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Chapter 5

The Prognostic Significance of the Expression Change of EGFR during Neoadjuvant Chemoradiotherapy in Patients with Rectal Carcinoma

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Additional information is available at the end of the chapter

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Abstract

Aim of the study: the aim of this retrospective study was to determine the prognostic impact of epidermal growth factor receptor (EGFR) expression changes during neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer.

Material and methods: fifty patients with locally advanced rectal cancer were evaluated. All the patients were administered the total dose of 44 Gy. Capecitabine was concomitantly administered in the dose 825 mg/m^2 in two daily oral administrations. Surgery was indicated 4–8 weeks from the chemoradiotherapy completion. EGFR expression in the pretreatment biopsies and in the resected specimens was assessed with immunohistochemistry.

Results: all 50 patients received radiotherapy without interruption up to the total planned dose. The median disease-free survival was 64.9 months, and median overall survival was 76.4 months. Increased EGFR expression was found in 12 patients (26.1%). A statistically significant shorter overall survival ($p < 0.0001$) and disease-free survival ($p < 0.0001$) were found in patients with increased expression of EGFR compared with patients where no increase in the expression of EGFR during neoadjuvant chemoradiotherapy was observed.

Conclusions: the overexpression of EGFR during neoadjuvant chemoradiotherapy for locally advanced rectal adenocarcinoma is associated with significant shorter overall survival and disease-free survival.

Keywords: rectal cancer, radiotherapy, chemotherapy, neoadjuvant treatment, targeted treatment

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1. Introduction

Colorectal cancer is one of the most common cancers in developed countries. The incidence of rectal adenocarcinoma represents approximately 30% of all colorectal cancers. The rectal adenocarcinoma typically develops distant metastasis, and the local relapses in presacral area could be identified in 50% of the cases in clinical stage III [1]. The incidence of local relapses could be reduced by radiotherapy. Meta-analysis of 22 clinical studies demonstrated that neoadjuvant or adjuvant radiotherapy significantly reduced the local relapse incidence compared to surgery alone [2]. A neoadjuvant chemoradiotherapy followed by total mesorectal excision is the current standard of the treatment in patients with locally advanced rectal adenocarcinoma. Neoadjuvant chemoradiation has shown a lower incidence of local recurrence and better toxicity profile compared to adjuvant therapy, but no survival benefit was shown [2]. The combination of radiotherapy with 5-flurouracil (5-FU) or capecitabine has demonstrated a higher number of pathological complete remissions and lower incidence of local relapses compared to the treatment with radiotherapy alone [3]. The main prognostic factors of rectal adenocarcinoma are clinical stage, radicality of surgery, pretreatment concentration of CEA, tumor grade, angioinvasion, and mucinous histology. Epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), oncoprotein p53, and survivin were studied as the potential new biomarkers for rectal adenocarcinoma. The aim of this retrospective study was to determine the prognostic impact of EGFR expression changes during neoadjuvant chemoradiotherapy in patients with locally advanced rectal adenocarcima.

1.1. Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR/HER1/erbB-1) is a 170-kDa transmembrane glycoprotein. EGFR belongs to the ErbB family of the tyrosine kinase receptors [4]. More than 10 ligands are known to bind to the EGFR, including epidermal growth factor (EGF), amphiregulin, epiregulin, neuregulin, transforming growth factor alpha (TGF-α), betacellulin, and Heparin-binding EGF-like growth factor (HB-EGF) [5]. EGFR could be activated by the ionizing radiation, too. Ligand binding results in homodimerization of two EGFR molecules or heterodimerization of an EGFR molecule with another member of the ErbB family. After dimerization and internalization, autophosphorylation of the intracellular tyrosine kinase domain occurs, which activates different intracellular transduction pathways. The results are cell proliferation, acceleration of cell repopulation, and apoptosis inhibition. These related transduction pathways include Ras/Raf/MAPK, PI3K/AKT, JAK/STAT, or PLC/PKC. The major signaling route is the Ras/Raf/MAPK pathway that results in cell proliferation. The PI3K/AKT pathway activates PI3K/AKT, causing apoptosis inhibition [6]. EGFR could be directly translocated to the cell nucleus with a direct activation of transcription factors [7]. EGFR is very important for the reparation of normal epidermal cells. The most important mechanism of the increased activity of EGFR is its overexpression in cancer cells. The other mechanisms include increased production of EGFR ligands,

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activation mutation of EGFR receptor, and loss of intracellular regulation mechanisms or EGFR1 gene amplification. The activation of EGFR on cell surface leads to progression of cell cycle, cell proliferation, angiogenesis, and apoptosis inhibition. It is also associated with more aggressive properties of cancer cells and resistance to the radiotherapy or chemotherapy [8, 9]. The overexpression of EGFR is responsible for the increased motility of the cancer cells [10].

1.2. EGFR and his role in radiotherapy

The reparation, redistribution, repopulation, and reoxygenation are the basic mechanisms of interaction between radiation and cells. EGFR is important for reparation of the damage cells caused by radiation. EGFR is directly translocated to the cell nucleus with a direct activation of transcription factors and results in cells reparation [7]. Similarly, activation of EGFR by radiation results in the activation of Ras/Raf/MAPK pathways with increased expression of DNA reparation genes (Rad51, ATM, XRCC1) [11]. EGFR has influence on the redistribution of cells after radiation. It was found that EGFR inhibitors could cause the redistribution of the cell cycle by G1 phase blockade [12]. Radiobiological studies confirmed the critical role of EGFR in cytoprotective and pro-proliferative response of tumor cells after irradiation. The increased EGFR expression after radiotherapy is related to accelerated repopulation of cancer cells [13]. Increased tumor repopulation during radiotherapy leads to recovery of clonogenic tumor cells, thereby causing counterproductivity to radiation therapy alone [14].

1.2.1. The prognostic significant of EGFR expression in rectal cancer

The overexpression of EGFR is observed in 50–60% of rectal carcinoma and is associated with worse prognosis [15–17]. Azria evaluated the prognostic significance of EGFR expression in pretreatment biopsy on 77 patients with rectal cancer treated by neoadjuvant radiotherapy. The expression of EGFR was observed in 56% patients. In median follow-up of 36 months, it was observed that significantly high number of the local recurrences occurred in patients with overexpression of EGFR above 25% in multivariate analysis (HR 7.18; $p = 0.037$) [18]. Another study evaluated 92 patients with locally advanced rectal carcinoma treated by neoadjuvant chemoradiotherapy. The EGFR expression was observed in 71% of the patients. The patients with overexpression of EGFR had significantly shorter overall survival ($p = 0.013$), significantly shorter disease-free survival ($p = 0.002$) and significantly shorter survival without distance metastases ($p = 0.003$) compared with patients without EGFR expression [19]. Giralt in his study presented a total of 87 patients treated for the locally advanced rectal cancer by neoadjuvant treatment. EGFR overexpression was observed in 52 cases (60% of patients). The patients with overexpression of EGFR had significantly less pathological complete response ($p = 0.006$), shorter DFS compared to patients without EGFR overexpression ($p = 0.003$) [20].

1.2.2. EGFR inhibitors

The two dominant EGFR inhibition strategies under clinical investigation are used. One group of EGFR inhibitors are small molecules called tyrosine kinase inhibitors (TKI): gefitinib and

erlotinib are used in treatment in patients with non-small lung cancer as a palliative treatment. The other possibility of EGFR inhibition is adoption of monoclonal antibodies that bind to extracellular domain of EGFR. Cetuximab and panitumumab are the most commonly used inhibitors in metastatic colorectal cancer. Cetuximab is a chimeric mouse anti-EGFR monoclonal antibody that first received US Food and Drug Administration approval in 2004 for the treatment of irinotecan-refractory colorectal cancer [21]. Panitumumab is a fully human anti-EGFR antibody. With the development of molecular biology, it was found that an important predictive factor for anti-EGFR monoclonal antibodies is the status of K-RAS gene [22]. K-RAS belongs to the RAS genes family. The other members of RAS genes family are N-RAS and H-RAS genes. The products of RAS genes are regulatory proteins that regulate pathways after EGFR activation. Mutation of K-RAS gene is observed in 30–50% cases of colorectal cancer. Cetuximab and panitumumab were evaluated in treating patients with metastatic colorectal cancer with FOLFOX and FOLFIRI regimen. The best results were observed in the group of patients with wild type of RAS genes.

1.2.3. The combination of neoadjuvant chemoradiotherapy and anti-EGFR treatment

Monoclonal anti-EGFR antibodies have shown efficiency in the treatment of metastatic colorectal cancer. Neoadjuvant treatment of rectal cancer has been the topic of several clinical studies of I/II phases evaluating the benefits of monoclonal antibodies against EGFR combined with chemotherapy. The chemotherapy regimens include 5-FU, capecitabine, oxaliplatine, or irinotecan. The doses of radiation were in the range of 45–50.4 Gy. The primary point was to use the number of pathologically completed responses as the predictor of longer disease-free survival (DFS) and overall survival (OS) [23–26]. More DFS and OS dates are observed in treatment of cetuximab than panitumumab. Eleven clinical studies showed average number of pCR in only 10.7% (range 0–25%) of cases [27–37]. On the other hand, the percentage of pCR in separate chemoradiotherapy was 13.5% in 3157 patients in meta-analysis of clinical studies II/III phase [38]. The occurrence of toxicity grade III/IV was described in 30% in combination of neoadjuvant chemotherapy and cetuximab. The most common side effect was diarrhea, while leucopenia, anemia, and elevation of liver transaminases were infrequently observed. The acneiformic rash was observed in 87% of cases but predominantly in grade I/II. Hypersensitization reactions after infusional application of cetuximab were observed in 5–10% of cases. Panitumumab was evaluated in neoadjuvant treatment of rectal cancer with chemotherapy and radiotherapy in clinical study II phase. A total of 60 patients were treated. The percentage of pCR was 21 [39]. It seems that the results of combination cetuximab and chemotherapy in metastatic colorectal cancer or combination of cetuximab and radiotherapy in locally advanced squamous cell carcinoma of the head and neck could not be interpolated to the neoadjuvant treatment of locally advanced rectal cancer [21, 22, 40]. Some studies evaluated the prognostic significant of K-RAS mutation status in neoadjuvant treatment of rectal cancer. A study mentioned above evaluated the influence of panitumumab in neoadjuvant treatment of rectal cancer and failed to demonstrate the prognostic significance of K-RAS gene mutation state and response rate [39]. An EXPERT study evaluated 161 patients with locally advanced rectal cancer. The treatment combined neoadjuvant chemoradiotherapy (potentiated by capecitabine) and CAPOX regimen before and after chemoradiotherapy for both study arms. Cetuximab has been adopted

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in one arm in all phases of treatment. The patients with wild-type K-RAS treated with cetuximab have shown longer OS (HR 0.27; p = 0.034) compared to patients treated without cetuximab [41]. The following study demonstrated a higher percentage of pCR (37 vs. 11%) in patients in wild-type K-RAS compared to those with K-RAS mutations. In that study, a total of 39 patients with locally rectal cancer were treated with neoadjuvant chemoradiotherapy and cetuximab. K-RAS mutation status was observed in 6 patients, and the other 30 patients had wild-type K-RAS gene [42]. On the other hand, some studies have shown that no prognostic influence of mutation status of K-RAS gene on patients was observed [33, 43]. An interesting fact is the lower incidence of K-RAS mutation status in rectal carcinoma (12–30%) compared to colon carcinoma [43, 44]. The results of studies evaluating the influence of anti-EGFR antibodies with the combination of neoadjuvant chemoradiotherapy for rectal cancer are not satisfactory [27–37]. More options of how to better individualize the treatment of patients with EGFR inhibitors are under investigation. One of them is the research about the dynamics of EGFR expression during the neoadjuvant chemoradiotherapy, which is the focus of our study.

2. Materials and methods

A total of 50 patients with locally advanced rectal cancer were evaluated in our study. The median age was 61.4 years (range 40–78 years). TNM stage II was described in 28 and TNM stage III in 22 patients. The anatomical localization was as follows: 24 patients lower rectum (<5 cm from the anal verge), 24 patients middle rectum (>5–10 cm), and 2 patients upper rectum (above 10 cm from the anal verge). All patients had a histologically verified adenocarcinoma in a pretreatment biopsy: 3 patients grade I, 38 patients grade II, and 9 patients grade III. Pretreatment concentration of CEA was evaluated in 29 patients. Median CEA level was 3.2 (0.5–377) μg/L. Eleven patients had the elevation of CEA.

2.1. Treatments

Neoadjuvant treatment consisted of external beam radiation and chemotherapy. The source of radiation was a linear accelerator Elekta Precise or Elekta Synergy (Elekta, Sweden). The photon energy was 15 MeV. Patients were treated in supine position with full bladder (**Figure 1**). The localization was held on RTG simulator with AP projection. Then patients absolved the planning CT with reconstruction of slices of thickness 5 mm. The contouring of targeted volumes and organs at risk was performed by planning system PrecisPlan 2.15 (**Figure 2**). Patients were irradiated by 3D conformal radiotherapy technique or IMRT using segmented fields (**Figure 3**). A total dose of 44 Gy in 22 fractions (single dose 2 Gy) was administered. The target volume consisted of rectum with tumor, mesorectum, and pelvic regional lymph nodes. All patients were treated by using one targeted volume. The organs at risk were bladder and bowel sac. The verification was performed once a week with the help of cone-beam CT or portal image. Capecitabine was concomitantly administered with a dosage of 825 mg/m^2 twice daily by oral administrations for the whole duration of radiotherapy, including weekends. Surgery was performed 4–8 weeks after the end of chemoradiotherapy.

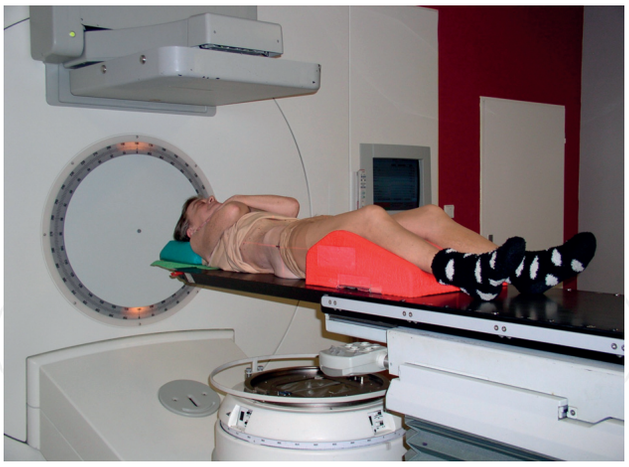


Figure 1. Supine position in irradiated patient in our department of oncology.

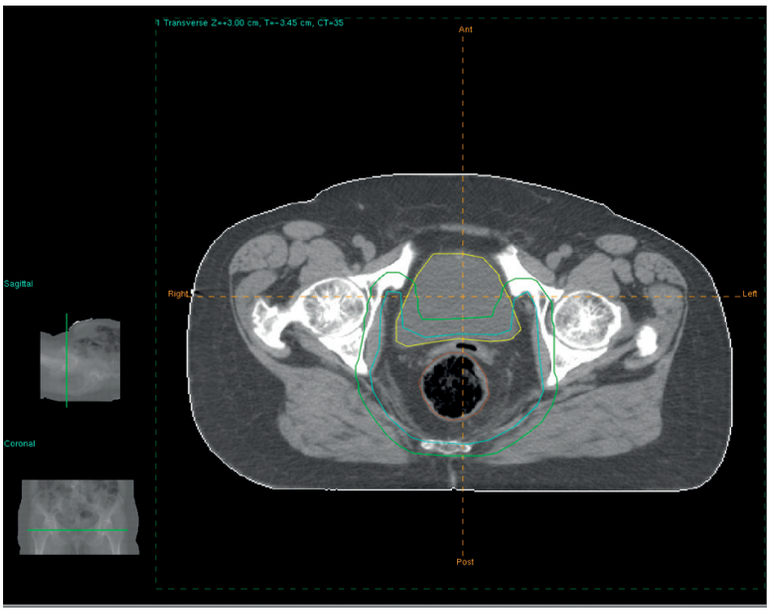


Figure 2. Targeted volumes in radiotherapy of rectal cancer.

2.2. Immunohistochemical determination of EGFR

Routinely fixed, paraffin-embedded blocks of pretreatment biopsies and resected specimens were cut in 3 μm sections. Slides were deparaffinized with xylene and rehydrated and subsequently treated with proteinase K for antigen retrieval. Endogenous peroxidase activity was blocked with peroxidase block solution with 3% hydrogen peroxide. Sections were incubated in complete medium for 30 min at room temperature with EGFR pharmDx monoclonal mouse anti-human IgG1 antibody (EGFR pharmDx™, DakoCytomation, Denmark). A labeled polymer-HRP was then applied and incubated for 30 min. DAB+ substrate-chromogen solution was used for visualization after 10 min incubation, after which slides were counterstained with hematoxylin. As a control for EGFR expression, EGFR pharmDx control slides containing section of two pelleted, formalin-fixed, paraffin-embedded human cell lines were used: one representing a moderate level

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of EGFR protein expression and the other no EGFR expression. Specimens were examined under a light microscope. All slides were assessed for EGFR expression by a trained pathologist who was blinded for tumor response data. The evaluation was semiquantitative as the color intensity of at least 1% of tumor cells was assessed as follows: 0 = none, 1+ = mild, 2+ = moderate, 3+ = strong (**Figure 4**).

2.3. Statistical analysis methods

Disease-free survival (DFS) and overall survival (OS) were counted from the date of the start therapy and analyzed using the Kaplan–Meier method. Relationship between the level of

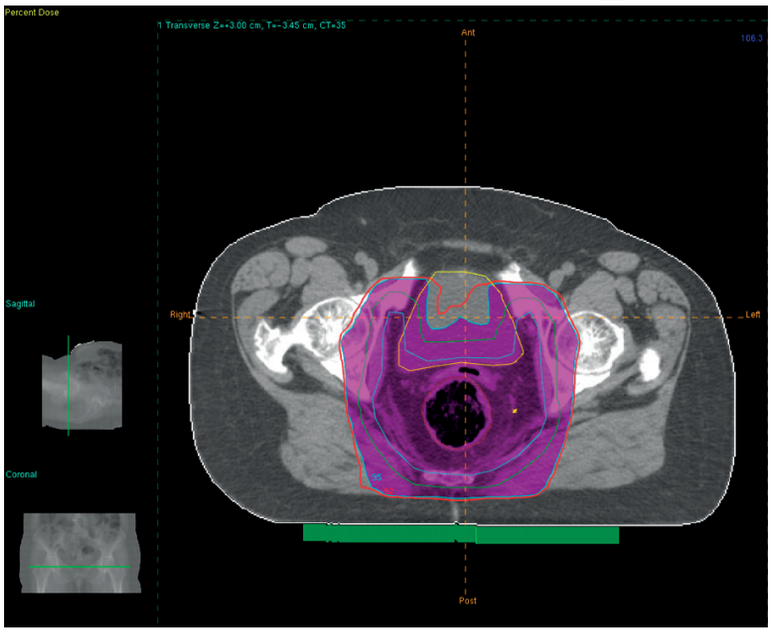


Figure 3. Isodose plane of the radiotherapy of rectal cancer.

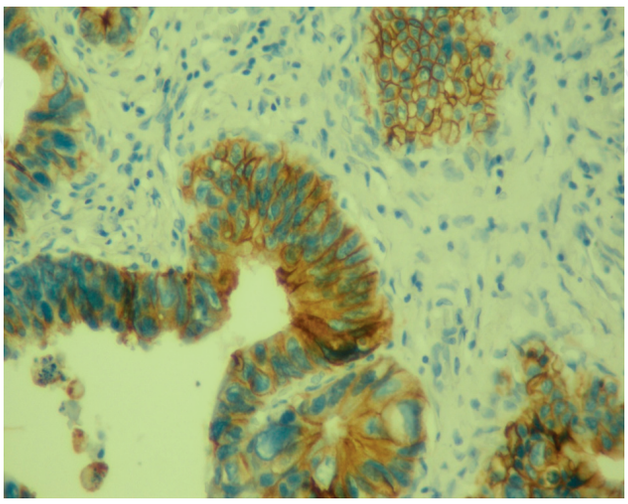


Figure 4. EGFR expression score 3+. Magnification 200x.

EGFR expression and clinical/histopathological characteristic were analyzed using the chi-squared test. Fisher exact test was used on a four-field table when the number of cases was fewer than 10. The prognostic significance of EGFR expression in biopsies and resected specimens and prognostic significance of increased EGFR expression during neoadjuvant chemoradiotherapy on treatment outcomes was assessed by the log-rank test. Multivariate analysis was performed using the Cox regression. We considered $p < 0.05$ to be statistically significant. All statistical analyses were performed using the NCSS 9 statistical software program (NCSS, USA).

3. Results

All 50 patients received radiotherapy without interruption up to the total planned dose. No patient died during the treatment. Concomitant chemotherapy was discontinued prematurely in 4 patients because of hematologic and gastrointestinal toxicity. No patient was hospitalized because of acute treatment toxicity. Non-hematological toxicity evaluation did not achieve grade III or grade IV. The most common types of toxicity were gastrointestinal complaints observed in 44 patients, of them 16 have had nausea and vomiting grade I or II. Hematological toxicity in general was expressed in 25 patients. Anemia grade I was found in 9 patients, grade II in 10 patients, grade III in 1 patient. Grade I leukopenia was found in 11 cases and grade II in 2 patients. One patient has had a grade II thrombocytopenia.

Surgery was conducted in all the patients following 4–8 weeks from neoadjuvant chemoradiotherapy completion. The median time between chemoradiotherapy completion and surgery was 44 days (6.3 weeks). In 30 patients, sphincter-saving surgery was performed, and 20 patients underwent amputation of the rectum. No patient was assessed by the surgeon and found inoperable. R0 resection was performed in 47 patients, and microscopically positive margin was described by a pathologist in 3 patients. No patient was left surgically macroscopic residue. According to the pathological TNM classification, 14 patients were postoperatively in the first clinical stage, 24 patients in the second clinical stage, and 8 patients in the third clinical stage. In four patients, pCR was achieved. Downstaging was described in 30 patients. Progression was reported in four patients. At the date of analysis, median follow-up was 51.3 months.

3.1. Overall survival

To the date of analysis, 21 patients died, and 29 were alive. The median of OS was 76.4 months (95% CI: 57.3–76.9). The 3-year OS evaluated in all patients was 92% (**Figure 5**).

3.2. Disease-free survival

At the time of assessment, recurrence occurred in 25 patients, while the other 25 patients had no signs of recurrence. A local recurrence was found in 8 patients, and generalization of disease was reported in 17 patients. The most common sites of metastases were the liver (8 patients) and lungs (7 patients). One patient suffered from brain metastases, and metastatic involvement of

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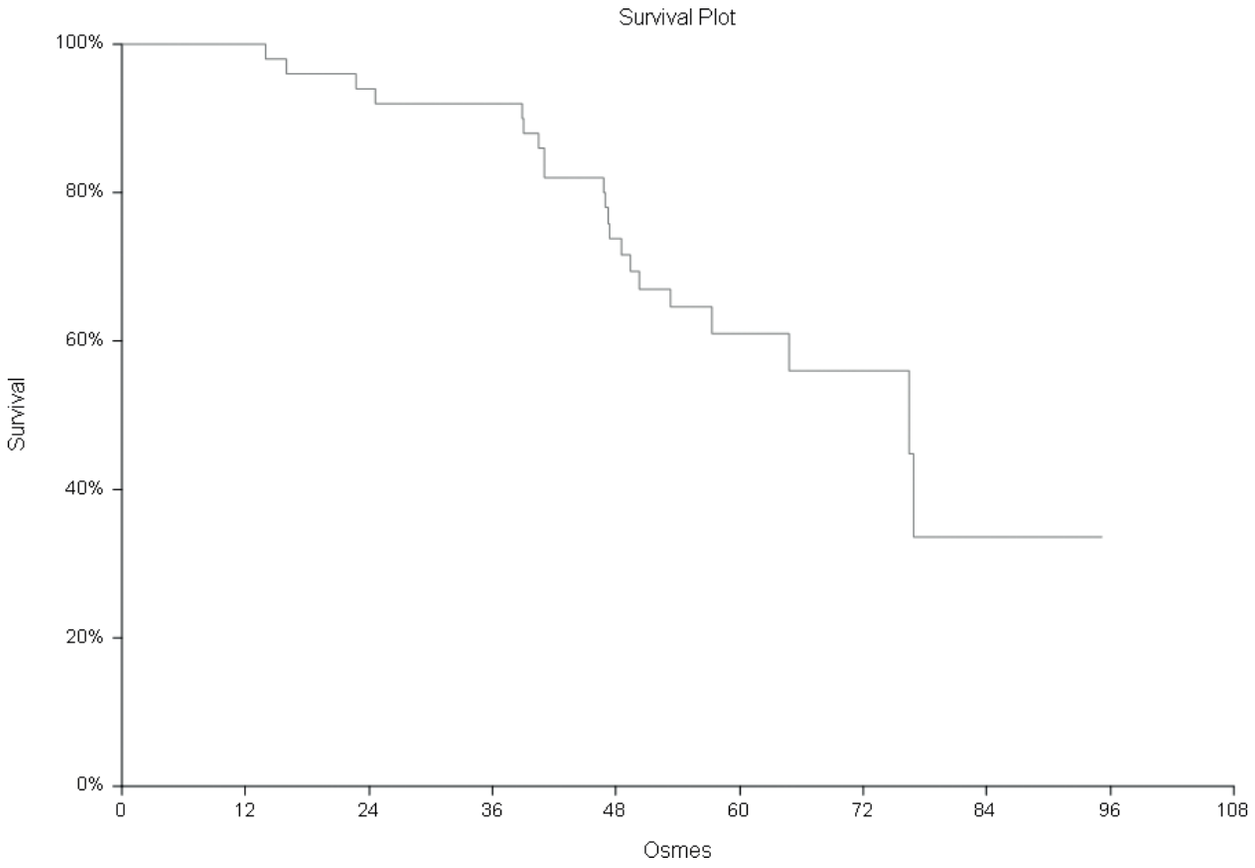


Figure 5. Overall survival (in months) in patients treated by chemoradiotherapy for rectal cancer.

retroperitoneal lymph nodes was found in another patient. The median of DFS was 64.9 months (95% CI 26.4–67.8). The 3-year DFS evaluated in all patients was 56% (**Figure 6**).

3.3. EGFR expression

EGFR expression was examined by both endobiopsy and surgical resection after neoadjuvant chemoradiotherapy. Endobiopsy EGFR was examined in all 50 patients. EGFR 1+ was observed in 18 patients, EGFR 2+ in 5 patients, and EGFR 3+ in 5 patients. Overall, EGFR expression was detected in 28 patients, while 22 patients were not detected with EGFR expression in endobiopsy. EGFR expression was examined and evaluated in 46 patients in the resection. In four patients, EGFR expression was not examined in resection because pCR after neoadjuvant chemoradiotherapy had been achieved. EGFR 1+ was found in 8 patients, EGFR 2+ in 11 patients, and EGFR 3+ in 4 patients. Overall, EGFR expression was detected in 23 patients. In 23 patients, no expression of EGFR was detected in the resection samples. Forty-six patients were enrolled into the evaluation of EGFR expression changes. In four patients, no change expression of EGFR was detected because it had achieved pCR after neoadjuvant chemoradiotherapy. Increased EGFR expression was found in 12 patients. In 34 patients, no increased expression of EGFR was observed (23 patients without any change of EGFR expression, 11 patients with a decrease of EGFR expression).

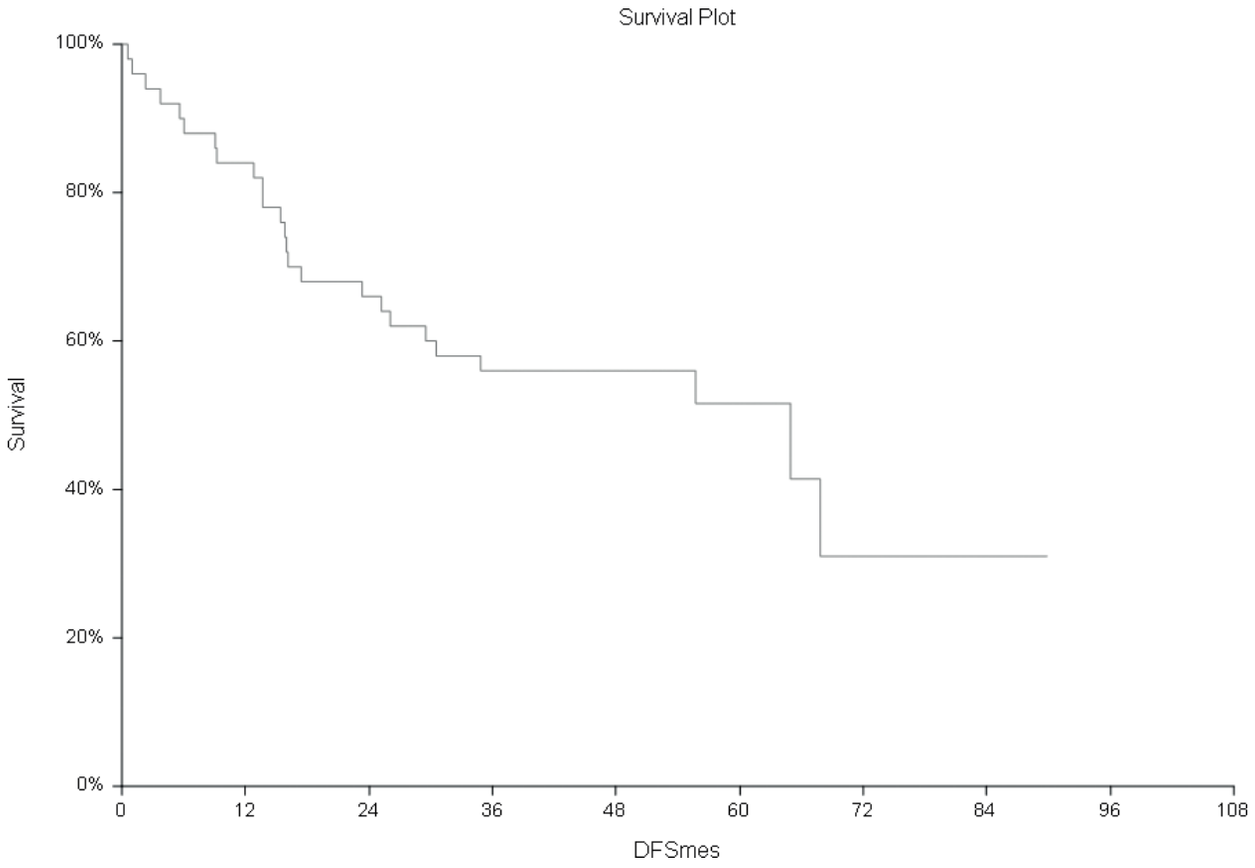


Figure 6. Disease-free survival (in months) in patients treated by chemoradiotherapy for rectal cancer.

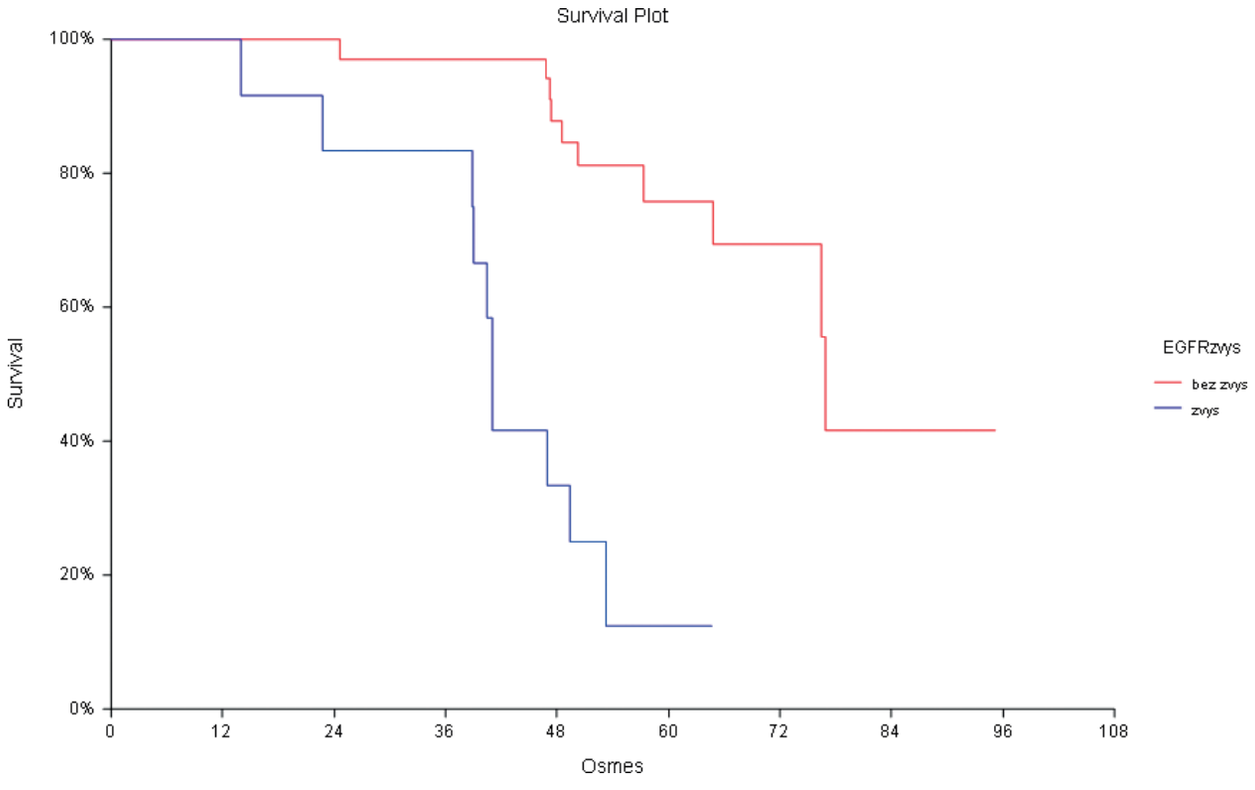


Figure 7. Overall survival (in months) in patients with increased EGFR expression (blue line) and patients without increased EGFR expression (red line) after chemoradiotherapy.

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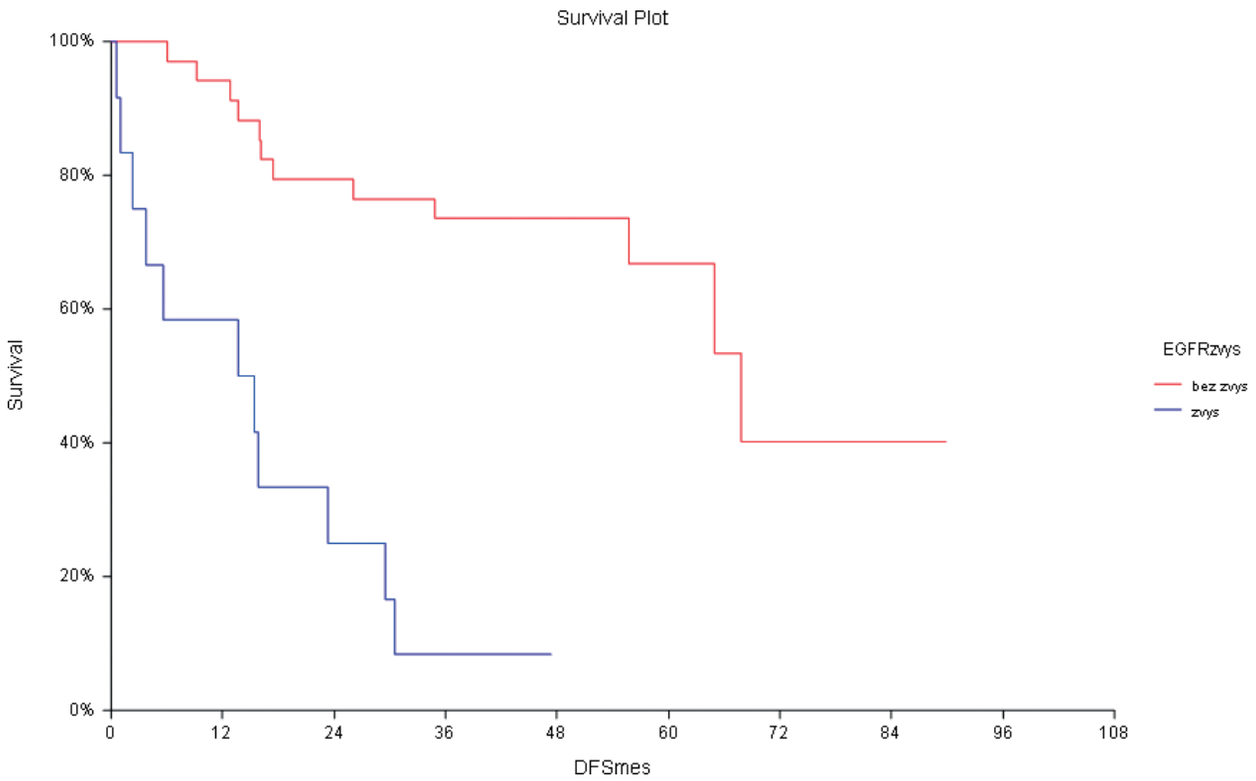


Figure 8. Disease-free survival (in months) in patients with increased EGFR expression (blue line) and patients without increased EGFR expression (red line) after chemoradiotherapy.

The increase of EGFR expression during neoadjuvant treatment had significant impact on OS and DFS. The median of OS in patients with increase of EGFR expression was 41.1 months (95% CI 39.1–47.0). Median of OS in patients without increase of EGFR expression was 76.9 months (95% CI 76.4–76.9). Log-rank: $p < 0.001$ (**Figure 7**). The median of DFS in patients with increase of EGFR expression was 13.7 months (95% CI 3.8–15.8). Median of DFS in patients without increase of EGFR expression was 67.2 months (95% CI 55.7–67.8). Log-rank: $p < 0.001$ (**Figure 8**).

4. Discussion

The results of the present study demonstrated significantly inferior DFS and OS in patients with tumors that had increased EGFR expression after neoadjuvant chemoradiotherapy. The increased EGFR expression after radiotherapy is related to accelerated repopulation of cancer cells [13]. Increased tumor repopulation during radiotherapy leads to recovery of clonogenic tumor cells, thereby causing counterproductivity to radiation therapy alone [14]. The repopulation of clonogenic tumor cells is therefore undesirable phenomenon in treatment using the radiation. We demonstrated increased expression of EGFR in 12 patients, that is, 26.1% of all evaluated patients. In 2012, a retrospective study was conducted in 53 patients with locally advanced rectal cancer treated by neoadjuvant chemoradiotherapy. The aim of the study was similar to our study. During chemoradiotherapy, 14 patients (26%) had an increase in EGFR expression. Patients with increased EGFR expression during treatment had

significantly shorter DFS (HR 3.02, 95% CI 1.15–7.98, p = 0.003) and OS (HR 2.86, 95% CI 1.10–7.40, p = 0.005) than patients with either no change or decreased EGFR expression. In this study, patients were treated with radiotherapy (total dose 50.4 Gy) and chemotherapy (continual administration of 5-FU) [45]. Both studies demonstrated the prognostic influence of change of EGFR expression on DFS and OS in two different groups of patients treated in two different cancer centers. EGFR was evaluated in different pathology laboratories. In the group of 53 patients, radiotherapy was potentiated by continuous 5-FU and in our group by capecitabine. In both studies, the prognostic significance of EGFR dynamics was confirmed. Therefore, they cannot be considered to be simple coincidence but a proven link. In 2014, we published the comparison of both studies with actual follow-up. A total of 103 patients were evaluated. In patients without increasing EGFR expression, there was significantly longer DFS (HR 3.51, 95% CI 1.62–7.61, p < 0.0001) and OS (HR 3.40, 95% CI 1.64–7.04, p < 0.0001, OBR) compared with patients with increase of EGFR. The patients with increase of expression of EGFR had significantly shorter 5-year DFS (20.9 vs. 63.3%, p < 0.0001) and OS (23.3 vs. 68.8%, p < 0.0001) compared with patients with either no change or decreased EGFR expression (**Figures 9** and **10**) [46].

The overexpression of EGFR is observed in 50–60% of rectal carcinoma and is associated with worse prognosis [15–17]. Some studies demonstrated the prognostic influence of EGFR expression on outcomes [18–20]. The most frequent approach of EGFR determination is immunohistochemical (IHC) reaction. This approach was used in most studies. The advantages of IHC determination are simplicity, rapidity of execution, and conservation of tissues morphology.

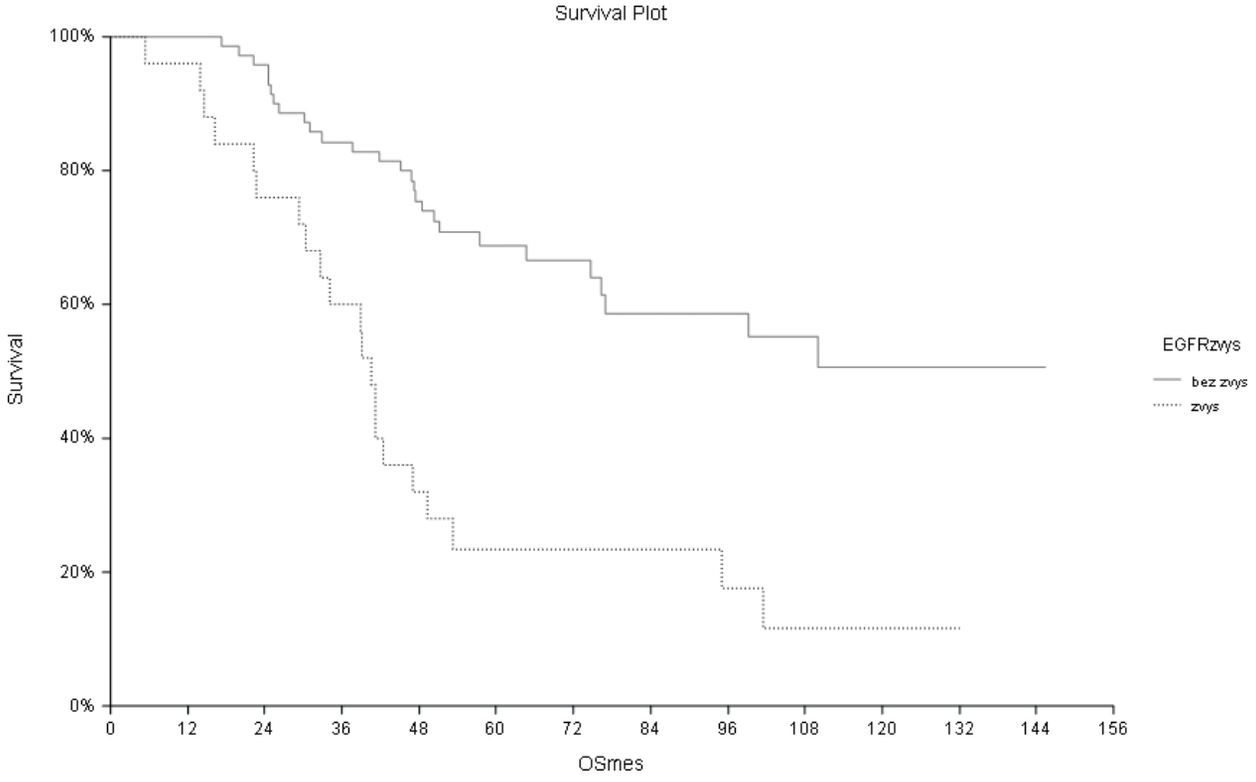


Figure 9. Overall survival (in months) in patients with increased EGFR expression (dotted line) and patients without increased EGFR expression (full line) after chemoradiotherapy.

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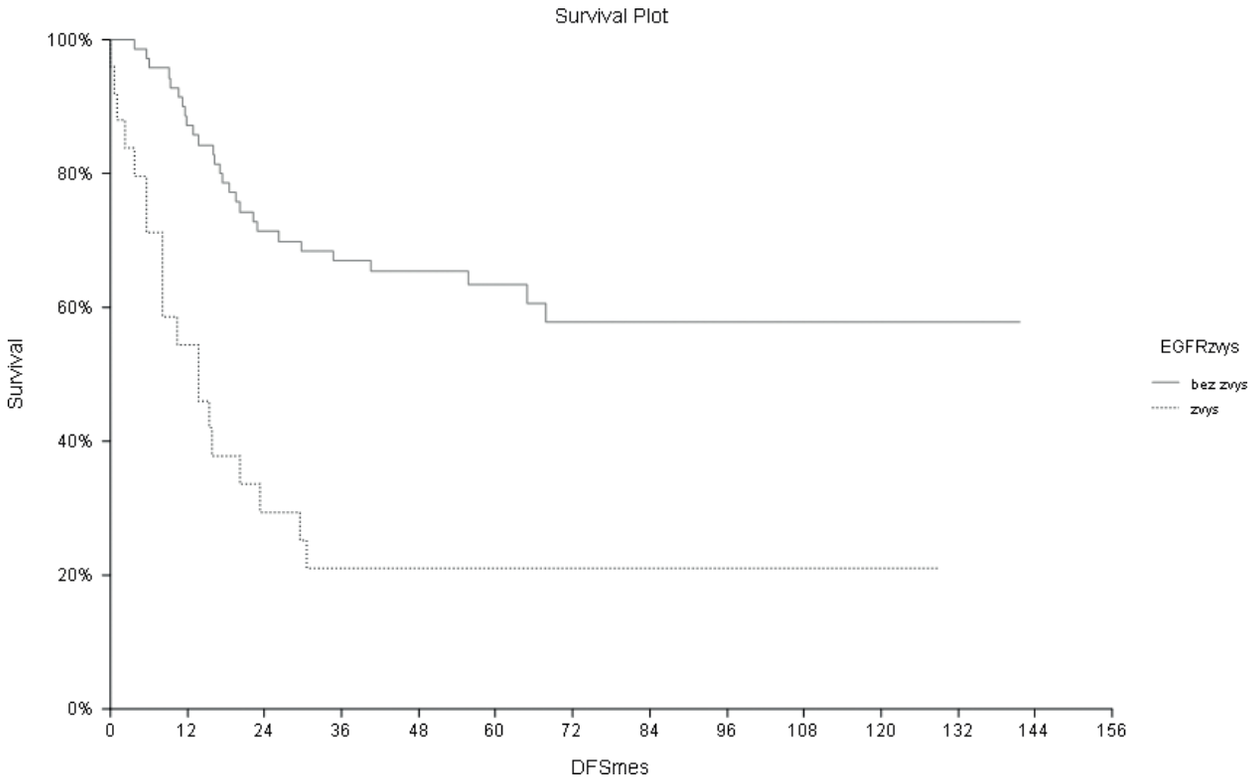


Figure 10. Disease-free survival (in months) in patients with increased EGFR expression (dotted line) and patients without increased EGFR expression (full line) after chemoradiotherapy.

The disadvantages are subjective interpretation by pathologist and existence of more scoring systems for determining EGFR expression. The evaluation of EGFR expression is based on percent range, color intensity, or its combination [21]. Neoadjuvant treatment of rectal cancer has been the topic of several clinical studies I/II phases evaluating the benefits of monoclonal antibodies against EGFR combined with chemotherapy. Eleven clinical studies showed average number of pCR only 10.7% (range 0–25%) of cases [27–37]. The explanation of this will need further understanding of the interaction between radiotherapy, EGFR inhibitors, and cytostatics. Initial studies of this topic showed that prolonged exposure of head and neck cancer cells to EGF could increase the effects of radiation [47, 48]. The reason of radiosensitivity was probably through EGF-induced EGFR degradation. Another early study demonstrated that anti-EGFR antibodies increased radiation-induced apoptosis [49]. Other studies showed the inverse correlation between EGFR expression and response to radiotherapy [50–52]. This relationship between EGFR expression and lower response to radiotherapy was confirmed in human head and neck cancer [53]. EGF is known to induce cyclin D1 expression, a protein that is required for progression from the G1 to S phase. Studies of EGFR signaling inhibition have demonstrated proliferation inhibition of cells in G1 phase [12]. EGFR inhibitors commonly produce cytostatic effects rather than cytotoxicity [54, 55]. The interest of new approach combining the EGFR inhibitors and radiation was generated by the experimental studies that demonstrated of radiation-induced EGFR activity in vitro. Confluent cells in culture treated with ionizing radiation rapidly show increased levels of phosphorylated EGFR [56–59]. The result is cellular proliferation and DNA-damage repair capability. The phenomenon is known as accelerated repopulation. Cetuximab

inhibits this radiation-activated of DNAPK, as well as EGFR nuclear import, DNA repair, and radiation survival [60]. Various preclinical studies demonstrated that EGFR inhibitors increased radiosensitivity in both in vitro and in vivo [10–12, 61, 62]. The most important role is the interaction between chemotherapy and EGFR inhibitors. Nyati discussed in his review whether the results of the combination of neoadjuvant chemoradiotherapy with EGFR inhibitors could be seen in the suboptimal sequence of administered treatment that might lead to an antagonistic rather than a potentiating effect [63]. Administration of EGFR inhibitors before the cytostatic scan arrested the cell cycle in the G1 phase, which can affect the attenuation of the effects of subsequently administered cytostatics, with an impact on other phases of the cell cycle. It is cytostatics used for the treatment of colorectal cancer (5-FU, capecitabine) that have the most highlighted effect on the cell cycle in the S/G2/M phases of the cell cycle [29]. This would lead to the hypothesis that giving chemotherapy before an EGFR inhibitor would be more effective than reverse schedule. A study has evaluated the optimum sequencing for the combination of gemcitabine and gefitinib. It demonstrated that gemcitabine followed by gefitinib was superior to the opposite drug order [64]. Other studies showed similar results with better effect of sequention cytostatics–EGFR inhibitors than vice versa [65, 66]. It is not clear why the sequence of cytostatics before EGFR inhibitor is crucial in the case of cytotoxic agents that are not necessarily S-phase specific. Another mechanism of interaction between cytostatics and EGFR inhibitors is modulation of the EGFR-induced pathway. EGFR phosphorylation occurs in response to various cytotoxic drugs, including oxaliplatine, 5-FU, and irinotecan [67]. The phosphorylation of EGFR by oxaliplatine or 5-FU treatment alone correlates with the inhibition of cell viability and cell growth by gefitinib [67]. EGFR phosphorylation can lead to the EGFR degradation and dead cells, under condition of prolonged cellular stress. The reason is the persistent deoxyribonucleotide pool depletion. The EGFR degradation is dependent on the activation of the proteosome [68]. The other mechanism of synergy between chemotherapy and EGFR inhibitors is through the inhibition of DNA repair. Cytostatics induce various types of DNA damage (strand breaks, DNA adducts, inter- and intra-strand crosslink). The repair of cisplatine induces DNA inter-strand crosslink inhibited by gefitinib [69, 70].

The results of the combination of neoadjuvant treatment and EGFR inhibitors were not successful [27–37]. Similarly, EGFR inhibitors did not demonstrate better outcomes in adjuvant treatment of colorectal cancer. The phase III clinical study evaluated a total of 2686 patients with colorectal cancer treated with the combination of FOLFOX and cetuximab or FOLFOX alone. The primary aim was to evaluate the overall survival. The addition of cetuximab did not demonstrate longer survival compare to chemotherapy alone in median follow-up of 28 months [71]. The other studies evaluating the importance of neoadjuvant or adjuvant treatment with EGFR inhibitors in rectal adenocarcinoma should be performed in future. The study of change of EGFR expression during neoadjuvant chemoradiotherapy is to better individualize the treatment. Our study would be to define the population of patients with increases of EGFR expression after neoadjuvant chemoradiotherapy. In this group of patients (a total about 25% of studied patients), we assumed that phenomenon acceleration repopulation is applied. This phenomenon is observed in a smaller number of cases than in patients with squamous cell head and neck cancer. The patients with the increased EGFR expression would benefit from additional anti-EGFR therapy after surgery. Future prospective study could adopt not only immunohistochemistry ex vivo as in our

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study but also whole body immunochemistry in vivo by using PET/EGFR. PET detection of EGFR would facilitate the evaluation of EGFR expression not only after but also during the course of neoadjuvant chemoradiotherapy [72].

In our study, we described local relapse in eight patients who represented 16% of all the patients. The CAO/ARO/AIO-94 study comparing neoadjuvant to adjuvant chemoradiotherapy presented local relapse in 7.1% of patients. The reason is that total mesorectal excision was not used in some of the patients. The significance of TME was conclusively demonstrated in clinical studies [73, 74]. The TME surgical standard treatment of rectal carcinoma was defined. We further observed distant metastases in 17 patients who represented 34% of the patients. The cause is a fact of the early subclinical systemic dissemination in the time of diagnosis [75]. This hypothesis confirmed the results of clinical studies with approximately 30% incidence of distance metastases [76–78].

In our study, the patients relatively tolerated the treatment well. We did not demonstrate the death during the neoadjuvant chemoradiotherapy. In four patients, we stopped capecitabine administration due to the hematological toxicity. The most common type of toxicity was a gastrointestinal toxicity. This fact is caused by radiation to the pelvic area and adverse events of capecitabine. In addition, the symptoms could be caused by the presence of tumor. The rectal carcinoma presents along with hemorrhage, tenesmus, pelvic pain, and diarrhea. These symptoms dominated in patients treated with chemoradiotherapy.

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