ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 3, pp. 227-245. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © O. L. Polanovski, E. N. Lebedenko, S. M. Deyev, 2012, published in Biokhimiya, 2012, Vol. 77, No. 3, pp. 289-311.

REVIEW

ERBB Oncogene Proteins as Targets for Monoclonal Antibodies

O. L. Polanovski[1], E. N. Lebedenko[2], and S. M. Deyev[2,3]*

[1]*Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, 119991 Moscow, Russia*
[2]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117997 Moscow, Russia; fax: (495) 335-7103; E-mail: deyev@ibch.ru*
[3]*Nizhny Novgorod State Medical Academy, pl. Minina i Pozharskogo 10/1, 603005 Nizhny Novgorod, Russia*

Received November 14, 2011

Abstract—General properties of the family of tyrosine kinase ERBB receptors are considered in connection with their role in the generation of cascades of signal transduction in normal and tumor cells. Causes of acquisition of oncogene features by genes encoding these receptors and their role in tumorigenesis are analyzed. Anti-ERBB monoclonal antibodies approved for therapy are described in detail, and mechanisms of their antitumor activity and development of resistance to them are reviewed. The existing and the most promising strategies for creating and using monoclonal antibodies and their derivatives for therapy of cancer are discussed.

DOI: 10.1134/S0006297912030029

Key words: EGFR, HER2/neu, receptor tyrosine kinases ERBB1-4, signaling cascades, Herceptin

Such integral cellular processes as division, proliferation, differentiation, and apoptosis in eukaryotic cells are finely coordinated with the involvement of protein phosphorylation and dephosphorylation under the influence of protein kinases and protein phosphatases. About one third of proteins encoded by the human genome are phosphorylated, and disorders in phosphorylation of various proteins can cause a failure in the complicated system of intracellular signal transduction and result in pathological transformation of cells.

Protein kinases are involved in the signal transduction from the cell membrane to the nucleus. The first transmembrane protein shown to have tyrosine kinase properties was epidermal growth factor receptor EGFR (ERBB1, HER1). Now mammals are known to have at least 58 receptor tyrosine kinases (RTK), which are subdivided into 20 families [1-3]. These cellular receptors have a common structural scheme: the intracellular part is represented by rather conservative subdomains of tyrosine kinase, which are separated by a transmembrane domain from the receptor inherent extracellular part of the molecule [4]. The extracellular domains of RTKs have significant differences, which promote the selective activation of RTKs through interactions with a number of natural ligands.

We have under consideration the family of RTKs that in mammals is represented by four receptors, ERBB1-4. Normally, ERBB receptors are involved in growth, differentiation, migration, and apoptosis of epidermal cells. Disorders in the regulation of ERBB receptors lead to uncontrolled growth of cells characteristic of some epidermal tumors. The signaling network initiated by interaction of the ERBB family receptors with ligands and its key elements regulating the direction and rate of the signal transduction play an important role in pathogenesis of tumor diseases.

Overexpression of ERBB receptors in many tumor cells as compared with cells of the normal tissues seems to be promising for successful use of these receptors as targets for diagnosis of diseases and a selective affecting the

Abbreviations: ADCC, antibody-dependent cell cytotoxicity; BC, breast cancer; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERBB, avian erythroblastic leukemia viral oncogene homolog B (also EGFR/HER); ERKs, extracellular signal-regulated kinases; GPCRs, G-protein coupled receptors; HER, human epidermal growth factor receptor; IGF-IR, insulin-like growth factor 1 receptor; JNKs, c-Jun-*N*-terminal protein kinases; mAbs, monoclonal antibodies; MAPKs, mitogen-activated protein kinases; NLS, nuclear localization signal; NRG, neuregulin; NSCLC, non-small cell lung cancer; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; SCCHN, squamous cell carcinoma of head and neck; STATs, proteins of signal transduction and activators of transcription; TGFα and TGFβ1, transforming growth factors α and β1; VEGF and VEGFR, vascular endothelial growth factor and its receptor.

* To whom correspondence should be addressed.

tumor by monoclonal antibodies (mAbs) [5, 6]. At present around 30 mAb preparations are approved for clinical application, and the most of them are specific for surface cellular receptors including ERBB [6, 7]. We have considered some clinical aspects of using mAbs for therapy of oncologic diseases, molecular mechanisms that determine their efficiency, and also causes of appearance of resistance to treatment with anti-ERBB antibodies and approaches to overcome it. The prospects of creating and using anti-ERBB monoclonal antibodies of the new generation in the treatment of tumor diseases are discussed.

STRUCTURE AND BIOLOGICAL ROLE OF RECEPTOR TYROSINE KINASES OF THE ERBB FAMILY

A complicated network of transduction of intracellular signals mediated by ERBB1-4 receptors (avian erythroblastic leukemia viral oncogene homolog B) or EGFR/HER2/neu/HER1-4 (epidermal growth factor receptor/human epidermal growth factor receptor) consists of several levels (Fig. 1). The first level includes various natural polypeptide ligands (epidermal growth factors), which interact with ERBB receptors incorporated in the cell membrane and activate their kinase activity. Activated homo- and heterodimers of the receptors interact with adaptor proteins located in the cytoplasm, which, in turn initiate cascades of signal transduction. The cascades including a multiplicity of proteins are united in a complicated network. This is the second level of the fine mechanisms regulating the rate of signal transduction. On the third, the epigenetic level, the signals reach transcription factors and repressors involved in the regulation of expression of genes responsible for the main processes of cell life (homeostasis, proliferation, differentiation, migration, and apoptosis). This complicated system of signal transduction is characterized by extreme

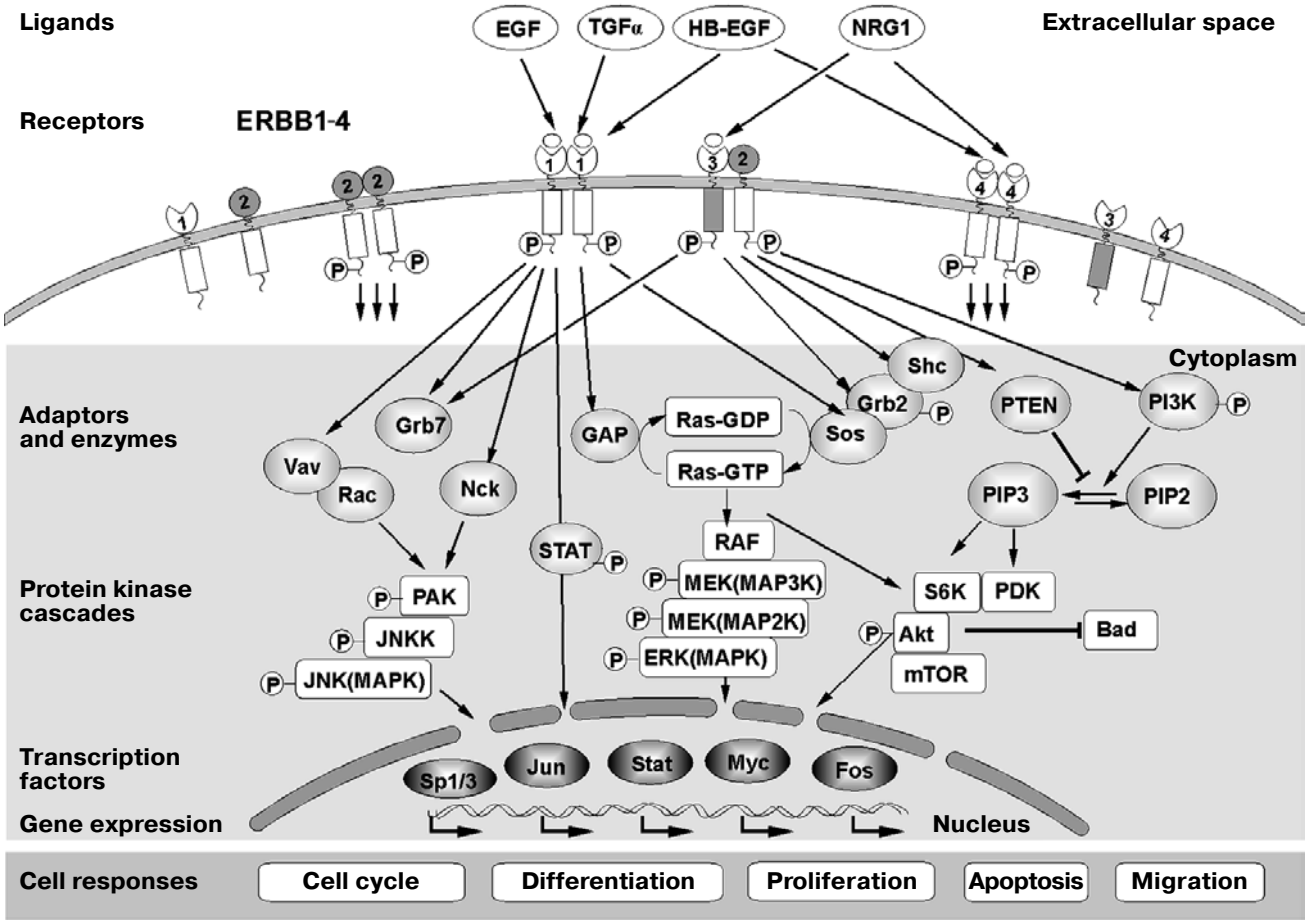

Fig. 1. Scheme of the signaling network of ERBB receptors. For better visualization, the signal transduction is exemplified by two functionally active dimers (ERBB1/ERBB1 and ERBB2/ERBB3) and three main descending signaling pathways (PAK/JNK, Ras/MAPK, and PI3K/Akt). Of 11 ligands interacting with ERBB receptors, the following are shown: epidermal growth factor (EGF), transforming growth factor-α (TGFα), heparin-binding EGF-like growth factor (HB-EGF), and neuregulin-1 (NRG1). Monomers and some functionally active dimers of ERBB1-4 are shown.

stability and by ability to function independently of external and internal perturbations. The stability of this multilevel system is promoted by a modular structure with conservative core processes, a systemic control with positive and negative feedbacks, redundancy, and, although paradoxical, by an ability to rapidly change [4].

Interaction of ERBB receptors with natural ligands and activation of the tyrosine kinase function. Receptors ERBB1-4 (EGFR/HER1-4) of mammals are members of one of 20 families of transmembrane receptor tyrosine kinases (RTK) [1]. Similarly to the majority of RTKs, they consist of the receptor inherent extracellular *N*-cellular domain, the solitary transmembrane α-helical part, and the cytoplasmic tyrosine kinase *C*-terminal domain (Fig. 1). ERBB receptors are located on the cell surface as functionally inactive monomers, which are in equilibrium with a small population of inactive receptor domains.

In normal cells activation of the tyrosine kinase function of ERBB is strictly regulated by natural polypeptide ligands (Fig. 2) that interact with the extracellular domain of the receptor. Epidermal growth factor (EGF) [9] was the first ligand detected, and for its discovery Stanley Cohen was awarded the Nobel Prize in 1986. The ligand interaction with ERBB receptors is not strictly selective [4]. EGF preferentially binds with the ERBB1 receptor, whereas neuregulins bind with ERBB3, 4. The ligand affinity for the receptors can be significantly different, thus neuregulins NRG3/4 have a high affinity for ERBB4 and neuregulins NRG1β/2β for ERBB3 and ERBB4 [4, 8]. In the absence of ligands ERBB receptors do not have tyrosine kinase activity. Note that no natural ligand has been found for ERBB2 (HER2/neu) (Fig. 2), and ERBB3 even upon binding with a ligand does not have protein kinase activity because of structural imperfections in the tyrosine kinase domain; therefore, these two receptors do not work separately but form functionally active heterodimers [8].

Extracellular domains of ERBB receptors (except HER2/neu) in the absence of ligand are shown to be in a tethered conformation supported by intramolecular interaction of subdomains II and IV (Fig. 3, a and b) [10]. In this conformation the receptors are able only for a reversible symmetric dimerization and do not have kinase activity (an intramolecular auto-inhibition). The addition of the ligand to subdomains I and III induces significant conformational rearrangements in the extracellular domain of the receptor, which passes into the open state and is dimerized due to intermolecular interaction of subdomains of the same name II/II and IV/IV (Fig. 3b) [10]. As differentiated from the majority of tyrosine kinase receptors in which transphosphorylation of juxtamembrane regions results in a further activation of the kinase domain, ERBB receptors are known to have an allosteric mechanism of kinase activation [11]. The addition of ligand to two neighboring molecules of EGFR was recently shown not only to stabilize the dimer of the extracellular domains, but also to induce asymmetric conformational changes in the juxtamembrane region of the intracellular kinase domains of the receptor [12]. One of the kinase domains becomes an activator, and the other acts as a recipient that acquires the kinase activity as a result of the asymmetric dimerization [12]. A similar mechanism of activation has been shown for ERBB4 [11].

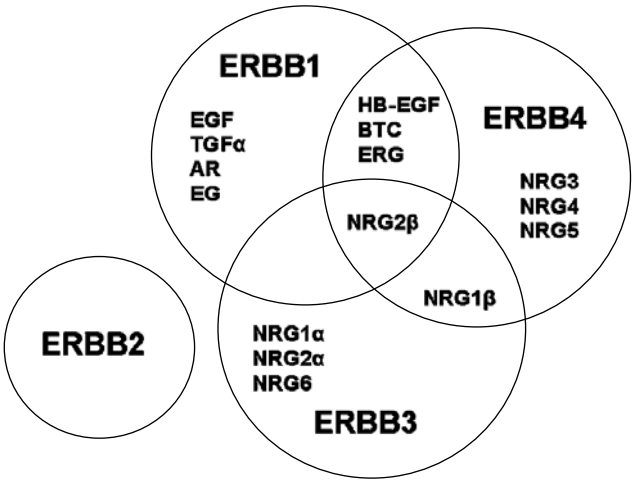

Fig. 2. Scheme of cross-interaction of ligands with ERBB1-4 receptors. Adapted from [8] (Wilson, K. J., Gilmore, J. L., Foley, J., Lemmon, M. A., and Riese, D. J., 2nd. (2009) *Pharmacol. Ther.*, **122**, 1-8) by permission of Elsevier, copyright (2009).

Note that, as differentiated from the corresponding domains of ERBB1, 3, and 4, the extracellular domain ERBB2 (HER2/neu) has an open conformation (Fig. 3, a and b) and normally is capable of producing functionally active heterodimers with other ERBB receptors and intensifying the signal without a preliminary binding with a ligand. In some carcinomas ERBB2 is overexpressed, which results in a sharp increase in the concentration of this receptor on the surface of tumor cells promoting formation of functionally active homo- and heterodimers of ERBB2 and uncontrolled transduction of the signal [13].

Interaction of ERBB receptors with adaptors and signaling proteins. Intracellular *C*-terminal domains of ERBB receptors are the first and the most important substrates of activated RTKs. Autophosphorylation of tyrosine residues on the *C*-terminus of the tyrosine kinase domains of ERBB receptors results in formation of docking sites interacting with adaptor and signaling molecules containing regions of homology-2 with the Src oncogene (SH2) and phosphotyrosine-binding domains (Polo box domains, PBD) [14] (Fig. 4). The set of tyrosine residues phosphorylated upon ERBB1-4 activation varies depending on the receptor and at the lower degree on the ligand [15, 16], and this determines the receptor interaction with cytoplasmic proteins and, as a result, the signal direction and intensity (Fig. 4). The interaction of receptors with adaptor proteins Grb2 and Shc and such signaling pro-

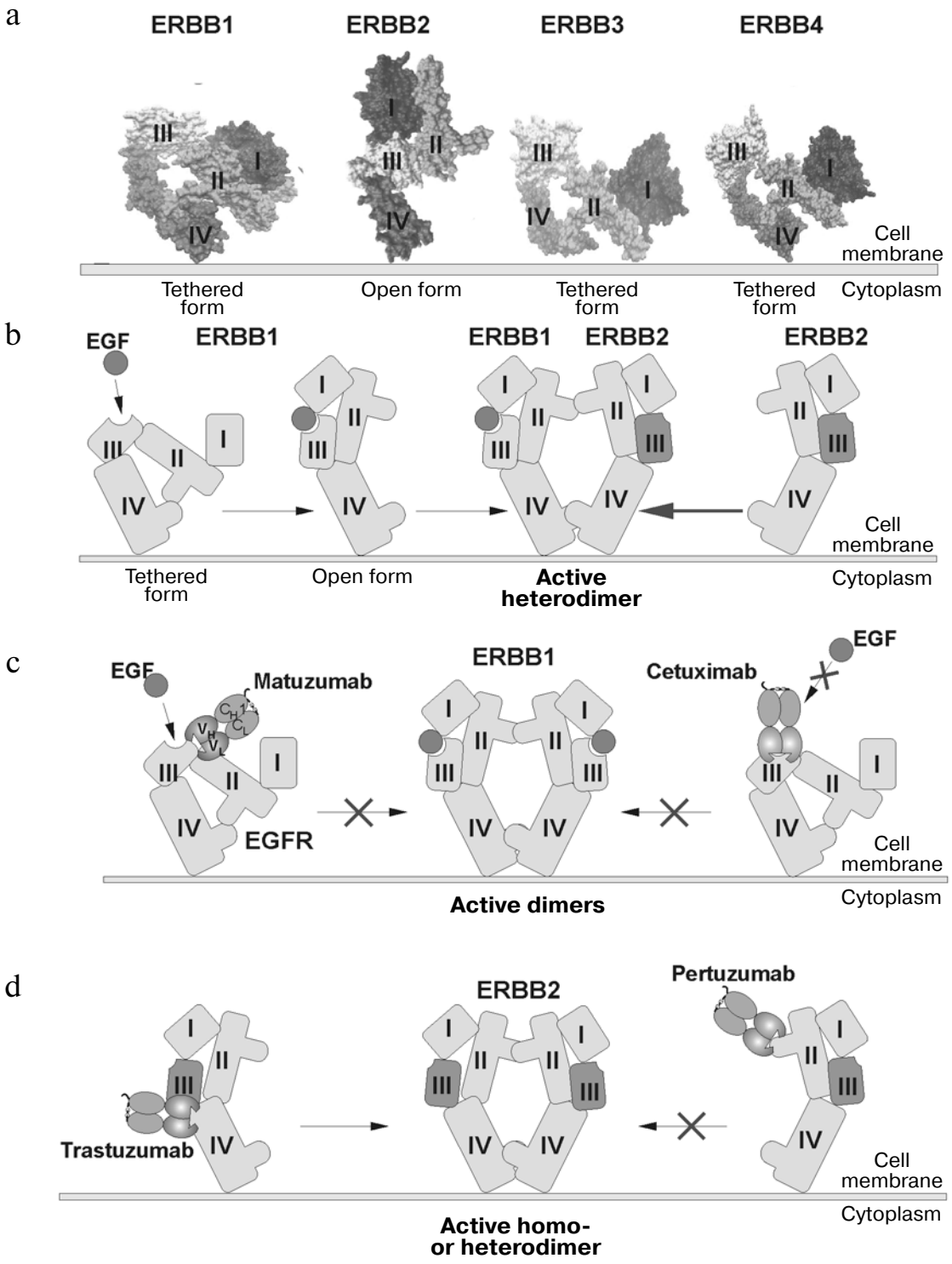

Fig. 3. Structures of extracellular domains of ERBB1-4 receptors and mechanisms of inhibition of their dimerization by monoclonal antibodies with different specificity. a) Structures of extracellular domains of ERBB1-4 without ligands; b) scheme of an active heterodimer formation; c, d) schemes of inhibitory mechanisms of receptor dimerization by antibodies specific to different epitopes of ERBB1 and ERBB2, respectively. Modified from [10] (Lemmon, M. A. (2009) *Exp. Cell Res.*, **315**, 638-648) by permission of Elsevier, copyright (2009), and from [81] (Schmiedel, J., Blaukat, A., Li, S., Knochel, T., and Ferguson, K. M. (2008) *Cancer Cell*, **13**, 365-373) by permission of Elsevier, copyright (2008).

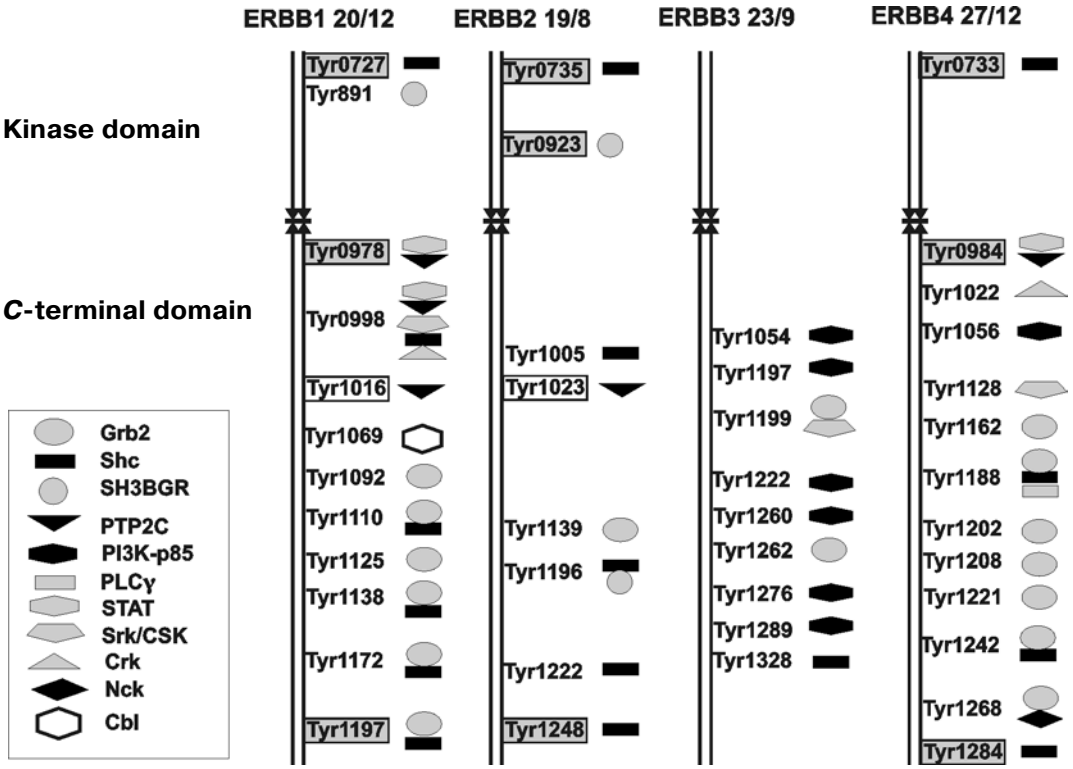

Fig. 4. Scheme of interaction of adaptor proteins with phosphotyrosine residues in the *C*-terminal and kinase domains of the ERBB1-4 receptors. Only phosphotyrosine residues involved in contacts with adaptor proteins are shown. Adapted from [15] (Schulze, W. X., Deng, L., and Mann, M. (2005) *Mol. Syst. Biol.*, **1**, 2005-2008) by permission of Macmillan Publishers Ltd: [Mol. Syst. Biol.], copyright (2005).

teins as phospholipase Cγ (PLCγ), phosphatidylinositol-3-kinase (PI3K), proteins of signal transduction, and activators of transcription (STATs) triggers the subsequent cascades of signaling pathways mediating different cellular processes [1, 4] (Fig. 1). Thus, activated ERBB receptors are the key point in the signal transduction from the environment into the cell.

Homodimers ERBB1 and ERBB4 can interact with many adaptor protein and lipid kinases that are signal transducers (Fig. 4). Thus, the homodimer ERBB1 upon autophosphorylation of some tyrosine residues on the *C*-terminus of the molecule interacts with adaptor phosphotyrosine-binding proteins Grb2 and Shc and activates them via phosphorylation. Later, these proteins responsible for phosphorylation of the Ras protein trigger reactions of the cascade of mitogen-activated protein kinase (MAPK) (Fig. 1). Another direct substrate of ERBB1 is the signal transducer and activator of transcription STAT5. ERBB1 cannot directly activate the signaling pathway PI3K/Akt, but it can influence it by triggering the Ras/MAPK signaling cascade.

It has been shown that EGFR/ERBB1 plays a key role in development of epithelial cells of different tissues and organs. Knockout of the *ERBB1* gene induces aberrant proliferation, migration, and differentiation of epithelial cells of lungs, skin, intestine, and placenta and causes lethal imperfections of the brain and neurodegeneration [17]. Another member of this family, ERBB4, is functionally similar to ERBB1 and is associated with differentiation of epithelial cells. As an activated dimer, ERBB4 interacts with the adaptor proteins Grb2 and Shc, the activator of transcription STAT5, and, as discriminated from ERBB1, can activate the PI3K/Akt signaling cascade [15, 18].

The ERBB3 receptor is not an autonomous member of the family: it fails to produce homodimers having kinase activity, but it can auto-associate into inactive oligomers that are destroyed on the receptor binding with a ligand (neuregulin) [19].

ERBB2 does not bind with any ligand (Fig. 2), but it is a preferential partner for producing heterodimers with three other members of the ERBB family and upon phosphorylation binds with many phosphotyrosine-binding proteins, including the adaptor proteins Grb2 and Shc [20] (Fig. 4). Heterodimers containing ERBB2 have a higher affinity for growth factors than other heterodimers due to the low dissociation rate of the receptor–ligand complex [20], and that are also characterized by a slow endocytosis and rapid recycling of the receptor [21].

ERBB2-containing heterodimers and especially ERBB2/3 are the most effective signal transducers in the cascade chains [22]. These heterodimers initiate strong

mitogenic signals that lead to a synchronous and prolonged triggering of a multiplicity of signaling cascades, promote accelerated cell proliferation and migration, and decelerate the entrance into apoptosis. Thus, ERBB2 is a natural enhancer in the complicated system of signal transduction mediated by ERBB receptors.

Signal transduction cascades mediated by ERBB receptors. Even on the level of signal transduction from ERBB receptors to cytoplasmic proteins, it is obvious that not a simple linear pathway of signal transduction into the cell from the solitary receptor has been formed in mammals during evolution but rather a complicated signaling network mediated by four functionally different membrane receptors capable of being activated upon interaction with different ligands and of triggering different signaling pathways [2]. Among these pathways, it is necessary to set off the main directions of the signal transduction whose damage on different levels can result in tumorigenesis.

Signaling pathway Akt/PI3K, or a survival pathway (Fig. 1). In response to stimulation with growth factors or various external stresses (thermal shock, ischemia, hypoxia, hypoglycemia, oxidative stress), the serine-threonine kinase Akt influences cell proliferation and apoptosis [23]. The Akt/PI3K signaling cascade starts from interaction of phosphorylated tyrosine residues of ERBB (Fig. 4) with the regulatory domain p85 of PI3K kinase [24, 25]. As a result of a conformational rearrangement, the catalytic domain p110 of PI3K kinase becomes enzymatically active. Then PI3K kinase catalyzes the transformation of phosphatidylinositol diphosphate (PIP2) to triphosphate (PIP3) necessary for the subsequent phosphorylation of Akt under the influence of protein kinases PDK and S6K.

The key role in regulation of the Akt cascade belongs to phosphatase and tensin homolog PTEN, which dephosphorylates an excess of phosphatidylinositol-3-phosphate to the diphosphate (PIP3 → PIP2), inhibiting phosphorylation of Akt and preventing the signal transduction.

Kinase PI3K can also be activated by proteins Ras (see below) and through other signaling pathways (VEGFR, cytokines, insulin) [24, 25] (Fig. 5).

Further, kinase Akt is involved in many various events, and they all display antiapoptotic and proliferative

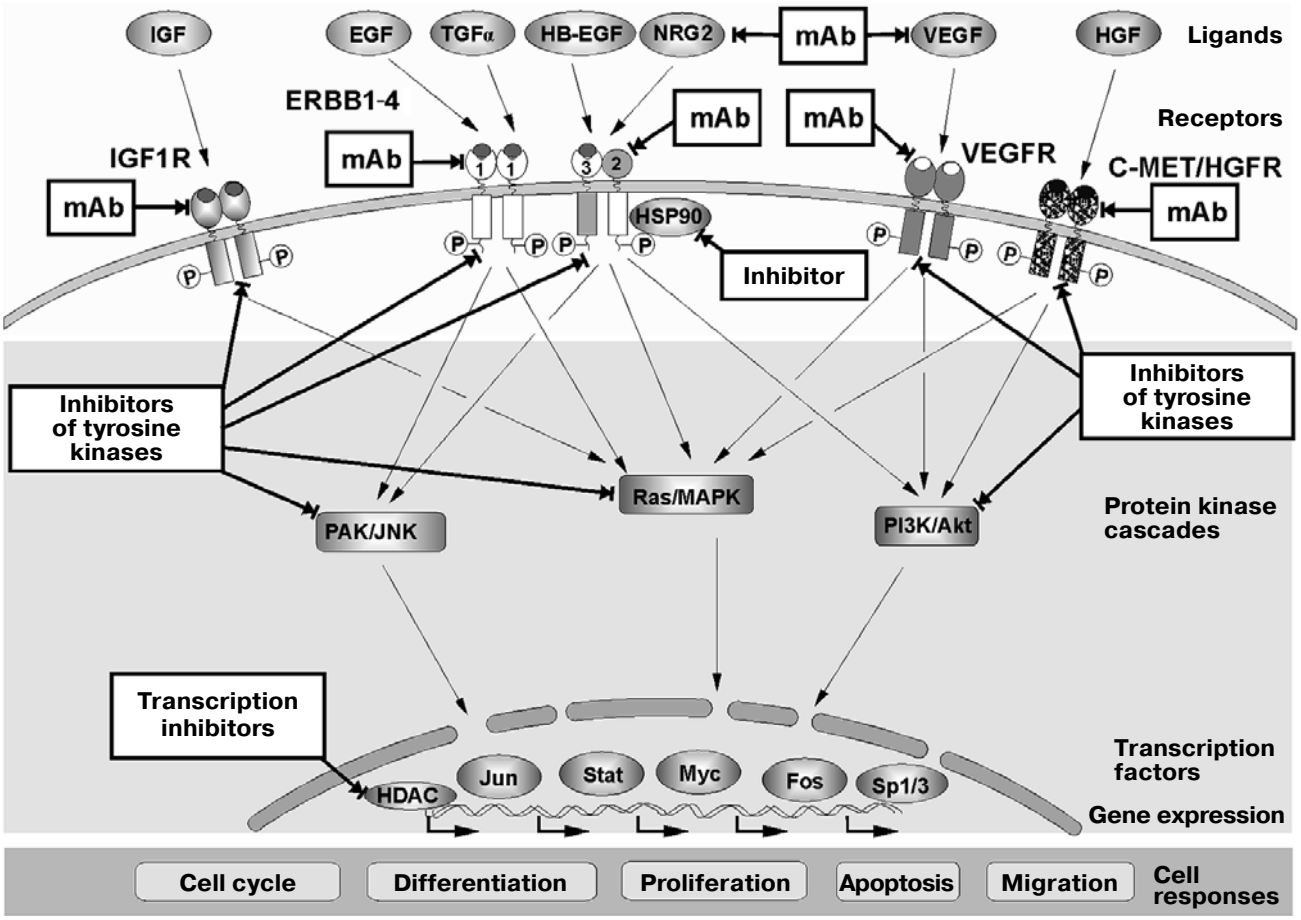

Fig. 5. Scheme of overlapping signaling pathways activated by membrane receptor protein kinases ERBB1-4, IGF1R, VEGFR, and cMET/HGFR. Points of action of antitumor therapeutic agents are indicated.

effects and contribute to metabolic processes, protein synthesis, regulation of transcription and cell cycle, and also of apoptosis [23]. Akt exerts its influences through phosphorylation of many various substrates, including the mammalian target of rapamycin (mTOR), transcription factors FKHRL1 and nuclear factor-kappa B (NF-κB), cycle checkpoint kinase-1 (Chk1), and also apoptosis proteins: BCL2 antagonist of cell death (BAD), caspase-9, and apoptosis signal-regulating kinase-1 (ASK1) [26].

Proteins of the Akt signaling cascade (Akt, PI3K, PTEN) are important prognostic markers of oncologic diseases and targets for therapeutic interventions [27].

Signaling pathway Ras/ERK/MAPK as a pathway of cell proliferation and differentiation (Fig. 1). Mitogen-activated protein kinases (MAPKs) belong to the class of serine-threonine protein kinases, and they are activated in response to various external exposures and transmit signals from the cell surface to the cell nucleus. MAPKs are subdivided into three main families: extracellular signal-regulated kinases (ERKs), c-Jun *N*-terminal protein kinases (JNKs), and p38 kinases. ERK1 and ERK2 act as a central component of the Ras/ERK/MAPK signaling cascade responsible for cell growth and differentiation. In addition to receptors of the ERBB family, this cascade can be activated by G-protein coupled receptors (GPCRs) and by other RTKs. Activation of ERKs is triggered as a result of interaction of an activated ERBB receptor with the adaptor proteins Shc and growth factor receptor bound protein-2 (Grb2), which, in turn, attract for further events the son of sevenless protein (SOS) (Fig. 1). The resulting complex provides for the replacement of GDP bound with the Ras protein by GTP and the activation of Raf kinase, which activates a three-step cascade of successive phosphorylation of MAP kinase kinase kinase (MAP3K/MEKK), MAP kinase kinase (MAP2K/MEK), and MAPK (Fig. 1). In turn, MAPK activates ERK1 and ERK2 via phosphorylation on threonine and tyrosine residues in the conservative motif TEY [28].

Some members of the Ras/ERK/MAPK signaling pathway (proteins of the Ras and Raf families) were initially identified as protooncogenes, and they are important prognostic markers of oncologic diseases and targets for therapeutic interventions.

Signaling pathway JNK/SAPK, or the stress pathway (Fig. 1). Members of the JNK/SAPK family play the major role in regulation of cell responses under conditions of stress or inflammation and also during neuronal differentiation and during apoptosis [29]. JNKs can be activated through RTKs, cytokines, or GPCRs. This process is much more complicated and less studied than the activation of ERKs, and it involves a large set of proteins including more than 10 MAPKKs [29]. As differentiated from the Ras/ERK/MAPK signaling pathway, the initial stage of JNK activation occurs under the influence of a protein Vav (initially identified in humans as a protooncogene), which belongs to the Dbl family of guanidine exchange factors and interacts with the Rho/Rac family of GTPases [30]. Upon activation, JNK/SAPK is translocated into the nucleus where it phosphorylates some transcription factors, including c-Jun and p53 [31]. Moreover, JNK phosphorylates and stabilizes heat shock factor-1 (HSF1) and thus contributes to the cell protection against stress. The JNK/SAPK stress signaling pathway seems to play an important role in pathogenesis of some neurodegenerative, inflammatory, and oncologic diseases; therefore, identification of crucial components of this pathway may reveal new therapeutic targets for treatment of these diseases [29].

Recent data have shown that the signal from ligand-activated tyrosine kinase receptors can be transduced not only via cascades involving protein kinases and transcription factors, but also by a direct transfer of receptors or their fragments into the nucleus [32]. As early as 5 min after activation by EGF, ERBB1 can be detected in the nucleus [33]. All four ERBBs have been shown to contain in the transmembrane domain nuclear localization signal (NLS) amino acid sequences responsible for the nuclear localization of these proteins [34]. NLS sequences contain three clusters enriched with Arg and Lys in the region of 645-657. Mutations Arg/Ala or Lys/Ala sharply decrease the nuclear localization of the receptors.

Regulation and stability of the ERBB signaling network. Primary mechanisms of interrupting or weakening the signal include receptor inactivation under the influence of phosphatases and also its internalization by endocytosis and subsequent proteolytic degradation [35]. On the *C*-terminus of the ERBB1 molecule there is a site of recognition of ubiquitin ligase Cbl (Fig. 4), which is responsible for ubiquitinylation of the receptor and its subsequent rapid degradation in lysosomes [36]. The major mechanism of ERBB1 internalization is clathrin-dependent endocytosis, which can lead to the backward migration of activated receptors from caveolae or to irreversible degradation of ERBB1 [37]. The binding with EGF accelerates endocytosis of the receptor and concurrently diminishes the recycling/degradation ratio resulting in interruption of the signal [38]. Hyperphosphorylation of the internalized ERBB1 is also shown, and it retains the enzymatic activity and interacts with effector proteins that are signal transducers (Shc, Grb2, SOS). And the efficiency of ERBB1 is sufficient for activation of the main signaling pathways leading to cell proliferation and survival. These processes occur with involvement of the APPL family proteins, in particular, with EGF, which is the major ligand of ERBB1 inducing the transfer of APPL1 into the nucleus, where this protein interacts with histone acetyltransferase and controls gene expression. In contrast, the binding of ERBB1 with TGFα promotes rapid recycling and the receptor return onto the cell membrane as an inactivated monomer [37].

The ERBB2 receptor does not have sites for interaction with ubiquitin ligase Cbl (Fig. 4), so this receptor

undergoes slow endocytosis and is characterized by a rapid return onto the cell membrane [21]. Note that ERBB1 as a component of the heterodimer with ERBB2 also escapes ubiquitinylation and rapid degradation in lysosomes [39].

In many cases, the stability of all elements of the signaling network is important and often decisive for its functioning. Supporting the native structure of ERBB2 and protection against proteolytic cleavage are provided under the influence of the chaperon HSP90 and the co-chaperon CDC37, which protect ERBB2 against degradation by the 26S proteasome complex [40]. The chaperon not only stabilizes the ERBB2 structure. Interacting with the kinase domain of ERBB2, the chaperon decreases the kinase activity and limits production of active ERBB2 heterodimers with other receptors, e.g. with ERBB3 [41]. Inhibition of glycolysis or respiration in mitochondria of isolated myocytes causes a rapid dissociation of ERBB2 from the complex with the chaperon and degradation of this receptor. Note that the action of the antibiotic geldanamycin (an inhibitor of HSP90) used in cancer therapy is based on the same effect. This result suggests the dependence of epidermal growth factors on the energy potential of the cell [42].

Note that during recent years many experimental data have accumulated indicating that the activity of *ERBB* oncogenes is controlled on the genetic level with involvement of transcription factors and also of repressors interacting with *ERBB* promoters [43] or with promoters of the genes encoding proteins of the signaling cascades [44]. Thus, the transcription factor FOXP3 inhibits transcription of the *ERBB2* gene via direct binding with the *ERBB2* gene promoter [43]. In fact, 90% of mice carrying the mutant gene *Foxp3* are characterized by a spontaneous appearance of mammary gland tumors, and ~60% of these are malignant [43].

Posttranslational (epigenetic) modifications and, in particular, aberrant methylation of *ERBB* promoters play an important role in the regulation of *ERBB* genes and malignant transformation of cells. Thus, in some cases hypermethylation of the *ERBB4* promoter completely suppresses this gene, which results in a malignant tumorigenesis with poor prognosis [45].

TRANSFORMATION OF *ERBB* GENES INTO ONCOGENES

Disorders in the structure and regulation of ERBB receptors result in uncontrolled growth of cells and are characteristic for many epithelial tumors and also for some other diseases. These disorders are as follows: hyperexpression of the receptors on the cell surface, mutations, incorrect localization, autocrine secretion of ligands, and impaired endocytosis. During early stages of breast cancer development in tumor cells, genetic "breaks" are accumulated that manifest themselves in a disturbed regulation of expression of potential oncogenes and suppressors of tumor growth. These changes lead to a further increase in the instability of the tumor cell genome, disorders in cell differentiation, tumor growth, and metastasis [46].

Instability of the tumor cell genome and amplification of the *ERBB* genes. The *ERBB2* gene, which is one of the first identified human oncogenes, is located in the 17q12 locus. The amplification of this gene and overexpression of the corresponding receptor are observed in 20-30% of malignant breast tumors [47]. The tumor cells with overexpressed *ERBB2* have gene amplification in the wide region of 17q12-q21 ($5.7 \cdot 10^6$ bp), and in this region, in addition to the *ERBB2* gene, the genes *TOP2*, *BRCA1* (breast cancer 1), *GRB7*, and also the gene *STAT3* (signal transducer and activator of transcription 3) are also localized. The expression levels of these genes are increased in tumors overexpressing *ERBB2* and thus induce a rapid and aggressive tumor progression [48]. Moreover, the *ERBB2* amplification is associated with specific changes in some loci of other chromosomes (11q13, 16q22-q24, and 18q21) that, in turn, can influence the amplification of oncogene *ERBB2* and accelerate tumor growth [46]. The *ERBB2* gene hyperexpression can be also a result of disorders in functions of transcription factors involved in its regulation [43]. The status of gene *HER2/neu* (*ERBB2*) is one of major traits for identification of breast tumor subtypes, disease prognosis, and choice of treatment approaches [49].

The *ERBB1* gene is located in the 7p12 locus, the amplification of which associated with receptor overexpression, and genetic instability of the tumor cells is characteristic for malignant tumors of head and neck, colorectal cancer, breast carcinomas, and non-small cell lung cancer [50-52]. Until recently, this gene status had not been used as a prognostic trait in breast cancer, but an increased expression of this gene found in ~40% of mammary gland tumors that in the most cases are hormone-dependent allows us to consider it as an important prognostic marker. An increased expression of the *ERBB1* gene is also observed in 80% of cases of triple negative breast cancer (TNBC), a recently described class of aggressive mammary gland tumors characterized by the absence of hormonal dependence and overexpression of the *ERBB2* gene [53]. The *ERBB1* gene amplification is 0-14% for a random sample of tumors and up to 28% for carcinomas [54].

Data on the status of the *ERBB3* (genetic locus 12q13) and *ERBB4* (genetic locus 2q33) genes in human tumors and on their prognostic value are mixed [55, 56]. These loci are amplified in 75 and 37% of breast cancer cases, respectively [55]. The *ERBB3* overexpression especially combined with *ERBB2* or *ERBB1* hyperexpression is associated with a rapid progression of ovary, prostate, and lung cancer and with poor prognosis for the patient

[56], whereas a high expression of *ERBB4* is associated with better survival and response to treatment [55].

Spontaneous somatic mutations. ERBB receptors can acquire features of oncogene proteins also as a result of somatic mutations arising in tumor cells [52]. Such mutations are found in virtually every functional domain of ERBB receptors – the extracellular, ligand-bound, perimembrane, and protein kinase intracellular domains. The elongated deletion del2-7 of the extracellular domain EGFRvIII characteristic for 30-50% of gliomas leads to a constitutive ligand-independent dimerization and the subsequent activation of the receptor [57]. Mutations affecting the contact between subdomains II and IV lead to increase in the binding affinity of EGF and TGFα and to spontaneous dimerization [10]. Rare missense mutations have been detected recently in the perimembrane regulatory region of ERBB1, and two of them are constitutively active possibly due to a stabilizing influence during receptor dimerization.

Activating mutations of *ERBB1* and *ERBB2* in the kinase domain induce a ligand-independent increase in the signaling activity, resistance to treatment with tyrosine kinase inhibitors, deceleration of internalization, and degradation of the internalized receptor [58, 59]. Small deletions or insertions in the P-loop of the *ERBB1* kinase domain not affecting the reading frame are found in 10-13% of patients with non-small cell lung cancer (NSCLC). Similar somatic mutations of *ERBB2* were found in 5% of patients with NSCLC, in 3-5% of patients with gastrointestinal tract carcinomas, and in <5% of patients with breast carcinoma [60, 61]. Cells of multiform glioblastoma, which is one of the most aggressive brain tumors, were found to have duplication of the EGFR kinase domain associated with kinase constitutive activation and with a malignant course of the disease [62].

Autocrine stimulation of RTKs. Expression of activating mutants of ERBB2 not only increases the signal transduction but also induces some pro-tumor growth factors and changes the tumor microenvironment [63]. Thus, it has been recently shown that expression of a mutant *ERBB2* in epithelial cells of mammary gland activates the autocrine transforming growth factor TGFβ1 and ligands TGFα and amphiregulin of the ERBB1 receptor and also the vascular endothelium growth factor VEGF [63].

Considering the signaling network mediated by the ERBB receptors and its role in carcinogenesis on the whole, we must note that disorders in the regulation of this very complicated system leading to carcinogenesis can occur at its every level [2, 46, 64] (Fig. 1). In fact, both the receptors themselves and the majority of signaling proteins and adaptors (BRAF, KRAS, HRAS, NRAS) have been identified as oncogenes or their homologs [65] and are characterized by hyperexpression and somatic mutations in tumor cells [48]. The ERBB receptors and also many components of this cascade, especially kinases (kinases BRAF, KRAS, HRAS, NRAS, Akt, MEK1, PI3K, phosphatase PTEN, chaperon HSP90), are diagnostic markers and therapeutic targets in oncologic diseases [27, 48, 54]. Consideration in detail of these oncogenes, except for the ERBB receptors, is beyond the limits of the present review, but we cannot fully neglect them when combined approaches to cancer therapy are under discussion.

ERBB RECEPTORS AS TARGETS FOR THERAPEUTIC INTERVENTIONS

During the last decade, many new therapeutic targets have been found that belong to signaling cascades of ERBB receptors and that play an important role in malignization of epithelial cells. Various kinase inhibitors have been developed having a wide spectrum of activity and narrow specific ones acting on proteins of the ERBB signaling network at virtually all levels of the signal transduction [66, 67] (Fig. 5); however, the greatest efforts of researchers have been concentrated on finding approaches for inhibition of the ERBB signaling system just at the signal entrance, i.e. at the receptor level.

The main therapeutic strategies for inhibition of the ERBB signaling network on the receptor level include the following approaches: selective inhibition of autocrine ligands (TGFα and HB-EGF) of ERBB receptors by mAbs [68]; selective inhibition of ERBB receptors by mAbs [69]; inhibition of autophosphorylation and kinase activity of ERBB receptors by low molecular mass kinase inhibitors with different degrees of specificity [66, 67, 70]; inhibition of HSP90 by the antibiotic geldanamycin to increase the rate of endocytosis and degradation of ERBB2 [40]; inhibition of transcription of genes encoding ERBB receptors [71]; inhibition of so-called sheddases – enzymes "shedding" the ectodomain of ERBB receptors from the cancer cell surface [72].

Among 11 kinase inhibitors approved by the FDA for clinical application, three are specific to ERBB receptors: two preparations, gefitinib (Iressa®) and erlotinib (Tarceva®), are reversible inhibitors of EGFR kinase activity, and a new generation drug lapatinib (Tykerb®) irreversibly inhibits EGFR and ERBB2 [73]. The following preparations are now subjects of stage I and II clinical trials: canertinib capable of irreversible inhibiting ERBB1-4 due to blocking the ATP-binding site of the receptors, and pelitinib that irreversibly inhibits ERBB1, 2, 4 due to binding with the tyrosine kinase domain. The so-called new "multi-target" kinase inhibitors, which can simultaneously block different signaling pathways, also attract increasing attention. They are, in particular, exemplified by the preparation vandetanib inhibiting the kinase activity of VEGFR and EGFR [74]. As a rule, low molecular mass inhibitors of RTKs have a low specificity to tumor cells, high toxicity, and induce development of drug resistance when used for a long time, which is asso-

Monoclonal antibodies to ERBB receptors (EGFR/HER) and some other cell surface receptors triggering overlapped signaling pathways

Molecular target	Antibody name in USAN*	Commercial or working name/ producers	Antibody format/ K_d (M)	Epitope/action mechanism	Use for disease therapy	Introduction into clinical practice	Source
1	2	3	4	5	6	7	8
ERBB1 (EGFR)	Cetuximab	Erbitux®/ ImClone (USA), Bristol-Myers Squibb (USA)	chim. IgG1 (IMC225)/ $1 \cdot 10^{-10}$	subdomain III/ inhibits binding with ligand, ADCC	CRC, NSCLC, SCCHN	FDA 2004	[78]
	Panitumumab	Vectibix®/ Amgen (USA)	hum. mAbs IgG2 (E7.6.3)/ $5 \cdot 10^{-11}$	subdomain III of EGFR	CRC, NSCLC, BC	FDA 2006 (CRC); CT, phase II/III (NSCLC, BC)	[79]
	Nimotuzumab	Theraloc®/ Oncoscience (Germany), TheraCIM/ CIMYM Biosciences (Canada)	hmnz. mAbs (h-R3)/$1 \cdot 10^{-9}$	–"–	SCCHN, NSCLC, PGC, SC	CT finished	[80]
	Matuzumab	EMD72000/ Merck KGaA (Germany)	hmnz. mAbs 425/$3.4 \cdot 10^{-10}$	subdomain III (epitope other than of Cetuximab)	OC, PGC, EC	–"–	[79, 81]
	Zalutumumab	HuMax-EGFr/ Genmab (Denmark)	hum. IgG1	subdomain III of EGFR	SCCHN	CT, phase II/III	[77]
	Necitumumab	IMC-11F8/ ImClone (USA)	–"–	–"–	NSCLC	–"–	[77]
EGFR(del2–7)/ EGFRvIII	–	mAbs 806	mAbs 806	subdomain II/III	glioma	CT, phase I/II	[82]
ERBB1 + CD64	–	MDX-447/ Medarex (USA), Merck KGaA (Germany)	hmnz. bispecific $(Fab)_2$	EGFR subdomain III/ ADCC (CD64)	SCCHN	–"–	[83]
ERBB2 (HER2/neu)	Trastuzumab	Herceptin®/ Genentech (USA)	hmnz. IgG1 mAbs 4D5	subdomain IV/ ADCC, shedding inhibitor	HER2/ neu(+) BC	FDA 1998	[84]
	Trastuzumab emtansine	Genentech (USA)	IgG1, conjugate with DM1	subdomain IV/ immunotoxin, meiosis inhibitor	–"–	CT, phase III	[77]
	Pertuzumab	–"–	hmnz. IgG2 mAbs 2C4	subdomain II/ dimerization inhibitor	HER2/neu(+) mBC	–"–	[85]
ERBB2 + CD3	Ertumaxomab	CD3-HER2/neu MOAB/TRION Pharma (Germany)	chim. mouse/ rat IgG2a/ IgG2b	subdomains II and III/ ADCC, phagocytosis	HER2/neu(+/–) cancer	CT, phase I	[86]

(Contd.)

1	2	3	4	5	6	7	8
VEGFα	Bevacizumab	Avastin®/ Genentech (USA)	hmnz. IgG1 mAbs	/binds with ligand, VEGFR antagonist	HER2/neu(–) mBC	FDA 2004	[77]
	Ranibizumab	Lucentis®/ Genentech, Novartis (USA)	hmnz. IgG1 Fab	–"–	AMD	FDA 2006	[77]
VEGFR1	Icrucumab	IMC-18F1/ ImClone (USA)	hum. IgG1	/binds with receptor, inhibits angiogenesis		CT, phase II	[87]
VEGFR2	Ramucirumab	IMC-1121B/ Im-Clone, Eli Lilly (USA)	–"–	–"–	mAS, BC, HCC	CT, phase III	[77]
IGF1R	Dalotuzumab	MK-0646/Merck (USA), Pierre Fabre (France)	hmnz. IgG1	/binds with receptor, inhibits signaling pathway PI3K/Akt	BC, PC, PGC, MM	–"–	[88]
	Cixutumumab	IMC-A12/ ImClone (USA)	hum. IgG1	–"–	CRC, PC, solid tumors	–"–	[88]
c-Met (HGFR)	Rilotumumab	AMG-102/ Amgen (USA)	–"–	/binds with receptor, inhibits binding with HGF ligand		CT, phase II	[87]
	Onartuzumab	MetMAb/ Genentech (USA)	hmnz. IgG1	–"–		–"–	[87]

Notes: AMD, age-related macular dystrophy; AS, adenocarcinoma of the stomach; BC, breast cancer; chim., chimeric; CRC, colorectal carcinoma; CT, clinical trials; EC, esophagus cancer; HGF, hepatocyte growth factor; HCC, hepatocellular carcinoma; m, metastasizing form; hmnz., humanized; hum., human; MM, multiple myeloma; NSCLC, non-small cell lung cancer; OC, ovary cancer; PGC, pancreatic gland cancer; PC, prostate cancer; SC, stomach cancer; SCCHN, squamous cell carcinoma of head and neck.

* The US-adopted nomenclature of monoclonal antibodies and their fragments is now used throughout the world. USAN, US Adopted Names; www.ama-assn.org, see also [6]; the names are corrected according to the site Internet http://cme.nci.nih.gov/drugdictionary.

ciated with activation of lower mediators of signal transduction or with activation of bypassing signaling pathways [74]. Moreover, the efficiency of low molecular mass inhibitors of RTKs depends on polymorphism of the ERBB receptor genes. Thus, the mutation T790M, which by an order increases the affinity of EGFR for ATP, is associated with a patient's resistance to treatment with competitive inhibitors gefitinib and erlotinib [75].

Antitumor monoclonal antibodies specific to ERBB receptors. An increased concentration of ERBB receptors on the surface of various tumor cells compared to the basal level on healthy tissue cells and also their key role in the signal transduction allowed these receptors to be used as selective targets for mAbs specific to extracellular domains of ERBB. The selective effect of mAbs on tumor cells is underlain by some different mechanisms, such as attracting immune system cells to the tumor (antibody-dependent cell cytotoxicity (ADCC)), direct interruption of the signal via competitive binding with the receptor, disturbance of the receptor dimerization, and targeted delivery of toxins or other agents [6, 76]. About ten mAbs preparations have been approved for clinical use in oncology, and 13 preparations have passed into stage III of clinical trials in 2011 [77]. The majority of these therapeutic mAbs are specific to the ERBB family receptors (table). As discriminated from low molecular mass inhibitors, therapeutic mAbs are much less toxic for the organism. Moreover, mAbs, in addition to be used as acting agents, are also widely used as targeting modules for creating multifunctional antitumor compounds [6, 89-93].

Monoclonal antibodies specific to ERBB1. ERBB1 receptor is the main target for therapy of some tumors hyperexpressing this receptor. For therapy of patients with metastatic colorectal carcinoma (mCRC), NSCLC, and some other tumors (table), anti-ERBB1 antibodies cetuximab and panitumumab are used. Cetuximab is a chimeric (mouse/human) immunoglobulin G1, which is a derivative of the mouse mAbs C225. The binding affinity of this antibody is two orders of magnitude higher than the binding affinity of natural ligands of ERBB1. The binding of cetuximab with subdomain III of the extracellular part of ERBB1 causes internalization and subsequent degradation of the receptor without its phosphorylation and activation, and this results in lowering the number of receptors on the cell surface and prevents activation of the corresponding signaling pathways [69]. Cetuximab also interacts with the mutant receptor EGFRvIII (del2-7) inducing the internalization of 50% of the ligand–receptor complexes and decreasing by 80% phosphorylation of EGFRvIII. Cetuximab inhibits ERBB1 binding with endogenous growth factors, suppresses cell mobility and metastasizing, induces apoptosis of cancer cells, and also inhibits generation of pro-angiogenic factors VEGF and interleukin-8.

Panitumumab is the first human antibody approved for clinical application; it has a high affinity for the receptor ($K_d = 5 \cdot 10^{-11}$ M), binds with subdomain III of the extracellular part of ERBB1, and disturbs the interaction of this receptor with ligands, preventing the activation of the receptor kinase domain and thus interrupting the signal transduction [79]. As discriminated from cetuximab, panitumumab does not induce ADCC mechanisms because it belongs to the IgG2 isotype. Due to the full human sequence of the protein, panitumumab is well tolerated by patients and does not cause allergic reactions or anaphylaxis. However, such high binding affinity (table) seems to be a cause of frequent pronounced skin reactions in patients treated with this preparation [80].

Panitumumab decelerates progression of tumors (mCRC) and increases the survival of patients. Nevertheless, panitumumab is not recommended as an addition to chemotherapy in patients with mCRC with progressing metastases during the late IIIB phase because this combination does not decelerate the tumor growth, but it enhances the toxicity of chemotherapy [94].

Clinical trials of the next generation of mAbs specific to the ERBB1 receptor are now complete. These mAbs include preparations matuzumab and nimotuzumab (table), which are humanized IgGs interacting with subdomain III of the extracellular fragment of the ERBB1 receptor. The epitope of matuzumab (derivative of mouse mAbs 425) is different from the cetuximab epitope: matuzumab does not inhibit the interaction of the receptor with the ligand but sterically prevents local conformational changes and correct rearrangements of the domains, which are designed to promote high affinity binding of the ligand with following activation of phosphorylation (Fig. 3c) [81].

Matuzumab has a very high affinity and specificity for ERBB1 (table). Clinical trials of matuzumab have shown promising results for patients with progressing cancer of pancreas when given combined with the cytostatic gemcitabine [79]. Matuzumab was given to 18 patients together with the mitosis inhibitor paclitaxel, and an improvement was observed in four patients with NSCLC in stages IIIB and IV, and complete cure was observed in one of these patients [95].

Clinical trials of nimotuzumab on patients with brain tumors and with squamous cell carcinoma of head and neck (SCCHN) have shown a significant increase in survival of the patients with SCCHN treated with high doses of this antibody in addition to radiotherapy. The patients tolerated this combined therapy well, and skin complications were virtually absent [80].

The third generation of mAbs specific to the ERBB1 receptor is represented by entirely human monoclonal IgG antibodies binding with the subdomain III. Preparations zalutumumab and necitumumab (table) based on these mAbs are subjects of initial stages of trials [77]. Clinical trials of the MDX-447 preparation (table) belonging to a new promising type of mAbs have started. MDX-447 is a bispecific antibody of the $(Fab)_2$ format with a high affinity for the ERBB1 and Fc-receptors (CD64 antigen) of cytotoxic cells [83]. The mAb 806 is also very interesting (table) because it is specific to a constitutively active deletion mutant of ERBB1, EGFR(del2-7)/EGFRvIII (see above), and it is capable of binding with the open form of the receptor and preventing its dimerization [82].

Monoclonal antibodies specific to ERBB2. The humanized monoclonal anti-ERBB2 antibody trastuzumab (Herceptin®) [84] (table) was the first preparation of mAbs approved by FDA for therapy of cancer. Trastuzumab given at early stages of breast cancer is effective in 20-30% of patients with cancer cells overexpressing *ERBB2*. On combined therapy with trastuzumab and cytostatic chemical preparations (especially taxanes and vinorelbine), the efficiency increases to 50-80% [96]. However, the long-term treatment with trastuzumab is associated with cardiotoxicity and some other side effects [97], and many patients become resistant to the therapy [97, 98], making it necessary to use a combined treatment or change the therapeutic strategy.

The humanized monoclonal anti-ERBB2 antibody pertuzumab (Omnitarg®) (table) binds with the dimerization arm of the subdomain II of the extracellular part of ERBB2 receptor (Fig. 3d) [85]. As discriminated from trastuzumab, which interacts with the ERBB2 epitope located in subdomain IV, pertuzumab sterically prevents formation of heterodimers ERBB2/ERBB1 and ERBB2/ERBB3, thus inhibiting the signal transduction in the cascade chains. Despite the synergic action of these

therapeutic antibodies, pertuzumab does not statistically decrease the survival of tumor cells resistant to trastuzumab [97].

Therapeutic use of antibodies revealed their insufficient efficiency, and this is a significant problem. To strengthen the effect on cancer cells, antibodies are conjugated with various toxins [90, 99, 100]. Clinical trials have ended for the bifunctional preparation trastuzumab emtansine (table), which is a conjugate of antibodies with a meiosis-inhibiting toxic compound of the maytansinoid class (table) for treatment of locally progressing or metastasizing HER2/neu-positive breast cancer [77].

The trastuzumab and pertuzumab preparations have been created for therapy of tumors with *ERBB2* hyperexpression and are inefficient in many cancers with a low level of this receptor on the cell surface. To treat such tumor cells, a trifunctional antibody ertumaxomab (table) has been created with a unique hybrid isotype (mouse IgG2a/rat IgG2b) specific to ERBB2 and the CD3 receptor of T-cells [86]. The specificity of ertumaxomab to tumor cells is determined by the mouse mAbs 2502A interacting with the epitope (subdomains II and III) different from epitopes recognized by trastuzumab and pertuzumab. High toxicity of this preparation is caused by the ability of constant domains of the rat antibody to attract the (CD3+)-T-cells to the tumor. However, preclinical testing has shown that strong side reactions caused by this preparation are associated with the xenogenous origin of the antibodies [86], and before its introduction for clinical use it must be humanized.

Action mechanisms of monoclonal antibodies specific to ERBB receptors. Molecular mechanisms of trastuzumab and cetuximab action have been studied in detail [101, 102]. Humanized or human antibodies of the IgG format can usually induce mechanisms of antibody-dependent cell cytotoxicity (ADCC) for killing cancer cells. In model experiments on "nude" mice and upon using in clinical practice, trastuzumab has been shown to display not only cytostatic effect but also cytotoxic properties due to activation of natural killers expressing Fc-receptors. Mice with the Fc-receptor (−/−) phenotype retain only a weak sensitivity to trastuzumab (<30%). The efficiency of therapy with trastuzumab in human breast cancer depends on polymorphism of the receptor FcγRIIIa (CD16), which is a characteristic activating receptor of various killer cells involved in ADCC processes. Treatment with cetuximab is also associated with a pronounced contribution of the ADCC mechanisms to the therapeutic efficiency of these antibodies.

The constant domain of IgG2 has a low binding affinity for Fc-receptors of killer cells; therefore, antibodies of this format (panitumumab, pertuzumab (table)) cannot, as a rule, induce ADCC. On the contrary, trifunctional chimeric antibody ertumaxomab constructed using the constant domains of mouse and rat immunoglobulins are capable of binding with FcγRI and FcγRIII receptors. Due to specificity to CD3, ertumaxomab retargets the (CD3+)-T-cells of the immune system to the tumor and stimulates the release of proinflammatory cytokines (IL-6, IFNγ, TNFα). Due to the chimeric constant domain, ertumaxomab can also concurrently attract to the tumor and activate FcγRI- and FcγRIII-positive cells of the immune system (monocytes, macrophages, natural killers, dendritic cells), thus promoting phagocytosis of tumor cells. Ertumaxomab is highly cytotoxic not only for tumor cells with hyperexpressed *ERBB2*, but also for cells of carcinomas of mammary gland, lung, and large intestine that have a low density of ERBB2 receptor on the surface [103]. Another powerful mechanism of killing pathogenic cells, complement-dependent cytotoxicity (CDC), is not characteristic for individual therapeutic antibodies but can manifest itself on use together with antibodies specific to different ERBB1 epitopes (e.g. cetuximab + matuzumab) [104].

On binding with the fourth subdomain of the ERBB2 extracellular domain [105], trastuzumab decreases the effective concentration of the ERBB2 on the cell membrane due to competition with other ERBB receptors that form heterodimers with ERBB2. In fact, trastuzumab effectively inhibits formation of the ERBB2 heterodimer with ERBB1 but does not prevent its interaction with ERBB3 [106].

Another mechanism of trastuzumab action is based on blocking the signal transduction from activated ERBB2 heterodimers [107]. In this case, the target is phosphatase PTEN (see above), which acts as a natural regulator of signal transduction from ERBB2. Trastuzumab increases the PTEN concentration on the cell membrane and concurrently enhances the phosphatase activity of PTEN, which results in a rapid dephosphorylation of PIP3 (Fig. 1), inhibition of the PDK/AKT cascade, and suppression of proliferation. Patients with a low level of PTEN in mammary gland tumor cells respond much worse to treatment with trastuzumab [107]. Overexpression of *ERBB2* in mammary gland cancer cells in experiments on mice increases blood supply to the tumor tissue. Trastuzumab was shown to decrease angiogenesis [108]. This occurs most effectively when mitosis inhibitors (e.g. paclitaxel) are concurrently used.

Combined effect of mAbs on ERBB receptors. The combined effect on tumor cells of two antibodies specific to different epitopes of the ERBB receptor was observed much earlier, but the mechanism of this synergism was unclear. Studies on the effect of two antibodies specific to ERBB1 and ERBB2 receptors on corresponding xenografted tumors have shown that this effect is due to more rapid endocytosis and subsequent degradation of the receptor [109]. The authors supposed that the concurrent interaction with ERBB receptors of two kinds of antibodies specific to different epitopes should result in

formation of spatial lattices, which should undergo significantly more rapid endocytosis than receptors bound with one kind of antibody [109]. Another group of authors succeeded in complete elimination of a xenografted tumor in model animals using Sym004, which is a mixture of two anti-EGFR antibodies specific to non-overlapping epitopes of the EGFR extracellular domain III [110]. The internalization and degradation of EGFR are shown to be similarly accelerated under the influence on tumor cells of a mixture of cetuximab and immunoglobulin specific to human IgG [110]. Similar results have been obtained for a pair of mAbs specific to different epitopes of the ERBB2 receptor [111].

Note that the effect of trastuzumab on tumor cells is not always associated with *ERBB2* hyperexpression. Cells with a low level of *ERBB2* expression, the growth of which is not suppressed under conditions of long-term treatment with trastuzumab, have been recently shown to acquire sensitivity to anti-ERBB1 mAbs cetuximab and the low molecular mass inhibitor of ERBB1 gefitinib [112].

MOLECULAR MECHANISMS OF RESISTANCE TO THERAPEUTIC ANTIBODIES SPECIFIC TO ERBB RECEPTORS AND APPROACHES FOR OVERCOMING IT

Clinical studies have shown that only some of patients respond to treatment with antibodies specific to the ERBB receptors, even if their tumors display hyperexpression of these markers. Such resistance to the treatment can be primary, or acquired and mechanical, or apparent. The resistance to trastuzumab and cetuximab has been studied most systematically and in detail [102, 113], but researchers still continue to reveal new aspects of this problem [98]. Notwithstanding essential differences in functioning of the ERBB1 and ERBB2 receptors, some general mechanisms of resistance have been established that manifest themselves under the direct action on them. Mutations in the genes encoding the ERBB receptors [75] and/or in the genes encoding effectors of the subsequent signaling pathways [114] have been recently found in the majority of patients with primary resistance to anti-ERBB antibodies. Finding mechanisms of the resistance to anti-ERBB antibodies is an urgent and very important problem. The main mechanisms of resistance to anti-ERBB antibodies have been established, but many problems remain unclear.

In particular, the activation of alternative RTKs activating the same signaling cascades as the ERBB receptors (Fig. 5) is a mechanism of resistance to anti-ERBB antibodies. The branching and redundancy of signaling pathways is a prerequisite for switching the signal in the case of blocking one kind of receptors onto other receptors triggering the same signaling cascade. Thus, in trastuzumab-resistant (but not in trastuzumab-sensitive) tumor cells the receptor of insulin-like growth factor 1 (IGF-IR) (Fig. 5) is capable of interacting with the ERBB2 receptor producing heterodimers and inducing its phosphorylation and the following signal transduction [97]. The inhibition of IGF-IR by antibodies dalotuzumab or cixutumumab (table) restores the cell sensitivity to trastuzumab [88]. The triggering of bypassing signaling cascades through IGF-IR also correlates with the resistance to therapy directed to other RTKs including EGFR and mTOR [88]. In fact, bispecific antibodies binding with the EGFR and IGF-IR receptors inhibit tumor cells more strongly than each of these antibodies used separately [115].

Another bypassing signaling pathway can be activated during therapy with anti-ERBB1 antibodies or with low molecular mass inhibitors. Upon inhibition of the ERBB1 receptor, the amplification of the Met-oncogene is shown to trigger the signaling pathway that is usually activated by ERBB3. The c-Met receptor of hepatocyte growth factor (HGFR) (Fig. 5) forms heterodimers with ERBB3 and, via involvement of cascade proteins src and PI3K, completely replaces the function of signal transduction by the inhibited ERBB1 [116]. Antibodies rilotumumab and onartuzumab specific to c-Met recover the sensitivity of the tumor cells to treatment with anti-ERBB1 [87].

Researchers pay special attention to the contribution of vascular endothelium growth factor receptor (VEGFR) to the development of tumor cell resistance to anti-ERBB therapy [117]. On one hand, similarly to other RTK cases, the resistance can be caused by activation of the bypassing signaling pathway Akt/PI3K mediated through the VEGFR1 receptor [118]. Using mAbs specific to VEGFR-1 can restore the tumor cell sensitivity to the action of anti-ERBB antibodies [118]. On the other hand, ERBB oncogenes are known to activate functioning of the transcription factors Sp1 and Ap2, which in turn activate the *VEGFα* gene promoter [119]. The increase in the *VEGFα* gene expression stimulates angiogenesis and promotes tumor blood supply and acceleration of its growth [119].

For suppression of tumor angiogenesis, a specific to VEGFα humanized antibody bevacizumab (table) is used, which is approved by the FDA for clinical use and especially required in ophthalmology [77]. For the same purpose, human IgGs icrucumab and ramucirumab have been developed that are specific, respectively, to VEGFR1 and VEGFR2 (table). They are now at the stage of clinical trials for therapy of metastasizing stomach adenocarcinoma, breast cancer, and hepatocellular carcinoma [77, 87]. The first experience of clinical use of two antibodies with different specificity, trastuzumab and bevacizumab, was rather successful and stimulated further investigations [120]. The combined injection of cetuximab and bevacizumab decelerates the tumor

growth and suppresses the growth of tumor cells in experiments on animals [121]. Clinical application of bevacizumab, especially combined with other antibodies and also with inhibitors of tyrosine kinases and therapeutic agents, improves the condition of patients with various tumors (mCRC, NSCLC, BC, metastatic renal carcinoma) [122]. Note that the combined injection of anti-EGFR and anti-VEGFR antibodies is observed only if patients have no activating mutations in the *KRAS* gene [122]. Fundamental studies on angiogenesis and its association with tumor transformation are so important that the National Institutes of Health of the USA has declared clinical studies on angiogenesis to be a priority trend for the coming decade.

Resistance to anti-ERBB antibodies is often caused by a constitutive activation of mediators of the lower signaling pathways. Thus, activating mutations of *KRAS* or *PIK3CA* (Fig. 1) are associated with the loss of response to addition of anti-ERBB1 antibodies to the standard chemotherapy. Tumors with detectable wild type *KRAS* are sensitive to panitumumab and cetuximab, whereas mutations in codons 12 and 13 of *KRAS* gene exon 2 result in stabilization of the functionally active complex RAS–GTP and an uninterrupted entrance of the signal by the RAF-MAPK pathway (Fig. 1) [123]. Because such tumors are completely resistant to the action of anti-ERBB1 antibodies and represent about 43% in patients with CRC, it is necessary to carefully select patients before prescribing treatment with these mAbs [124].

Resistance to trastuzumab can also be caused by a significant change in the expression level of the PTEN phosphatase gene with resulting disorders in the regulation of the PI3K/Akt signaling cascade (Fig. 1) and amplifying in signal transduction both *in vitro* and *in vivo* [107]. Inhibitors of protein kinase PI3K restore the sensitivity to trastuzumab [107]. These results can be used for diagnostics: a low level of PTEN in the tumor tissue predicts resistance to trastuzumab. Inhibitors of PI3K can be used as a basis for creating new therapeutic agents for patients with tumors resistant to trastuzumab and having a low level of PTEN.

An increase in the activity of Src kinases and in signal transduction into the nucleus is also observed in some cetuximab-resistant lines [125].

An increased expression of genes encoding ligands of the ERBB receptor, which is a target of the therapeutic antibody, can also enhance the signal transduction and resistance to therapeutic antibodies. Thus, resistance to trastuzumab can arise on hyperexpression in tumor cells of transforming growth factor α (TGFα), which is a natural ligand of ERBB1 [101]. The efficiency of therapy with anti-EGFR and anti-VEGFR antibodies decreases under conditions of hypoxia due to increased expression of genes encoding such pro-angiogenic factors as FGF and PDGFβ, which can VEGF-independently re-stimulate the tumor angiogenesis [126].

The resistance of tumor cells can be also caused by disorders in formation of functional dimers of ERBB receptors. The hyperexpression of *EGFR* and an increase in tyrosine kinase activity in cetuximab-resistant cells have been recently shown to result in HER2/neu and HER3 activation and in triggering signaling cascades via the heterodimer HER2/neu/HER3 [127]. Thus, just HER3 deprived of the kinase activity plays an important role in the development of tumor resistance to antibodies targeted to EGFR or HER2. The anti-HER2/neu antibody pertuzumab (table) preventing the dimerization of the receptor can restore the tumor cell sensitivity to anti-EGFR-antibodies [128].

The resistance to treatment with RTK inhibitors (both low molecular mass inhibitors and specific antibodies) also arises due to genetic instability of tumor cells and appearance in the ERBB receptors of specific mutations responsible for the tyrosine kinase activity of the receptors. The EGFR T790M mutation is detected in 50% of cases with acquired resistance [102]. This mutation leads to a significant increase in the affinity of ATP binding with the receptor and to a complete resistance to the ATP-competitive low molecular mass inhibitors gefitinib and erlotinib. Using the irreversible noncompetitive inhibitor of ERBB1 and ERBB2 lapatinib and its combination with cetuximab allows clinicians to overcome this type of resistance.

Large deletions of the extracellular domain of EGFR and HER2/neu also can be a cause of resistance to therapy. EGER variant III (EGFRvIII) contains a deletion in the reading frame of exons 2-7 (6-273 amino acid residues) of the extracellular domain that results in a ligand-independent constitutive activation of this receptor [102]. This mutation is not characteristic for normal tissues, but is detected in 40% of glioblastomas and also in some other tumors. This deletion is associated with resistance to the therapeutic antibody cetuximab despite the ability of this antibody to bind with its epitope in domain III. The receptor EGFRvIII can be neutralized by the specially created antibody mAb-h806 (mAb806; table), which is specific to this form of EGFR [82].

An even longer deletion is found in the case of the HER2/neu receptor (ERBB2). This p95HER2 isoform is deprived of the extracellular domain, which is responsible for auto-inhibition of RTKs and, therefore, is a constitutively active kinase and a powerful oncogene insensitive to therapeutic antibodies [98]. Earlier the full-size ERBB2 receptor was shown to undergo proteolysis in the tumor cell by metalloproteases (sheddases) [129]. The therapeutic antibody trastuzumab inhibits this process [130]. Later it was shown that the p95HER2 isoform could also appear as a result of an alternative transcription of *HER2* [131]. The relative contributions of these two mechanisms are still unknown.

Resistance to trastuzumab can also appear as a result of an increase in ERBB2 receptor stability upon the inter-

action with the chaperon HSP90 [40]. In a model of trastuzumab-resistant p95-HER2-hyperexpressing tumors, the long-term injection *in vivo* of HSP90 inhibitors, e.g. the antibiotic geldanamycin (Fig. 5), caused a stable decrease in the expression of ERBB2 and its imperfect form p95-HER2 and inhibited the activation of Akt followed by induction of apoptosis [132].

In some cases, resistance to therapeutic antibodies has not a molecular but rather a mechanical nature. The ERBB receptors are not always easily available for therapeutic antibodies targeted to them. Thus, in solid tumors the extracellular matrix makes difficult the diffusion of therapeutic antibodies and masks their receptor targets. On model tumors with hyperexpressed HER2/neu oncomarker, it has been shown that the intra-tumor expression of a peptide hormone relaxin leads to degradation of the extracellular matrix proteins and as a result improves the therapeutic effect of trastuzumab [133].

During the last 10-15 years researchers' optimism for using monoclonal antibodies for therapy of cancer was often changed to pessimism. Extreme variability of tumor cells and their ability to escape every time the influence of a newly created compound (antibodies, kinase inhibitors) can be compared only with the ability of pathogenic microorganisms to develop new protective mechanisms against antibiotics. At present, monoclonal antitumor antibodies are not agents capable of curing cancer, and the patient's life can be prolonged by them for some months but not for years. Nevertheless, it can be concluded that researchers have overcome a difficult initial stage of creating mAbs and their clinical use, and are again at the beginning of a long task of creating a new generation of antitumor compounds based on monoclonal antibodies (including those specific to ERBB receptors). In fact, many years of a persistent work of many research groups has resulted in detailed knowledge of the signaling network activated by ERBB receptors; molecular mechanisms of tumor transformation are detected, molecular mechanisms of mAbs action on tumor cells are established, causes of tumor resistance to therapy, including those associated with genotypic features of individual patients, are discovered. And it becomes clear that the determination of an individual molecular profile of each patient's disease [134, 135] and an integral approach to treatment of the tumor [52] are strategic trends in therapy of cancer. And in both cases monoclonal antibodies specific to tumor markers are as before the basis for compounds created for diagnostics, targeted therapy, and monitoring of treatment [76, 100, 110, 136-139], as well as an important component of the therapeutic regulation on combined treatment of various tumors [140-144]. Obviously, to develop these approaches further basic studies of molecular mechanisms of carcinogenesis, searches for new diagnostic markers and therapeutic targets, creation of drugs, and optimization of their delivery are required.

Great progress has been seen during the last decade in the engineering of antibodies with desired properties [6, 91]. Techniques allowing researchers to manage functions of the constant domain of the antibodies are a new developing trend [145]. A wide-scale screening of antitumor antibodies is also performed to reveal new predictably effective targets for therapeutic antibodies purposed for treatment of solid tumors [146]. The development of nanotechniques, in particular, the creation of various nanoparticles has resulted in formulation of a new approach in medicine – theranostics [147, 148], i.e. uniting in a single nanoconstruction of functions required for diagnostics of disease, therapy, and monitoring of treatment. And in this case antibodies are one of main components providing for accurate targeting of the multifunctional construction to pathogenic tissues [136-139].

Basic studies on resistance to treatment with anti-ERBB antibodies are now on a new upward flight [98, 113, 140]. Elucidation of fine molecular mechanisms and identification of general mediators of resistance to anti-ERBB antibodies is promising for more efficient use in all aspects of various influences of these multifunctional molecules on the tumor cell and the patient's organism [149, 150]. The therapeutic potential of monoclonal antibodies to ERBB oncogene proteins is far from exhaustion and, no doubt, will play an important role in the person-oriented medicine of the twenty-first century.

This work was supported by the Russian Foundation for Basic Research (projects Nos. 12-04-00757-a, 09-04-01298-a, 11-04-12091-ofi-m-2011) and by the Russian Federation Ministry of Education and Science for 2009-2013 (projects Nos. 16.740.11.0497, 16.512.11.2053, 16.523.12.3009, 11.G34.31.0017).

REFERENCES

1. Lemmon, M. A., and Schlessinger, J. (2010) *Cell*, **141**, 1117-1134.
2. Amit, I., Wides, R., and Yarden, Y. (2007) *Mol. Syst. Biol.*, **3**, 151.
3. Severin, E. S., and Savateeva, M. V. (2011) *Acta Naturae*, **3**, 20-29.
4. Citri, A., and Yarden, Y. (2006) *Nat. Rev. Mol. Cell Biol.*, **7**, 505-516.
5. Deyev, S. M., and Lebedenko, E. N. (2008) *BioEssays*, **30**, 904-918.
6. Deyev, S. M., and Lebedenko, E. N. (2009) *Acta Naturae*, **1**, 32-50.
7. Reichert, J. M., and Valge-Archer, V. E. (2007) *Nat. Rev. Drug Discov.*, **6**, 349-356.
8. Wilson, K. J., Gilmore, J. L., Foley, J., Lemmon, M. A., and Riese, D. J., 2nd (2009) *Pharmacol. Ther.*, **122**, 1-8.
9. Carpenter, G., and Cohen, S. (1990) *J. Biol. Chem.*, **265**, 7709-7712.
10. Lemmon, M. A. (2009) *Exp. Cell Res.*, **315**, 638-648.
11. Bae, J. H., and Schlessinger, J. (2010) *Mol. Cells*, **29**, 443-448.

12. Jura, N., Endres, N. F., Engel, K., Deindl, S., Das, R., Lamers, M. H., Wemmer, D. E., Zhang, X., and Kuriyan, J. (2009) *Cell*, **137**, 1293-1307.
13. Citri, A., Gan, J., Mosseson, J., Vereb, G., Szollosi, J., and Yarden, Y. (2004) *EMBO Rep.*, **5**, 39-43.
14. Pawson, T. (2004) *Cell*, **116**, 191-203.
15. Schulze, W. X., Deng, L., and Mann, M. (2005) *Mol. Syst. Biol.*, **1**, 2005-2008.
16. Kaushansky, A., Gordus, A., Budnik, B. A., Lane, W. S., Rush, J., and MacBeath, G. (2008) *Chem. Biol.*, **15**, 808-817.
17. Miettinen, P. J., Berger, J. E., Meneses, J., Phung, Y., Pedersen, R. A., Werb, Z., and Derynck, R. (1995) *Nature*, **376**, 337-341.
18. Engelman, J. A., Janne, P. A., Mermel, C., Pearlberg, J., Mukohara, T., Fleet, C., Cichowski, K., Johnson, B. E., and Cantley, L. C. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 3788-3793.
19. Berger, M. B., Mendrola, J. M., and Lemmon, M. A. (2004) *FEBS Lett.*, **569**, 332-336.
20. Jones, R. B., Gordus, A., Krall, J. A., and MacBeath, G. (2006) *Nature*, **439**, 168-174.
21. Austin, C. D., De Maziere, A. M., Pisacane, P. I., van Dijk, S. M., Eigenbrot, C., Sliwkowski, M. X., Klumperman, J., and Scheller, R. H. (2004) *Mol. Biol. Cell*, **15**, 5268-5282.
22. Wallasch, C., Weiss, F. U., Niederfellner, G., Jallal, B., Issing, W., and Ullrich, A. (1996) *EMBO J.*, **14**, 4267-4275.
23. Kane, L. P., and Weiss, A. (2003) *Immunol. Rev.*, **192**, 7-20.
24. Krasilnikov, M. A. (2000) *Biochemistry (Moscow)*, **65**, 59-67.
25. Cantley, L. C. (2002) *Science*, **296**, 1655-1657.
26. Los, M., Maddika, S., Erb, B., and Schulze-Osthoff, K. (2009) *BioEssays*, **31**, 492-495.
27. Wang, Y., Kristensen, G. B., Helland, A., Nesland, J. M., Borresen-Dale, A. L., and Holm, R. (2005) *Am. J. Clin. Pathol.*, **124**, 392-401.
28. Subramaniam, S., and Unsicker, K. (2006) *Neuroscience*, **138**, 1055-1065.
29. Heasley, L. E., and Han, S. Y. (2006) *Mol. Cells*, **21**, 167-173.
30. Bustelo, X. R. (2000) *Mol. Cell. Biol.*, **20**, 1461-1477.
31. Das, M., Jiang, F., Sluss, H. K., Zhang, C., Shokat, K. M., Flavell, R. A., and Davis, R. J. (2007) *Proc. Natl. Acad. Sci. USA*, **104**, 15759-15764.
32. Ni, C. Y., Murphy, M. P., Golde, T. E., and Carpenter, G. (2001) *Science*, **294**, 2179-2181.
33. Orth, J. D., Krueger, E. W., Weller, S. G., and McNiven, M. A. (2006) *Cancer Res.*, **66**, 3603-3610.
34. Hsu, S. C., and Hung, M. C. (2007) *J. Biol. Chem.*, **282**, 10432-10440.
35. Abella, J. V., and Park, M. (2009) *Am. J. Physiol. Endocrinol. Metab.*, **296**, e973-e984.
36. Haglund, K., Sigismund, S., Polo, S., Szymkiewicz, I., Di Fiore, P. P., and Dikic, I. (2003) *Nat. Cell Biol.*, **5**, 461-466.
37. Huang, F., Kirkpatrick, D., Jiang, X., Gygi, S., and Sorkin, A. (2006) *Mol. Cell*, **21**, 737-748.
38. Wiley, H. S. (2003) *Exp. Cell Res.*, **284**, 78-88.
39. Sorkin, A., and Goh, L. K. (2008) *Exp. Cell. Res.*, **314**, 3093-3106.
40. Citri, A., Kochupurakkal, B. S., and Yarden, Y. (2003) *Cell. Cycle*, **3**, 51-60.
41. Xu, W., Yuan, X., Beebe, K., Xiang, Z., and Neckers, L. (2007) *Mol. Cell. Biol.*, **27**, 220-228.
42. Peng, X., Guo, X., Borkan, S. C., Bharti, A., Kuramochi, Y., Calderwood, S., and Sawyer, D. B. (2005) *J. Biol. Chem.*, **280**, 13148-13152.
43. Zuo, T., Wang, L., Morrison, C., Chang, X., Zhang, H., Li, W., Liu, Y., Wang, Y., Liu, X., Chan, M. W., Liu, J. Q., Love, R., Liu, C. G., Godfrey, V., Shen, R., Huang, T. H., Yang, T., Park, B. K., Wang, C. Y., Zheng, P., and Liu, Y. (2007) *Cell*, **129**, 1275-1286.
44. Hsu, M. C., Chang, H. C., and Hung, W. C. (2006) *J. Biol. Chem.*, **281**, 4718-4725.
45. Das, P. M., Thor, A. D., Edgerton, S. M., Barry, S. K., Chen, D. F., and Jones, F. E. (2010) *Oncogene*, **29**, 5214-5219.
46. Ellsworth, R. E., Ellsworth, D. L., Patney, H. L., Deyarmin, B., Love, B., Hooke, J. A., and Shriver, C. D. (2008) *BMC Cancer*, **8**, 297.
47. Slamon, D. J., Godolphin, W., Jones, L. A., Holt, J. A., Wong, S. G., Keith, D. E., Levin, W. J., Stuart, S. G., Udove, J., Ullrich, A., and Press, M. F. (1989) *Science*, **244**, 707-712.
48. Perou, C. M., Sorlie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Rees, C. A., Pollack, J. R., Ross, D. T., Johnsen, H., Akslen, L. A., Fluge, O., Pergamenschikov, A., Williams, C., Zhu, S. X., Lonning, P. E., Borresen-Dale, A. L., Brown, P. O., and Botstein, D. (2000) *Nature*, **406**, 747-752.
49. Harbeck, N., Pegram, M. D., Ruschoff, J., and Mobus, V. (2010) *Breast Care* (Basel), **5**, 3-7.
50. Reis-Filho, J. S., Pinheiro, C., Lambros, M. B., Milanezi, F., Carvalho, S., Savage, K., Simpson, P. T., Jones, C., Swift, S., Mackay, A., Reis, R. M., Hornick, J. L., Pereira, E. M., Baltazar, F., Fletcher, C. D., Ashworth, A., Lakhani, S. R., and Schmitt, F. C. (2006) *J. Pathol.*, **209**, 445-453.
51. Knosel, T., Schluns, K., Stein, U., Schwabe, H., Schlag, P. M., Dietel, M., and Petersen, I. (2004) *Neoplasia*, **6**, 23-28.
52. Zhang, Z., Stiegler, A. L., Boggon, T. J., Kobayashi, S., and Halmos, B. (2010) *Oncotarget*, **1**, 497-514.
53. Dawson, S. J., Provenzano, E., and Caldas, C. (2009) *Eur. J. Cancer*, **45**, 27-40.
54. Moelans, C. B., de Weger, R. A., Monsuur, H. N., Vijzelaar, R., and van Diest, P. J. (2010) *Mod. Pathol.*, **23**, 1029-1039.
55. Sassen, A., Rochon, J., Wild, P., Hartmann, A., Hofstaedter, F., Schwarz, S., and Brockhoff, G. (2008) *Breast Cancer Res.*, **10**, R2.
56. Sithanandam, G., and Anderson, L. M. (2008) *Cancer Gene Therap.*, **15**, 413-448.
57. Pedersen, M. W., Meltorn, M., Damstrup, L., and Poulsen, H. S. (2001) *Ann. Oncol.*, **12**, 745-760.
58. Sharma, S. V., Bell, D. W., Settleman, J., and Haber, D. A. (2007) *Nat. Rev. Cancer*, **8**, 169-181.
59. Wang, S. E., Narasanna, A., Perez-Torres, M., Xiang, B., Wu, F. Y., Yang, S., Carpenter, G., Gazdar, A. F., Muthuswamy, S. K., and Arteaga, C. L. (2006) *Cancer Cell*, **10**, 25-38.
60. Lee, J. W., Soung, Y. H., Seo, S. H., Kim, S. Y., Park, C. H., Wang, Y. P., Park, K., Nam, S. W., Park, W. S., Kim, S. H., Lee, J. Y., Yoo, N. J., and Lee, S. H. (2006) *Clin. Cancer Res.*, **12**, 57-61.
61. Stephens, P., Hunter, C., Bignell, G., Edkins, S., Davies, H., Teague, J., Stevens, C., O'Meara, S., Smith, R., Parker, A., Barthorpe, A., Blow, M., Brackenbury, L., Butler, A.,

Clarke, O., Cole, J., Dicks, E., Dike, A., Drozd, A., Edwards, K., Forbes, S., Foster, R., Gray, K., Greenman, C., Halliday, K., Hills, K., Kosmidou, V., Lugg, R., Menzies, A., Perry, J., Petty, R., Raine, K., Ratford, L., Shepherd, R., Small, A., Stephens, Y., Tofts, C., Varian, J., West, S., Widaa, S., Yates, A., Brasseur, F., Cooper, C. S., Flanagan, A. M., Knowles, M., Leung, S. Y., Louis, D. N., Looijenga, L. H., Malkowicz, B., Pierotti, M. A., Teh, B., Chenevix-Trench, G., Weber, B. L., Yuen, S. T., Harris, G., Goldstraw, P., Nicholson, A. G., Futreal, P. A., Wooster, R., and Stratton, M. R. (2004) *Nature*, **431**, 525-526.

62. Ozer, B. H., Wiepz, G. J., and Bertics, P. J. (2010) *Oncogene*, **29**, 855-864.
63. Wang, S. E., Yu, Y., Criswell, T. L., Debusk, L. M., Lin, P. C., Zent, R., Johnson, D. H., Ren, X., and Arteaga, C. L. (2010) *Oncogene*, **29**, 3335-3348.
64. Riese, D. J., 2nd, Gallo, R. M., and Settleman, J. (2007) *BioEssays*, **29**, 558-565.
65. Kolch, W. (2002) *Expert Opin. Pharmacother.*, **3**, 709-718.
66. Zhang, J., Yang, P. L., and Gray, N. S. (2009) *Nat. Rev. Cancer*, **9**, 28-39.
67. Duckett, D. R., and Cameron, M. D. (2010) *Expert Opin. Drug Metab. Toxicol.*, **6**, 1175-1193.
68. Lindzen, M., Lavi, S., Leitner, O., and Yarden, Y. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 12559-12563.
69. Martinelli, E., de Palma, R., Orditura, M., de Vita, F., and Ciardiello, F. (2009) *Clin. Exp. Immunol.*, **158**, 1-9.
70. Imyanitov, E. N., and Moiseyenko, V. M. (2011) *Hered. Cancer Clin. Pract.*, **9**, 5-21.
71. Bivona, T. G., Hieronymus, H., Parker, J., Chang, K., Taron, M., Rosell, R., Moonsamy, P., Dahlman, K., Miller, V. A., Costa, C., Hannon, G., and Sawyers, C. L. (2011) *Nature*, **471**, 523-526.
72. Dempsey, P. J., Garton, K., and Raines, E. W. (2002) *Mol. Interv.*, **2**, 136-141.
73. Zeineldin, R., Muller, C. Y., Stack, M. S., and Hudson, L. G. (2010) *J. Oncol.*, **2010**, 414676.
74. Gossage, L., and Eisen, T. (2010) *Clin. Cancer Res.*, **16**, 1973-1978.
75. Yun, C. H., Mengwasser, K. E., Toms, A. V., Woo, M. S., Greulich, H., Wong, K. K., Meyerson, M., and Eck, M. J. (2008) *Proc. Natl. Acad. Sci. USA*, **12**, 2070-2075.
76. Gaborit, N., Larbouret, C., Vallaghe, J., Peyrusson, F., Bascoul-Mollevi, C., Crapez, E., Azria, D., Chardes, T., Poul, M. A., Mathis, G., Bazin, H., and Pelegrin, A. (2011) *J. Biol. Chem.*, **286**, 11337-11345.
77. Reichert, J. M. (2011) *MAbs*, **3**, 76-99.
78. Li, S., Schmitz, K. R., Jeffrey, P. D., Wiltzius, J. J., Kussie, P., and Ferguson, K. M. (2005) *Cancer Cell*, **7**, 301-311.
79. Socinski, M. A. (2007) *Clin. Cancer Res.*, **13**, 4597-4601.
80. Ramakrishnan, M. S., Eswaraiah, A., Crombet, T., Piedra, P., Saurez, G., Iyer, H., and Arvind, A. S. (2009) *MAbs*, **1**, 41-48.
81. Schmiedel, J., Blaukat, A., Li, S., Knochel, T., and Ferguson, K. M. (2008) *Cancer Cell*, **13**, 365-373.
82. Johns, T. G., Adams, T. E., Cochran, J. R., Hall, N. E., Hoyne, P. A., Olsen, M. J., Kim, Y. S., Kim, Y. S., Rothacker, J., Nice, E. C., Walker, F., Ritter, G., Jungbluth, A. A., Old, L. J., Ward, C. W., Burgess, A. W., Wittrup, K. D., and Scott, A. M. (2004) *J. Biol. Chem.*, **279**, 30375-30384.
83. Argyriou, A. A., and Kalofonos, H. P. (2009) *Mol. Med.*, **15**, 183-191.
84. Carter, P., Presta, L., Gorman, C. M., Ridgway, J. B., Henner, D., Wong, W. L., Rowland, A. M., Kotts, C., Carver, M. E., and Shepard, H. M. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 4285-4289.
85. Franklin, M. C., Carey, K. D., Vajdos, F. F., Leahy, D. J., de Vos, A. M., and Sliwkowski, M. X. (2004) *Cancer Cell*, **5**, 317-328.
86. Kiewe, P., Hasmuller, S., Kahlert, S., Heinrigs, M., Rack, B., Marme, A., Korfel, A., Jager, M., Lindhofer, H., Sommer, H., Thiel, E., and Untch, M. (2006) *Clin. Cancer Res.*, **12**, 3085-3091.
87. Sierra, J. R., Cepero, V., and Giordano, S. (2010) *Mol. Cancer*, **9**, 75.
88. Li, R., Pourpak, A., and Morris, S. W. (2009) *J. Med. Chem.*, **27**, 4981-5004.
89. Deyev, S. M., Waibel, R., Lebedenko, E. N., Schubiger, A. P., and Pluckthun, A. (2003) *Nature Biotechnol.*, **21**, 1486-1492.
90. Edelweiss, E., Balandin, T. G., Ivanova, J. L., Lutsenko, G. V., Leonova, O. G., Popenko, V. I., Sapozhnikov, A. M., and Deyev, S. M. (2008) *PLoS One*, **3**, e2434.
91. Deyev, S. M., and Lebedenko, E. N. (2009) *Bioorg. Khim.*, **35**, 761-778.
92. Lebedenko, E. N., Balandin, T. G., Edelweiss, E. F., Georgiev, O., Moiseeva, E. S., Petrov, R. V., and Deyev, S. M. (2007) *Dokl. Akad. Nauk*, **414**, 408-411.
93. Semenyuk, E. G., Stremovskiy, O. A., Edelweiss, E. F., Shirshikova, O. V., Balandin, T. G., Buryanov, Y. I., and Deyev, S. M. (2007) *Biochimie*, **89**, 31-38.
94. Hecht, J. R., Mitchell, E., Chidiac, T., Scroggin, C., Hagenstad, C., Spigel, D., Marshall, J., Cohn, A., McCollum, D., Stella, P., Deeter, R., Shahin, S., and Amado, R. G. (2008) *J. Clin. Oncol.*, **27**, 672-680.
95. Schittenhelm, M. M., Kollmannsberger, C., Oechsle, K., Harlow, A., Morich, J., Honecker, F., Kurek, R., Storkel, S., Kanz, L., Corless, C. L., Wong, K. K., Bokemeyer, C., and Heinrich, M. C. (2009) *Mol. Cancer Ther.*, **8**, 481-489.
96. Montemurro, F., Valabrega, G., and Aglietta, M. (2004) *Expert Opin. Pharmacother.*, **5**, 81-96.
97. Nahta, R., and Esteva, F. J. (2006) *Cancer Lett.*, **232**, 123-138.
98. Wilken, J. A., and Maihle, N. J. (2010) *Ann. N. Y. Acad. Sci.*, **1210**, 53-65.
99. Yazynin, S., Lange, H., Mokros, T., Deyev, S., and Lemke, H. (1999) *FEBS Lett.*, **452**, 351-354.
100. Serebrovskaya, E. O., Edelweiss, E. F., Stremovskiy, O. A., Lukyanov, K. A., Chudakov, D. M., and Deyev, S. M. (2009) *Proc. Natl. Acad. Sci. USA*, **106**, 9221-9225.
101. Valabrega, G., Montemurro, F., and Aglietta, M. (2007) *Ann. Oncol.*, **18**, 977-984.
102. Hopper-Borge, E. A., Nasto, R. E., Ratushny, V., Weiner, L. M., Golemis, E. A., and Astsaturov, I. (2009) *Expert Opin. Ther. Targets*, **13**, 339-362.
103. Jager, M., Schoberth, A., Ruf, P., Hess, J., and Lindhofer, H. (2009) *Cancer Res.*, **69**, 4270-4276.
104. Dechant, M., Weisner, W., Berger, S., Peipp, M., Beyer, T., Schneider-Merck, T., Lammerts van Bueren, J. J., Bleeker, W. K., Parren, P. W., van de Winkel, J. G., and Valerius, T. (2008) *Cancer Res.*, **68**, 4998-5003.
105. Cho, H. S., Mason, K., Ramyar, K. X., Stanley, A. M., Gabelli, S. B., Denney, D. W., Jr., and Leahy, D. J. (2003) *Nature*, **421**, 756-760.

106. Wehrman, T. S., Raab, W. J., Casipit, C. L., Doyonnas, R., Pomerantz, J. H., and Blau, H. M. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 19063-19068.
107. Nagata, Y., Lan, K. H., and Zhou, X. (2004) *Cancer Cell*, **6**, 117-127.
108. Izumi, Y., Xu, L., Di Tomaso, E., Fukumura, D., and Jain, R. K. (2002) *Nature*, **416**, 279-280.
109. Friedman, L. M., Rinon, A., Schechter, B., Lyass, L., Lavi, S., Bacus, S. S., Sela, M., and Yarden, Y. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 1915-1920.
110. Pedersen, M. W., Jacobsen, H. J., Koefoed, K., Hey, A., Pyke, C., Haurum, J. S., and Kragh, M. (2010) *Cancer Res.*, **70**, 588-597.
111. Emde, A., Pradeep, C. R., Ferraro, D. A., Ben-Chetrit, N., Sela, M., Ribba, B., Kam, Z., and Yarden, Y. (2011) *Oncogene*, **30**, 1631-1642.
112. Wilken, J. A., Webster, K. T., and Maihle, N. J. (2010) *J. Ovarian Res.*, **3**, 7.
113. Kruser, T. J., and Wheeler, D. L. (2010) *Exp. Cell Res.*, **316**, 1083-1100.
114. De Reynies, A., Boige, V., Milano, G., Faivre, J., and Laurent-Puig, P. (2008) *J. Clin. Oncol.*, **26**, 2228-2230.
115. Lu, D., Zhang, H., Koo, H., Tonra, J., Balderes, P., Prewett, M., Corcoran, E., Mangalampalli, V., Bassi, R., Anselma, D., Patel, D., Kang, X., Ludwig, D. L., Hicklin, D. J., Bohlen, P., Witte, L., and Zhu, Z. (2005) *J. Biol. Chem.*, **280**, 19665-19672.
116. Engelman, J. A., Zejnullahu, K., Mitsudomi, T., Song, Y., Hyland, C., Park, J. O., Lindeman, N., Gale, C. M., Zhao, X., Christensen, J., Kosaka, T., Holmes, A. J., Rogers, A. M., Cappuzzo, F., Mok, T., Lee, C., Johnson, B. E., Cantley, L. C., and Janne, P. A. (2007) *Science*, **316**, 1039-1043.
117. Wheeler, D. L., Dunn, E. F., and Harari, P. M. (2010) *Nat. Rev. Clin. Oncol.*, **7**, 493-507.
118. Bianco, R., Rosa, R., Damiano, V., Daniele, G., Gelardi, T., Garofalo, S., Tarallo, V., de Falco, S., Melisi, D., Benelli, R., Albini, A., Ryan, A., Ciardiello, F., and Tortora, G. (2008) *Clin. Cancer Res.*, **14**, 5069-5080.
119. Viloria-Petit, A., Crombet, T., Jothy, S., Hicklin, D., Bohlen, P., Schlaeppi, J. M., Rak, J., and Kerbel, R. S. (2001) *Cancer Res.*, **61**, 5090-5101.
120. Pegram, M., Yeon, C., and Ku, N. C. (2004) *Breast Cancer Res. Treat.*, **88** (Suppl. 1), S124.
121. Prichard, C. N., Kim, S., Yazici, Y. D., Doan, D. D., Jasser, S. A., Mandal, M., and Myers, J. N. (2007) *Laryngoscope*, **117**, 674-679.
122. Tol, J., and Punt, C. J. (2010) *Clin. Ther.*, **32**, 437-453.
123. Allegra, C. J., Jessup, J. M., Somerfield, M. R., Hamilton, S. R., Hammond, E. H., Hayes, D. F., McAllister, P. K., Morton, R. F., and Schilsky, R. L. (2009) *J. Clin. Oncol.*, **27**, 2091-2096.
124. Vaughn, C. P., Zobell, S. D., Furtado, L. V., Baker, C. L., and Samowitz, W. S. (2011) *Genes Chromosomes Cancer*, **50**, 307-312.
125. Wheeler, D. L., Iida, M., Kruser, T. J., Nechrebecki, M. M., Dunn, E. F., Armstrong, E. A., Huang, S., and Harari, P. M. (2009) *Cancer Biol. Ther.*, **8**, 696-703.
126. Dempke, W. C., and Heinemann, V. (2009) *Eur. J. Cancer*, **45**, 1117-1128.
127. Sergina, N. V., Rausch, M., Wang, D., Blair, J., Hann, B., Shokat, K. M., and Moasser, M. M. (2007) *Nature*, **445**, 437-441.
128. Wheeler, D. L., Huang, S., Kruser, T. J., Nechrebecki, M. M., Armstrong, E. A., Benavente, S., Gondi, V., Hsu, K. T., and Harari, P. M. (2008) *Oncogene*, **27**, 3944-3956.
129. Pupa, S. M., Menard, S., Morelli, D., Pozzi, B., de Palo, G., and Colnaghi, M. I. (1993) *Oncogene*, **8**, 2917-2923.
130. Molina, M. A., Codony-Servat, J., Albanell, J., Rojo, F., Arribas, J., and Baselga, J. (2001) *Cancer Res.*, **61**, 4744-4749.
131. Anido, J., Scaltriti, M., Bech-Serra, J. J., Santiago-Josefat, B., Todo, F. R., Baselga, J., and Arribas, J. (2006) *EMBO J.*, **25**, 3234-3244.
132. Chandarlapaty, S., Scaltriti, M., Angelini, P., Ye, Q., Guzman, M., Hudis, C. A., Norton, L., Solit, D. B., Arribas, J., Baselga. J., and Rosen, N. (2010) *Oncogene*, **29**, 325-334.
133. Beyer, I., Li, Z., Persson, J., Liu, Y., van Rensburg, R., Yumul, R., Zhang, X. B., Hung, M. C., and Lieber, A. (2011) *Mol. Ther.*, **19**, 479-489.
134. Siena, S., Sartore-Bianchi, A., di Nicolantonio, F., Balfour, J., and Bardelli, A. (2009) *J. Natl. Cancer Inst.*, **101**, 1308-1324.
135. Slavina, E. G., Chertkova, A. I., Zabotina, T. N., Gan'shina, I. P., and Lichinitser, M. R. (2006) *Byul. Eksp. Biol. Med.*, **141**, 361-363.
136. Nikitin, M. P., Zdobnova, T. A., Lukash, S. V., Stremovskiy, O. A., and Deyev, S. M. (2010) *Proc. Natl. Acad. Sci. USA*, **107**, 5827-5832.
137. Generalova, A. N., Sizova, S. V., Zdobnova, T. A., Zarifullina, M. M., Artemyev, M. V., Baranov, A. V., Oleinikov, V. A., Zubov, V. P., and Deyev, S. M. (2011) *Nanomedicine* (Lond.), **6**, 195-209.
138. Lapotko, D. (2011) *Cancers* (Basel), **3**, 802-840.
139. Zdobnova, T. A., Lebedenko, E. N., and Deyev, S. M. (2010) *Acta Naturae*, **3**, 30-50.
140. Hecht, E. M., Mitchell, E., Chidiac, T., Scroggin, C., Hagenstad, C., Spigel, D., Marshall, J., Cohn, A., McCollum, D., Stella, P., Deeter, R., Shahin, S., and Amado, R. G. (2009) *J. Clin. Oncol.*, **27**, 672-680.
141. Cutsem, E. V. (2008) *J. Clin. Oncol.*, **26** (Suppl.), Abstract 2.
142. Zhukov, N. V., and Tyulyandin, S. A. (2008) *Biochemistry (Moscow)*, **73**, 605-618.
143. Besova, N. S. (2010) *Sovrem. Onkol.*, **12**, 43-51.
144. Yin, W., Jiang, Y., Shen, Z., Shao, Z., and Lu, J. (2011) *PLoS One*, **6**, e21030.
145. Zhang, N., Liu, L., Dumitru, C. D., Cummings, N. R., Cukan, M., Jiang, Y., Li, Y., Li, F., Mitchell, T., Mallem, M. R., Ou, Y., Patel, R. N., Vo, K., Wang, H., Burnina, I., Choi, B. K., Huber, H. E., Stadheim, T. A., and Zha, D. (2011) *MAbs*, **3**, 289-298.
146. Kurosawa, G., Sumitomo, M., Ukai, Y., Subere, J., Muramatsu, C., Eguchi, K., Tanaka-Hashiba, M., Sugiura, M., Ando, M., Sato, N., Morita, M., Inaba, K., Morigaki, S., Takasaki, A., Akahori, Y., Miyakawa, S., Uyama, I., Maeda, K., Shiroki, R., Hoshinaga, K., Mizoguchi, Y., Hattori, Y., Sugioka, A., Sugiura, M., and Kurosawa, Y. (2011) *Cancer Sci.*, **102**, 175-181.
147. Hartman, K. B., Wilson, L. J., and Rosenblum, M. G. (2008) *Mol. Diagn. Ther.*, **12**, 1-14.
148. McCarthy, J. R. (2009) *Nanomedicine*, **4**, 693-695.
149. Brand, T. M., Iida, M., and Wheeler, D. L. (2011) *Cancer Biol. Ther.*, **11**, 777-792.
150. Abelev, G. I., and Eraizer, T. L. (2008) *Biochemistry (Moscow)*, **73**, 487-498.