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The prognostic significance of epidermal growth factor receptor expression in patients with anal carcinoma

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The aim of the present retrospective study was to evaluate the prognostic significance of epidermal growth factor receptor (EGFR) expression in patients treated with radiotherapy or concomitant chemoradiotherapy for squamous cell anal cancer (SCAC)

Patients and methods: A total of 17 patients with SCAC (clinical stages I-III) were studies. All patients were treated with radiotherapy (total dose range 40 – 68 Gy), 13 patients received concomitant chemotherapy (7 patients mitomycin/5-fluorouracil, 5 patients cisplatine/5-fluorouracil, 1 patient cisplatine weekly). EGFR expression in the pretreatment biopsieswas assessed with imunohistochemistry.

Patients with EGFR expression had significantly shorter progression free survival (PFS) (p=0.0109; HR 9.38, 95% CI 1.75 – 50.35) and overall survival (OS) (p=0.0351; HR 7.11, 95% CI 1.4 – 36.13) than patients without expression EGFR. The 4-year PFS in patients with increased EGFR expression was only 28.57% (95% CI 17.07 – 62.04%) compared to 87.5% (95% CI 64.58 - 100%) in patients without EGFR expression. The 4-year OS in patients with increased EGFR expression was only 50.0% (95% CI 15.35 – 84.65%) compared to 87.5% (95% CI 64.58 – 100.0%) in patients without EGFR expression.

Patients with expression EGFR had significantly shorter PFS and OS compared with patients without EGFR expression.

Key words: anal carcinoma, epidermal growth factor receptor, chemoradiotherapy, overall survival, progression free survival

Squamous cell carcinoma of the anal canal (SCAC) is relative rare gastrointestinal malignancy. It represents less than 2.5% of all gastrointestinal cancer [1]. Essential prognostic factors include the clinical stage, involvement of regional lymph nodes, tumor grade, age and sex [2]. In the past, the standard treatment of anal cancer was abdominoperineal resection with an approximate 40–70% 5-year survival [3]. In 1974, Nigro observed complete remission in patients treated with concomitant chemoradiotherapy [4]. The current standard treatment for invasive SCAC is the combination of radiotherapy and chemotherapy, based on the findings of several randomized studies [5-10]. Additional prognostic and predictive factors have been sought in line with the development of molecular biology in order to improve the results and better individualize the therapy. The epidermal growth factor receptor (EGFR) signaling pathway is one of the most widely studied ones. EGFR is a 170 kDa transmembrane glycoprotein [11]. After ligand binding, two extracellular domains of EGFR homodimerize, or an EGFR domain heterodimerizes with another ErbB family member. Dimer internalization is followed by autophosphorylation of the intracellular tyrosine kinase domain, which activates cytoplasmic transduction protein cascades; these induce cell proliferation, acceleration of cell repopulation, and inhibit apoptosis [12]. Overexpression of EGFR has been found in most solid tumors and is associated with more aggressive behavior of the cancer cells, worse response to radiotherapy or chemotherapy and increases the motility of cancer cells [13-15]. Radiobiological studies have confirmed the critical role of EGFR in terms of the cytoprotective and pro-proliferative response of cancer cells after irradiation. An induced increase in EGFR expression after radiotherapy can be associated with accelerated repopulation

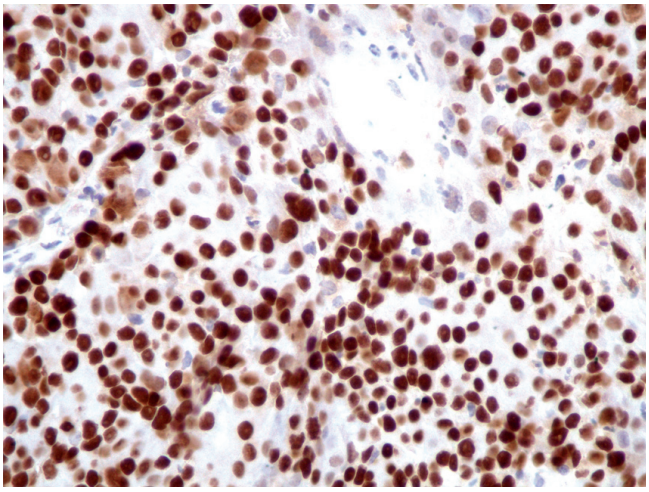

Figure 1. Expression of p63 positivity in squamous cell of anal carcinoma. Magnification 400x.

of cancer cells [16, 17]. Accelerated repopulation of cancer cells resulting from increased EGFR expression induced by ionizing radiation represents a potential mechanism of therapeutic failure [18-20]. The aim of this retrospective study is to evaluate the prognostic significance of EGFR expression in patients with SCAC treated with radiotherapy or concomitant chemoradiotherapy.

Patients and methods

Patient characteristics. Between January 2003 and December 2010, 17 patients (3 men and 14 women) with anal cancer were treated at the Department of Oncology, Regional Hospital Liberec, Czech republic. The median age was 57 (range 40 – 81) years. One patient had clinical stage I, 10 patients had clinical stage II, and 6 patients had clinical stage III. All patients had histologically verified squamous cell carcinoma in pretreatment biopsy: 1 patient grade I, 10 patients grade II, and 6 patients grade III. The median of pretreatment concentration of hemoglobin was 128 (range 94 – 155) g/L, leukocytes 7.3 (range 4.9 – 11.6)*10^9/L, and thrombocytes 266 (range 156 – 492) *10^9/L.

Treatment. Radiotherapy was indicated in all 17 patients. Fifteen patients received radiotherapy in two targeted volumes. The first one included irradiation of pelvic lymph nodes, inguinal lymph nodes, and the tumor. The boost volume included to involved inguinal lymph nodes and the tumor of the anus. Irradiation using a single target volume was indicated in 2 patients (patients with a worse performance status). Boost with using of brachytherapy was indicated in 1 patient (dose 2x8 Gy), and boost with using of electron beam was indicated also in 1 patient (dose 2x5 Gy). All patients received radiotherapy using with megavoltage photon beams (6 or 15 MV). The source of radiation was a linear accelerator (Elekta Synergy or Elekta Precise, Elekta Sweden). Patients were irradiated using 3D conformal radiotherapy or IMRT technique using segmented fields. The total applied dose ranged between 40 Gy – 68 Gy in daily fractionation of 1.8 Gy – 2.0 Gy; median dose was 60 Gy. Concomitant chemotherapy was administered to 13 patients. The combination of mitomycin C and 5-fluorouracil (5-FU) was used most commonly, in 7 patients in total. The combination of cisplatin and 5-FU was used in 5 patients, and cisplatin monotherapy in the weekly regimen was indicated in 1 patient.

Imunohistochemical evaluate. Processed paraffin blocks were cut using a microtome to obtain sections 3 μm thick. Standard deparaffinization was performed using xylene with subsequent rehydration. Subsequently, proteolytic reaction of the tissue was carried out upon addition of proteinase K. Endogenous peroxidase reaction was inhibited by applying 3% hydrogen peroxide solution. The tissue sections were then incubated at ambient temperature with murine monoclonal antibody IgG1 against EGFR. The monoclonal antibody formed part of a commercially supplied kit (Dako EGFR PharmDx™, Denmark). Furthermore, the sample was incubated for approx. 30 min with marked HRP polymer and for additional 10 minutes with DAB+ dye solution. The samples were then colored using hematoxylin. Control preparations supplied in the commercial kit were used as negative control of EGFR expression. The samples were read in an light microscope Olympus BX 60. The preparations were read by an experienced pathologist not acquainted with therapeutic results of the patients. We evaluated the total membrane EGFR expression. The evaluation was semiquantitative, and coloring intensity of at least 1% cancer cells was evaluated: 0 = none; 1 = weak; 2 = moderate; 3 = strong. In all patients were confirmed the squamous cell carcinoma with imunodetection of p63 (DAK-p63, Dako, Denmark, Fig. 1).

Statistical analysis methods. The software Number Cruncher Statistical System NCSS 9 (Kaysville, Utah, USA) was used for statistical assessments. Overall survival (OS) = time from diagnosis to death or to the last visit in surviving patients. Progression-free survival (PFS) = time from radiotherapy or chemoradiotherapy completion to a relapse or progression of the disease or to the last visit in patients with no relapses. Overall survival and progression-free survival were calculated using the Kaplan-Meier method. The log-rank test was used to evaluate any effect of EGFR expression on the therapeutic results (OS, PFS). All statistical tests were evaluated using the significance level $\alpha = 0.05$.

Results

All 17 patients underwent radiotherapy. Concomitant chemotherapy was completed by 13 patients in total. The therapy was temporarily discontinued for 2 to 14 days in the total of 5 patients due to toxicity. The therapy was terminated prematurely by 2 fractions (boost dose 4 Gy) in 1 patient, again due to toxicity. Eight patients were hospitalized for supportive

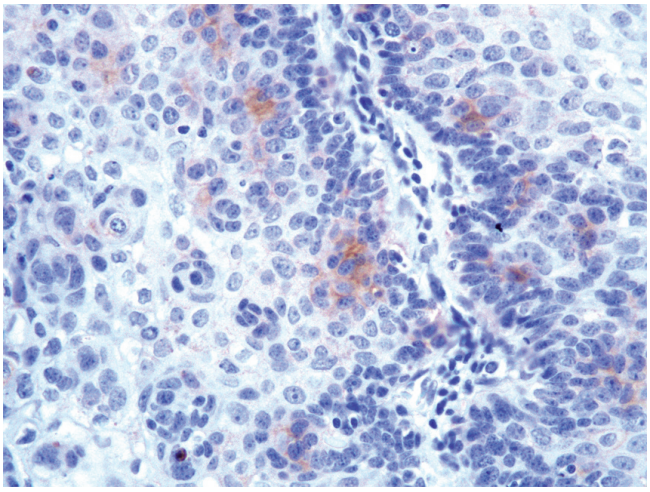

Figure 2. Epidermal growth factor receptor staining score 1+. Magnification 400x

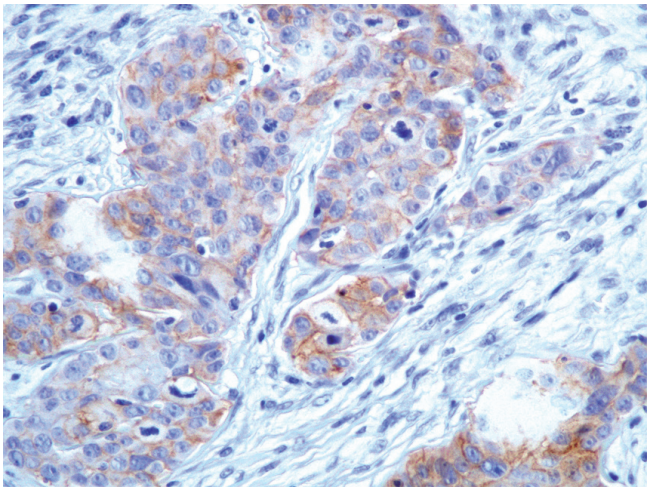

Figure 3. Epidermal growth factor receptor staining score 2+. Magnification 400x

therapy. No patient died during therapy. Toxicity was the most common type of acute toxicity, which affected all patients. According to RTOG scale, grades I and II were described in 5 patients, and grade III in 9 patients. Grade IV was found in 3 patients. Acute gastrointestinal toxicity was described in 15 patients; grades I and II were found in 13 patients, and grade III in 2 patients. Genitourinary acute toxicity was observed in 14 patients in total, grades I and II occurred in 11 patients, grade III was observed in 3 patients. As regards evaluation of hematologic toxicity, grade I of anemia was found in 7 patients, and grade II in 1 patient. Leucopenia grade I was observed in 3 patients, grade II in 3 patients, and grade III in 1 patient. Thrombocytopenia grade II occurred in 2 patients. The median nadir of hemoglobin was 116 (range 92 – 128) g/L, leucocytes 4.0 (range 2.2 – 8.0)*10^9/L, and thrombocytes 167 (range 60 – 492) *10^9/L.

Partial response with subsequent full clinical remission was observed in 15 patients. Full clinical remission occurred between 4 weeks to 18 months from completion of chemoradiotherapy or radiotherapy alone. Stabilized disease was described in 1 patient. Progression after therapy occurred in 1 patient. At the time of assessment (30 Juny 2015) median follow-up was 57 months (4.7 years). During follow-up, recurrence occurred in 6 patients: 3 local and 3 generalized diseases (metastases in retroperitoneal lymph nodes in 2 cases and metastatic lung involvement in 1 case). Six patients died until the date of evaluation. The 4-year progression free survival (PFS) was 61.36% (95% CI 37.00 – 85.73%) and 4-year overall survival (OS) was 76.47% (95% CI 56.31 – 96.63%).

EGFR expression was examined in 16 patients; the assessment was not done in 1 patient due to insufficient amount of histological material. EGFR expression was not observed in 8 patients; EGFR expression was found in 8 patients (4 patients EGFR 1+, 4 patients EGFR 2+, no patient was EGFR 3+ expression, Tab. 1 and Fig. 2 and 3). The prognostic significance of EGFR expression was evaluated in terms of PFS and OS. Patients with EGFR expression had significantly shorter PFS (p=0.0109; HR 9.38, 95% CI 1.75 – 50.35) and OS (p=0.0351; HR 7.11, 95% CI 1.4 – 36.13) than patients without expression EGFR (Fig. 4 and 5). The 4-year PFS in patients with increased EGFR expression was only 28.57% (95% CI 17.07 – 62.04%) compared to 87.5% (95% CI 64.58 – 100%) in patients without EGFR expression. The 4-year OS in patients with increased EGFR expression was only 50.0% (95% CI 15.35 – 84.65%) compared to 87.5% (95% CI 64.58 – 100.0%) in patients without EGFR expression.

Discussion

EGFR expression in anal cancer was evaluated by several studies. The first study was published by Alvarez in 2006 who evaluated EGFR in 38 patients. He observed increased EGFR expression in 21 patients in total, which presents 55% [21]. Several other studies were then published, finding EGFR expression between 58% – 100% [22-25]. In our study we observed EGFR expression in one half of the patients. EGFR expression 1+ was observed in 4 patients, and similarly also EGFR expression 2+. Van Damme described EGFR expression 1+ in 7 patients, EGFR expression 2+ in 12 patients, and EGFR expression 3+ in 17 patients. In total, he observed EGFR expression in 83.7% patients [23]. We observed no EGFR

Table 1. EGFR expression score in biopsies

EGFR expression	0	1	2	3	not applicable
Number of patients	8	4	4	0	1

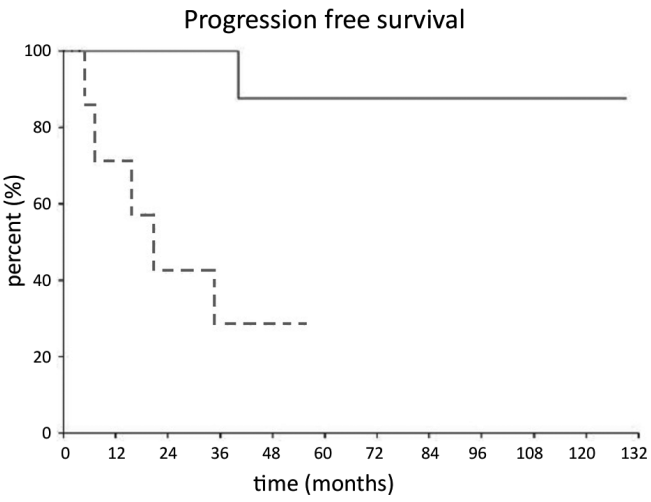

Figure 4. Progression free survival (in months) in patients who had expression EGFR (broken line) and patients with no EGFR expression (solid line).

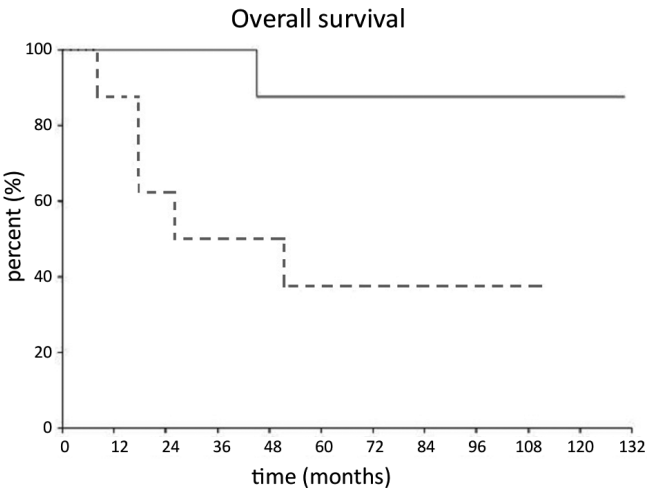

Figure 5. Overall survival (in months) patients who had expression EGFR (dotted line) and patients with no EGFR expression (broken line).

expression 3+ in our study. We use the semiquantitative evaluation technique based on the immunohistochemical reaction. The different results of EGFR expression may have been caused by the use of different antibodies, different immunohistochemical techniques or different expression scoring systems. Potential subjective influence on EGFR expression evaluation using immunohistochemistry should also be emphasized. Our study demonstrated a significant prognostic significance of EGFR expression on overall survival and progression-free survival. In his study in 30 patients, Ajani did not demonstrate a significant correlation between EGFR expression grade and diseases-free survival [26]. Similarly, Alvarez did not observe any dependence between EGFR expression and clinical and pathological parameters [21]. Our study could be criticized for its lower number of evaluated patients. This fact is related to the rare incidence of this disease in the population of central Europe. At our Department of Oncology, 2-3 cases of anal cancer are diagnosed every year on average. Based on the facts above, we could ask whether EGFR function can be inhibited during oncology therapy of SCAC. The first group of inhibitors of EGFR presented small molecular inhibitors of the tyrosine kinase domain of the receptor (TKI). The oral TKIs gefitinib and erlotinib have been applied most and currently, they are use in particular for palliative therapy of non-small cell lung cancer [27]. Monoclonal antibodies against EGFR extracellular receptor domain are another group; among these, cetuximab and panitumumab have been used most, predominantly in the therapy of metastatic colorectal cancer [28-33]. With the development of molecular biology revealed that the K-RAS oncogene status, and later the entire RAS complex, was a principal predictive factor for the use of anti-EGFR therapy in metastatic colorectal cancer [29, 34]. RAS oncogenes code for regulatory proteins that exert a significant effect on the signal pathway triggered by EGFR activation. These proteins may be present in tumors either in their normal, non-mutated forms (wild type), or with an mutation. In this case the regulatory protein RAS is activated permanently irrespective of EGFR inhibition. Several studies have been done with the aim to determine the frequency of K-RAS mutation in anal cancer. Three studies evaluated the determination of K-RAS mutation in 146 patients in total. None of the assessments showed the presence of the mutated K-RAS form [25, 35]. A larger study evaluated 193 biopsies, demonstrating the K-RAS mutation in only 3 cases [36]. In SCAC, similarly as in spinocellular head and neck cancer, the frequency of K-RAS mutations seems to be much less common than in colorectal cancer where the mutation is found in 30-50% cases [37, 38]. The predictive effect of K-RAS mutation was evaluated by a small study in 7 patients with metastatic anal cancer treated with cetuximab. K-RAS mutation was demonstrated in 2 patients. Patients with mutated K-RAS progressed, unlike the group with non-mutated K-RAS where therapeutic response or stabilization of the disease was observed [39]. Several clinical studies were also done to evaluate the benefit of antiEGFR therapy in anal cancer. The phase II clinical study ECOG 3205 was presented at ASCO 2012, which evaluated the efficacy and safety of combined cetuximab and chemoradiotherapy in patients with anal cancer without any immune system disorder. The first results showed 2-year survival with no signs of a relapse in 92% patients, and safety was assessed as acceptable by the authors. The authors presented a similar therapy also in immunosuppressed patients with HIV infection where 2-year survival without a relapse was achieved in 80% patients (AMC 045 study) [40]. On the contrary, another phase II study, ACCORD 16, was terminated prematurely due to high toxicity of combined cetuximab and chemoradiotherapy using the combination of cisplatin and 5-FU [41]. Similarly, yet another study demonstrated high toxicity of cetuximab therapy [42]. At present,

evaluation of any benefit of cetuximab or panitumumab in the therapy of metastatic anal cancer is available mostly in the form of case reports [39, 43-45]. As mentioned above, worse tolerance of the therapy is a problem in the combination of antiEGFR therapy and chemoradiotherapy. Other possible ways are thus being sought to offer higher individualization of the therapy. Our study could contribute to identification of patients who may benefit from antiEGFR therapy combined with chemoradiotherapy.

Conclusions

Patients with expression EGFR had significantly shorter PFS and OS compared with patients without EGFR expression.

References

[1] SIEGEL R, NAISHADHAM D, JEMAL A. Cancer statistics. Ca Cancer J Clin 2013; 63: 11–30 http://dx.doi.org/10.3322/caac.21166

[2] BILIMORIA KY, BENTREM DJ, ROCK CE, STEWART AK, KO CY et al. Outcomes and prognostic factors for squamous-cell carcinoma of the anal cancal: Analysis of patiens from the National Cancer Database. Dis Colon Rectum 2009; 52: 624 http://dx.doi.org/10.1007/DCR.0b013e31819eb7f0

[3] RYAN DP, COMPTON CC, MAYER RJ. Carcinoma of the anal canal. N Eng J Med 2000; 342: 792–800 http://dx.doi.org/10.1056/NEJM200003163421107

[4] NIGRO ND, VAITKEVICIUS VK, CONSIDINE B. Combined therapy for cancer of the anal canal: a preliminary report. Dis Colon Rectum 1974; 17: 354–356 http://dx.doi.org/10.1007/BF02586980

[5] Epidermoid Anal CANCER: results from the UKCCCR randomized trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. UKCCCR Anal Cancer Trial Working Party. UK Coordinating Comimittee on Cancer Research. Lancet 1996; 348: 1049–1054 http://dx.doi.org/10.1016/S0140-6736(96)03409-5

[6] BARTELINK H, ROELOFSEN F, ESCHWENGE F, ROUGIER P, BOSSET JF et al. Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of phase III randomized trial of the EORTC and Gastrointestinal Cooperative Groups. J Clin Oncol 1997; 15: 2040–2049

[7] NORTHOVER J, GLYNNE-JONES R, SEBAG-MONTEFIORE D, JAMES R, MEADONS H et al. Chemoradiation for the treatment of epidermoid anal cancer: 13-year follow up of the first randomized UKCCCR Anal Cancer Trial (ACT I). Br J Cancer 2010; 102: 1123–1128 http://dx.doi.org/10.1038/sj.bjc.6605605

[8] FLAM M, JOHN M, PAJAK TF, PETRELLI N, MYERSON R et al. Role of mitomycin in combination with fluorouracil and radiotherapy, and salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. J clin Oncol 1996; 14: 2527–2539

[9] GUNDERSON LL, WINTER KA, AJANU JA, PEDERSEN JE, MOUGHAN J et al. Long-term update of US GI intergroup RTOG 98–11 phase III trial for anal carcinoma: survival, relapse, and colostomy failure with concurrent chemoradiation involving fluorouracil/mitomycin versus fluorouracil/cisplatine. J clin Oncol 2012; 30: 4344–4351 http://dx.doi.org/10.1200/JCO.2012.43.8085

[10] JAMES RD, GLYNNE-JONES R, MEADOWS HM, CUNNINGHAM D, MYINT AS et al. Mitomycin or cisplatine chemoradiation with or without maintance chemotherapy for treatment of squamous-cell carcinoma of the anus (ACT II): a randomised, phase 3, open-label, 2x2 factorial trial. Lancet 2013; 30: 4344–4351

[11] WILLET CG, DUDA DG, CZITO BG, BENDELL JC, CLARK JN et al. Targeted therapy in rectal cancer. Oncology 2007; 21: 1055–1065

[12] UBERALL I, KOLAR Z, TROJEC R, BERKOVCOVA J, HAJDUCH M. The status and role of ErbB receptors in human cancer. Exp Mol Pathol 2008; 84: 79–89 http://dx.doi.org/10.1016/j.yexmp.2007.12.002

[13] AKIMOTO T, HUNTER NR, BUCHMILLER L, MASON K, ANG KK et al. Inverse relationship between epidermal growth factor expression and radiocurability of murine carcinomas. Clin Cancer Res 1999; 5: 2884–2890

[14] LIANG K, ANG KK, MILAS L, HUNTER N, FAN Z. The epidermal growth factor receptor mediates radioresistance. Int J Radiat Biol Phys 2003; 57: 246–254 http://dx.doi.org/10.1016/S0360-3016(03)00511-X

[15] VERBEEK BS, ANDRIAANSEN-SLOT SS, VROOM TM, BECKERS T, RIJKSEN G. Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. FEBS Lett 1998; 425: 145–150 http://dx.doi.org/10.1016/S0014-5793(98)00224-5

[16] WITHERS HR, TAYLOR JM, MACIEJEWSKI B. The hazard of accelerated tumour clonogen repopulation during radiotherapy. Acta Oncol 1988; 27: 131–146 http://dx.doi.org/10.3109/02841868809090333

[17] BAUMANN M, PETERSEN C, EICHLER W. Mechanism of repopulation in experimental squamos cell carcinoma. In: Kogelnik HD, Lukas P, Sedlmayer F. Progress in radiation-oncology, vol.7. Bologna, Monduzzi; 2002, pp. 417–422

[18] BEGG AC. Prediction of repopulation rates and radiosensitivity in human tumours. Int J Radiat Biol 1994; 65: 103–108 http://dx.doi.org/10.1080/09553009414550141

[19] FOWLER JF. Rapid repopulation in radiotherapy: a debate on mechanism. The phantom of tumor treatment-continually rapid proliferation inmasked. Radiother Oncol 1991; 22: 156–158 http://dx.doi.org/10.1016/0167-8140-(91)90017-B

[20] SCHMITDT-ULLRICH RK, CONTESSA JN, DENT P, MIKKELSEN RB, VALERIE K et al. Molecular mechanism of radiation-induced accelerated repopulation. Radiat Oncol Investig 1999; 7: 321–330 http://dx.doi.org/10.1002/(SICI)1520-6823(1999)7:6<321::AID-ROI2>3.0.CO;2-Q

[21] ALVAREZ G, PERRY A, TAN BR, WANG HL. Expression of epidermal growth factor receptor in squamous cell carcinomas of the anal canal is independent of gene amplification.

Mod Pathol 2006; 19: 942–949 http://dx.doi.org/10.1038/modpathol.3800608

[22] LE LH, CHETTY R, MOORE MJ. Epidermal growth factor receptor expression in anal carcinoma. Am J Clin Pathol 2005; 124: 20–23 http://dx.doi.org/10.1309/X4UADHVN317-V2XMW

[23] VAN DAMME N, DERON P, VAN ROY N, DEMETTER P, BOLS A et al. Epidermal growth factor receptor and K-RAS status in two cohorts of squamous cell carcinomas. BMC Cancer 2010; 10: 189 http://dx.doi.org/10.1186/1471-2407-10-189

[24] WALKER F, ABRAMOWITZ L, BENABDERRAHMANE D, DUVAL X, DESCATOIRE V et al. Growth factor receptor expression in anal squamous lesions: modifications associated with oncogenic human papillomavirus and human immunodefiuency virus. Hum Pathol 2009; 40: 1517–1527 http://dx.doi.org/10.1016/j.humpath.2009.05.010

[25] ZAMPINO MG, MAGNI E, SONZOGNI A, RENNE G. K-ras status in squamous cell anal carcinoma (SCC): it's time for target-oriented treatment? Cancer Chemother Pharmacol 2009; 65: 197–1999 http://dx.doi.org/10.1007/s00280-009-1117-3

[26] AJANI JA, WANG X, IZZO JG, CRANE CH, ENG C et al. Molecular biomarkers correlate with disease-free survival in patiens with anal canal carcinoma treated with chemoradiation. Dig Dis Sci 2009; 55: 1098–1105 http://dx.doi.org/10.1007/s10620-009-0812-6

[27] SHEPHERD FA, PEREIRA JR, CIULEANU T, TAN EH, HIRSH V et al. Erlotinib in Previously treated Non-Small-Cell Lung Cancer. N Engl J Med 2005; 353: 123–132 http://dx.doi.org/10.1056/NEJMoa050753

[28] CUNNINGHAM D, HUMBLET Y, SIENA S, KHAYAT D, BLEIBERG H et al. Cetuximab monotherapy and cetuximab plus irinotekan in irinotekan-refractory metastatic colorectal cancer. N Engl J Med 2004; 351: 337–345 http://dx.doi.org/10.1056/NEJMoa033025

[29] VAN CUTSEM E, KOHNE CH, LANG I, FOLPRECHT G, NOWACKI MP et al. Cetuximab plus irinotecan, fluorouracil, and leucovorine as fist-line treatment for metastatic colorectal cancer: update analysis of overall survival according to tumor KRAS and BRAF station status. J Clin Oncol 2011; 29: 2011–2019 http://dx.doi.org/10.1200/JCO.2010.33.5091

[30] BOKEMEYER C, BONDARENKO I, MAKHSON A, HARTMANN JT, APARICIO J et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 2009; 27: 663–671 http://dx.doi.org/10.1200/JCO.2008.20.8397

[31] Maugham TS, ADAMS RA, SMITH CG, MEADE AM, SEYMOUR MT et al. Addition of cetuximab to oxaliplatine-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. Lancet 2011; 377: 2013–2114

[32] TVEIT KM, GUREN T, GLIMELIUS B, PFEIFFER P, SORBYE H et al. Phase III trial of cetuximab with continuous or intermittent fluorouracil, luecovorine, and oxaliplatine (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. J Clin Oncol 2012; 30: 1755–1762 http://dx.doi.org/10.1200/JCO.2011.38.0915

[33] VAN CUTSEM E, PEETERS M, SIENA S, HUMBLET Y, HENDLISZ A et al. Open.label phase III trial of panitumumab plus bets supportive care compared with best supportive care alone in patiens with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 2007; 25: 1658–1664 http://dx.doi.org/10.1200/JCO.2006.08.1620

[34] OLINER KS, DOUILLARD JY, SIENA S, TABARNERO J, BURKES R et al. Analysis of KRAS/NRAS and BRAF station in the phase III PRIME study of panitumumab (pmab) plus FOLFOX versus FOLFOX as first-line treatment (tx) for metastatic colorectal cancer (mCRC). J Clin Oncol 2013; 31(Suppl): Abstract 3511

[35] PALIGA A, ONERHEIM R, GOLOGAN A, CHONG G, SPATZ A et al. EGFR and K-ras gene mutation status in squamous cell anal carcinoma: a role for concurrent radiation and EGFR inhibitors? Br J Cancer 2012; 107: 1864–1868 http://dx.doi.org/10.1038/bjc.2012.479

[36] SERUP-HANSEN E, LINNEMANN D, HOGDAL E, GEERTSEN PF, HAVSTEEN H. KRAS and BRAF mutation in anal carcinoma. APMIS 2015; 123: 53 – 59 http://dx.doi.org/10.1111/apm.12306

[37] CHANG SE, BHATIA P, JOHNSON NW, MORGAN PR, MCCORMICK F et al. Ras mutation in United Kingdom examples of oral malignances are infrequent. Int J Cancer 1991; 48: 409–412 http://dx.doi.org/10.1002/ijc.2910480318

[38] WANG WY, CHEIN YC, WONG YK, WONG YK, LIN YL et al. Effects of KRAS status mutation and polymorphismus on the risk and prognosis of oral squamous cell carcinoma. Head Neck 2012; 34: 663–666 http://dx.doi.org/10.1002/hed.21792

[39] LUKAN N, STROBEL P, WILLER A, KRIPP M, DINTER D et al. Cetuximab-based treatment of metastatic anal cancer: correlation of response with K-RAS mutational status. Oncology 2009; 77: 293–299 http://dx.doi.org/10.1159/000259615

[40] GARG M, LEE JY, KACHNIC LA, CATALANO PJ, HENRY DH et al. Phase II trials of cetuximab (CX) plus cisplatine (CDDP), 5-fluorouracil (5-FU) and radiation (RT) in imunocompetent (ECOG 3205) and HIV-positive (AMC045) patients with squamous cell carcinoma of tha anal canal (SCAC): Safety and preliminary efficacy results (abstract). ASCO Meeting Anstracts 2012; 30: 4030

[41] DEUTSCH E, LEMANSKI C, PIGNON JP, LEVY A, DELAROCHEFORDIERE A et al. Unexpected toxicity of cetuximab combined with conventional chemoradiotherapy in patients with locally advanced anal cancer: results of the UNICANCER ACCORD 16 phase II trial. Ann Oncol 2013; 24: 2834–2838 http://dx.doi.org/10.1093/annonc/mdt368

[42] OLIVATTO LO, VIERA FM, PEREIRA BV, VICTORINO AP, BEZERRA M et al. Phase I study of cetuximab in combination with 5-fluorouracil, cisplatine, and radiotherapy in patients with locally advanced anal carcinoma. Cancer 2013; 119: 2973–2980 http://dx.doi.org/10.1002/cncr.28045

[43] SAIF MW, KONTNY E, SYRIGOS KN, SHAHROKNI A. The Role of EGFR inhibitors in the treatment of metastatic anal canal carcinoma: A Case series. J of Oncol 2011; 2011: 125467 http://dx.doi.org/10.1155/2011/125467

[44] BARMETTLER H, KOMMINOTH P, SCHMID M, DUERR D. Efficacy of cetuximab in combination with FOLFIRI in patient with KRAS wild-type metastatic anal cancer. Case Rep Oncol 2012; 5: 428–433 http://dx.doi.org/10.1159/000341371

[45] BAMBA T, SUDA T, NAKANO M, TERASHIMA T, UMEZU H et al. Pathologically complete response for inresectable stage IV rectal cancer using systemic chemotherapy with panitumumab. A Case report. Gan To Kagaku Ryomo 2012; 39: 311–315