

# Molecular Profiling of EGFR Status to Identify Skin Toxicity in Colorectal Cancer: A Clinicopathological Review

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**ABSTRACT:** Colorectal cancer (CRC) represents an important health problem, being the third most common type of cancer. In Romania, the CRC incidence has doubled over the years. Both environmental factors and genetic susceptibility are very important for the pathogenesis of CRC. The epidermal growth factor receptor (EGFR) plays an extremely important role in CRC tumorigenesis. Overexpression or dysregulation of EGFR pathway molecules are frequently associated with tumor aggressiveness and patient response to treatment. Based on these considerations, EGFR became one of the first targets of molecular therapies used in CRC. At present, cetuximab and panitumumab are considered to be essential in the treatment of patients with metastatic colorectal cancer expressing the KRAS wild-type gene and EGFR. The main adverse effect for both cetuximab and panitumumab is skin toxicity, present in approximately 80% of patients. The risk of secondary infections, in particular of bacterial infections, is also increased. Cases of staphylococcal infection associated with skin peeling, cellulite, erysipelas, and even Staphylococcus sepsis, were reported. For a long time cutaneous toxicity has been a positive predictor in the efficacy of anti-EGFR treatment, but compliance with treatment and the quality of life of patients with metastatic CRC decreases in the presence of these skin reactions. That is why we emphasize the necessity and importance of using a modern method (molecular analysis of gene polymorphisms possibly supplemented by targeted confocal laser endomicroscopy) to identify a molecular diagnosis, in order to foresee and prevent the appearance of skin reactions and to manage skin toxicity.

**KEYWORDS:** Confocal laser endomicroscopy, EGFR polymorphisms, skin toxicity.

## Introduction

Colorectal cancer is not only the most common digestive cancer but also a global burden expected to increase by 60% until 2030. However, the survival rate associated with colorectal cancer is higher than other cancers of the gastrointestinal tract, due to organised screening programs, improved perioperative care and therapeutic management [1]. The incidence of colorectal cancer is higher in Europe, North America, Australia and New Zealand. The increased incidence in industrialized countries can be explained by the more frequent involvement of environmental factors, including diet and genetic predisposition, in the pathogenesis of cancer [2-5].

CRC treatment requires a multidisciplinary approach: surgery, chemotherapy and/or radiotherapy. Current data emphasizes the importance of using targeted molecular therapies in order to inhibit vascular endothelial growth

factor (bevacizumab) or epidermal growth factor receptor (cetuximab, panitumumab) [6]. These drugs are currently used in the management of metastatic colorectal cancer, increasing the survival rate of these patients. Actual data highlights the shift to individualized therapies based on predictive biomarkers, with the management of adverse effects generated by these drugs.

Angiogenesis is an important process underlying tumor growth and later the progression to metastasis. This process takes place due to the interaction between numerous growth factors and signalling molecules [7]. The individualized therapies depend on the detailed assessment of angiogenesis performed with the purpose of identifying angiogenic factors [8]. Also, cutaneous toxicity and early tumor shrinkage are predictors of anti-EGFR therapy efficacy. Furthermore, another challenge is to manage adverse effects without interfering with the response to treatment [9-10].

This article reviews and summarizes current literature regarding the impact of severe adverse dermatological reactions observed in patients receiving anti-EGFR therapy, with focus on highlighting successes and barriers of confocal laser endomicroscopy in skin reactions evaluation, hence increasing quality of life by reducing the progress to skin toxicity.

### Impact of dermatologic side effects in patients treated with anti-EGFR

Used for a long-time as a positive predictive factor, cutaneous toxicity resulting from monoclonal antibodies anti-EGFR (cetuximab, panitumumab) can take multiple forms. The more severe effects can have a significant impact on the quality of life in patients with colorectal cancer, up to 30% of these patients being obliged to stop the anti-EGFR treatment [9-10].

The occurrence of skin toxicity approaches 80% of patients treated with cetuximab, the most common adverse effects being papulopustular rash, fissures, xerosis, blepharitis, pruritus, paronychia, changes of the hair growth and hypo/hyperpigmentation. The EGFR overexpression is detected in the epidermis (specifically in the basal keratinocytes) and in hair follicles [9-13] (Fig.1,2,3).

Skin toxicity is most commonly located at the level of the scalp, face, and trunk, and begins either in the first 3 weeks with cetuximab, or during the second cure [14].

Only 10-20% of these patients have severe cutaneous toxicity. Still, those severe skin manifestations have a dramatic effect on the psychosocial, physical and emotional state of these patients, also with high financial implications [9].

In most cases, skin lesions overlap with infections, most commonly bacterial infections. Patients with staphylococcal infection have massive skin lesions, sometimes necrotizing fasciitis and/or sepsis [15].

The current oncological strategy is based on the development of a personalized treatment to increase the survival rate and improve the quality of life [16]. However, this new era of oncology has led to numerous adverse skin reactions.

For the diagnosis and management of adverse effects we emphasize the importance of working in a multidisciplinary team involving an oncologist, a dermatologist and a gastroenterologist. Several systems for assessing the impact on quality of life are currently being used: The National Cancer Institute's Combinatorial Criteria for Adverse Events (CTCAE), the Functional Assessment of Cancer Therapy Epidermal Growth Factor Inhibitor Receptor 18 (FACT-EGFRI-18) Scale, and Dermatologic Reaction Targeted Therapy [16-18].

According to CTCAE, skin rash is divided in: grade 1 (~40% of cases), grade 2-moderate (~40% of cases), and grade 3-severe (~20% of cases) [14].

The identification and application of methods for assessing the severity of the skin reactions are necessary and of great importance in the administration of personalized treatments, in establishing the optimal doses to control both the cancerous process and the adverse effects.

Interestingly, the good response to treatment of the cancer patients receiving EGFR has been associated with the severity of skin toxicity [19].

Managing skin toxicity ensures patient adherence to treatment, preventing discontinuation of treatment and relapse [9].



Fig.1. Vasculitis grade 3



Fig.2. Vasculitis grade 4



**Fig.3. Papulopustular skin rash**



**Fig.4. Acneiform eruption without comedons**



**Fig. 6. Acnelike rashes, changes of the hair growth**



**Fig.5. Xerosis, papulopustular skin rash**

### Genome wide association with skin toxicity

Although many studies highlight the association between anti-EGFR treatments and cutaneous toxicity, the severity of these manifestations could not be quantified to date with predictive factors [21].

The anti-EGFR antibodies, cetuximab and panitumumab, frequently cause cutaneous toxicity. Current genetic studies are exploring a number of predictive factors by analyzing EGFR polymorphisms and genetic mutations. A wide approach to the genome may lead to discovering new single nucleotide polymorphisms (SNPs), which may be predictive biomarkers [17].

Even though cetuximab has been proved efficient in the treatment of patients with metastatic CRC without mutations in the RAS gene, randomized clinical trials suggest a much higher efficacy in patients with colorectal cancer presenting the wild-type KRAS gene [2,3].

Current literature suggests a much better response rate for treatment if the tumor has shrunk early during the treatment with cetuximab. Also, patients with early cutaneous toxicity have a much higher survival rate [22].

In colorectal cancer, monoclonal antibodies directed against the epidermal growth factor receptor have been shown to be active antitumor agents. EGFR is a transmembrane glycoprotein tyrosine kinase receptor belonging to the HER1 family, found on the surface of epithelial cells. EGFR activation occurs following the interaction of several factors such as EGF, EGF-like that convert heparin-binding growth factor, epiregulin and growth factor- $\alpha$ . This interaction might be followed by autophosphorylation of the intracellular domain and receptor dimerizations. Through the activation of these signaling pathways cell proliferation and inhibition of apoptosis might appear. Dysregulated activation of EGFR or overexpression of this signal lead to solid tumors growth, such as colorectal cancer, genitourinary cancers, head and neck cancer or glioblastoma [20].

Predictive biomarkers include intron 1 polymorphisms with EGFR overexpression, the presence of EGFR ligand in serum and the presence of inflammatory lymphokines.

The mechanism of severe cutaneous toxicity induced by cetuximab has not been fully elucidated, but pharmacogenomic factors and SNPs are suspected to act as a trigger in cutaneous toxicity. These skin reactions appear to be associated with high levels of EGFR, most commonly located in the epidermis.

A recent research paper identified and validated SNP rs849142 polymorphisms as a potential biomarker related to skin reactions. This study has shown the association with acneiform eruption triggered by cetuximab treatment. Except for the acneiform aspect of the eruption, it differs from the usual acne eruption in regard to etiology and lack of comedones [14] (Fig.4,5,6). As to the cause of skin toxicity, the following can be highlighted as causes: alterations in the chemokine or cytokine system, defects in the cutaneous barrier due to infections or xeroses and disorders in the differentiation of keratinocytes [21].

In patients with head or neck squamous cell carcinoma being treated with Cetuximab, the EGFR-R 521K genotype revealed an obvious association between cutaneous toxicity and clinical efficacy of cetuximab.

## **In vivo molecular imaging of colorectal cancer with Confocal Laser Endomicroscopy**

Confocal laser endomicroscopy (CLE) is one of the latest tools which enables in vivo observation of different tissues and cellular structures at a higher resolution similar to histopathological examination. It provides an optical digital biopsy or real-time histopathological evaluation of the examined tumors. Topical or systemic contrast agents may be used [24-25].

An extremely important element that facilitates tumor examination at molecular level is the association of CLE with fluorescein-labeled antibodies or peptides that can bind specifically to a particular target [26].

Increasing the number of its clinical applications and the possibility for molecular examination of tumors enabled CLE to open new opportunities for fundamental and clinical research [24,27].

The CLE techniques, both endoscope based (eCLE) and probe based (pCLE), are available in the Research Center of Gastroenterology and Hepatology Craiova. Our previous studies have already described with success the presence and expression patterns of several biomarkers (CD44, CD133, CD166, CD31, CD105) proposed for the CLE assessment of colorectal cancer patients [28,29,30]. In the study regarding the assessment of CD44 expression patterns in colorectal cancer the eCLE dedicated system used was the EC-3870 CIFK, Pentax, Tokyo, Japan whose components are based on the integration of a scaled-down confocal laser microscope mounted in the tip of a conventional scope. During the investigation, the argon ion laser discharges an excitation wavelength of 488nm with a maximum laser power output of  $\leq 1\text{mW}$  at the tissue surface. The maximum depth of imaging is  $250\mu\text{m}$  from the surface of the mucosa. The resulting optical sections have a lateral resolution of  $0.7\mu\text{m}$  for a  $7\mu\text{m}$  thick slice and a field of view of  $475\times 475\mu\text{m}$ . CD44 is proposed as one of the main colorectal cancer stem cell markers being a transmembrane glycoprotein and a distinctive adhesion molecule. The monoclonal antibody used in the above mentioned study is labeled with Alexa-Fluor 488 and directed against human CD44/PGP-1, it recognizes both cell surface-expressed and soluble form of human CD44 as an antigen (Phagocyte glycoprotein 1) [28]. (Fig.7,8)

Other recent studies have focused on the development of modern techniques of highlighting overexpression of EGFR and have focused their efforts on making correlations about the association of overexpression of EGFR with the severity of cutaneous toxicity [26].

The EGFR is a transmembrane tyrosine kinase receptor [26]. Dysregulations in the EGFR activation are correlated with the aggressive nature of the tumor. Current studies highlight overexpression of EGFR (24-80%) in the case of colorectal adenomas and overexpression of EGFR (25-97%) in invasive colorectal cancer. EGFR thus becomes a target for molecular therapies [31].

However, the level of EGFR expression detected by IHC does not appear to be a clinical predictor of response to treatment with monoclonal antibodies.

Recent trials place CLE as an advanced optical instrument which leads to a paradigm shift in detecting gastrointestinal tumors by including molecular images along with macroscopical images [32].

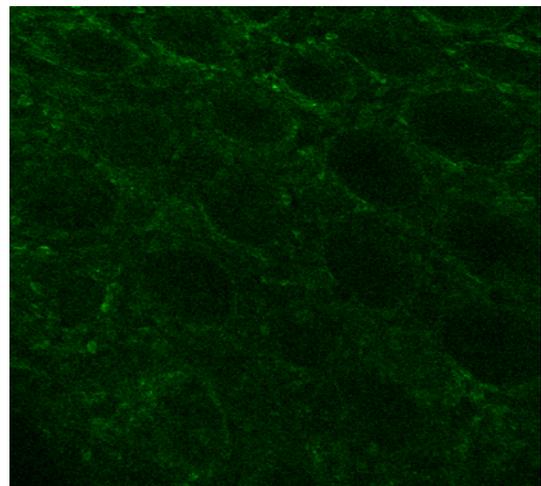
In vivo molecular images of EGFR expression could be visualized after topical application of fluorescein, anti-EGFR monoclonal antibody. The study used Alexa Fluor 488 to mark colorectal neoplasia in vivo. After topical application of the AF 488 spray, 5ml to the colorectal tumor formation, CLE images were recorded after 10 and 15min after application [26-28].

By topical application of fluorescein, the intensity of the EGFR signal can be quantified.

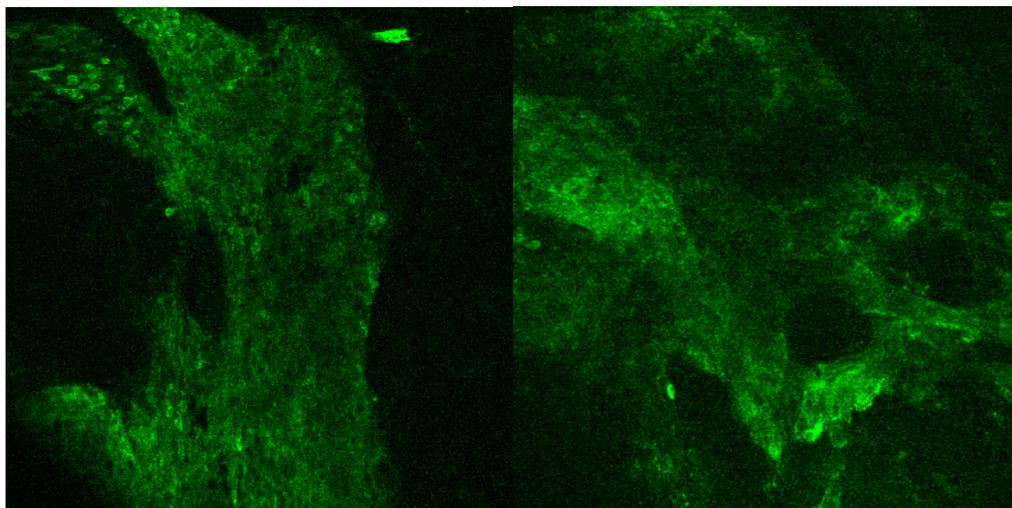
Currently, the selection of candidates for cetuximab therapy depends mostly on IHC. The

IHC result may be influenced by the tissue sample fixation and processing. Moreover, EGFR immunoreactivity is often altered, due to the removal of this antigen from its natural microenvironment [27,31-33].

Obtaining in vivo images using CLE provides a more comprehensive image of EGFR expression in his natural microenvironment and allows performing multiple optical biopsies. Also, the literature underlines the importance of optical biopsies, by presenting important complications following biopsy for IHC, mentioning a risk of bleeding and other potential complications. All this reinforces the importance of analysing EGFR expression through CLE [26,27,31-33].



**Fig.7. Confocal Laser Endomicroscopy image of normal mucosa visualized with fluorescently labeled anti- CD44 antibody. A well defined layout of the mucosal structures, with normal size and regular arrangement of crypts can be observed**



**Fig.8. Confocal Laser Endomicroscopy image of tumour tissue visualized with fluorescently labeled anti-CD44 antibody. The mucosal architecture reveals irregular arrangement with destroyed crypts and unrecognizable structures**

## Conclusions

Skin toxicity is the main adverse effect related to EGFR inhibitors in patients with metastatic colorectal cancer. In patients with colorectal cancer the severity of the skin reactions is closely related to therapeutic compliance and the appropriate management of therapy which impacts the patients' quality of life. A new approach to the management of cutaneous toxicity is needed. The evaluation of EGFR expression through genetic studies and CLE techniques settle an early molecular diagnosis and prevent subsequent severe skin reactions, leading to optimal adjustment of the treatment dosage, improving quality of life and outcomes in patients with colorectal cancer. Notably, in cases where EGFR is overexpressed and severe cutaneous toxicity is expected, these modern investigations (EGFR polymorphisms and CLE) may help provide cutaneous treatment in advance of skin toxicity events, avoiding further lesions.

Particular importance should be attributed to the preventive treatment of skin reactions, increasing the quality of life and preventing the discontinuation of oncology therapy due to serious skin complications.

## Conflict of interests

The authors declare that they have no conflict of interest.

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For the original data presented here, all study participants provided informed written consent prior to study enrollment for data sharing. The study has been approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania.

## References

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, 2017, 66(4):683-691.
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M. Cancer incidence and mortality worldwide: Sources, methods and major patterns. *Int J Cancer*, 2015, 136(5):E359-386.
3. Fornasier G, Francescon S, Baldo P. An Update of Efficacy and Safety of Cetuximab in Metastatic Colorectal Cancer: A Narrative Review. *Adv Ther*, 2018, 35(10):1497-1509.
4. Matsuda T., Zhang M. Comparison of time trends in colorectal cancer mortality (1990-2006) in the world from WHO mortality database. *Jpn J Clin Oncol*. 2009, 39(11):777-778.

5. Jemal A, Bray F, Center M, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*, 2011, 61(2):69-90.
6. Cook KM, Figg WD. Angiogenesis inhibitors: current strategies and future prospects. *CA Cancer J Clin*, 2010, 60(4):222-243.
7. de Castro Junior G, Puglisi F, de Azambuja E, El Saghier NS, Awada A. Angiogenesis and cancer: A cross-talk between basic science and clinical trials (the "do ut des" paradigm). *Crit Rev Oncol Hematol*, 2006, 59(1):40-50.
8. Rasheed S, McDonald PJ, Northover JM, Guenther T. Angiogenesis and hypoxic factors in colorectal cancer. *Pathol Res Pract*, 2008, 204(7):501-510.
9. De Tursi M, Zilli M, Carella, C Auriemma M, Lisco MN, Di Nicola M, Di Martino G, Natoli C, Amerio P. Skin toxicity evaluation in patients treated with cetuximab for metastatic colorectal cancer: a new tool for more accurate comprehension of quality of life impacts. *Onco Targets Ther*, 2017, 2017(10):3007-3015.
10. Joshi SS, Ortiz S, Witherspoon JN, Rademaker A, West DP, Anderson R, Rosenbaum SE, Lacouture ME. Effects of epidermal growth factor receptor inhibitor-induced dermatologic toxicities on quality of life. *Cancer*, 2010, 116(16):3916-3923.
11. Urban C, Anadkat MJ. A review of cutaneous toxicities from targeted therapies in the treatment of colorectal cancers. *J Gastrointest Oncol*, 2013, 4(3):319-327.
12. Agero AL, Dusza SW, Benvenuto-Andrade C, Busam KJ, Myskowski P, Halpern AC. Dermatologic side effects associated with the epidermal growth factor receptor inhibitors. *J Am Acad Dermatol*, 2006, 55(4):657-670.
13. Yamazaki N, Oomuku Y, Mishiho I, Soeda J. Pre-emptive skin treatments to prevent skin toxicity caused by anti-EGFR antibody: the real-world evidence in Japan. *Future Oncology*, 2018, 14(30):3163-3174.
14. Froelich M, Stintzing S, Kumbrink J, Grünwald TGP, Mansmann U, Heinemann V, Kirchner T, Jung A. The DNA-polymorphism rs849142 is associated with skin toxicity induced by targeted anti-EGFR therapy using cetuximab. *Oncotarget*, 2018, 9(54):30279-30288.
15. D'Alessio A, Cecchini S, Di Mauro D, Geroli L, Villa S, Quadri A, Resta D, Fortugno C. Cutaneous Toxicity from Epidermal Growth Factor Receptor Inhibitors: Would a Subcutaneous Desensitization be Helpful? Case Report. *Tumori Journal*, 2016, 102(2\_suppl):S65-S68.
16. Barbu M, Nițipir C, Voiosu T, Giurcăneanu C. Impact of dermatologic adverse reactions on QOL in oncologic patients: results from a single-center prospective study. *Romanian Journal of Internal Medicine*, 2018, 56(2):96-101.
17. Baas J, Krens L, Bohringer S Linda Mol, Cornelis Punt, Henk-Jan Guchelaar, Hans Gelderblom. Genome wide association study to identify predictors for severe skin toxicity in colorectal cancer patients treated with cetuximab. *PLoS ONE*, 2018, 13(12):e0208080.

18. The ASCO Post. 2019. How to Measure the Impact of Dermatologic and Mucosal Adverse Events on Symptom Burden and Quality of Life [online]. Available at „<http://www.ascopost.com/issues/december-15-2013/how-to-measure-the-impact-of-dermatologic-and-mucosal-adverse-events-on-symptom-burden-and-quality-of-life/>” [Accessed: february 6, 2019].
19. Petrelli F, Borgonovo K, Barni S. The predictive role of skin rash with cetuximab and panitumumab in colorectal cancer patients: a systematic review and meta-analysis of published trials. *Target Oncol*, 2013, 8(3):173-181.
20. Abdel-Rahman O, Fouad M. Correlation of cetuximab-induced skin rash and outcomes of solid tumor patients treated with cetuximab: a systematic review and meta-analysis. *Crit Rev Oncol Hematol*, 2015, 93(2):127-135.
21. Holcman M, Sibilia M. Mechanisms underlying skin disorders induced by EGFR inhibitors. *Mol Cell Oncol*, 2015, 2(4):e1004969.
22. Kogawa T, Doi A, Shimokawa M, Fouad TM, Osuga T, Tamura F, Mizushima T, Kimura T, Abe S, Ihara H, Kukitsu T, Sumiyoshi T, Yoshizaki N, Hirayama M, Sasaki T, Kawarada Y, Kitashiro S, Okushiba S, Kondo H, Tsuji Y. Early skin toxicity predicts better outcomes, and early tumor shrinkage predicts better response after cetuximab treatment in advanced colorectal cancer. *Target Oncol*, 2014, 10(1):125-133.
23. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med*, 2009, 360(14):1408-1417.
24. Goetz M, Kiesslich R. Advances of endomicroscopy for gastrointestinal physiology and diseases. *Am J Physiol Gastrointest Liver Physiol*, 2010, 298:G797-G806.
25. Polglase AL, McLaren WJ, Skinner SA, Kiesslich R, Neurath MF, Delaney PM. A fluorescence confocal endomicroscope for in vivo microscopy of the upper-and the lower-GI tract. *Gastrointestinal Endoscopy*, 2005, 62(5):686-695.
26. Liu J, Zuo X, Li C, Yu T, Gu X, Zhou C, Li Z, Goetz M, Kiesslich R, Li Y. In vivo molecular imaging of epidermal growth factor receptor in patients with colorectal neoplasia using confocal laser endomicroscopy. *Cancer Lett*, 2013, 330(2):200-207.
27. Ștefănescu D, Streba C, Cârțână E, Săftoiu A, Gruionu G, Gruionu L. Computer Aided Diagnosis for Confocal Laser Endomicroscopy in Advanced Colorectal Adenocarcinoma. *PLoS ONE*. 2016, 11(5):e0154863.
28. Cherciu IF, Ivan ET, Burtea DE, Calita M, Pirici D, Mărgăritescu C, Saftoiu A. Targeted Confocal Laser Endomicroscopy (CLE) for the Assessment of Putative Cancer Stem Cell Markers in Colorectal cancer-A Pilot Study. *Surgery, Gastroenterology and Oncology*, 2017, 22.303.10.21614/sgo-22-4-303.
29. Cârțână T, Săftoiu A, Gruionu LG, Gheonea DI, Pirici D, Georgescu CV, Ciocâlțeu A, Gruionu G. Confocal laser endomicroscopy for the morphometric evaluation of microvessels in human colorectal cancer using targeted anti-CD31 antibodies. *PLoS One*, 2012, 7(12):e52815.
30. Ciocâlțeu A, Săftoiu A, Pirici D, Georgescu CV, Cârțână T, Gheonea DI, Gruionu LG, Cristea C, Gruionu G. Tumor neoangiogenesis detection by confocal laser endomicroscopy and anti-CD105 antibody: Pilot study. *World journal of gastrointestinal oncology*, 2015, 7(11):361-368.
31. Goetz M, Ziebart A, Foersch S, Vieth M, Waldner MJ, Delaney P, Galle PR, Neurath MF, Kiesslich R. In Vivo Molecular Imaging of Colorectal Cancer With Confocal Endomicroscopy by Targeting Epidermal Growth Factor Receptor. *Gastroenterology*, 2010, 138(2):435-446.
32. Goetz M, Hoetker M, Diken M, Galle P, Kiesslich R. In vivo molecular imaging with cetuximab, an anti-EGFR antibody, for prediction of response in xenograft models of human colorectal cancer. *Endoscopy*, 2013, 45(06):469-477.
33. Goldstein NS, Armin M. Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system. *Cancer*, 2001, 92(5):1331-1346.

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