ANTICANCER RESEARCH *36*: 4421-4426 (2016)
doi:10.21873/anticanres.10985

Review

The Role of Cetuximab in the Induction of Anticancer Immune Response in Colorectal Cancer Treatment

LUBOS HOLUBEC[1,2], JIRI POLIVKA JR.[1,3], MARTIN SAFANDA[2], MICHAL KARAS[4] and VACLAV LISKA[1]

[1]Biomedical Center, and Departments of [3]Histology and Embryology, and [4]Haematology and Oncology, Faculty of Medicine Plzen, Charles University in Prague, Pilsen, Czech Republic; [2]Department of Clinical Oncology, Na Homolce Hospital, Prague, Czech Republic

Abstract. *Monoclonal antibodies binding the epidermal growth factor receptor (EGFR), such as cetuximab or panitumumab, are widely used targeted therapeutics for the treatment of patients with colorectal cancer. The clinical significance of these drugs has so far been associated with combined chemotherapy or radiation. It has been shown that these treatment strategies have their clinical limitations and do not fully exploit the immunomodulatory effect of these drugs. In this review, we discuss the mechanisms of immunomodulation together with the anticancer immune response to the monoclonal antibodies targeted to the EGFR. The combination of anti-EGFR monoclonal antibodies with other immunotherapeutic treatment modalities certainly brings new opportunities for targeted therapy in patients with colorectal cancer.*

During the past 10 years, there have been fundamental discoveries in oncogenic transformation which have changed the current view of the diagnosis and treatment of cancer (1, 2). It is receded from reductionist theory, in which cancer is seen as a homogeneous population of tumor cells interacting on the autocrine and paracrine level thereby determining the biological activity of the tumor. More and more scientific articles from basic as well as applied research actually highlight the tumor complex theory in which a tumor is considered to be a complex tissue (3). Whereas the tumor parenchyma consists of cancer cells, the stroma consists partly of non-cancerous cellular components and non-cellular parts. The tumor mass includes a number of non-cancerous cells and structures of the extracellular matrix, affected by local conditions and aberrant signalization of tumor cells, which in turn leads to the feedback influence of tumor cells themselves. The non-cancerous cellular components of the tumor microenvironment include tumor stem cells with unlimited generational potential, cancer-associated fibroblasts (CAF) that form the extracellular matrix, endothelial cells and pericytes involved in pathological angiogenesis, and immune cells affecting the interaction among tumor and immunocompetent cells (3-5). Non-cellular components of the tumor microenvironment are predominantly composed of extracellular matrix containing cytokines, growth and angiogenic factors and other bioactive molecules that are produced and secreted by both cancerous and non-cancerous cells. The communication between tumor and other cells is very intense and actively modifies the biological activity of the tumor itself and its sensitivity to anticancer treatment (1, 6).

The last two decades of intensive research focused on cancer immunology revealed the dual role of the immune system during the process of carcinogenesis. The immune system contributes to the elimination of newly transformed tumor cells and to the eradication of the residual tumor population after treatment. On the other hand, many experiments clearly demonstrated the supportive role of the immune system in survival, growth, and spread of a variety of tumors, including colorectal cancer (7, 8). A better understanding of the importance of the immune system for tumor growth and survival is reflected in the development of new anticancer therapeutic approaches, such as the application of monoclonal antibodies against tumor antigens and growth factor receptors, adoptive transfer of tumor-specific cytotoxic T-lymphocytes, active immunization with tumor vaccines, and

Correspondence to: Jiri Polivka Jr., Department of Histology and Embryology, Faculty of Medicine in Plzen, Charles University in Prague, Husova 3, 301 66 Plzen, Czech Republic. E-mail: polivkajiri@gmail.com

Key Words: Colorectal cancer, cetuximab, immune response, targeted therapy, immunomodulation, review.

modulation of the tumor microenvironment *e.g.* monoclonal antibodies against cytotoxic T–lymphocyte-associated antigen 4 (CTLA4) or programmed cell death 1 (PD1) (1, 9).

In this review, we discuss the mechanisms of immunomodulation together with the anticancer immune response to targeting monoclonal antibodies.

Monoclonal Antibodies and Anticancer Immunity

Immunotherapy aims to stimulate the immune system of the patient to reject and destroy the tumor, which is affected indirectly by the patient´s immune cells (10, 11). Immunotherapeutics can be divided into two groups. The first are monoclonal antibodies that bind to specific receptors and cell antigens, thereby blocking transmission of information and cell proliferation through signaling pathways. Monoclonal antibodies also activate the immune system response after binding to the target receptor. The examples of therapeutics in this group are monoclonal antibodies blocking receptors of proliferative signaling pathways *e.g.* epidermal growth factor receptor (EGFR) and those binding to specific cellular antigens *e.g.* cluster of differentiation 20 (CD20) (12-14). Immune effects are also observed using monoclonal antibodies that bind to growth factors *e.g.* vascular endothelial growth factor (VEGF) and other endogenous mediators *e.g.* receptor activator of nuclear factor kappa B ligand (RANKL).

The second group of immunotherapeutics includes substances exclusively affecting the immune system. Their effect depends on the inhibition of immune-response blockage (affecting ligands of CTLA4 or PD1), or conversely, on the specific activation of the immune system (10, 15). The principal biological effects of monoclonal antibody-mediated immune response are the activation of complement and immune cell-mediated cytotoxicity which is antibody-dependent (antibody dependent cellular cytotoxicity, ADCC). In the following text, we focus on monoclonal IgG antibodies that block the proliferative signaling pathways by interference with the EGFR. Specifically, we focus on extracellular inhibitors, whose main advantage is the ability to activate the immune system response against tumor (1, 16).

Mechanism of Action of Antibodies to EGFR in Colorectal Carcinoma

The EGFR is stimulated by transforming growth factor (TGF-α) as well as epidermal growth factor (EGF) (17). Cetuximab and panitumumab are monoclonal antibodies against human EGFR. They act as functional antagonists of the EGF and TGF ligands and are thus inhibitors of the EGFR-dependent signaling pathways EGFR/phosphatidylinositol 3-kinase (PI3K)/protein kinase-B (AKT)/mammalian target of rapamycin (mTOR) and EGFR/retrovirus-associated DNA sequences (RAS)/proto-oncogene serine threonine-protein kinase (RAF)/mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK), and the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (12, 18-21). Signal blockage leads to inhibition of cancer cell division in the G1 phase because of the lack of transcription factors, which ultimately leads to cell apoptosis (22). Moreover, both these monoclonal antibodies induce an immune response against cells whose receptors they bind. While cetuximab is a chimeric IgG1 antibody, panitumumab is a fully human IgG2 antibody. This distinction is very important for activation of the immune response. Even though panitumumab binds to EGFR with higher activity than does cetuximab, the IgG2 isotype of monoclonal antibody has a significantly lower immunogenicity for poor binding to fragment crystallizable (Fc) receptor-gamma (FcγR) (23). An immunoglobulin Fc region provides the antibody with the ability to interact with receptors expressed by effector immune cells, or with complement. In contrast to IgG1 antibodies (cetuximab), IgG2 antibodies do not have such a significant ability to induce an immune response by ADCC or by other immune mechanisms.

Cetuximab and its Immune Interactions in Tumor Complex of Colorectal Cancer

Cetuximab is a chimeric antibody with an antigen-binding region of murine origin. Other parts of the heavy and light chains are of human origin (24-26). Typical therapeutic monoclonal antibody consists of two identical fragment antigen-binding (Fab) fragments and one Fc fragment (Figure 1). Fab fragments serve to bind tumor antigen, while the Fc fragment mediates binding and activation of immune cells [macrophages, natural killer (NK) cells, cytotoxic T-lymphocytes, *etc.*]. Monoclonal antibody cetuximab may, therefore, affect the immune response in the tumor complex by various forms of interaction. In the first case, upon binding of the monoclonal antibody to the specific target structure in the tumor cell, binding of the first component of complement (C1q) to Fc fragments of the monoclonal antibody occurs. This results in activation of the classical complement pathway, during which the membrane of the transformed tumor cell is attacked by the complex of complement components C5 to C9, while releasing chemotactic fragments C3a and C5a. Formation of the membrane-lytic complex (membrane attack complex) penetrates the cytoplasmic membrane and this ultimately kills the tumor cell. Simultaneously released chemoattractant lead to accumulation of leukocytes and the initiation of antitumor immune responses. This mechanism is also called complement-dependent cytotoxicity (1, 27).

The second possible mechanism of tumor cell destruction is called antibody-dependent cellular cytotoxicity (ADCC).

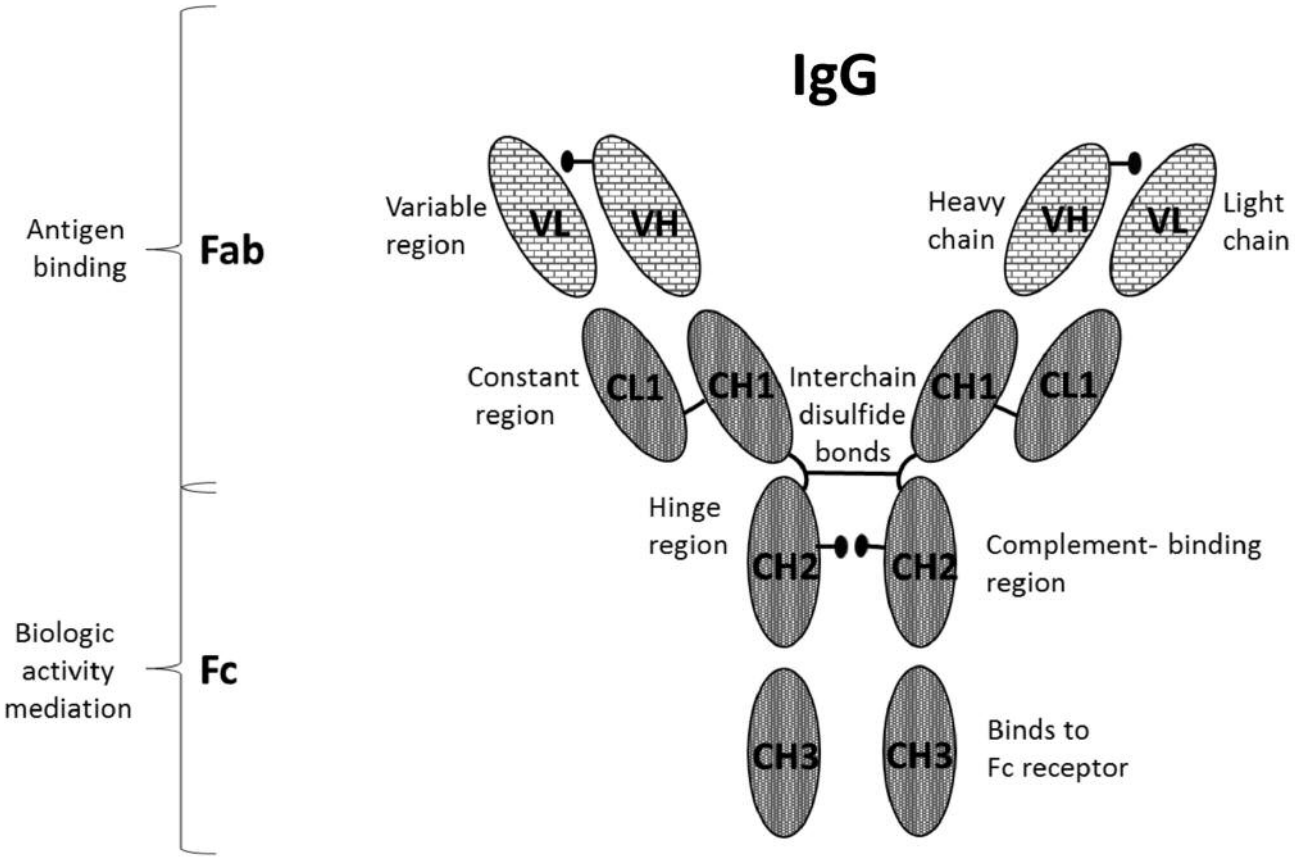

Figure 1. *Immunoglobulin structure: Immunoglobulin G (IgG).*

During this reaction, the tumor cells with bound antibodies (*e.g.* cetuximab) are recognized by NK cells *via* Fcγ receptors (FcγRIIIA=CD16). This leads to activation of NK cells and cytotoxic T-lymphocytes and subsequent effects of cytotoxic agents that damage the membrane of tumor cells (*e.g.* perforin or granzyme B). Moreover, the C3b fragment is produced during the activation of complement which acts as an opsonin for damaged tumor cells and allows phagocytosis by binding to the C3b receptor of macrophages (1, 28-30). This mechanism of immune system activation is called complement-dependent cell-mediated cytotoxicity (Figure 2).

Perspectives on the Use of Cetuximab Immune Response in Colorectal Cancer Treatment

Colorectal carcinoma has generally been regarded as an immunoresistant tumor. In the light of recent research findings, it has become clear that this presumption is not true. The possibility for specific immune system modulation appears to be crucial not only for prognosis, but also for the prediction of response to therapy in patients with colorectal cancer. Tumor-infiltrating lymphocytes ($CD8^+$ and $CD45^+$ T-cells) are increasingly considered to be an independent prognostic factor. Regulatory T-lymphocytes (Tregs) that are responsible for the optimization of the immune response appear to be an optimal predictive factor for monitoring the effect of immunomodulatory therapy (31-33). The determination of the *RAS* mutation status is a powerful predictive factor for the response to the synergistic effect of antibodies to EGFR in combination with chemotherapy. However, *RAS* status is inappropriate for monitoring the activity of the immune response to antibodies EGFR (especially cetuximab) in patients with colorectal cancer. The determination of single nucleotide polymorphisms, variations of individual nucleotides in the DNA sequence (34-36), seems to be more promising. Mutations in Fc fragment domains (especially FcγR2A and FcγR3A) correlate well with an objective response to cetuximab in combination therapy as well as in monotherapy.

Another promising predictive factor is the activity of NK cells and cells involved in ADCC. Sophisticated

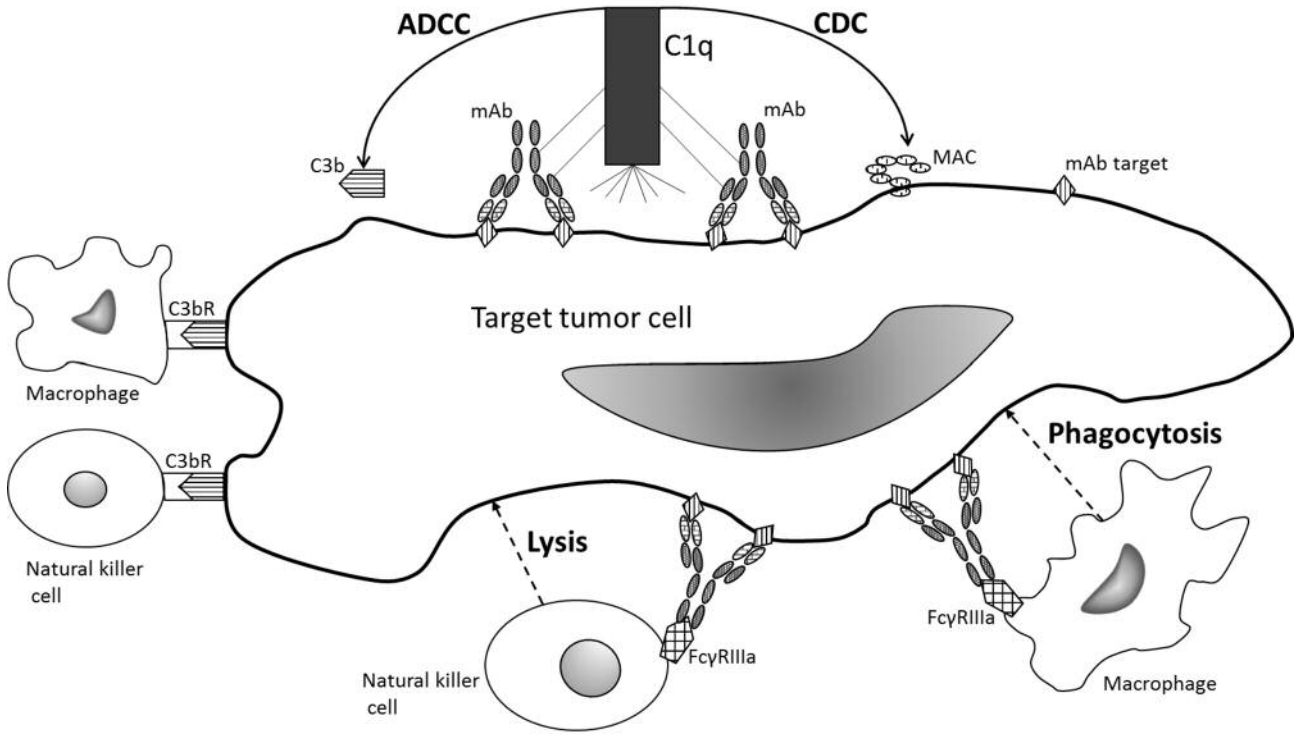

Figure 2. *A schematic model of the effect of monoclonal antibody cetuximab through the immune system. ADCC: Antibody-dependent cellular cytotoxicity, CDC: complement-dependent cytotoxicity, mAb: monoclonal antibody, MAC: membrane attack complex.*

laboratory methods for the determination of NK cell and ADCC activity from peripheral blood samples of individual patients with colorectal cancer are currently being standardized. This activity appears to be an independent prognostic and predictive factor for monitoring of various forms of immunomodulatory treatment, especially the ADCC activity of cetuximab (34, 35, 37-40). These methods are completely independent from the determination of *RAS* mutation and EGFR expression on the surface of tumor cells. Currently there are more than 40 phase I, and III clinical trials evaluating the effectivity of immunomodulatory agents in patients with colorectal cancer in adjuvant as well as palliative treatment settings. The combination of cetuximab with monoclonal antibodies targeted to CTLA4 and PD1 antigens (*in vitro* studies; *in vivo* especially in patients with head and neck tumors and lung cancer) is especially promising. Furthermore, there are numerous studies which are focused on the combination of cetuximab with various vaccines (autologous tumor cells, dendritic cells, adoptive cell therapy *etc.*) or combination with granulocyte-macrophage colony-stimulating factor and several types of interleukin (14, 41-44).

Summary

The clinical significance of cetuximab treatment in patients with colorectal cancer or other cancer types (head and neck tumors, lung tumors) has so far been associated with combined chemotherapy or with radiation. It was shown that these treatment strategies have their clinical limitations and do not fully exploit the immunomodulatory effect of cetuximab, particularly in the induction of ADCC response (45). The combination of cetuximab with other immunotherapeutic treatment modalities certainly opens-up new opportunities for targeted therapy in patients with colorectal cancer.

Conflicts of Interest

The Authors declare that they have no conflict of interests regarding the publication of this article.

Acknowledgements

This work was supported by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.

References

1 Pernot S, Terme M, Voron T, Colussi O, Marcheteau E, Tartour E and Taieb J: Colorectal cancer and immunity: What we know and perspectives. World J Gastroenterol *20*: 3738-3750, 2014.

2 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144*: 646-674, 2011.

3 Pitt JM, Marabelle A, Eggermont A, Soria J-C, Kroemer G and Zitvogel L: Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. Ann Oncol pii: mdw168, 2016.

4 Hui L and Chen Y: Tumor microenvironment: Sanctuary of the devil. Cancer Lett *368*: 7-13, 2015.

5 Ishii G, Ochiai A and Neri S: Phenotypic and functional heterogeneity of cancer-associated fibroblast within the tumor microenvironment. Adv Drug Deliv Rev *99*: 186-196, 2016.

6 Tang H, Qiao J and Fu Y-X: Immunotherapy and tumor microenvironment. Cancer Lett *370*: 85-90, 2016.

7 Krawczyk PA and Kowalski DM: Genetic and immune factors underlying the efficacy of cetuximab and panitumumab in the treatment of patients with metastatic colorectal cancer. Contemp Oncol (Pozn) *18*: 7-16, 2014.

8 Munn DH and Bronte V: Immune suppressive mechanisms in the tumor microenvironment. Curr Opin Immunol *39*: 1-6, 2016.

9 Monteverde M, Milano G, Strola G, Maffi M, Lattanzio L, Vivenza D, Tonissi F, Merlano M and Lo Nigro C: The relevance of ADCC for EGFR targeting: A review of the literature and a clinically-applicable method of assessment in patients. Crit Rev Oncol Hematol *95*: 179-190, 2015.

10 Mahoney KM, Rennert PD and Freeman GJ: Combination cancer immunotherapy and new immunomodulatory targets. Nat Rev Drug Discov *14*: 561-584, 2015.

11 Pennock GK and Chow LQM: The evolving role of immune checkpoint inhibitors in cancer treatment. The Oncologist *20*: 812-822, 2015.

12 Pietrantonio F, Cremolini C, Petrelli F, Di Bartolomeo M, Loupakis F, Maggi C, Antoniotti C, de Braud F, Falcone A and Iacovelli R: First-line anti-EGFR monoclonal antibodies in panRAS wild-type metastatic colorectal cancer: A systematic review and meta-analysis. Crit Rev Oncol Hematol *96*: 156-166, 2015.

13 Ahmadzadeh V, Tofigh R, Farajnia S and Pouladi N: The Central Role for Microenvironment in B-cell malignancies: recent insights into synergistic effects of its therapeutic targeting and anti-CD20 antibodies. Int Rev Immunol *35*: 136-155, 2016.

14 Hughes PE, Caenepeel S and Wu LC: Targeted therapy and checkpoint immunotherapy combinations for the treatment of cancer. Trends Immunol *37*: 462-476, 2016.

15 Azoury SC, Straughan DM and Shukla V: Immune checkpoint inhibitors for cancer therapy: clinical efficacy and safety. Curr Cancer Drug Targets *15*: 452-462, 2015.

16 Seo Y, Ishii Y, Ochiai H, Fukuda K, Akimoto S, Hayashida T, Okabayashi K, Tsuruta M, Hasegawa H and Kitagawa Y: Cetuximab-mediated ADCC activity is correlated with the cell surface expression level of EGFR but not with the KRAS/BRAF mutational status in colorectal cancer. Oncol Rep *31*: 2115-2122, 2014.

17 Sotelo MJ, García-Paredes B, Aguado C, Sastre J and Díaz-Rubio E: Role of cetuximab in first-line treatment of metastatic colorectal cancer. World J Gastroenterol *20*: 4208-4219, 2014.

18 Voigt M, Braig F, Göthel M, Schulte A, Lamszus K, Bokemeyer C and Binder M: Functional dissection of the epidermal growth factor receptor epitopes targeted by panitumumab and cetuximab. Neoplasia *14*: 1023-1031, 2012.

19 Polivka J and Janku F: Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. Pharmacol Ther *142*: 164-175, 2014.

20 Polivka J, Pesta M and Janku F: Testing for oncogenic molecular aberrations in cell-free DNA-based liquid biopsies in the clinic: Are we there yet? Expert Rev Mol Diagn *15*: 1631-1644, 2015.

21 Polivka J Jr., Polivka J, Rohan V, Topolcan O and Ferda J: New molecularly targeted therapies for glioblastoma multiforme. Anticancer Res *32*: 2935-2946, 2012.

22 Imai K and Takaoka A: Comparing antibody and small-molecule therapies for cancer. Nat Rev Cancer *6*: 714-727, 2006.

23 Mellor JD, Brown MP, Irving HR, Zalcberg JR and Dobrovic A: A critical review of the role of Fc gamma receptor polymorphisms in the response to monoclonal antibodies in cancer. J Hematol Oncol *6*: 1, 2013.

24 Yazdi MH, Faramarzi MA, Nikfar S and Abdollahi M: A Comprehensive Review of Clinical Trials on EGFR Inhibitors Such as Cetuximab and Panitumumab as Monotherapy and in Combination for Treatment of Metastatic Colorectal Cancer. Avicenna J Med Biotechnol *7*: 134-144, 2015.

25 Holubec L, Liska V, Matejka VM, Fiala O, Dreslerova J, Mrazkova P, Treska V and Finek J: The role of cetuximab in the treatment of metastatic colorectal cancer. Anticancer Res *32*: 4007-4011, 2012.

26 Sotelo Lezama MJ, Sastre Valera J and Díaz-Rubio García E: Impact of cetuximab in current treatment of metastatic colorectal cancer. Expert Opin Biol Ther *14*: 387-399, 2014.

27 Gancz D and Fishelson Z: Cancer resistance to complement-dependent cytotoxicity (CDC): Problem-oriented research and development. Mol Immunol *46*: 2794-2800, 2009.

28 Schoppy DW and Sunwoo JB: Immunotherapy for head and neck squamous cell carcinoma. Hematol Oncol Clin North Am *29*: 1033-1043, 2015.

29 Wang W, Erbe AK, Hank JA, Morris ZS and Sondel PM: NK Cell-mediated antibody-dependent cellular cytotoxicity in cancer immunotherapy. Front Immunol *6*: 368, 2015.

30 Bakema JE and van Egmond M: Fc receptor-dependent mechanisms of monoclonal antibody therapy of cancer. Curr Top Microbiol Immunol *382*: 373-392, 2014.

31 Niesen J, Stein C, Brehm H, Hehmann-Titt G, Fendel R, Melmer G, Fischer R and Barth S: Novel EGFR-specific immunotoxins based on panitumumab and cetuximab show *in vitro* and *ex vivo* activity against different tumor entities. J Cancer Res Clin Oncol *141*: 2079-2095, 2015.

32 Ma T, Liu H, Sun X, Gao L, Shi J, Zhao H, Jia B, Wang F and Liu Z: Serial *in vivo* imaging using a fluorescence probe allows identification of tumor early response to cetuximab immunotherapy. Mol Pharm *12*: 10-17, 2015.

33 Deschoolmeester V, Baay M, Van Marck E, Weyler J, Vermeulen P, Lardon F and Vermorken JB: Tumor-infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. BMC Immunol *11*: 19, 2010.

34 Press OA, Zhang W, Gordon MA, Yang D, Lurje G, Iqbal S, El-Khoueiry A and Lenz H-J: Gender-related survival differences associated with EGFR polymorphisms in metastatic colon cancer. Cancer Res *68*: 3037-3042, 2008.

35 Liu G, Tu D, Lewis M, Cheng D, Sullivan LA, Chen Z, Morgen E, Simes J, Price TJ, Tebbutt NC, Shapiro JD, Jeffery GM, Mellor JD, Mikeska T, Virk S, Shepherd LE, Jonker DJ, O'Callaghan CJ, Zalcberg JR, Karapetis CS and Dobrovic A: Fc-γ receptor polymorphisms, cetuximab therapy, and survival in the NCIC CTG CO.17 trial of colorectal cancer. Clin Cancer Res *22*: 2435-2444, 2016.

36 Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, Azuma M, Chang H-M, Borucka E, Lurje G, Sherrod AE, Iqbal S, Groshen S and Lenz H-J: FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. J Clin Oncol *25*: 3712-3718, 2007.

37 Seidel UJE, Schlegel P and Lang P: Natural killer cell mediated antibody-dependent cellular cytotoxicity in tumor immunotherapy with therapeutic antibodies. Front Immunol *4*: 76, 2013.

38 Brower V: ASCO Reveals additional promising results with immunotherapies. J Natl Cancer Inst 107, pii: djv295, 2015.

39 Medico E, Russo M, Picco G, Cancelliere C, Valtorta E, Corti G, Buscarino M, Isella C, Lamba S, Martinoglio B, Veronese S, Siena S, Sartore-Bianchi A, Beccuti M, Mottolese M, Linnebacher M, Cordero F, Di Nicolantonio F and Bardelli A: The molecular landscape of colorectal cancer cell lines unveils clinically actionable kinase targets. Nat Commun *6*: 7002, 2015.

40 Derer S, Glorius P, Schlaeth M, Lohse S, Klausz K, Muchhal U, Desjarlais JR, Humpe A, Valerius T and Peipp M: Increasing FcγRIIa affinity of an FcγRIII-optimized anti-EGFR antibody restores neutrophil-mediated cytotoxicity. MAbs *6*: 409-421, 2014.

41 Okada Y, Miyamoto H, Goji T and Takayama T: Biomarkers for predicting the efficacy of anti-epidermal growth factor receptor antibody in the treatment of colorectal cancer. Digestion *89*: 18-23, 2014.

42 Weinberg BA, Marshall JL, Hartley M and Salem ME: A paradigm shift from one-size-fits-all to tailor-made therapy for metastatic colorectal cancer. Clin Adv Hematol Oncol *14*: 116-128, 2016.

43 Maurel J and Postigo A: Prognostic and predictive biomarkers in colorectal cancer. from the preclinical setting to clinical practice. Curr Cancer Drug Targets *15*: 703-715, 2015.

44 Jacobs J, Smits E, Lardon F, Pauwels P and Deschoolmeester V: Immune checkpoint modulation in colorectal cancer: What's new and what to expect. J Immunol Res *2015*: 158038, 2015.

45 Noguchi T, Ritter G and Nishikawa H: Antibody-based therapy in colorectal cancer. Immunotherapy *5*: 533-545, 2013.

Received June 22, 2016
Revised July 7, 2016
Accepted July 11, 2016