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Single Case

Multimodality Treatment of a Colorectal Cancer Stage IV Patient with FOLFOX-4, Bevacizumab, Rigvir Oncolytic Virus, and Surgery

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Keywords
Colorectal cancer · Oncolytic virus · ECHO-7 virus · Angiogenesis inhibitor · Bevacizumab · Rigvir · Multimodality treatment

Abstract
Colorectal cancer is one of the most commonly diagnosed cancers worldwide. The treatment consists of surgical resection, systemic chemotherapy, and new biological agents. One more recently emerging treatment option is oncolytic virotherapy. Although the use of the new treatment methods shows improved overall and progression-free survival, in general, even with the new treatments, mortality remains high and combinations of treatments should be sought to treat patients with colorectal cancer. Here we report a stage IV colorectal cancer patient who received multimodality treatment including bevacizumab, FOLFOX-4, surgery, and the oncolytic virus Rigvir. The patient shows complete pathological remission and remains stable 7.7 years after initial diagnosis. The possible benefits of combining Rigvir oncolytic virus and bevacizumab should be investigated since in vitro research suggests that anti-angiogenesis agents improve viral distribution by altering the microenvironment of the tumor.

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Introduction

Colorectal cancer is the third most common tumor type in the world with high mortality [1]. Approximately 50% of patients will develop liver metastases during the disease. Of all patients with liver metastases, only 10–20% are resectable [2]. The main treatment of metastatic colorectal cancer is surgical resection; if resection is not possible, systemic chemotherapy is usually prescribed [2]. First- and second-line treatments include fluorouracil, leucovorin, and irinotecan (FOLFIRI), infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX), or capecitabine plus oxaliplatin [3]. In the second-line treatment the standard chemotherapy is combined with targeted drugs, for example, bevacizumab, which is considered effective regarding overall survival and progression-free survival [3]. According to the AJCC Staging Manual, only 5.7% of stage IV colorectal cancer patients are expected to survive 5 years, with the 50% overall survival being less than 1 year [4]. The patient described in this case report was diagnosed 7.7 years ago and currently shows no evidence of disease.

It has been suggested that bevacizumab-containing regimens benefit the patient by improving the pathological response for colorectal liver metastases and the degree and incidence of hepatic injury is decreased. The pathological response is considered a reliable criterion to assess the future treatment of patients and efficacy of new postoperative treatments since it has been shown that the pathological response correlates with survival [5, 6]. Even with new treatments becoming available, the survival of metastatic colorectal cancer patients is low and new strategies should be sought. In vitro studies suggest that patients could benefit from the use of immunotherapy agents in combination with oncolytic viruses [7].

Oncolytic viruses are a novel class of treatment that is currently extensively studied. These viruses selectively infect cancer cells, which leads to the lysis of the infected cancer cells, while the healthy cells remain intact. The mechanism by which viruses can infect cancer cells vary; viral replication is carried out intracellularly and it is known that altered signaling in cancer cells creates favorable conditions for viral replication. Some of the oncogenic proteins might also facilitate the replication of the virus [8]. It has been noted that cancers that are resistant to immunotherapy, chemotherapy, and radiation therapy might still be susceptible for oncolytic viruses [9].

Rigvir is an oncolytic, nonpathogenic ECHO-7 virus selected and adapted for melanoma that has not been genetically modified, approved and registered in Latvia since 2004 for cutaneous melanoma [10]. In a retrospective study it has been shown that patients treated with Rigvir showed significant improvement in overall survival and had a 4.39- to 6.57-fold lower mortality ratio [10]. In vitro studies show that Rigvir reduced cell viability in various cell lines of human origin [11].

The aim of this report is to show the positive effect of Rigvir oncolytic virus treatment in combination with FOLFOX-4, anti-VEGF-A antibody therapy (bevacizumab), and surgery of a colorectal cancer stage IV patient. A preliminary report has been published [12].

Case Report

A man born in 1974 was acutely diagnosed at 35 years of age in October 2010 with bowel obstruction and underwent Hartmann's type resection of the sigmoid colon. During the surgery the malignancy was found – poorly differentiated rectal adenocarcinoma stage IV (pT3N2bM1b G3 R0). Histological examination showed rectal adenocarcinoma cells with high mitotic activity, infiltration in mesorectal tissue, malignant cells invading nerves, and growth

within the perineural space (Fig. 1). Metastases were found in sigmoid mesenteric lymph nodes; from 10 lymph nodes, 7 were involved with disease. Before being diagnosed, the patient complained about abdominal pain and constipation, which had lasted for about a year. He had not received any treatment before hospitalization. The patient has no family history of colorectal cancer.

An abdominal computed tomography (CT) scan in November 2010 showed an unresectable solitary liver metastasis in the 8th segment of the right lobe with the largest diameter being 7.9 cm (Fig. 2a). The patient was treated with FOLFOX-4 palliative chemotherapy (8 infusions) plus the biological agent bevacizumab (4 infusions). The administration frequency was every 2 weeks for 4 months [12]. In addition, considering the grave prognosis, starting December 2010, the patient also received Rigvir therapy; 11 intramuscular administrations (2 mL solution containing $>10^6$ $TCID_{50}$/mL in 0.9% sodium chloride) during 13 months intermittently between the chemotherapy cycles. Physical examination and blood test assessment were carried out during Rigvir therapy.

A CT scan in January 2011 showed that the metastasis was reduced in size by 50%, now with the largest diameter being 3.8 cm (Fig. 2b). The liver metastasis had changed from unresectable to resectable. In March 2011, the patient underwent a partial hepatectomy; segment 8 of the liver was resected. After the surgery, in April 2011, the patient started an adjuvant postoperative chemotherapy with 4 infusions of FOLFOX-4 for a 2-month period [12].

After the treatment, follow-up CT scans were made from 2011 to 2016. Abdomen, retroperitoneal, pelvic, and thoracic CT scans showed a residual 2.5 cm formation at the surgery site in the right hepatic lobe and mesenteric lymph nodes of less than 1 cm (November 2011), the largest of them with calcifications in the left hypogastric area (February 2012; March 2013) (Fig. 2e, f, h; Fig. 3a). The liver, spleen, and the right side of the kidney contained multiple, cystic structures. Fibrotic changes were observed in both lungs (March 2013). The formation at the surgery site and lymph nodes decreased in size and stabilized by the end of 2016. No nodular structures in the lungs were found (Septemper 2015). No evidence of metastasis was found in November 2016 (Fig. 2m; Fig. 3b). The partially calcinated structure located in the pelvis or hypogastrium could be a granuloma, which is mostly constant in all CT scans. In conclusion, the radiological findings (repeated CT scans) and pathological findings (from the removed liver metastasis [12]) show that the patient has reached complete response to the applied therapies.

Serum clinical chemistry parameters were recorded and graded according to NCI CTCAE. Values above grade 1 were not observed during Rigvir therapy. Serum clinical chemistry parameters after chemotherapy and bevacizumab are not available for analysis.

The patient described here was diagnosed with stage IV colorectal cancer in October 2010. Seven years on, after complete pathological remission the patient shows no evidence of disease at the time of writing.

Discussion

The patient also underwent genetic screening for predominant somatic mutations in KRAS codons 12, 13, 61, BRAF V600E, TP53 exon 5, 6, 7, 8, PIK3CA exon 9, 10, and PTEN whole coding sequence. The screening showed 1 mutation Q61H located in the KRAS codon 61 [12]. (While those authors write G61H, the correct codon appears to be Q61H [13].) KRAS is reported to be an oncogene playing an important role in carcinogenesis, which is located downstream of the epidermal growth factor receptor (EGFR) with high incidence in colorectal

cancer [14]. Treatments targeting the EGFR are more effective towards wild-type KRAS cases than KRAS mutations [14, 15]. Bevacizumab was used instead of cetuximab due to a mutation in Q61H, which might play a role in resistance to cetuximab [12].

The positive effect of combining bevacizumab and oncolytic viruses in in vivo models has been reported (reviewed in [16]). Kurozumi et al. [17] found that the angiogenesis inhibitor cRGD reduced inflammation, leucocyte infiltration, and vascular hyperpermeability when used prior to injection of the oncolytic virus hrR3, thus exerting additive or synergistic effects. It was suggested that the oncolytic virus induces an inflammatory response and leukocyte infiltration, which in turn enhances virus clearance from tumor. Angiogenesis inhibitors reduce these responses and the efficacy of the oncolytic viruses are enhanced [17]. Libertini et al. [18] reported increased viral distribution of oncolytic adenovirus in thyroid carcinoma when used in combination with bevacizumab. One of the main aspects influencing the efficacy of oncolytic viruses is the incomplete viral dissemination caused by extracellular matrix, acidosis, enhanced proteolytic activity, and hypoxia. It is suggested that hypoxia induces angiogenesis in neoplastic formations; the lack of normal vessels increases interstitial fluid pressure, causes incomplete viral dissemination, and reduces viral entry into the tumor (reviewed in [18]). Improved distribution of hrR3 oncolytic herpes virus in human gastric cancer xenografts in combination with bevacizumab and persistent viral distribution in pancreatic cancer xenografts in combination with erlotinib has also been observed (reviewed in [16]).

A recent report suggests a synergistic effect of the anti-VEGF-A monoclonal antibody bevacizumab and herpes simplex oncolytic virus HF10 [16]. A possible explanatory mechanism for this synergy lies in the enhanced viral distribution due to inhibition of angiogenesis and higher vascular permeability to the virus [16]. Oncolytic vesicular stomatitis virus has been tested in 2 models in immune competent rats using a colorectal cancer cell line. In the liver metastases model, vesicular stomatitis virus caused an increase in median overall survival compared to control (110 vs. 25 days). In the lung metastases model, the median survival was 10 days in comparison to 7 days in the control group; no long-term survival was observed [19].

Positive effects of bevacizumab in combination with a conditionally replicative adenovirus CRAd-S-pk7 has been reported both in vitro and in vivo. Treatment of glioblastoma tumors with bevacizumab that was followed by adenovirus CRAd-S-pk7 injection showed increased distribution and replication of virus as well as a decreased level of collagen IV – an essential part of the extracellular matrix, which inhibits the distribution of viral particles in the tumor [20]. It is also noted that to successfully develop new treatments for cancer, a combination of drugs could be used that affect different aspects of tumor microenvironment and biology, thus improving the clinical benefit [4]. Taken together, the focus in these studies is on the microenvironment of the tumor that is altered by angiogenesis inhibitors and allows better viral distribution.

Recently, a phase 2 clinical study of FOLFOX5/bevacizumab with and without pelareorep virus in patients with metastatic colorectal cancer reported an improved objective response rate of 53 versus 35%, respectively. However, no statistical difference was observed in the overall survival (median 19.2 vs. 20.1 months), and progression-free survival was lower in the pelareorep arm (median 7 vs. 9 months) [21].

The present case report shows complete pathological remission in a patient who was treated with a combination of chemotherapy, an anti-angiogenesis agent, surgery, and oncolytic virotherapy with Rigvir. The patient was diagnosed more than 7.7 years ago with stage IV colorectal cancer (in October 2010) and since September 2011 there is no evidence of disease. To our knowledge, this is the first clinical case to show such a multimodality treatment.

Recent in vitro and in vivo studies suggest a possible benefit of combining an oncolytic virus with an anti-angiogenesis agent in cancer treatment [16]. Meta-analysis of randomized studies comparing the efficacy and safety of bevacizumab with and without chemotherapy in first-line treatment of metastatic colorectal cancer showed that the median overall survival in the XELOX group and in the FOLFOX/bevacizumab (studies NO16966 and ITACA) combination group was 21.3 and 20.8 months, respectively [22]. The present patient has lived for more than 90 months since the diagnosis. Future research should focus on different aspects of cancer microenvironment and biology in order to find combinations that increase the efficacy of treatment methods [17]. Since mortality from metastatic colorectal cancer is extremely high, it is important that new possible treatment combinations are being investigated.

Statement of Ethics

Consent to publication was obtained from the patient.

Disclosure Statement

The authors declare that they have no competing interests.

Author Contributions

A.T., A.R., L.B., and P.A. made substantial contributions to acquisition of data, analysis, and interpretation of data and drafted the manuscript. E.O. and S.N. carried out the radiology and histology analysis. R.E. was the attending oncologist of the patient. All authors have read and approved the final version for publication.

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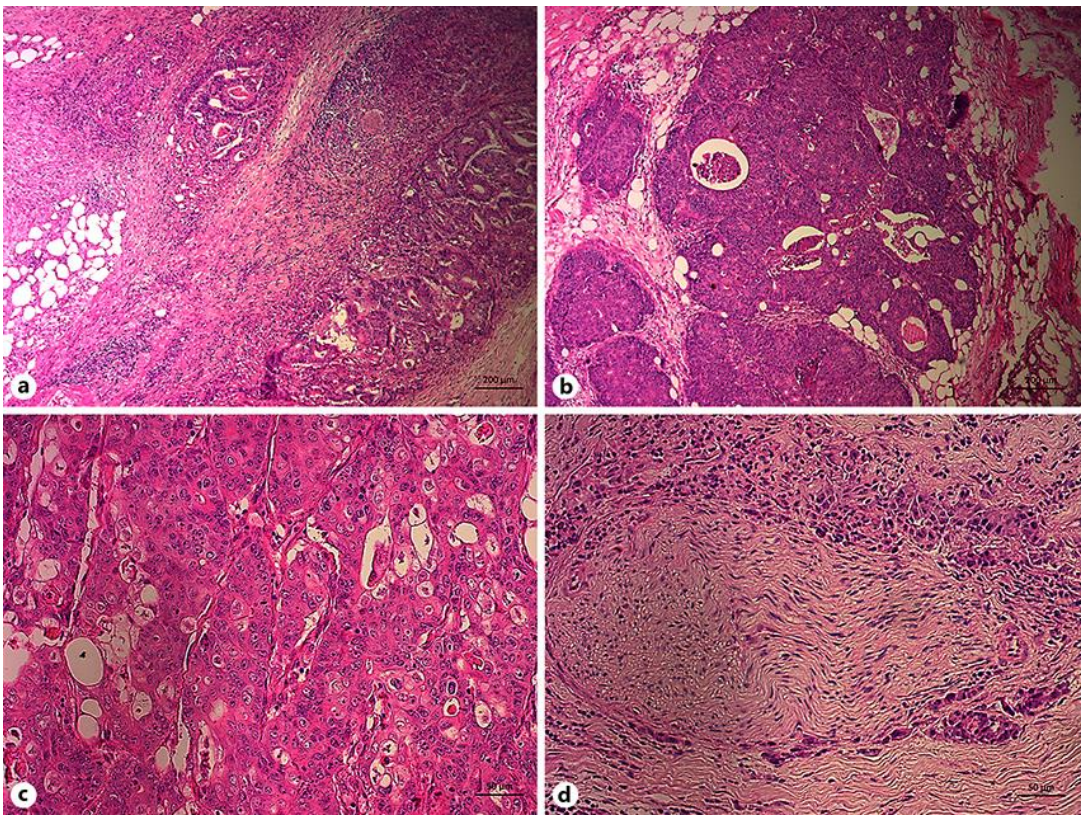

Fig. 1. Rectal adenocarcinoma cells. Hematoxylin and eosin stain. **a** Rectal adenocarcinoma infiltration of mesorectal tissue. **b** Rectal adenocarcinoma grade 3 with solid differentiation and some microscopic microabscesses. Scale bar, 200 μm (**a**, **b**). **c** Differently sized rectal adenocarcinoma cells with high mitotic activity and some mitoses. **d** Malignant cells invade nerves and grow within the perineural space. Scale bar, 50 μm (**c**, **d**).

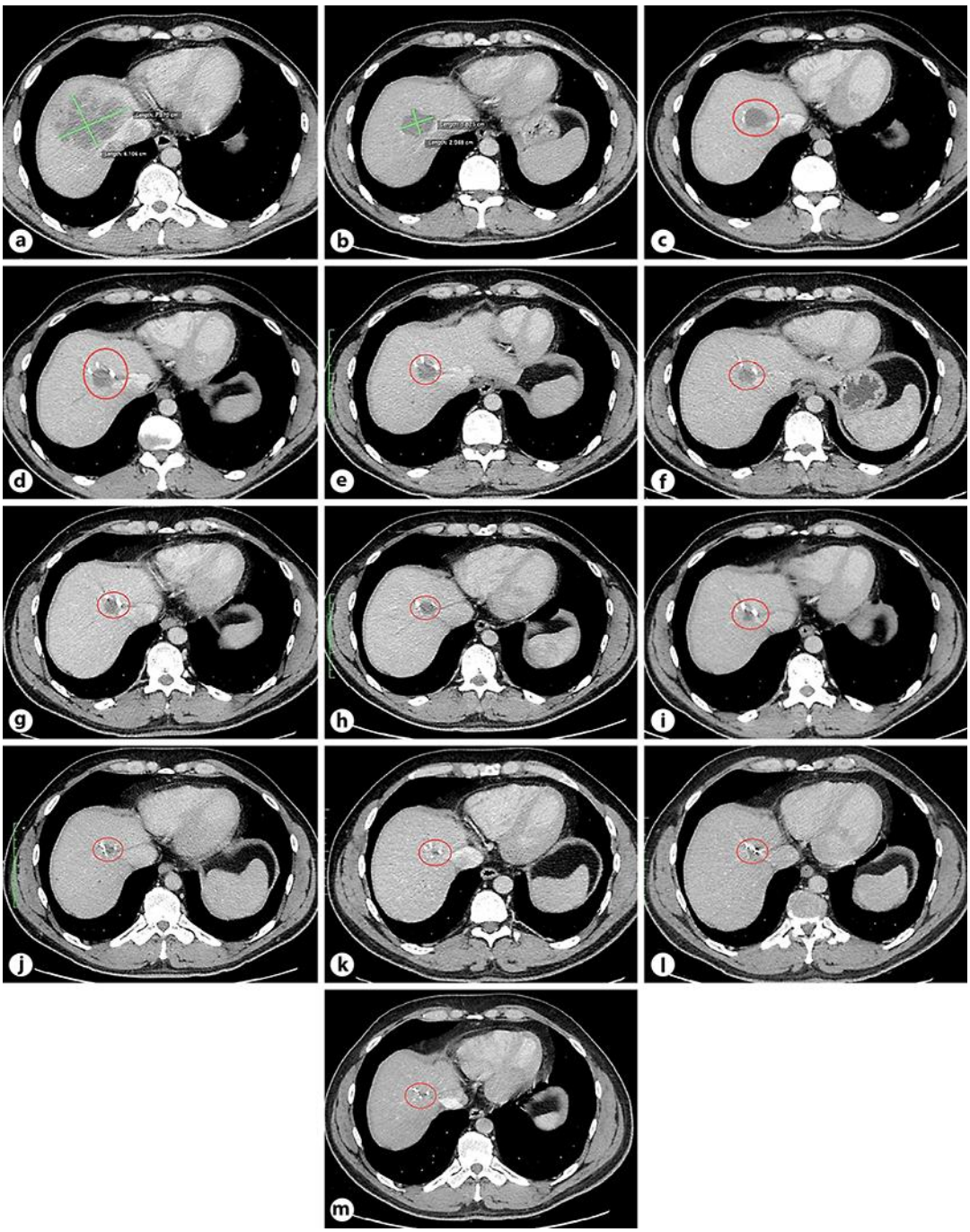

Fig. 2. Liver contrast-enhanced CT late-phase scans show that from November 2010 (**a**) to January 2011 (**b**) a liver metastasis decreased in size by 50%, 2 months after the start of treatment with FOLFOX-4, bevacizumab, and Rigvir. After partial hepatectomy and postoperative treatment, the residual formation decreased in size and stabilized. **c**, **d** August 26, 2011. **e** November 25, 2011. **f** February 25, 2012. **g** August 31, 2012. **h** March 15, 2013. **i** September 27, 2013. **j** August 29, 2014. **k** September 2, 2015. **l** August 26, 2016. **m** November 26, 2016.

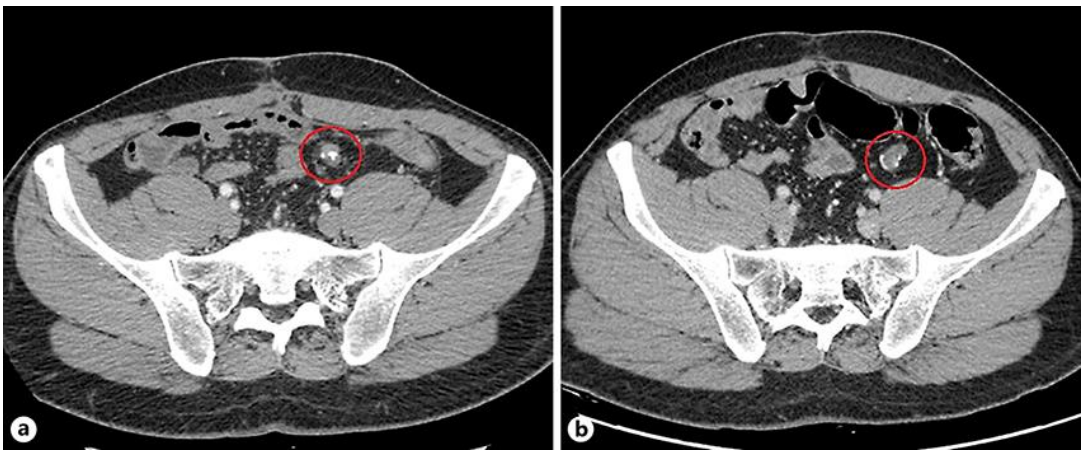

Fig. 3. **a** Contrast-enhanced pelvic CT scan shows a residual formation after surgery and during postoperative chemotherapy on November 25, 2011. **b** Pelvic CT scan 5 years after treatment shows that the formation is stabilized (November 26, 2016).