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
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 chemotactic 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).
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
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.

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