV, Block NL, Nadji M, Szepeshazi K, Zarandi M, Vidaurre I, Perez R, Halmos G, Szalontay L. Antagonists of growth hormone-releasing hormone (GHRH) reduce prostate size in experimental benign prostatic hyperplasia. *Proc Natl Acad Sci USA* 2011; **108**: 3755-3760 [PMID: 21321192 DOI: 10.1073/pnas.1018086108]

61 **Radulovic S**, Schally AV, Reile H, Halmos G, Szepeshazi K, Groot K, Milovanovic S, Miller G, Yano T. Inhibitory effects of antagonists of bombesin/gastrin releasing peptide (GRP) and somatostatin analog (RC-160) on growth of HT-29 human colon cancers in nude mice. *Acta Oncol* 1994; **33**: 693-701 [PMID: 7946450]

62 **Schwartsmann G**, DiLeone LP, Horowitz M, Schunemann D, Cancella A, Pereira AS, Richter M, Souza F, da Rocha AB, Souza FH, Pohlmann P, De Nucci G. A phase I trial of the bombesin/gastrin-releasing peptide (BN/GRP) antagonist RC3095 in patients with advanced solid malignancies. *Invest New Drugs* 2006; **24**: 403-412 [PMID: 16505950 DOI: 10.1007/s10637-006-6886-5]

63 **Guillemin R**, Brazeau P, Böhlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* 1982; **218**: 585-587 [PMID: 6812220]

64 **Schally AV**, Varga JL, Engel JB. Antagonists of growth-hormone-releasing hormone: an emerging new therapy for cancer. *Nat Clin Pract Endocrinol Metab* 2008; **4**: 33-43 [PMID: 18084344 DOI: 10.1038/ncpendmet0677]

65 **Fürstenberger G**, Senn HJ. Insulin-like growth factors and cancer. *Lancet Oncol* 2002; **3**: 298-302 [PMID: 12067807]

66 **Busto R**, Schally AV, Varga JL, Garcia-Fernandez MO, Groot K, Armatis P, Szepeshazi K. The expression of growth hormone-releasing hormone (GHRH) and splice variants of its receptor in human gastroenteropancreatic carcino-

mas. *Proc Natl Acad Sci USA* 2002; **99**: 11866-11871 [PMID: 12186980 DOI: 10.1073/pnas.182433099]
67 **Hohla F**, Buchholz S, Schally AV, Seitz S, Rick FG, Szalontay L, Varga JL, Zarandi M, Halmos G, Vidaurre I, Krishan A, Kurtoglu M, Chandna S, Aigner E, Datz C. GHRH antagonist causes DNA damage leading to p21 mediated cell cycle arrest and apoptosis in human colon cancer cells. *Cell Cycle* 2009; **8**: 3149-3156 [PMID: 19755849]
68 **Rick FG**, Seitz S, Schally AV, Szalontay L, Krishan A, Datz C, Stadlmayr A, Buchholz S, Block NL, Hohla F. GHRH antagonist when combined with cytotoxic agents induces S-phase arrest and additive growth inhibition of human colon cancer. *Cell Cycle* 2012; **11**: 4203-4210 [PMID: 23095641 DOI: 10.4161/cc.22498]
69 **Havt A**, Schally AV, Halmos G, Varga JL, Toller GL, Horvath JE, Szepeshazi K, Köster F, Kovitz K, Groot K, Zarandi M, Kanashiro CA. The expression of the pituitary growth hormone-releasing hormone receptor and its splice variants in normal and neoplastic human tissues. *Proc Natl Acad Sci USA* 2005; **102**: 17424-17429 [PMID: 16299104 DOI: 10.1073/pnas.0506844102]
70 **Hohla F**, Moder A, Mayrhauser U, Hauser-Kronberger C, Schally AV, Varga JL, Zarandi M, Buchholz S, Huber R, Aigner E, Ritter M, Datz C. Differential expression of GHRH receptor and its splice variant 1 in human normal and malignant mucosa of the oesophagus and colon. *Int J Oncol* 2008; **33**: 137-143 [PMID: 18575759]
71 **Engel J**, Emons G, Pinski J, Schally AV. AEZS-108 : a targeted cytotoxic analog of LHRH for the treatment of cancers positive for LHRH receptors. *Expert Opin Investig Drugs* 2012; **21**: 891-899 [PMID: 22577891 DOI: 10.1517/13543784.2012.685128]
72 **Schally AV**, Nagy A. Chemotherapy targeted to cancers through tumoral hormone receptors. *Trends Endocrinol Metab* 2004; **15**: 300-310 [PMID: 15350601 DOI: 10.1016/j.tem.2004.07.002]
73 **Patel YC**. Molecular pharmacology of somatostatin receptor subtypes. *J Endocrinol Invest* 1997; **20**: 348-367 [PMID: 9294784]
74 **Reisine T**, Bell GI. Molecular biology of somatostatin receptors. *Endocr Rev* 1995; **16**: 427-442 [PMID: 8521788]
75 **Hohla F**, Buchholz S, Schally AV, Krishan A, Rick FG, Szalontay L, Papadia A, Halmos G, Koster F, Aigner E, Datz C, Seitz S. Targeted cytotoxic somatostatin analog AN-162 inhibits growth of human colon carcinomas and increases sensitivity of doxorubicin resistant murine leukemia cells. *Cancer Lett* 2010; **294**: 35-42 [PMID: 20156671 DOI: 10.1016/j.canlet.2010.01.018]
76 **Szepeshazi K**, Schally AV, Halmos G, Armatis P, Hebert F, Sun B, Feil A, Kiaris H, Nagy A. Targeted cytotoxic somatostatin analogue AN-238 inhibits somatostatin receptor-positive experimental colon cancers independently of their p53 status. *Cancer Res* 2002; **62**: 781-788 [PMID: 11830533]
77 **Cai RZ**, Karashima T, Guoth J, Szoke B, Olsen D, Schally AV. Superactive octapeptide somatostatin analogs containing tryptophan at position 1. *Proc Natl Acad Sci USA* 1987; **84**: 2502-2506 [PMID: 2882520]
78 **Nagy A**, Schally AV, Halmos G, Armatis P, Cai RZ, Csernus V, Kovács M, Koppán M, Szepesházi K, Kahán Z. Synthesis and biological evaluation of cytotoxic analogs of somatostatin containing doxorubicin or its intensely potent derivative, 2-pyrrolinodoxorubicin. *Proc Natl Acad Sci USA* 1998; **95**: 1794-1799 [PMID: 9465096]
79 **Schally AV**. Luteinizing hormone-releasing hormone analogs: their impact on the control of tumorigenesis. *Peptides* 1999; **20**: 1247-1262 [PMID: 10573298]
80 **Szepeshazi K**, Schally AV, Halmos G. LH-RH receptors in human colorectal cancers: unexpected molecular targets for experimental therapy. *Int J Oncol* 2007; **30**: 1485-1492 [PMID: 17487370]

P- Reviewers: Mutoh M, Wang G **S- Editor**: Zhai HH
L- Editor: A **E- Editor**: Ma S

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

© 2014 Baishideng Publishing Group Inc. All rights reserved.