Treatment with Cetuximab, Bevacizumab and Irinotecan in Heavily Pretreated Patients with Metastasized Colorectal Cancer

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Abstract. *Background: Modern therapy algorithms for advanced colorectal cancer include the monoclonal antibodies bevacizumab and cetuximab. Routinely, these antibodies are given sequentially in combination with chemotherapy. The question whether a combination of bevacizumab and cetuximab is beneficial has not been answered. The results of the BOND-2 study showed that tumor drug resistance to irinotecan can be overcome by addition of both cetuximab and bevacizumab. Patients and Methods: Here, we present the cases of five patients who were heavily pretreated and already had received cetuximab (and in two cases also bevacizumab). These patients received a chemotherapeutic regimen consisting of irinotecan, cetuximab and bevacizumab. Results: The combination of these two antibodies with irinotecan surprisingly induced marked tumor response in four out of five patients. Conclusion: There are currently no published data concerning the question whether resistance against one monoclonal antibody can be overcome by the addition of another monoclonal antibody. These cases point to a possible novel treatment approach and provide an incentive for further experimental investigations. The treatment was well tolerated and should be considered as a further medical treatment strategy.*

Colorectal cancer (CRC) is one of the major causes of death from cancer, accounting for more than 55,000 deaths per year in the United States alone (1). Patients with irresectable or metastasized CRC usually receive chemotherapy. Modern chemotherapeutic regimens for metastatic CRC combine 5-fluorouracil (5-FU) and folinic acid (FA) with either irinotecan or oxaliplatin. The sequential use of infusional regimens of 5-FU, FA and irinotecan (FOLFIRI) and 5-FU, FA and oxaliplatin (FOLFOX) respectively, leads to a median overall survival time of 20-21 months (2). Recently, the vascular endothelial growth factor (VEGF) antibody bevacizumab (3) and the epidermal growth factor receptor (EGFR) antibody cetuximab (4-7) were licensed for the therapy of advanced CRC. These antibodies were shown to improve the efficacy of concomitant standard chemotherapy. Thus it is widely accepted that patients should receive all three cytotoxic drugs and both antibodies in the course of the disease. While several studies showed that the monoclonal antibody cetuximab can overcome resistance against irinotecan, there are no published data concerning the question whether resistance against one monoclonal antibody can be overcome by the addition of another monoclonal antibody.

A clinical problem in medical oncology is the heavily pretreated patient in a good overall health status who has already received standard combination therapies. The use of antibody combinations in patients who have already received both antibodies during previous therapy is not established. Addition of an antibody to break drug resistance has been studied as a therapeutic principle. Here, we present cases of heavily pretreated patients with metastasized colorectal cancer who had a response to a therapeutic regimen with both antibodies. These cases elucidate the possibility of breaking tumor drug resistance or restoring therapy response with antibody combinations, even in heavily pretreated patients. It is well documented that patients who are off a particular regimen for some time can respond to the same regimen if retreated at a later time. This could be another explanation for the observed therapy responses in some of the reported cases, which nevertheless makes the reported treatment strategy attractive for heavily pretreated patients in an overall good health status.

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Patients and Methods

Patients were selected for this report if they had metastatic colorectal adenocarcinoma and had already received at least irinotecan- and oxaliplatin-based chemotherapy for metastatic disease before starting a combination therapy with two antibodies (see below). Prior antibody therapy should have included at least either cetuximab or bevacizumab. Patients were required to have measurable disease by Response Evaluation Criteria in Solid Tumors Group (RECIST), an Eastern Cooperative Oncology Group PS of zero to one, and adequate hematological, hepatic and renal function before starting chemotherapy with two antibodies (cetuximab and bevacizumab) and irinotecan. As an additional marker, the reduction (~25%) of metabolic activity on combined positron-emission tomography with computed tomography (PET-CT) was regarded as a response criterion. The following protocol (BOND-2) was used (8): (i) cetuximab 250 mg/m^2 for 1 h, day 1 (exception: 400 mg/m^2 for 2 h on day 1 of the first cycle) 250 mg/m^2 for 1 h, day 8; (ii) irinotecan 180 mg/m^2, day 1 in 250 ml NaCl 0.9%, 30-45 min; (iii) bevacizumab 5 mg/kg in 100 ml NaCl 0.9% *i.v.* day 1. All steps were repeated on day 15.

Patients were not treated with this regimen if contraindications were present for each drug used in this investigation. Examples include major surgery within the previous 4 weeks, inadequately controlled hypertension (blood pressure >150/100 mmHg on antihypertensive medications), unstable angina, history of myocardial infarction or stroke within 6 months, clinically significant peripheral vascular disease, bleeding diathesis or coagulopathy, history of abdominal fistula or abscess within the past 6 months, history of gastrointestinal perforation, nonhealing wound or ulcer and lack of physical integrity of the upper gastrointestinal tract or malabsorption syndrome. Patients were informed of potential toxicities and written consent was obtained.

Results

Case 1. This male patient was diagnosed with metastasized rectal cancer in the year 2000 at the age of 50 years. Anterior resection of the rectum was performed in November 2000. Hepatic metastases were found and the tumor was classified as pT3 pN1 M1 (liver). Histological findings showed an undifferentiated adenocarcinoma of the rectum. Postoperative radiochemotherapy was initiated with 5-FU and a cumulative radiation dose of 50.4 Gy. In April 2001, the hepatic metastases in segments 7 and 8 were resected. In September 2001, new liver metastases were detected and treated locally with radiofrequency ablation. Multiple local ablations of liver metastases followed in March 2003, August 2003 and January 2004. In September 2002, pulmonary metastases were found and were removed *via* wedge resection (segments 4 and 9) through thoracoscopy. In December 2003, a carcinoma of the bladder was diagnosed and treated curatively with resection and intracavital chemotherapy. None of the histological specimens of metastases in the course of treatment for rectal cancer showed cells from the bladder tumor. Thus the carcinoma of the bladder was regarded as definitively treated.

A suspicious pulmonary lesion was seen in November 2004 and again a wedge resection (segment 6) was performed revealing new pulmonary metastases of the rectal cancer. A new hepatic lesion and two new pulmonary lesions were noted, and palliative chemotherapy was initiated. Chemotherapy with oxaliplatin, FA and 5-FU according to the FOLFOX-3 protocol was given for 8 cycles. Treatment was successful and an indivdualized two-step approach was chosen to first remove the hepatic metastases and then remove the pulmonary metastases after another set of four cycles of chemotherapy. Advanced hemihepatectomy was performed with en bloc resection of the diaphragm and removal of parts of the inferior vena cava. Chemotherapy was continued afterwards but unfortunately new liver metastases developed. Furthermore an orbital metastasis with involvement of the musculus rectus medialis of the right eye appeared. This tumor was removed and postoperative radiation therapy was initiated. Histological findings showed a metastasis of the rectal adenocarcinoma. Systemic chemotherapy with FOLFIRI plus bevacizumab was initiated and given from May until November 2006. The situation was stabilized through this chemotherapy but in November a progression of an orbital tumor mass was noted. Hepatic and pulmonary metastases remained stable. Chemotherapy was changed to FOLFIRI in combination with cetuximab and was given from November 2006 until February 2007. The orbital tumor mass increased in size and another surgical reduction of the orbital lesion was performed. Histological analysis revealed a recurrence of the metastasis of the rectal adenocarcinoma. The remaining orbital tumor mass steadily increased in size and extensive craniomaxillary surgery with exenteratio orbitae, lateral rhinotomy, orbita resection of the left side, craniotomy with exstirpation of the anterior skull base and duraplasty was performed. Due to the extent of the operation, chemotherapy was paused until June 2007.

Routine follow-up showed progressive hepatic and pulmonary lesions and a recurrence of a tumor mass in the ethmoid bone with affection of the optical nerve. The patient noted a decreasing visual field. No further surgical options remained at this point and so radiation therapy with a cumulative dose of 30 Gy was initiated. After radiation therapy, systemic chemotherapy was initiated again. Due to the slightly reduced overall status of the patient, a well-tolerable regimen was sought and it was decided on an individual basis to give systemic therapy with irinotecan, cetuximab and bevacizumab according to the BOND-2 protocol (8).

After four cycles of therapy, reduction of tumor size of all lesions (hepatic, pulmonary and ethmoid) was noted. Treatment was well tolerated: only grade III skin toxicity occurred, no diarrhea or neutropenia was seen. The visual field remained stable after radiation therapy. The overall clinical situation has improved with the combined antibody chemotherapy.

Case 2. The second patient was a man who was diagnosed with colon cancer in October 2001 at the age of 61 years. Right-sided hemicolectomy was performed, showing the pathological classification of a pT4 N0 M0 GII colon carcinoma of the cecal region. A paranephritic lesion was seen and had to be removed in a second operation in November 2001. Intraoperative radiation therapy was applied and part of the abdominal wall and the small intestine had to be resected during this intervention. The patient recovered quickly. Follow-up was unremarkable.

In 2002, the patient began to have right-sided abdominal discomfort and intraabdominal pressure. No significant changes on imaging studies were found. The patient began to have night sweats and a rise in tumor markers was noted in June 2003. Further examinations were initiated and finally revealed peritoneal metastases on laparoscopic exploration in November 2003. First-line chemotherapy according to the FOLFOX-3 protocol was started and given for eight cycles. Due to a progression of the metastases, chemotherapy was switched to the FOLFIRI protocol which was given from April to June 2004. In November 2004, renal insufficiency caused an interruption of the therapy. Another explorative laparotomy showed massive intraabdominal metastases, infiltration of the right kidney and an exulcerated tumor of the rectosigmoid. Nephrectomy, adhesiolysis and a deep anterior rectal excision were performed. From October 2004 to September 2005, the patient received chemotherapy with irinotecan, 5-FU and FA. Cetuximab was added in December 2005 due to a progression of the tumor. The disease remained stable until September 2006. Due to symptoms of angina pectoris after administration of 5-FU the chemotherapy was reduced in January 2006 to cetuximab and irinotecan alone. In September 2006, progression again occurred and the chemotherapy was switched to irinotecan, cetuximab and bevacizumab (according to the BOND-2 protocol).

Staging in December 2006 showed regression of the tumor in the right lower quadrant of the abdomen (as detected with MRI). Treatment was well tolerated, but due to increasing diarrhea, treatment was paused from January to March 2007. Intermittent anal bleeding was noted but endoscopy remained unremarkable. After two months without therapy a progression of the metastases was seen and a therapy with cetuximab and bevacizumab was initiated again in March 2007. Administration of irinotecan was omitted due to recurrent diarrhea. With this regimen, the metastases remained stable until September 2007. An infiltration of the abdominal wall was found and a surgical resection of the tumor mass on the abdominal wall was initiated.

Case 3. The patient was diagnosed at the age of 60 with synchronous rectal and colon cancer. A carcinoma of the rectum (pT3) and of the colon (pT2 pN2) were found in February 2002 and so a hemicolectomy and anterior rectal excision were performed after preoperative short-term radiation (5×5 Gy). Adjuvant chemotherapy with 5-FU/FA according to the Mayo protocol was given from May to July 2002 but new metachronous liver metastases were found in July 2002. Chemotherapy was changed to oxaliplatin, 5-FU and FA (FOLFOX-3 protocol) and was given from September to October 2002. In December 2002, liver segment resection was performed (segments 2, 4, 5 and 7). In the postoperative phase, chemotherapy was continued from January to July 2003. In October 2003, new pulmonary lesions were resected by atypical pulmonary resection and showed new metastases of the CRC. New hepatic metastases were also noted.

Palliative chemotherapy with irinotecan, 5-FU and FA was initiated and given from December 2003 to July 2004. Due to a progression of the disease, chemotherapy was switched to the FOLFOX-3 protocol again as an individual decision, but was stopped due to progressive disease after two months of therapy. From October 2004 to September 2005, palliative chemotherapy with irinotecan, 5-FU and FA and the antibody cetuximab was given. Progressive disease required another treatment regimen and so a combination therapy with cetuximab, bevacizumab, irinotecan, 5-FU and FA was begun. After four cycles, therapy was evaluated and showed only a mixed response, with a reduction of the metastatic load in the liver but progression of the pulmonary lesions.

From September 2005 on, multiple palliative chemotherapies with combinations of mitomycin c, capecitabin, oxaliplatin and Avastin were given, but progression of the disease was not impeded. Best supportive care was initiated and the patient died in 2006.

Case 4. In October 2003, the patient was aged 53 years and was diagnosed with rectal cancer. Synchronous hepatic metastases were noted and the primary tumor of the rectum was removed. In November 2003, palliative chemotherapy was initiated and six cycles of chemotherapy with oxaliplatin, 5-FU, FA and cetuximab were given until July 2004. Due to oxaliplatin-induced hemolysis, this regimen was stopped. From July to September 2004, two cycles of chemotherapy with cetuximab, 5-FU and FA were given. Due to progressive disease, four cycles of chemotherapy with irinotecan and 5-FU/FA were administered from October 2004 to May 2005. The chemotherapy regimen was switched to irinotecan, 5-FU and FA according to the FOLFIRI protocol and cetuximab was added after failure of irinotecan and 5-FU alone. The new regimen was given for four cycles from June to August 2005. Severe (grade IV) stomatitis developed and a combination of irinotecan, cetuximab and bevacizumab was initiated. From September 2005 until March 2006, this combination was used. After four cycles, partial remission was seen, but treatment efficacy was lost after eight cycles of therapy. As an individualized therapeutic decision mitomycin c and 5-FU were given, but due to another progression of the disease, chemotherapy was stopped and best supportive care was begun.

Case 5. At the age of 63, the patient was diagnosed with a colon carcinoma involving local lymph nodes. The tumor and local lymph nodes were resected and adjuvant treatment with oxaliplatin and 5-FU was administered for six months. In October 2006, lymph node metastases in the cervical region were diagnosed and verified through histological evaluation of biopsies. First-line chemotherapy with irinotecan, Xeloda and bevacizumab was begun. The patient recognized an enlargement of cervical lymph nodes in January 2007 and a PET-CT showed progression of the lymph node metastases to para-aortal and mediastinal regions. Second-line chemotherapy with Tomodex was initiated but did not stop tumor progression. Therefore, in an attempt to reduce tumor burden, the patient underwent surgical lymphadenectomy. Bilateral mediastinal retroclavicular lymphadenectomy, lymphadenectomy of the paratracheal and paraaortal region and resection of the lymph nodes in the left-sided paraaortal abdominal region were performed. After having evaluated the residual tumor burden, the patient continued again on chemotherapy, this time with irinotecan and cetuximab. From June to November 2007, the situation remained stable: two lesions in cervical lymph nodes remained unchanged. In December 2007, however a significant progression of intensity was seen in PET-CT in these two cervical lymph nodes and a marked increase in serum oncomarkers was noted. Therefore, a chemotherapy regimen with a combination of irinotecan, cetuximab and bevacizumab was initiated in January 2008 and given until March 2008. The subsequent evaluation showed a significant intensity reduction of 25% in the PET-CT. The clinical situation remained stable throughout chemotherapy: skin irritation (grade II) was the most dominant side-effect; a single episode of rectal bleeding occurred during therapy with irinotecan, cetuximab and bevacizumab, but endoscopy of the colon did not reveal any causative lesions. Chemotherapy with both antibodies was otherwise well tolerated.

Discussion

In medical oncology, an often encountered clinical problem is that of patients still in a good performance status after multiple standard chemotherapies (with sequential use of oxaliplatin, irinotecan, and 5-FU with concomitant administration of bevacizumab or cetuximab) and no further established treatment options. While cetuximab was intended for pretreated patients in a refractory setting (7, 9), bevacizumab was introduced as a front-line therapeutic (10). Pretreated patients however can also benefit from the addition of bevacizumab (11). In the BOND trial (12), cetuximab was used to break drug resistance against irinotecan. In the BOND-2 trial (8), tumor drug resistance against irinotecan was approached with the combination of cetuximab and bevacizumab in patients naïve to both antibodies. The effect of a "dual blockade" of the VEGF and EGF pathways after pretreatment with bevacizumab and cetuximab however remains unclear (13). Whether the addition of bevacizumab to cetuximab is able to overcome drug resistance against cetuximab is not known. Saltz *et al*. (8) showed that the combination of the antibodies cetuximab and bevacizumab can break tumor drug resistance in patients who had received irinotecan before. Two of our patients had never received bevacizumab before. Patients 1, 2 and 5 had already received both antibodies in combination with standard chemotherapy before, both antibodies were never given combined. The effects of the combination of bevacizumab, cetuximab and irinotecan however were profound. In patient 1, an effect of radiation therapy on the ethmoidal lesion is possible and thus may not be due to (immuno-) chemotherapy. Interestingly, no reduction of tumor mass was reached with either FOLFIRI plus bevacizumab or cetuximab in this patient. Based on this observation, the reduction of tumor mass can only be attributable to an increased effect of combining bevacizumab and cetuximab. Patient 3 had only little benefit from the combination therapy.

Another important aspect is visible in the other two patients. Addition of bevacizumab to the therapeutic regimen was able to break tumor drug resistance against cetuximab (plus standard chemotherapy). Thus the addition of the second antibody was able to restore treatment efficacy. Patient 3 never had a good response to any chemotherapy, at best the results were mixed responses.

In these cases, the patients had already received pretreatment with cetuximab (and bevacizumab). In four out of five cases, response to irinotecan was restored in a triple combination therapy with two different antibodies. Even in the case of a heavily pretreated patient, this approach seems to be successful. This gives rise to the hypothesis that triple combinations with two antibodies influencing different signaling pathways are able to break chemotherapy resistance in tumors. To our knowledge, no corresponding pre-clinical experimental data (*e.g*. studies in cell culture or animal models) have been published. Further experimental studies should be initiated to analyze and demonstrate the molecular background of our clinical observations. Successful retreatment of patients with a once successful treatment regimen could also be the explanation for the observed therapy responses in some of the reported patients. For the medical oncologist however, these cases highlight another possible treatment regimen for heavily pretreated patients, regardless of the underlying molecular mechanisms.

In summary, these cases demonstrate another possible treatment option for these patients. The combination of both antibodies seems to be effective for overcoming tumor drug resistance, or restoring therapy response. Adding bevacizumab to the treatment regimen seems to overcome

drug resistance against cetuximab (plus irinotecan). Even in patients pretreated with both antibodies, chemotherapy and radiation therapy, a response could be induced. In the light of emerging new antibodies [*e.g.* anti-PlGf antibodies, (14)] combinatory antibody therapies therefore could hold promise of amplifying therapeutical efficiency beyond single agent effects. However, cost effectivness remains a difficult topic in times of increasing therapy costs and lower budgets.

In conclusion, we think that the combination of two different antibodies which influence different signaling pathways should be considered in a carefully selected group of heavily pretreated patients. Our cases highlight the possibility of restoring response to an irinotecan-based chemotherapy with cetuximab through the addition of bevacizumab.

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