Targeted Inhibitors of Epidermal Growth Factor Receptor

Subjects: Oncology

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Members of the epidermal growth factor receptor (EGFR) family of tyrosine kinase receptors are major regulators of cellular proliferation, differentiation, and survival. In humans, abnormal activation of EGFR is associated with the development and progression of many cancer types, which makes it an attractive target for molecular-guided therapy. Two classes of EGFR-targeted cancer therapeutics include monoclonal antibodies (mAbs), which bind to the extracellular domain of EGFR, and tyrosine kinase inhibitors (TKIs), which mostly target the intracellular part of EGFR and inhibit its activity in molecular signaling. While EGFR-specific mAbs and three generations of TKIs have demonstrated clinical efficacy in various settings, molecular evolution of tumors leads to apparent and sometimes inevitable resistance to current therapeutics, which highlights the need for deeper research in this field.

epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) monoclonal antibodies (mAbs)

1. EGF Receptor Protein Family

In humans, the epidermal growth factor (EGF) receptor family (ERBB/HER) consists of four structurally related receptor tyrosine kinases (RTKs) that regulate proliferative cell signaling and play pivotal roles in both normal physiology and proliferative diseases like cancer [1]. The four family members are EGFR/ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4 proteins [2], which are encoded, respectively, by genes EGFR, ERBB2, ERBB3, and ERBB4 [3]. These genes are located on four different chromosomes, but their products share common structural organization, including an extracellular domain, lipophilic transmembrane region, intracellular domain with tyrosine kinase activity, and a carboxy-terminal region [4].

The ERBB/HER family members are expressed in epithelial, mesenchymal, and neuronal cells and in their cellular progenitors [5]. The family members play central roles in cell proliferation, survival, differentiation, adhesion, and migration. These molecules interconnect the inner and outer compartments of the cytoplasmic membrane and trigger the cellular responses to various external stimuli by transmitting the intracellular regulatory stimuli [6]. The activated ERBB/HER receptors form regulatory complexes in which components can enter the cytoplasm and promote downstream molecular pathways (**Figure 1**), including well-known oncogenic pathways of RAS-RAF-MEK-ERK and AKT-PI3K-mTOR signaling axes [7]. Furthermore, apart from dimerization, EGFR molecules can also form oligomers on the cell surface, both under the action of natural ligands or in their absence [8][9]. The phenomenon of EGFR oligomerization is thought to be important for intracellular signaling because it results in a

tight organization of kinase-active molecules in a manner that is optimal for autophosphorylation in trans between adjacent dimers [10].

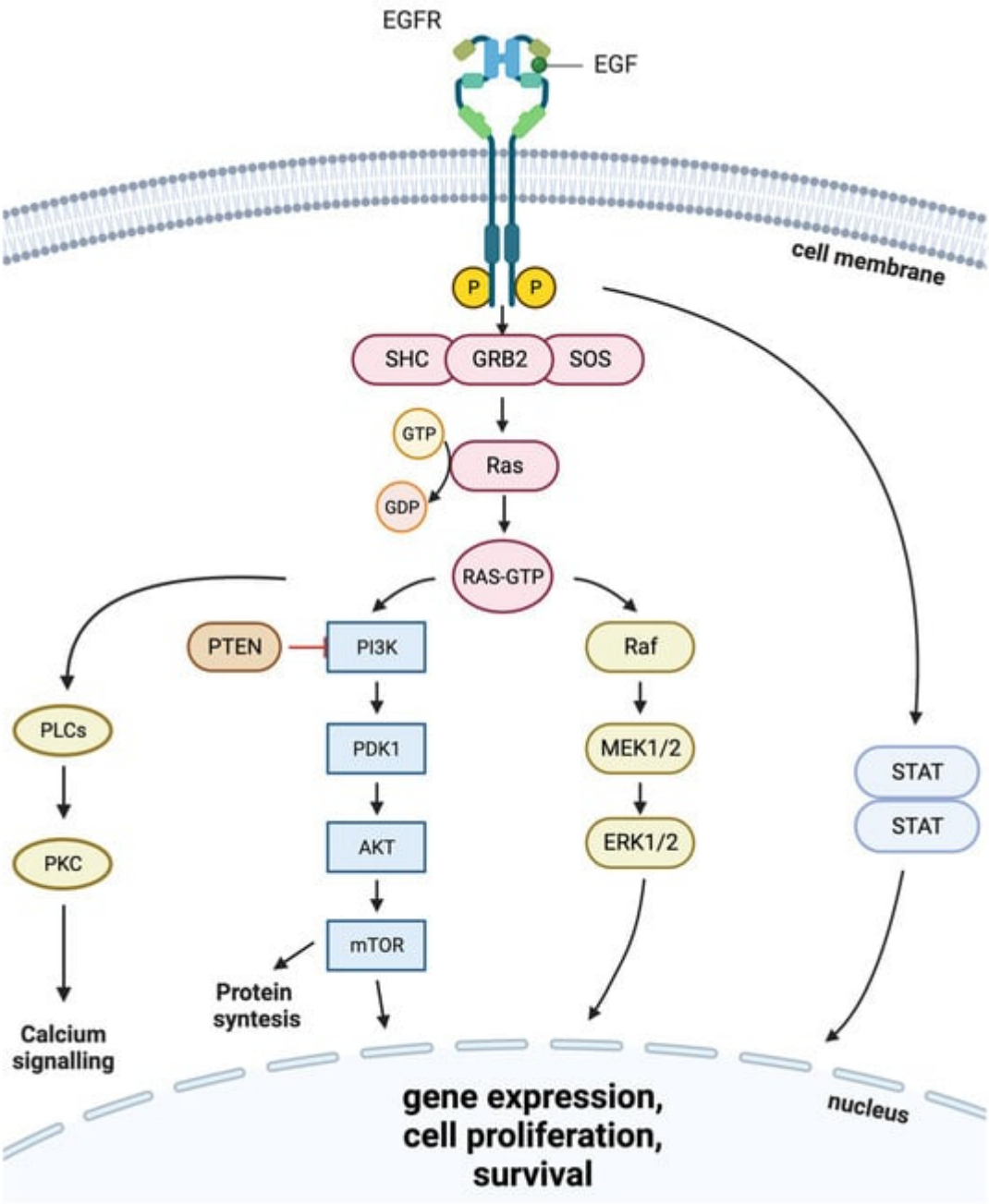

Figure 1. Intracellular signaling involving EGFR. The major regulatory pathways downstream of EGFR and other HER receptors are shown. Binding of specific ligands (e.g., EGF) leads to homo- or heterodimerization of receptors, thus resulting in conformational changes in the intracellular kinase domain, which results in phosphorylation and activation of the receptor. The signaling axes RAS-RAF-MEK-ERK and PI3K-AKT-mTOR, in turn, activate various downstream signaling pathways, thus leading to enhanced cell proliferation and survival. Created with BioRender.com (accessed on 1 November 2023).

Several growth factors are known to be able to bind ERBB/HER receptors and activate them. These are the members of the epidermal growth factor (EGF) family, which are generally classified into three groups. Representatives of the first one bind only to EGFR, which includes EGF [11], transforming growth factor alpha (TGF-α) [12], epigen (EPG) [13], and amphiregulin (AR) [14]. The second group has dual specificity of receptor binding and includes betacellulin (BTC) [15], heparin-binding epidermal growth factor (HB-EGF) [16], and epiregulin (EPR) [17]. The third group consists of neuregulins (NRG) and forms two subgroups depending on their ability to bind both HER3 and HER4 (NRG1 and NRG2 [18]) or only HER4 (NRG3 and NRG4 [19][20]) (**Figure 2**a).

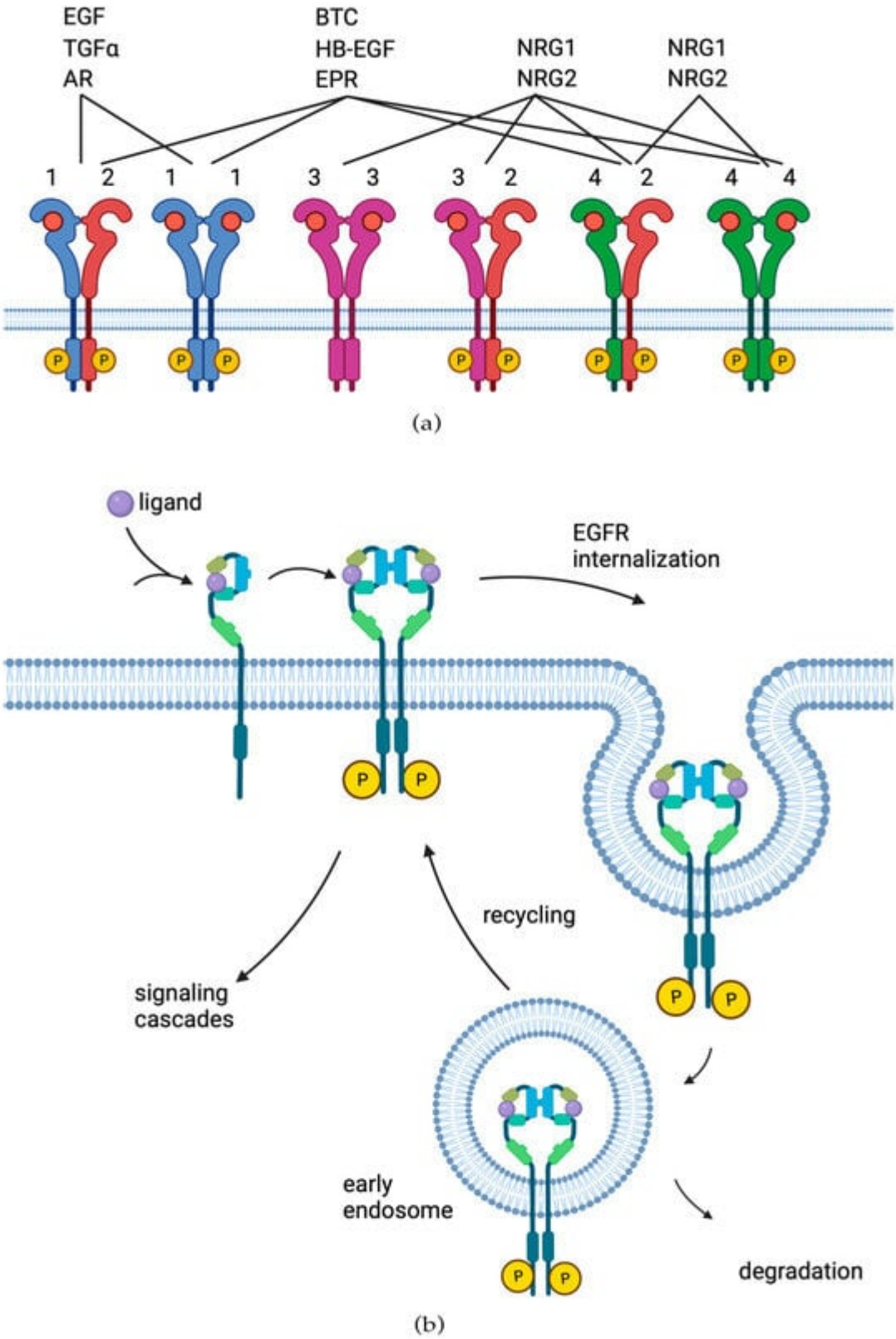

Figure 2. (**a**) Ligands that bind to common types of homo- and heterodimers formed by HER receptors. The following designations were used: 1—EGFR, 2—HER2, 3—HER3, and 4—HER4. (**b**) Dimerization, activation, and internalization of the EGFR receptor. Created with BioRender.com (accessed on 18 October 2023).

The inactivated forms of EGFR, HER3, and HER4 receptors exist in a pre-dimerized state. In turn, binding of the specific ligand causes rearrangement of the respective subunit of the receptor by turning the transmembrane domains. Activation leads to internalization of the receptor and trafficking to the early endosomal compartment of the cell. Next, endocytosis sorting occurs, whereby the receptor is either transported to the lysosome for further degradation or recycled to occupy a place in the cell membrane [21]. The family ligands affect receptor internalization in a different manner: upon EGF binding, the majority, but not all EGFRs, are continuously ubiquitinated and transported to lysosomes. HB-EGF and BTC also behave the same way. On the other hand, when subjected to the low pH of endosomes, TGF-α, EPR, and AR quickly separate from the receptor, which leads to de-ubiquitination of the receptor and its subsequent recycling to the plasma membrane (**Figure 2**b) [22].

In contrast with other HER family members, none of the ligands bind to HER2 [23]; it always exists in the dimerized state and acts as a preferred partner for heterodimerization with the other three ERBB/HER family members [24]. Also, HER2-containing heterodimers are characterized by higher affinity and broader ligand specificity than other heterodimeric ERBB/HER receptor complexes due to the slower dissociation rates of growth factors [25]. There was a controversy regarding HER3 pertaining to its kinase activity, and initially, it was posited that HER3 lacked kinase activity due to the absence of requisite residues [26].

2. EGFR Role in Cancer

Mutations and cases of overexpression of EGFR are especially frequently found in carcinomas and glioblastomas, tumors of epithelial and glial origin, respectively [27][28]. Worldwide, carcinomas are the most common type of cancer [29]. Overexpression of EGFR has been reported and implicated in the pathogenesis of many human malignancies, including head and neck [30], lung [31], breast [32], pancreatic [33], and colon cancer [34]. The EGFR-positive status of the tumor often correlates with poor prognosis and outcome, as it is beneficial for cancer cell proliferation [7][35]. EGFR overexpression was also shown to be associated with melanoma progression and promoted invasiveness and metastasis in this tumor type [36].

Mutations in the tyrosine kinase domain of EGFR were found in the majority of tumors that exhibited a positive response to treatment with EGFR-specific TKIs (**Figure 3**a in green) [37]. In some reports, the frequency of EGFR-activating mutations has strong ethnical specificity and varies by region, being as high as 46% in Asia versus only 8% in the Americas [38]. The two most common mutations of EGFR in NSCLC represent about 85–90% of all EGFR mutations [39]. The first one is a deletion of EGFR exon 19 (*del747–750*), which eliminates the leucine-arginine-glutamate-alanine motif in the tyrosine kinase domain of EGFR (*LREA deletion*), and the second one (*L858R*) is a thymine-to-guanine transversion, which results in the replacement of leucine with arginine in exon 21 codon 858 [40][41]. The third most frequent type of EGFR mutations in NSCLC is exon 20 insertions (ex20ins), which constitute 9% [42]–12% [43] of all EGFR mutations. In contrast to the other above mentioned mutations, Ex20ins is associated with poor response to treatment with TKIs [44]. It results in in-frame insertions, usually concentrated within or following the C-helix that dictates the activation status of EGFR [45]. In glioblastoma, the most frequently (~30%) occurring EGFR mutation is *EGFRΔIII* (EGFR variant III), which results from the in-frame deletion of 801 base pairs

spanning exons 2–7 of the coding sequence, resulting in ligand-independent activation of EGFR tyrosine kinase activity [46][47][48].

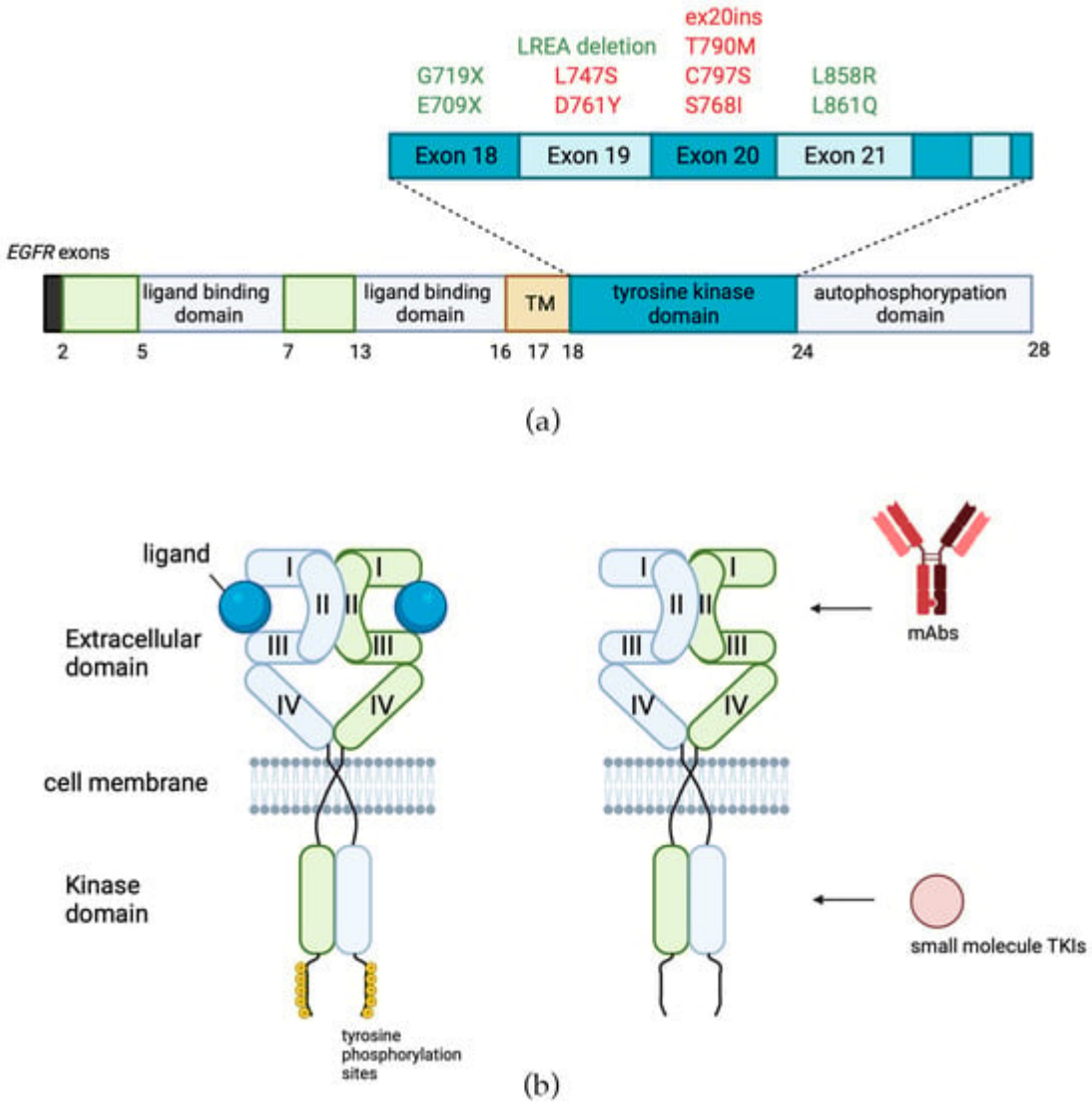

Figure 3. (**a**) Structure of EGFR gene. EGFR exons 18–21 encode the tyrosine kinase domain and may contain mutations, playing a crucial role in the development and progression of different cancers with a strong proven relationship to resistance (red) and sensitivity (green) to specific TKIs. (**b**) Domain view of EGFR protein. Left, a schematic diagram of ligand-bound dimerized EGFR. Right, sites of inhibition of EGFR activity by different targeted drugs (mAb: monoclonal antibodies; TKIs: tyrosine kinase inhibitors). Created with BioRender.com (accessed on 18 October 2023).

The *LREA* deletion of exon 19 of EGFR is shown to increase EGFR autophosphorylation and to activate downstream pathways AKT and STAT, thus promoting survival and cell growth [49]. With this mutation, the EGFR dimer exhibits increased stability as it tightens the molecular contacts of arginine ARG744 and asparagines ASP974 and ASP976 from the reciprocal monomers [50].

The 21st exon point mutation *L858R* is also a common activation mutation of *EGFR*, accounting for nearly 40% of all EGFR mutations. The *L858R* mutation locks the kinase in a constitutively active state by preventing the activation loop segment (residue 858 and flanking residues) from adopting the inactive, helical conformation, which leads to about 50-fold greater activity of the mutant EGFR [51].

EGFRvIII (EGFR with 2–7 exon deletion) lacks a ligand-binding domain and is constitutively active. It is the most common EGFR mutation occurring in glioblastoma [46]. *EGFRvIII* pathologic isoform does not contain amino acids 6-273 of wild-type EGFR, and it results in the formation of a new glycine residue at the junction site. This alteration imitates the effects of ligand binding and triggers changes in the receptor conformation by ultimately activating downstream signaling pathways [52].

In addition, the patients may have uncommon *EGFR* mutations. The use of next-generation sequencing (NGS) has become a novel diagnostic method for detection, which led to the identification of increasingly rare or atypical EGFR mutations. For example, *EGFR* fusion mutation *EGFR–SEPT14* was found in a patient with colorectal adenocarcinoma. The exon 24 of *EGFR* was fused to the exon 10 on *SEPT14* while retaining the EGFR tyrosine kinase domain. This tumor appeared to be sensitive to erlotinib treatment, and the patient developed a partial response following therapy [53].

In addition, activating mutations of downstream genes of regulatory kinases involved in the Ras/MAPK signaling pathway, such as KRAS, NRAS, and BRAF, are exceptionally frequent and appear in more than 90% of pancreatic, ~32% of lung, and ~52% of colon cancers [54].

3. EGFR-Targeted Therapies

Since EGFR is frequently overexpressed and/or mutated in multiple cancer types, it has prompted the development of a number of specific targeted therapeutics. Currently, there are two classes of EGFR-specific cancer drugs: monoclonal antibodies (mAbs), which bind to the extracellular domain of the transmembrane receptor and block its dimerization, and small-molecule tyrosine kinase inhibitors (TKIs), which bind to the adenosine triphosphate (ATP) binding site [55] (**Figure 3**b, **Table 1**). In turn, TKIs can be classified according to the mechanism of binding with the receptor tyrosine kinase domain: type I (binding with ATP site in mainly active conformation), type II (binding with ATP site plus back pocket, DFG(Asp855-Gly857)-out, in inactive conformation), type I½ (binding to a DFG-in, in inactive conformation), type III inhibitors binding to allosteric sites, and type IV inhibitors which generally form covalent adducts with their target protein [56][57]. EGFR-targeted drugs are currently widespread, globally approved, and are used worldwide for hundreds of thousands of patients per year.

Table 1. Characterization of EGFR-targeting inhibitors.

Tyrosine Kinase Inhibitors					
Drug	**Tumor Type**	**Therapeutic Indication**	**Molecular Target**	**Inhibitor Type**	**Molecular Markers of Efficiency**
•	*First Generation*				
Gefitinib	Advanced or metastatic NSCLC	First-line therapy for NSCLC carrying EGFR-activating mutations	EGFR: ATP-binding site	I	Activating mutations of *EGFR*: Exon 19 deletions; *L858R*
Erlotinib	Advanced or metastatic NSCLC, pancreatic cancer	First-line therapy for NSCLC carrying EGFR-activating mutations With gemcitabine: first-line treatment option for patients with locally advanced and metastatic pancreatic carcinoma	EGFR: ATP-binding site	I	Activating mutations of *EGFR*: Exon 19 deletions; *L858R*
Lapatinib	Metastatic breast cancer	With capecitabine: the treatment of HER2-positive MBC in patients who have previously received therapy (anthracycline, a taxane, trastuzumab) With letrozole: the treatment of postmenopausal women with hormone receptor positive MBC that overexpresses the HER2 receptor for whom hormonal therapy is indicated	ATP-binding site of EGFR and HER2	I½	HER2-positive status of tumor
•	*Second Generation*				
Afatinib	Metastatic NSCLC	First-line therapy for metastatic NSCLC carrying EGFR-activating mutations	ATP-binding site of EGFR, HER2, and HER4	IV	Activating mutations of *EGFR*: Exon 19 deletions; *L858R*
Neratinib	Breast cancer	Extended adjuvant treatment of patients with early stage HER2-positive breast cancer, to follow adjuvant trastuzumab based therapy With capecitabine: the treatment of patients with	ATP-binding site of EGFR, HER2, and HER4	IV	HER2-positive status of tumor

Tyrosine Kinase Inhibitors					
Drug	**Tumor Type**	**Therapeutic Indication**	**Molecular Target**	**Inhibitor Type**	**Molecular Markers of Efficiency**
		advanced or metastatic HER2-positive BC who have received two or more prior anti-HER2 based regimens in the metastatic setting			
Dacomitinib	Metastatic NSCLC	First-line therapy for metastatic NSCLC carrying EGFR-activating mutations	ATP-binding site of EGFR, HER2, and HER4	IV	Activating mutations of *EGFR*: Exon 19 deletions; *L858R*
•	*Third Generation*				
Osimertinib	Advanced or metastatic NSCLC	Adjuvant and first-line therapy for metastatic NSCLC carrying EGFR-activating mutations The treatment of adult patients with metastatic EGFR *T790M* mutation-positive NSCLC, whose disease has progressed on or after EGFR TKI therapy	ATP-binding site of the EGFR	IV	Activating mutations of *EGFR*: Exon 19 deletions; *L858R* The secondary *T790M* resistance mutation
Almonertinib	Advanced NSCLC	Adjuvant therapy for advanced NSCLC patients with *T790M*-mutant EGFR who had developed resistance to first- and second-generation EGFR TKIs like gefitinib and afatinib	ATP-binding site of the EGFR	IV	Activating mutations of *EGFR*: Exon 19 deletions; *L858R* The secondary *T790M* resistance mutation
Lazertinib	Advanced NSCLC	Treatment of locally advanced or metastatic NSCLC carrying EGFR *T790M* mutation	ATP-binding site of the EGFR	IV	Activating mutations of *EGFR*: Exon 19 deletions; *L858R* The secondary *T790M* resistance mutation
Furmonertinib	Locally advanced or metastatic NSCLC	Treatment of locally advanced or metastatic EGFR *T790M*+ NSCLC that developed after	ATP-binding site of the EGFR		The secondary *T790M* resistance mutation

Tyrosine Kinase Inhibitors					
Drug	**Tumor Type**	**Therapeutic Indication**	**Molecular Target**	**Inhibitor Type**	**Molecular Markers of Efficiency**
		progression on treatment with first-generation EGFR TKIs			
Monoclonal Antibodies					
Drug	**Tumor Type**	**Therapeutic Indication**	**Molecular Target**		**Molecular Markers of Efficiency**
Cetuximab	Advanced or metastatic SCCHN, metastatic CRC	With radiation therapy: treatment of locally or regionally advanced SCCHN With platinum-based therapy with fluorouracil: metastatic SCCHN Metastatic SCCHN progressing after platinum-based therapy With FOLFIRI: first-line treatment of KRASwt EGFR-overexpressing mCRC With irinotecan in patients who are refractory to irinotecan-based chemotherapy: treatment of KRASwt EGFR-overexpressing mCRC; as a single-agent in patients who have failed oxaliplatin-and irinotecan-based chemotherapy or who are intolerant to irinotecan	The binding site in domain III of EGFR		KRAS wild-type status of EGFR-overexpressing tumor
Panitumumab	Metastatic CRC	Single agent treatment of metastatic CRC with disease progression on or following fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens	The binding site in domain III of EGFR		RAS wild-type status of EGFR-overexpressing tumor
Necitumumab	Metastatic NSCLC	With gemcitabine and cisplatin: first-line treatment of patients with metastatic NSCLC	The binding site in domain III of EGFR		EGFR-overexpressing status of tumor

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Distinct from the Active State. J. Biol. Chem. 2020, 295, 13353–13362.

9. Mudumbi, K.C.; Burns, E.A.; Schodt, D.J.; Petrova, Z.O.; Kiyatkin, A.; Kim, L.W.; Mangiacapre, E.M.; Ortiz-Caraveo, I.; Ortiz, H.R.; Hu, C.; et al. Distinct Interactions Stabilize EGFR Dimers and Higher-Order Oligomers in Cell Membranes. bioRxiv 2023.

4. First Generation of EGFR-Targeted Drugs

10. Needham, S.R.; Roberts, S.K.; Arkhipov, A.; Mysore, V.P.; Tynan, C.J.; Zanetti-Domingues, L.C.; Kim, E.T.; Losasso, V.; Korovesis, D.; Hirsch, M.; et al. EGFR Oligomerization Organizes Kinase-Active Dimers into Competent Signalling Platforms. Nat. Commun. 2016, 7, 13307.

The function of tyrosine kinase enzymes (including those of the ERBB/HER receptor family) is transferring γ-phosphate of an ATP molecule to the tyrosine residue of the substrate, thus initiating signal transmission to further

11. Ogiso, H.; Ishitani, R.; Nureki, O.; Fukai, S.; Yamanaka, M.; Kim, J.-H.; Saito, K.; Sakamoto, A.; Inoue, M.; Shirouzu, M.; et al. Crystal Structure of the Complex of Human Epidermal Growth Factor and Receptor Extracellular Domains. Cell 2002, 110, 775–787.
12. Singh, B.; Coffey, R.J. From Wavy Hair to Naked Proteins: The Role of Transforming Growth Factor Alpha in Health and Disease. Semin. Cell Dev. Biol. 2014, 28, 12–21.
13. Schneider, M.R.; Yarden, Y. Structural Evolution of Epidermal Growth Factor/ErbB Ligands and Their Receptors. Cell Dev. Biol. 2014, 28, 57–61.
14. Berasain, C.; Avila, M.A. Amphiregulin. Semin. Cell Devo Biol. 2014, 28, 31–41.
15. Dunbar, A.J.; Goddard, C. Structure-Function and Biological Role of Betacellulin. Int. J. Biochem. Cell Biol. 2000, 32, 805–815.
16. Muraoka-Cook, R.S.; Sandahl, M.; Hunter, D.; Miraglia, L.; Earp, H.S. Prolactin and ErbB4/HER4 Signaling Interact via Janus Kinase 2 to Induce Mammary Epithelial Cell Gene Expression Differentiation. Mol. Endocrinol. 2008, 22, 2307–2321.
17. Sato, K.; Nakamura, T.; Mizuguchi, M.; Miura, K.; Tada, M.; Aizawa, T.; Gomi, T.; Miyamoto, K.; Kawano, K. Solution Structure of Epiregulin and the Effect of Its C-Terminal Domain for Receptor Binding Affinity. FEBS Lett. 2003, 553, 232–238.
18. Ozaki, M. Neuregulins and the Shaping of Synapses. Neuroscientist 2001, 7, 146–154.
19. Zhang, D.; Sliwkowski, M.X.; Mark, M.; Frantz, G.; Akita, R.; Sun, Y.; Hillan, K.; Crowley, C.; Brush, J.; Godowski, P.J. Neuregulin-3 (NRG3): A Novel Neural Tissue-Enriched Protein That Binds and Activates ErbB4. Proc. Natl. Acad. Sci. USA 1997, 94, 9562–9567.
20. Harari, D.; Tzahar, E.; Romano, J.; Shelly, M.; Pierce, J.H.; Andrews, G.C.; Yarden, Y. Neuregulin-4: A Novel Growth Factor That Acts through the ErbB-4 Receptor Tyrosine Kinase. Oncogene 1999, 18, 2681–2689.
21. Henriksen, L.; Grandal, M.V.; Knudsen, S.L.J.; van Deurs, B.; Grøvdal, L.M. Internalization Mechanisms of the Epidermal Growth Factor Receptor after Activation with Different Ligands. PLoS ONE 2013, 8, e58148.
22. Leblanc, J.A.; Sugiyama, M.G.; Antonescu, C.N.; Brown, A.I. Quantitative Modeling of EGF Receptor Ligand Discrimination via Internalization Proofreading. Phys. Biol. 2023, 20, 056008.
23. Landgraf, R. HER2 Therapy. HER2 (ERBB2): Functional Diversity from Structurally Conserved Building Blocks. Breast Cancer Res. 2007, 9, 202.
24. Tzahar, E.; Waterman, H.; Chen, X.; Levkowitz, G.; Karunagaran, D.; Lavi, S.; Ratzkin, B.J.; Yarden, Y. A Hierarchical Network of Interreceptor Interactions Determines Signal Transduction by Neu Differentiation Factor/Neuregulin and Epidermal Growth Factor. Mol. Cell. Biol. 1996, 16, 5276–5287.

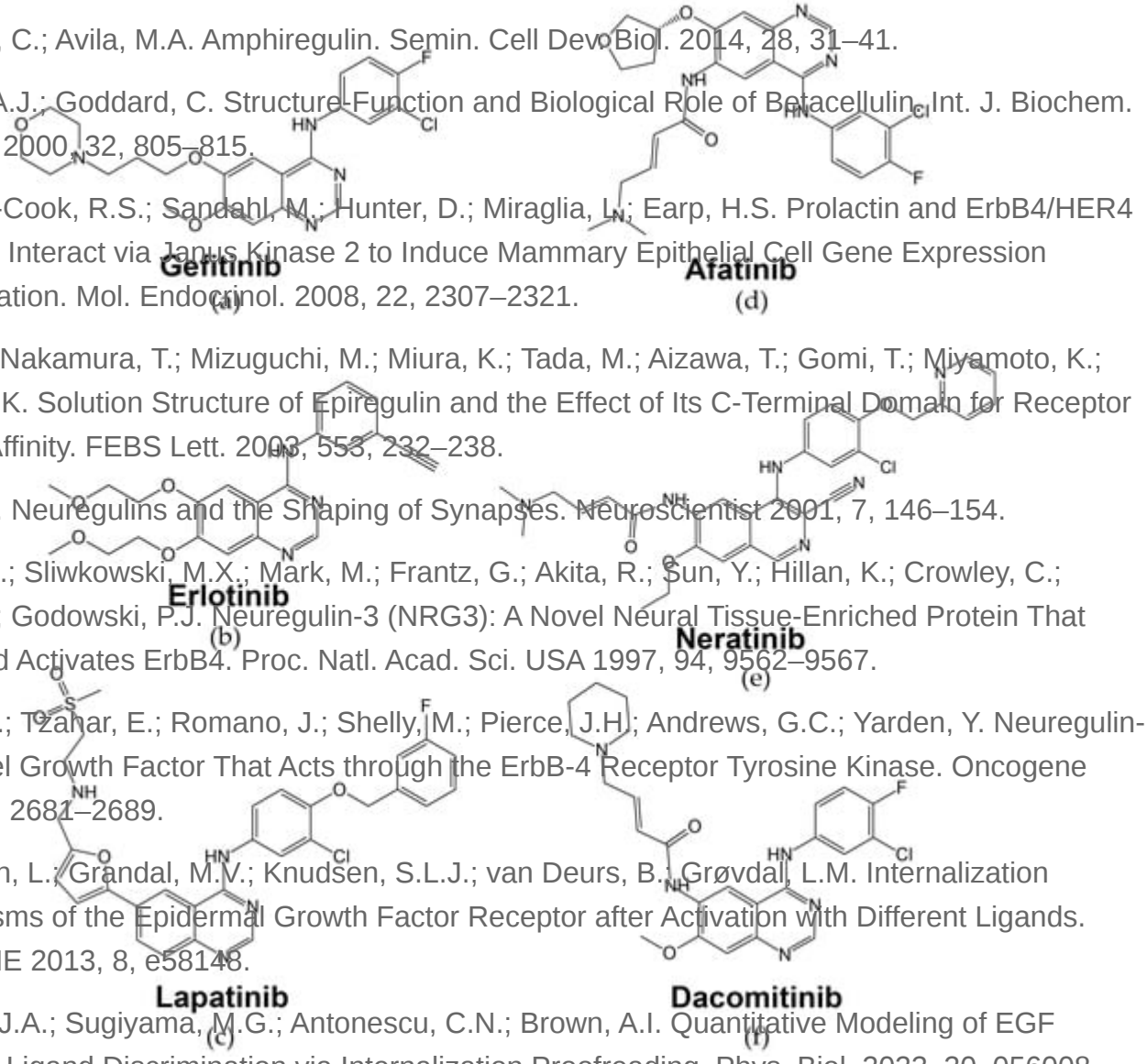

Figure 4. Molecular structures of members of the first and second generations of low molecular mass EGFR tyrosine kinase inhibitors.

low molecular mass EGFR tyrosine kinase inhibitors [58]

-Gefitinib, or ZD1839 (Iressa; Astra-Zeneca Pharmaceuticals), is an oral anilinoquinazolone with a structure formula presented in **Figure 4**a. By interacting with several amino acid residues, gefitinib takes up space in the ATP-binding site. The first nitrogen of the quinazoline ring creates a hydrogen bond with Met793 in the hinge region and interacts through a water molecule with Lys745, Met766, Leu788, Thr790, and Asp855 [59].

By inhibiting EGFR tyrosine phosphorylation, gefitinib affects downstream signaling cascades in the tumor cell. Many in vitro studies have been performed to determine cellular changes caused by gefitinib.

25. Jones, R.B.; Gordus, A.; Krall, J.A.; MacBeath, G. A Quantitative Protein Interaction Network for the ErbB Receptors Using Protein Microarrays. Nature 2006, 439, 168–174.

26. Jura, N.; Shan, Y.; Cao, X.; Shaw, D.E.; Kuriyan, J. Structural Analysis of the Catalytically Inactive Kinase Domain of the Human EGF Receptor 3. Proc. Natl. Acad. Sci. USA 2009, 106, 21608–21613.

27. Sharma, S.V.; Bell, D.W.; Settleman, J.; Haber, D.A. Epidermal Growth Factor Receptor Mutations in Lung Cancer. Nat. Rev. Cancer 2007, 7, 169–181.

28. Ohgaki, H.; Kleihues, P. Genetic Alterations and Signaling Pathways in the Evolution of Gliomas. Cancer Sci. 2009, 100, 2235–2241.

29. Hynes, N.E.; MacDonald, G. ErbB Receptors and Signaling Pathways in Cancer. Curr. Opin. Cell Biol. 2009, 21, 177–184.

30. Kalyankrishna, S.; Grandis, J.R. Epidermal Growth Factor Receptor Biology in Head and Neck Cancer. J. Clin. Oncol. 2006, 24, 2666–2672.

31. Bethune, G.; Bethune, D.; Ridgway, N.; Xu, Z. Epidermal Growth Factor Receptor (EGFR) in Lung Cancer: An Overview and Update. J. Thorac. Dis. 2010, 2, 48–51.

32. Al-Kuraya, K.; Schraml, P.; Torhorst, J.; Tapia, C.; Zaharieva, B.; Novotny, H.; Spichtin, H.; Maurer, R.; Mirlacher, M.; Köchli, O.; et al. Prognostic Relevance of Gene Amplifications and Coamplifications in Breast Cancer. Cancer Res. 2004, 64, 8534–8540.

33. Oliveira-Cunha, M.; Newman, W.G.; Siriwardena, A.K. Epidermal Growth Factor Receptor in Pancreatic Cancer. Cancers 2011, 3, 1513–1526.

34. Pabla, B.; Bissonnette, M.; Konda, V.J. Colon Cancer and the Epidermal Growth Factor Receptor: Current Treatment Paradigms, the Importance of Diet, and the Role of Chemoprevention. World J. Clin. Oncol. 2015, 6, 133–141.

35. Herbst, R.S. Review of Epidermal Growth Factor Receptor Biology. Int. J. Radiat. Oncol. Biol. Phys. 2004, 59, 21–26.

36. Pastwińska, J.; Karaś, K.; Karwaciak, I.; Ratajewski, M. Targeting EGFR in Melanoma—The Sea of Possibilities to Overcome Drug Resistance. Biochim. Biophys. Acta (BBA)-Rev. Cancer 2022, 1877, 188754.

37. Lynch, T.J.; Bell, D.W.; Sordella, R.; Gurubhagavatula, S.; Okimoto, R.A.; Brannigan, B.W.; Harris, P.L.; Haserlat, S.M.; Supko, J.G.; Haluska, F.G.; et al. Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib. N. Engl. J. Med. 2004, 350, 2129–2139.

38. Graham, R.P.; Treece, A.L.; Lindeman, N.I.; Vasalos, P.; Shan, M.; Jennings, L.J.; Rimm, D.L. Worldwide Frequency of Commonly Detected EGFR Mutations. Arch. Pathol. Lab. Med. 2018,

upregulated the expression of p-p38MAPK and p-JNK, suggesting stimulation of apoptosis potentially through the p38MAPK and AKT signaling pathways [70].

Despite demonstrated activity against HER2-positive cell lines, some cellular growth factors, such as NRG1 (neuregulin 1), have been found to have a negative effect on the action of lapatinib. In NCI-N87, the gastric carcinoma cell line, and OE19, esophageal adenocarcinoma cells, both sensitive to lapatinib, exposure to NRG1 together with lapatinib rescued cells from lapatinib-induced G1 cell cycle arrest and apoptosis.

Lapatinib, when used in combination with capecitabine, was approved by the FDA in 2007 for the treatment of HER2-positive metastatic breast cancer (MBC) in patients who have previously received therapy, including an anthracycline, a taxane, and trastuzumab. In a phase III study, OS times were 75.0 weeks for the combination of lapatinib and capecitabine treatment and 64.7 weeks for capecitabine treatment alone [71].

5. Second Generation of EGFR-Targeted Drugs

Emerging cases of secondary resistance to erlotinib and gefitinib forced researchers and the industry to develop new EGFR-specific therapeutics with the potential to overcome it. Second-generation EGFR TKIs were developed to address acquired resistance by inhibiting additional partner receptor tyrosine kinases (such as HER2) or irreversibly binding to the kinase domain and thereby abrogating downstream EGFR signaling.

Afatinib (Giotrif, BIBW2992, **Figure 4**d) was approved in 2013 for the treatment of metastatic NSCLC carrying activating EGFR exon 19 deletions or exon 21 *L858R* substitution [72]. Its mechanism of action is different from first-generation EGFR inhibitors erlotinib and gefitinib, as afatinib irreversibly inhibits autophosphorylation of EGFR, HER2, and HER4 receptors by forming irreversible covalent bonds with ATP-binding sites (**Table 1**) [73].

In cell culture studies, afatinib was more effective than erlotinib, gefitinib, or lapatinib in inhibiting the survival of lung cancer cell lines harboring wild-type (H1666) or *L858R/T790M* (NCI-H1975) EGFR, with IC_{50}s (half-maximal inhibitory concentration is drug concentration required for 50% inhibition) below 100 nM, whereas these cells were resistant to the first-generation drugs.

Afatinib was also proved to be more effective against NSCLC cell lines carrying the EGFR exon 19 deletion, most probably due to more efficient inhibition of EGFR phosphorylation [74]. In xenograft models, afatinib showed strong activities in EGFR *L858R/T790M* or HER2-overexpressing tumors [75]. In the head and neck squamous carcinoma cell line HN5 tumor xenograft, afatinib was found to be more effective in arresting tumor xenograft growth than three other TKIs with ERBB/HER-targeting activities (lapatinib, erlotinib, and neratinib) [76].

Phase III trials showed the efficacy of afatinib as first-line therapy in comparison with chemotherapy (pemetrexed/cisplatin in LUX-Lung 3 and gemcitabine/cisplatin in LUX-Lung 6 trials) in patients with metastatic lung adenocarcinoma carrying EGFR mutations. The drug significantly improved the objective response rate in

142, 163–167.

39. Castañeda-González, J.P.; Chaves, J.J.; Parra-Medina, R. Multiple Mutations in the EGFR Gene in Lung Cancer: A Systematic Review. Transl. Lung Cancer Res. 2022, 11, 2148–2163.
40. Choi, Y.W.; Jeon, S.Y.; Jeong, G.S.; Lee, H.W.; Jeong, S.H.; Kang, S.Y.; Park, J.S.; Choi, J.-H.; Koh, Y.W.; Han, J.H.; et al. EGFR Exon 19 Deletion Is Associated with Favorable Overall Survival After First-Line Gefitinib Therapy in Advanced Non-Small Cell Lung Cancer Patients. Am. J. Clin. Oncol. 2018, 41, 385–390.
41. Yermekova, S.; Orazgaliyeva, M.; Goncharova, T.; Rakhimbekova, F.; Dushimova, Z.; Vasilieva, T. Mutational Damages in Malignant Lung Tumors. Asian Pac. J. Cancer Prev. 2023, 24, 709–716.
42. Oxnard, G.R.; Lo, P.C.; Nishino, M.; Dahlberg, S.E.; Lindeman, N.I.; Butaney, M.; Jackman, D.M.; Johnson, B.E.; Jänne, P.A. Natural History and Molecular Characteristics of Lung Cancers Harboring EGFR Exon 20 Insertions. J. Thorac. Oncol. 2013, 8, 179–184.
43. Burnett, H.; Emich, H.; Carroll, C.; Stapleton, N.; Mahadevia, P.; Li, T. Epidemiological and Clinical Burden of EGFR Exon 20 Insertion in Advanced Non-Small Cell Lung Cancer: A Systematic Literature Review. PLoS ONE 2021, 16, e0247620.
44. Wang, F.; Li, C.; Wu, Q.; Lu, H. EGFR Exon 20 Insertion Mutations in Non-Small Cell Lung Cancer. Transl. Cancer Res. 2020, 9, 2982–2991.
45. Vyse, S.; Huang, P.H. Targeting EGFR Exon 20 Insertion Mutations in Non-Small Cell Lung Cancer. Signal Transduct. Target. Ther. 2019, 4, 5.
46. Rutkowska, A.; Stoczyńska-Fidelus, E.; Janik, K.; Włodarczyk, A.; Rieske, P. EGFRvIII: An Oncogene with Ambiguous Role. J. Oncol. 2019, 2019, 1092587.
47. Brennan, C.W.; Verhaak, R.G.W.; McKenna, A.; Campos, B.; Noushmehr, H.; Salama, S.R.; Zheng, S.; Chakravarty, D.; Sanborn, J.Z.; Berman, S.H.; et al. The Somatic Genomic Landscape of Glioblastoma. Cell 2013, 155, 462–477.
48. Zheng, Q.; Han, L.; Dong, Y.; Tian, J.; Huang, W.; Liu, Z.; Jia, X.; Jiang, T.; Zhang, J.; Li, X.; et al. JAK2/STAT3 Targeted Therapy Suppresses Tumor Invasion via Disruption of the EGFRvIII/JAK2/STAT3 Axis and Associated Focal Adhesion in EGFRvIII-Expressing Glioblastoma. Neuro-Oncology 2014, 16, 1229–1243.
49. Sharma, S.V.; Bell, D.W.; Settleman, J.; Haber, D.A. Epidermal Growth Factor Receptor Mutations in Lung Cancer. Nat. Rev. Cancer 2007, 7, 169–181.
50. Tsigelny, I.F.; Wheler, J.J.; Greenberg, J.P.; Kouznetsova, V.L.; Stewart, D.J.; Bazhenova, L.; Kurzrock, R. Molecular Determinants of Drug-Specific Sensitivity for Epidermal Growth Factor Receptor (EGFR) Exon 19 and 20 Mutants in Non-Small Cell Lung Cancer. Oncotarget 2015, 6, 6029–6039.

patients with brain metastases. In a combined analysis, PFS with afatinib was significantly improved in comparison with chemotherapy (8.2 months versus 5.4 months) [77].

-Neratinib (Nerlynx, HKI-272, **Figure 4**e [78]) is a second-generation HER2/EGFR/HER4 TKI [79]. It covalently combines with cysteine residues Cys-773 and Cys-805 of ATP-binding domains of HER1, HER2, and HER4, thus inhibiting the receptor function [80].

The anti-proliferative effects of neratinib were examined in vitro across a panel of 115 cancer cell lines by ATPlite 1step Luminescence Assay System for analysis of cell viability. In this panel, there were 22 cell lines harboring point mutations or amplifications of the *HER2* (*n* = 9), *HER3* (*n* = 10), or *EGFR* (*n* = 10) genes, and neratinib was proven to be the effective drug with IC_{50}s comparable to other TKIs in this study [81].

In xenograft models overexpressing HER2 (BT474) and EGFR (SKOV-3 and A431), neratinib dose-dependently inhibited tumor growth: almost by ~70–90% in xenografts of BT474, ~30–60% in xenografts of SK-OV-3, and ~32–44% in xenografts of A431 [79].

-Dacomitinib (Vizimpro, PF-00299804, **Figure 4**f [82]), another second-generation EGFR inhibitor, was approved by the FDA in 2018 as the first-line treatment of patients with metastatic NSCLC with EGFR-activating mutations (exon 19 deletion or exon 21 substitution *L858R*) (**Table 1**) [83]. This drug also has activity against EGFR, HER2, and HER4 receptors, which are inhibited through irreversible covalent binding of the drug at the edge of the ATP-binding cleft of tyrosine kinase domain [84]. For EGFR, irreversible inhibition is achieved by interacting with EGFR C797, similar to afatinib [85].

In NSCLC cell lines harboring endogenous EGFR *T790M* mutation, dacomitinib proved itself as an effective agent in vitro. In cell lines with *L858R* mutation or wild-type *EGFR*, dacomitinib had 10 times lower IC_{50} (μM) than reversible EGFR inhibitor gefitinib. For example, the IC_{50} of dacomitinib in the H3255 cell line carrying *L858R* mutation was 0.007 μM versus 0.075 μM for gefitinib. In wild-type *EGFR* cell lines H1819 and Calu-3, IC_{50} values of dacomitinib were 0.029 and 0.063 μM versus 0.42 and 1.4 μM for gefitinib, respectively [86].

6. Third Generation of EGFR-Targeted Drugs

First-generation EGFR-targeted low molecular mass therapeutics erlotinib and gefitinib have the disadvantage of being reversible inhibitors, and they are proven to be ineffective against the secondary EGFR mutations, such as the *T790M* substitution, which has been found in over 50% of EGFR-mutant NSCLC cases with acquired resistance to EGFR inhibitors [87].

-Osimertinib (Tagrisso™, AZD9291 AstraZeneca, **Figure 5**a) is an irreversible orally administered, EGFR-specific TKI with strong selectivity to *EGFR*-activating mutations as well as the secondary *T790M* resistance mutation in patients with advanced NSCLC (**Figure 5**a, **Table 1**) [88]. Osimertinib's mechanism of action is the formation of a covalent bond to the cysteine-797 residue in the EGFR ATP-binding site [89].

51. Yun, C.-H.; Boggon, T.J.; Li, Y.; Woo, M.S.; Greulich, H.; Meyerson, M.; Eck, M.J. Structures of Lung Cancer-Derived EGFR Mutants and Inhibitor Complexes: Mechanism of Activation and Insights into Differential Inhibitor Sensitivity. Cancer Cell 2007, 11, 217–227.
52. Garima, G.; Thanvi, S.; Singh, A.; Verma, V. Epidermal Growth Factor Receptor Variant III Mutation, an Emerging Molecular Marker in Glioblastoma Multiforme Patients: A Single Institution Study on the Indian Population. Cureus 2022, 14, e26412.
53. Li, Y.; Zhang, H.-B.; Chen, X.; Yang, X.; Ye, Y.; Bekaii-Saab, T.; Zheng, Y.; Zhang, Y. A Rare EGFR-SEPT14 Fusion in a Patient with Colorectal Adenocarcinoma Responding to Erlotinib. Oncologist 2020, 25, 203–207.
54. Cox, A.D.; Fesik, S.W.; Kimmelman, A.C.; Luo, J.; Der, C.J. Drugging the Undruggable RAS: Mission Possible? Nat. Rev. Drug Discov. 2014, 13, 828–851.
55. Gharwan, H.; Groninger, H. Kinase Inhibitors and Monoclonal Antibodies in Oncology: Clinical Implications. Nat. Rev. Clin. Oncol. 2016, 13, 209–227.
56. Roskoski, R. Small Molecule Inhibitors Targeting the EGFR/ErbB Family of Protein-Tyrosine Kinases in Human Cancers. Pharmacol. Res. 2019, 139, 395–411.
57. Roskoski, R. Classification of Small Molecule Protein Kinase Inhibitors Based upon the Structures of Their Drug-Enzyme Complexes. Pharmacol. Res. 2016, 103, 26–48.
58. Meng, Y.; Pond, M.P.; Roux, B. Tyrosine Kinase Activation and Conformational Flexibility: Lessons from Src-Family Tyrosine Kinases. Acc. Chem. Res. 2017, 50, 1193–1201.
59. Amelia, T.; Kartasasmita, R.E.; Ohwada, T.; Tjahjono, D.H. Structural Insight and Development of EGFR Tyrosine Kinase Inhibitors. Molecules 2022, 27, 819.
60. Suenaga, M.; Yamaguchi, A.; Soda, H.; Orihara, K.; Tokito, Y.; Sakaki, Y.; Umehara, M.; Terashi, K.; Kawamata, N.; Oka, M.; et al. Antiproliferative Effects of Gefitinib Are Associated with Suppression of E2F-1 Expression and Telomerase Activity. Anticancer Res. 2006, 26, 3387–3391.
61. Cohen, M.H.; Williams, G.A.; Sridhara, R.; Chen, G.; Pazdur, R. FDA Drug Approval Summary: Gefitinib (ZD1839) (Iressa) Tablets. Oncologist 2003, 8, 303–306.
62. Cohen, M.H.; Johnson, J.R.; Chen, Y.-F.; Sridhara, R.; Pazdur, R. FDA Drug Approval Summary: Erlotinib (Tarceva) Tablets. Oncologist 2005, 10, 461–466.
63. Shan, F.; Shao, Z.; Jiang, S.; Cheng, Z. Erlotinib Induces the Human Non-Small-Cell Lung Cancer Cells Apoptosis via Activating ROS-Dependent JNK Pathways. Cancer Med. 2016, 5, 3166–3175.
64. Kamashev, D.; Shaban, N.; Lebedev, T.; Prassolov, V.; Suntsova, M.; Raevskiy, M.; Gaifullin, N.; Sekacheva, M.; Garazha, A.; Poddubskaya, E.; et al. Human Blood Serum Can Diminish EGFR-Targeted Inhibition of Squamous Carcinoma Cell Growth through Reactivation of MAPK and EGFR Pathways. Cells 2023, 12, 2022.

Figure 5. Molecular structure of the third-generation irreversible low molecular mass EGFR tyrosine kinase inhibitors.

-Almonertinib, also known as Aumolertinib, HS-10296, or Ameile, is another low molecular mass TKI with high selectivity for EGFR-sensitizing and *T790M*-resistant mutations. Similar to osimertinib, it covalently and irreversibly binds to cysteine-797 at the ATP-binding site of EGFR (**Figure 5**b). The Growth Factor Receptor and Human Epidermal Growth Factor Receptor 2 Tyrosine Kinases Used in the Treatment of Breast Cancer. Clin. Ther. 2009, 31 Pt 2, 2332–2348.

-Lazertinib (YH25448, Leclaza) is an oral third-generation EGFR TKI developed primarily for the treatment of NSCLC (**Figure 5**c). It targets the EGFR molecules harboring *T790M* mutation and activating mutations such as deletion of exon 19 or L858R but is ineffective against wild-type EGFR tumors (**Table 1**) [91]. Similar to osimertinib, it forms a covalent bond with the C797 residue in the ATP-binding site [85].

-Furmonertinib or Alflutinib (AST2818, **Figure 5**d) is another third-generation TKI inhibitor that blocks EGFR with both activating mutations and secondary mutations such as *T790M* (**Table 1**) [92].

-Mobocertinib (TAK-788, AP32788, **Figure 5**e) is another third-generation EGFR inhibitor that was developed to treat NSCLC patients with EGFR exon 20 insertions [93]. Mobocertinib selectively targets EGFRex20ins variants by interacting with the C790 gatekeeper residue in the ATP-binding pocket of EGFR through its middle pyrimidine ring while also forming an irreversible covalent bond with the C797 residue [94].

7. Fourth Generation of EGFR-Targeted Drugs

Over time, patients treated with third-generation TKIs develop heterogeneous resistance to this therapy, which may be either EGFR-dependent or independent [95]. The primary cause of EGFR-dependent resistance is the emergence of a specific point mutation *C797S* in the ATP-binding cleft [96][97].

65. Rosell, R.; Carcereny, E.; Gervais, R.; Vergnenegre, A.; Massuti, B.; Felip, E.; Palmero, R.; Garcia-Gomez, R.; Pallares, C.; Sanchez, J.M.; et al. Erlotinib versus Standard Chemotherapy as First-Line Treatment for European Patients with Advanced EGFR Mutation-Positive Non-Small-Cell Lung Cancer (EURTAC): A Multicentre, Open-Label, Randomised Phase 3 Trial. Lancet Oncol. 2012, 13, 239–246.
66. Zhou, C.; Wu, Y.L.; Chen, G.; Feng, J.; Liu, X.-Q.; Wang, C.; Zhang, S.; Wang, J.; Zhou, S.; Ren, S.; et al. Final Overall Survival Results from a Randomised, Phase III Study of Erlotinib versus Chemotherapy as First-Line Treatment of EGFR Mutation-Positive Advanced Non-Small-Cell Lung Cancer (OPTIMAL, CTONG-0802). Ann. Oncol. 2015, 26, 1877–1883.
67. Wood, E.R.; Truesdale, A.T.; McDonald, O.B.; Yuan, D.; Hassell, A.; Dickerson, S.H.; Ellis, B.; Pennisi, C.; Horne, E.; Lackey, K.; et al. A Unique Structure for Epidermal Growth Factor Receptor Bound to GW572016 (Lapatinib): Relationships among Protein Conformation, Inhibitor off-Rate, and Receptor Activity in Tumor Cells. Cancer Res. 2004, 64, 6652–6659.
68. Ongko, J.; Setiawan, J.V.; Feronytha, A.G.; Juliana, A.; Effraim, A.; Wahjudi, M.; Antonius, Y. In-Silico Screening of Inhibitor on Protein Epidermal Growth Factor Receptor (EGFR). IOP Conf. Ser. Earth Environ. Sci. 2022, 1041, 012075.
69. Tevaarwerk, A.J.; Kolesar, J.M. Lapatinib: A Small Molecule Inhibitor of Epidermal Growth Factor Receptor and Human Epidermal Growth Factor Receptor 2 Tyrosine Kinases Used in the Treatment of Breast Cancer. Clin. Ther. 2009, 31 Pt 2, 2332–2348.
70. Liu, L.; Zhong, L.; Zhao, Y.; Chen, M.; Yao, S.; Li, L.; Xiao, C.; Shan, Z.; Gan, L.; Xu, T.; et al. Effects of Lapatinib on Cell Proliferation and Apoptosis in NB4 Cells. Oncol. Lett. 2018, 15, 235–242.
71. Cameron, D.; Casey, M.; Oliva, C.; Newstat, B.; Imwalle, B.; Geyer, C.E. Lapatinib plus Capecitabine in Women with HER-2-Positive Advanced Breast Cancer: Final Survival Analysis of a Phase III Randomized Trial. Oncologist 2010, 15, 924–934.
72. Dungo, R.T.; Keating, G.M. Afatinib: First Global Approval. Drugs 2013, 73, 1503–1515.
73. Wind, S.; Schnell, D.; Ebner, T.; Freiwald, M.; Stopfer, P. Clinical Pharmacokinetics and Pharmacodynamics of Afatinib. Clin. Pharmacokinet. 2017, 56, 235–250.
74. Banno, E.; Togashi, Y.; Kobayashi, Y.; Hayashi, H.; Mitsudomi, T.; Nishio, K. Afatinib Is Especially Effective against Non-Small Cell Lung Cancer Carrying an EGFR Exon 19 Deletion. Anticancer Res. 2015, 35, 2005–2008.
75. Li, D.; Ambrogio, L.; Shimamura, T.; Kubo, S.; Takahashi, M.; Chirieac, L.R.; Padera, R.F.; Shapiro, G.I.; Baum, A.; Himmelsbach, F.; et al. BIBW2992, an Irreversible EGFR/HER2 Inhibitor Highly Effective in Preclinical Lung Cancer Models. Oncogene 2008, 27, 4702–4711.

to overcome resistance mutations, fourth-generation drugs that bind to an allosteric site of EGFR are currently being developed and undergoing preclinical evaluation [98][99]. For example, after analyzing over 2.5 million chemical compounds, two non-ATP competitive compounds were discovered, EAI001 (EGFR allosteric inhibitor-1, **Figure 6**a) and EAI045 (EGFR allosteric inhibitor-45, **Figure 6**b), that target the allosteric site of EGFR and prevent its binding to ATP [99][100].

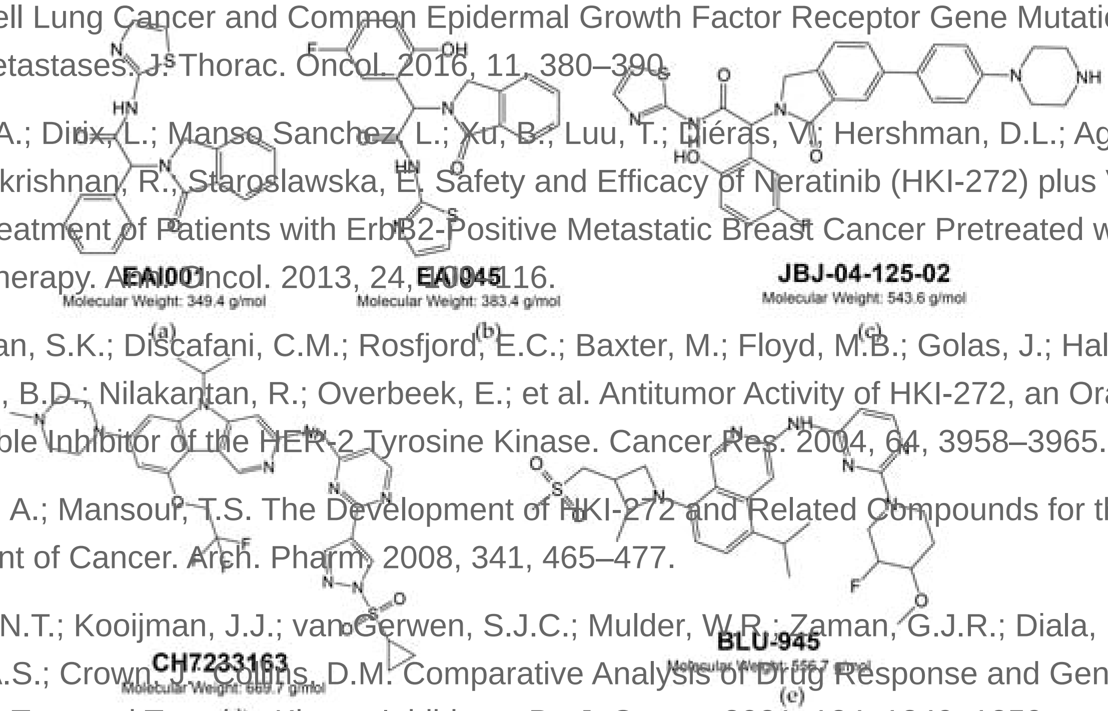

Figure 6. Structures of candidate molecules for the fourth-generation family of low molecular mass EGFR tyrosine kinase inhibitors.

Another reversible non-ATP competitive allosteric inhibitor of EGFR under investigation is JBJ-04-125-02 (**Figure 6**c), which is active against EGFR *L858R*, *L858R/T790M* or *L858R/T790M/C797S* Ba/F3 cells, but showed fewer activities against H1975 and H3255GR mutant EGFR cell models [101]. CH7233163 (**Figure 6**d) is another allosteric inhibitor of EGFR. It directly interacts with the gatekeeper residue T790M in addition to the P-loop and hinge regions and binds more extensively within the ATP-binding pocket [102]. CH7233163 has demonstrated activity both in vitro and in vivo against the following EGFR mutation models: *del19/T790M/C797S*, *L858R/T790M/C797S*, *del19/T790M*, *L858R/T790M*, *del19*, and *L858R*. In contrast to the previous allosteric inhibitors presented, it was also effective against *del19* EGFR-mutant models [102]. However, there were currently no clinical trial reports that could prove the clinical efficacy of the above mentioned fourth-generation EGFR inhibitors.

BLU-945 (**Figure 6**e) was obtained by Blueprint Medicines by optimizing the molecules from ~25,000 compound library of designed small-molecule kinase inhibitors and showed in vitro sub-nanomolar activities against the *EGFR T790M* and *EGFR T790M/C797S* mutants. In addition, it reduced EGFR phosphorylation in Ba/F3 cells in *L858R/T790M/C797S* mutants with IC_{50} = 3.2 nM and in *ex19del/T790M/C797S* mutants with IC_{50} = 4.0 nM. In

76. Young, N.R.; Soneru, C.; Liu, J.; Grushko, T.A.; Hardeman, A.; Olopade, O.I.; Baum, A.; Solca, F.; Cohen, E.E.W. Afatinib Efficacy against Squamous Cell Carcinoma of the Head and Neck Cell Lines In Vitro and In Vivo. Target. Oncol. 2015, 10, 501–508.

77. Schuler, M.; Wu, Y.-L.; Hirsh, V.; O'Byrne, K.; Yamamoto, N.; Mok, T.; Popat, S.; Sequist, L.V.; Massey, D.; Zazulina, V.; et al. First-Line Afatinib versus Chemotherapy in Patients with Non-Small Cell Lung Cancer and Common Epidermal Growth Factor Receptor Gene Mutations and Brain Metastases. J. Thorac. Oncol. 2016, 11, 380–390.

78. Awada, A.; Dirix, L.; Manso Sanchez, L.; Xu, B.; Luu, T.; Diéras, V.; Hershman, D.L.; Agrapart, V.; Ananthakrishnan, R.; Staroslawska, E. Safety and Efficacy of Neratinib (HKI-272) plus Vinorelbine in the Treatment of Patients with ErbB2-Positive Metastatic Breast Cancer Pretreated with Anti-HER2 Therapy. Ann. Oncol. 2013, 24, 109–116.

79. Rabindran, S.K.; Discafani, C.M.; Rosfjord, E.C.; Baxter, M.; Floyd, M.B.; Golas, J.; Hallett, W.A.; Johnson, B.D.; Nilakantan, R.; Overbeek, E.; et al. Antitumor Activity of HKI-272, an Orally Active, Irreversible Inhibitor of the HER-2 Tyrosine Kinase. Cancer Res. 2004, 64, 3958–3965.

80. Wissner, A.; Mansour, T.S. The Development of HKI-272 and Related Compounds for the Treatment of Cancer. Arch. Pharm. 2008, 341, 465–477.

81. Conlon, N.T.; Kooijman, J.J.; van Gerwen, S.J.C.; Mulder, W.R.; Zaman, G.J.R.; Diala, I.; Eli, L.D.; Lalani, A.S.; Crown, J.; Collins, D.M. Comparative Analysis of Drug Response and Gene Profiling of HER2-Targeted Tyrosine Kinase Inhibitors. Br. J. Cancer 2021, 124, 1249–1259.

82. Asami, K.; Atagi, S. Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors for Non-Small Cell Lung Cancer. World J. Clin. Oncol. 2014, 5, 646–659.

83. Nagano, T.; Tachihara, M.; Nishimura, Y. Dacomitinib, a Second-Generation Irreversible Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor (EGFR-TKI) to Treat Non-Small Cell Lung Cancer. Drugs Today 2019, 55, 231–236.

84. Garuti, L.; Roberti, M.; Bottegoni, G. Irreversible Protein Kinase Inhibitors. Curr. Med. Chem. 2011, 18, 2981–2994.

85. Duggirala, K.B.; Lee, Y.; Lee, K. Chronicles of EGFR Tyrosine Kinase Inhibitors: Targeting EGFR C797S Containing Triple Mutations. Biomol. Ther. 2022, 30, 19–27.

86. Engelman, J.A.; Zejnullahu, K.; Gale, C.-M.; Lifshits, E.; Gonzales, A.J.; Shimamura, T.; Zhao, F.; Vincent, P.W.; Naumov, G.N.; Bradner, J.E.; et al. PF00299804, an Irreversible Pan-ERBB Inhibitor, Is Effective in Lung Cancer Models with EGFR and ERBB2 Mutations That Are Resistant to Gefitinib. Cancer Res. 2007, 67, 11924–11932.

87. Arcila, M.E.; Oxnard, G.R.; Nafa, K.; Riely, G.J.; Solomon, S.B.; Zakowski, M.F.; Kris, M.G.; Pao, W.; Miller, V.A.; Ladanyi, M. Rebiopsy of Lung Cancer Patients with Acquired Resistance to EGFR

vivo experiments showed decreases in the T790M mutant *EGFR* nucleic acid-based and Ba/F3 xenografts.

BLU-701 is another compound developed by Blueprint Medicines that is a highly selective and potent inhibitor of EGFR with *ex19del*- or *L858R*-activating mutations and the *C797S* resistance mutation with nanomolar IC_{50} (~3.3 nM) [103][104]. At tolerated doses, oral administration of BLU-701 in mice led to significant and sustained regression of the PC9 ex19del tumor xenografts [103]. The safety and effectiveness of BLU-701 in patients with EGFR-mutated HARMONIA (NCT05153408) [105].

For two other fourth-generation EGFR inhibitors, JIN-A02 and BBT-176, the successful application in both in vitro and in vivo studies were published: JIN-A02 inhibited *ex19del/T790M/C797S* and *L858R/T790M/C797S* EGFR-mutant Ba/F3 cells (IC_{50} = 51.0 and 49.2 nM, respectively) and resulted in tumor regression in *ex19del/T790M/C797S* Ba/F3 xenograft mouse models [106]. The IC_{50} values of BBT-176 for Ba/F3 cells engineered to express EGFR *19Del/C797S*, EGFR *19Del/T790M/C797S*, and EGFR *L858R/C797S* and *L858R/T790M/C797S* were 42.3, 18.3, and 2.2 nM, respectively.

8. EGFR-Specific Therapeutic Monoclonal Antibodies

Soon after the discovery of the EGFR receptor in the 1980s, prof. John Mendelsohn noted that the addition of EGF, the ligand of the EGFR receptor, had a negative effect on the survival of the A431 tumor cell line, which contained large amounts of EGFR. The idea was that it was possible to stop EGFR-overexpressing tumor proliferation through interference with the EGFR signaling [107]. Because EGFR permeates the cell membrane, the idea arose that monoclonal antibodies could be an effective therapeutic against tumors with increased expression of this receptor. EGFR-specific mAbs function similarly by disrupting pro-tumor growth and survival signaling through binding to growth factor receptors, thus altering their activation state or preventing ligand binding [108].

-Cetuximab (Erbitux, Merck Serono) was the first monoclonal antibody targeting the EGFR receptor, a human–mouse chimeric anti-EGFR mAb with the human IgG1 constant region [109]. It exhibits a strong affinity for human EGFR and effectively hinders ligand binding, ultimately resulting in the suppression of receptor phosphorylation and downstream signaling pathways [110]. The primary effect of cetuximab binding to EGFR is steric blockage of ligand access to the binding site in domain III of the receptor (**Figure 3**b, **Table 1**).

It was found that the inhibition of cell growth induced by blocking EGFR activation of cetuximab deals with the induction of cell cycle arrest and apoptosis. Cetuximab induced cell accumulation in the G1 phase and increased the expression levels of cell cycle inhibitors $p27^{KIP1}$ and $p15^{INK4B}$ in human oral squamous cell carcinoma cell lines [111]. A similar effect was observed in SCCHN cell lines: cetuximab treatment decreased cell motility and enhanced cell arrest in the G1 phase; also, the accumulation of $p27^{KIP1}$ was observed following cetuximab treatment [112].

Inhibitors and Enhanced Detection of the T790M Mutation Using a Locked Nucleic Acid-Based Assay. Clin. Cancer Res. 2011, 17, 1169–1180.

88. Cross, D.A.E.; Ashton, S.E.; Ghiorghiu, S.; Eberlein, C.; Nebhan, C.A.; Spitzler, P.J.; Orme, J.P.; Finlay, M.R.V.; Ward, R.A.; Mellor, M.J.; et al. AZD9291, an Irreversible EGFR TKI, Overcomes T790M-Mediated Resistance to EGFR Inhibitors in Lung Cancer. Cancer Discov. 2014, 4, 1046–1061.

89. Leonetti, A.; Sharma, S.; Minari, R.; Perego, P.; Giovannetti, E.; Tiseo, M. Resistance Mechanisms to Osimertinib in EGFR-Mutated Non-Small Cell Lung Cancer. Br. J. Cancer 2019, 121, 725–737.

90. Yang, J.C.-H.; Camidge, D.R.; Yang, C.-T.; Zhou, J.; Guo, R.; Chiu, C.-H.; Chang, G.-C.; Shiah, H.-S.; Chen, Y.; Wang, C.-C.; et al. Safety, Efficacy, and Pharmacokinetics of Almonertinib (HS-10296) in Pretreated Patients with EGFR-Mutated Advanced NSCLC: A Multicenter, Open-Label, Phase 1 Trial. J. Thorac. Oncol. 2020, 15, 1907–1918.

91. Yun, M.R.; Kim, S.-Y.; Hong, M.H.; Kim, H.R.; Park, C.-W.; Kim, S.-Y.; Lee, S.S.; Koh, J.S.; et al. YH25448, an Irreversible EGFR-TKI with Potent Intracranial Activity in EGFR Mutant Non-Small Cell Lung Cancer. Clin. Cancer Res. 2019, 25, 2575–2587.

92. Zhang, S.S.; Ou, S.-H.I. Spotlight on Furmonertinib (Alflutinib, AST2818). The Swiss Army Knife (Del19, L858R, T790M, Exon 20 Insertions, "Uncommon-G719X, S768I, L861Q") Among the Third-Generation EGFR TKIs? Lung Cancer 2022, 13, 67–73.

93. Vasconcelos, P.E.N.S.; Kobayashi, I.S.; Kobayashi, S.S.; Costa, D.B. Preclinical Characterization of Mobocertinib Highlights the Putative Therapeutic Window of This Novel EGFR Inhibitor to EGFR Exon 20 Insertion Mutations. JTO Clin. Res. Rep. 2021, 2, 100105.

94. Arnold, A.; Ganti, A.K. Clinical Utility of Mobocertinib in the Treatment of NSCLC—Patient Selection and Reported Outcomes. OncoTargets Ther. 2023, 16, 559–569.

95. Wu, L.; Ke, L.; Zhang, Z.; Yu, J.; Meng, X. Development of EGFR TKIs and Options to Manage Resistance of Third-Generation EGFR TKI Osimertinib: Conventional Ways and Immune Checkpoint Inhibitors. Front. Oncol. 2020, 10, 602762.

96. Papadimitrakopoulou, V.A.; Wu, Y.-L.; Han, J.-Y.; Ahn, M.-J.; Ramalingam, S.S.; John, T.; Okamoto, I.; Yang, J.C.-H.; Bulusu, K.C.; Laus, G.; et al. Analysis of Resistance Mechanisms to Osimertinib in Patients with EGFR T790M Advanced NSCLC from the AURA3 Study. Ann. Oncol. 2018, 29, viii741.

97. Oxnard, G.R.; Hu, Y.; Mileham, K.F.; Husain, H.; Costa, D.B.; Tracy, P.; Feeney, N.; Sholl, L.M.; Dahlberg, S.E.; Redig, A.J.; et al. Assessment of Resistance Mechanisms and Clinical Implications in Patients with EGFR T790M-Positive Lung Cancer and Acquired Resistance to Osimertinib. JAMA Oncol. 2018, 4, 1527–1534.

98. Patnaik, A.; Swanson, K.D.; Csizmadia, E.; Solanki, A.; Landon-Brace, N.; Gehring, M.P.; Helenius, K.; Olson, B.M.; Pauly, M.G.; Chadwick, E.; et al. Cabozantinib Eradicates Advanced Murine Prostate Cancer by Activating Antitumor Innate Immunity. Cancer Discov. 2017, 7, 740–765.

99. Zhao, P.; Yao, M.-Y.; Zhu, S.-J.; Chen, J.-Y.; Yun, C.-H. Crystal Structure of EGFR T790M/C797S/V948R in Complex with EAI045. Biochem. Biophys. Res. Commun. 2018, 502, 332–337.

100. Jia, Y.; Yun, C.-H.; Park, E.; Ercan, D.; Manuia, M.; Juarez, J.; Xu, C.; Rhee, K.; Chen, T.; Zhang, H.; et al. Overcoming EGFR(T790M) and EGFR(C797S) Resistance with Mutant-Selective Allosteric Inhibitors. Nature 2016, 534, 129–132.

101. To, C.; Jang, J.; Chen, T.; Park, E.; Mushajiang, M.; De Clercq, D.J.H.; Xu, M.; Wang, S.; Cameron, M.D.; Heppner, D.E.; et al. Single and Dual Targeting of Mutant EGFR with an Allosteric Inhibitor. Cancer Discov. 2019, 9, 926–943.

102. Kashima, K.; Kawauchi, H.; Tanimura, H.; Tachibana, Y.; Ohba, T.; Tabata, T.; Sakamoto, H. CH7233163 Overcomes Osimertinib Resistant EGFR-Del19/T790M/C797S Mutation. Mol. Cancer Ther. 2020, 19, 2288–2297.

103. Conti, C.; Campbell, J.; Woessner, R.; Guo, J.; Timsit, Y.; Iliou, M.; Wardwell, S.; Davis, A.; Chicklas, S.; Hsieh, J.; et al. Abstract 1262: BLU-701 Is a Highly Potent, Brain-Penetrant and WT-Sparing next-Generation EGFR TKI for the Treatment of Sensitizing (Ex19del, L858R) and C797S Resistance Mutations in Metastatic NSCLC. Cancer Res. 2021, 81, 1262.

104. Tavera, L.; Schalm, S.; Campbell, J.; Guo, J.; Medendorp, C.; Chen, M.; Albayya, F.; Dineen, T.; Zhang, Z.; Iliou, M.; et al. Abstract 3328: Antitumor Activity of BLU-945 and BLU-701 as Single Agents and in Combination in EGFR L858R-Driven Models of NSCLC. Cancer Res. 2022, 82, 3328.

105. Spira, A.I.; Spigel, D.R.; Camidge, D.R.; De Langen, A.; Kim, T.M.; Goto, K.; Elamin, Y.Y.; Shum, E.; Reckamp, K.L.; Rotow, J.K.; et al. A Phase 1/2 Study of the Highly Selective EGFR Inhibitor, BLU-701, in Patients with EGFR-Mutant Non–Small Cell Lung Cancer (NSCLC). J. Clin. Oncol. 2022, 40, TPS9142.

106. Yun, M.R.; Yu, M.R.; Duggirala, K.B.; Lee, K.; Jo, A.; Seah, E.; Kim, C.; Cho, B.C. MA07.08 JIN-A02, a Highly Effective 4th Generation EGFR-TKI, Targeting EGFR C797S Triple Mutation in NSCLC. J. Thorac. Oncol. 2022, 17, S69–S70.

107. Kawamoto, T.; Sato, J.D.; Le, A.; Polikoff, J.; Sato, G.H.; Mendelsohn, J. Growth Stimulation of A431 Cells by Epidermal Growth Factor: Identification of High-Affinity Receptors for Epidermal Growth Factor by an Anti-Receptor Monoclonal Antibody. Proc. Natl. Acad. Sci. USA 1983, 80, 1337–1341.

108. Zahavi, D.; Weiner, L. Monoclonal Antibodies in Cancer Therapy. Antibodies 2020, 9, 34.

109. Galizia, G.; Lieto, E.; De Vita, F.; Orditura, M.; Castellano, P.; Troiani, T.; Imperatore, V.; Ciardiello, F. Cetuximab, a Chimeric Human Mouse Anti-Epidermal Growth Factor Receptor Monoclonal Antibody, in the Treatment of Human Colorectal Cancer. Oncogene 2007, 26, 3654–3660.

110. Goldstein, N.I.; Prewett, M.; Zuklys, K.; Rockwell, P.; Mendelsohn, J. Biological Efficacy of a Chimeric Antibody to the Epidermal Growth Factor Receptor in a Human Tumor Xenograft Model. Clin. Cancer Res. 1995, 1, 1311–1318.

111. Kiyota, A.; Shintani, S.; Mihara, M.; Nakahara, Y.; Ueyama, Y.; Matsumura, T.; Tachikawa, T.; Wong, D.T.W. Anti-Epidermal Growth Factor Receptor Monoclonal Antibody 225 Upregulates P27KIP1 and P15INK4B and Induces G1 Arrest in Oral Squamous Carcinoma Cell Lines. Oncology 2002, 63, 92–98.

112. Okuyama, K.; Suzuki, K.; Naruse, T.; Tsuchihashi, H.; Yanamoto, S.; Kaida, A.; Miura, M.; Umeda, M.; Yamashita, S. Prolonged Cetuximab Treatment Promotes p27Kip1-Mediated G1 Arrest and Autophagy in Head and Neck Squamous Cell Carcinoma. Sci. Rep. 2021, 11, 5259.

113. Kamashev, D.; Sorokin, M.; Kochergina, I.; Drobyshev, A.; Vladimirova, U.; Zolotovskaia, M.; Vorotnikov, I.; Shaban, N.; Raevskiy, M.; Kuzmin, D.; et al. Human Blood Serum Can Donor-Specifically Antagonize Effects of EGFR-Targeted Drugs on Squamous Carcinoma Cell Growth. Heliyon 2021, 7, e06394.

114. Information on Cetuximab (Marketed as Erbitux)|FDA. Available online: https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/information-cetuximab-marketed-erbitux (accessed on 17 November 2023).

115. Blick, S.K.A.; Scott, L.J. Cetuximab: A Review of Its Use in Squamous Cell Carcinoma of the Head and Neck and Metastatic Colorectal Cancer. Drugs 2007, 67, 2585–2607.

116. Voigt, M.; Braig, F.; Göthel, M.; Schulte, A.; Lamszus, K.; Bokemeyer, C.; Binder, M. Functional Dissection of the Epidermal Growth Factor Receptor Epitopes Targeted by Panitumumab and Cetuximab. Neoplasia 2012, 14, 1023–1031.

117. Kim, G.P.; Grothey, A. Targeting Colorectal Cancer with Human Anti-EGFR Monoclonocal Antibodies: Focus on Panitumumab. Biologics 2008, 2, 223–228.

118. Fala, L. Portrazza (Necitumumab), an IgG1 Monoclonal Antibody, FDA Approved for Advanced Squamous Non-Small-Cell Lung Cancer. Am. Health Drug Benefits 2016, 9, 119–122.

119. Lu, D.; Zhang, H.; Koo, H.; Tonra, J.; Balderes, P.; Prewett, M.; Corcoran, E.; Mangalampalli, V.; Bassi, R.; Anselma, D.; et al. A Fully Human Recombinant IgG-like Bispecific Antibody to Both the Epidermal Growth Factor Receptor and the Insulin-like Growth Factor Receptor for Enhanced Antitumor Activity. J. Biol. Chem. 2005, 280, 19665–19672.

120. Kuenen, B.; Witteveen, P.O.; Ruijter, R.; Giaccone, G.; Dontabhaktuni, A.; Fox, F.; Katz, T.; Youssoufian, H.; Zhu, J.; Rowinsky, E.K.; et al. A Phase I Pharmacologic Study of Necitumumab (IMC-11F8), a Fully Human IgG1 Monoclonal Antibody Directed Against EGFR in Patients with Advanced Solid Malignancies. Clin. Cancer Res. 2010, 16, 1915–1923.