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Review Article

EGFR^{vIII}: An Oncogene with Ambiguous Role

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Epidermal growth factor receptor variant III (EGFR^{vIII}) seems to constitute the perfect therapeutic target for glioblastoma (GB), as it is specifically present on up to 28–30% of GB cells. In case of other tumor types, expression and possible role of this oncogene still remain controversial. In spite of EGFR^{vIII} mechanism of action being crucial for the design of small active anticancer molecules and immunotherapies, i.e., CAR-T technology, it is yet to be precisely defined. EGFR^{vIII} is known to be resistant to degradation, but it is still unclear whether it heterodimerizes with EGF-activated wild-type EGFR (EGFR^{WT}) or homodimerizes (including covalent homodimerization). Constitutive kinase activity of this mutated receptor is relatively low, and some researchers even claim that a nuclear, but not a membrane function, is crucial for its activity. Based on the analyses of recurrent tumors that are often lacking EGFR^{vIII} expression despite its initial presence in corresponding primary foci, this oncogene is suggested to play a marginal role during later stages of carcinogenesis, while even in primary tumors EGFR^{vIII} expression is detected only in a small percentage of tumor cells, undermining the rationality of EGFR^{vIII}-targeting therapies. On the other hand, EGFR^{vIII}-positive cells are resistant to apoptosis, more invasive, and characterized with enhanced proliferation rate. Moreover, expression of this oncogenic receptor was also postulated to be a marker of cancer stem cells. Opinions regarding the role that EGFR^{vIII} plays in tumorigenesis and for tumor aggressiveness are clearly contradictory and, therefore, it is crucial not only to determine its mechanism of action, but also to unambiguously define its role at early and advanced cancer stages.

1. EGFR: Parental Gene of EGFR^{vIII}

Epidermal growth factor receptor (EGFR/ErbB1/HER1) is a member of a tyrosine kinase receptor family, also including ErbB2/HER2/Neu, ErbB3/HER3, and ErbB4/HER4 [1]. All these receptors are transmembrane glycoproteins with a molecular mass ranging from 170 to 185 kDa [2]. Activation of ErbB receptor may be triggered by one of 13 ligands, such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregulin, betacellulin, epiregulin, neuregulin 1–6, heparin-binding EGF-like growth factor (HB-EGF), or epigen, with the first five being EGFR-specific [3]. It is not clear how EGFR is activated and triggers a cascade of downstream signaling in cells. Generally, its activation involves ligand binding and subsequent receptor dimerization; however, it was also indicated that receptor may dimerize regardless of ligand presence [4, 5]. Intriguingly, dimers formed in such a ligand-independent manner remain inactive till the ligand is finally bound [4]. Activation of EGF receptor may induce signal in Ras/Raf/MAPK, PI3K/AKT, JAK/STAT, or PLC/PKC pathways [6, 7], having an impact on a variety of cellular processes, including proliferation, metabolism, apoptosis, cell survival, or differentiation [8, 9]. Termination of signaling cascade occurs after receptor internalization, mostly in clathrin-dependent endocytosis, leading to its trafficking into early endosomes. Further, receptor may be either transported back to the cell membrane or degraded in late endosomes and lysosomes [10].

Gene encoding EGFR is located on a short arm of chromosome 7 (p11.2) and consists of 28 exons [11]. Mature EGFR protein (1186 amino acids) is formed from a precursor one (1210 amino acids) following the removal of the *N*-terminal part [12]. From the *N* to the *C*-terminal end, EGFR is composed of extracellular domain involved in ligand binding and receptor dimerization (exons 1–16), hydrophobic transmembrane domain (exon 17), and intracellular domain with tyrosine kinase activity that is flanked by the linker region and the *C*-terminal part of the receptor (exons 18–28) (Figure 1(a)) [13]. Twelve out of 20 tyrosine residues of intracellular domain were demonstrated to undergo phosphorylation and these bind membrane-bound or cytoplasmic effector proteins that are recruited following receptor activation [14, 15].

2. EGFR^{vIII} Alteration in Cancer

Overexpression of EGF receptor was detected in many tumor types and demonstrated to be associated with cancer cell resistance to chemo-, radio-, and/or hormone therapy. This receptor is often mutated in certain tumors, especially in extracellular and tyrosine kinase domains [16], resulting in elevated or prolonged EGFR signaling [17, 18]. Such abnormal signaling is associated not only with enhanced proliferation and apoptosis inhibition in tumor cells, but also with metastasis and angiogenesis [19, 20]. In case of glioblastoma (GB), EGFR amplification is in the majority of cases accompanied by gene rearrangements. Such alterations involve deletion of particular exons or exon parts and are designated as EGFR^{vI} (deletion of *N*-terminal part), EGFR^{vII} (deletion of exons 14 and 15), EGFR^{vIII} (deletion of exons 2–7), EGFR^{vIV} (deletion of exons 25–27), and EGFR^{vV} (deletion of exons 25–28) [21–24]. One of the most commonly detected variants in GB cells is EGFR^{vIII} [25–29] (Figure 1(b)).

Despite being mentioned in several articles [30], alternative splicing does not constitute key mechanisms for EGFR^{vIII} expression in glioblastoma and other tumor types. There are only single reports indicating that this phenomenon may be involved in EGFR^{vIII} generation in head and neck squamous cell carcinoma (HNSCC), but it is still not considered the major mechanism. Gene encoding EGFR is amplified in approximately 50% of GB patients, and in 50–60% of cases, amplification is accompanied by EGFR^{vIII} expression that is tumor cell-specific, making this oncogenic protein a perfect therapeutic target [22, 31, 32]. Expression of mutated receptor is also detected in few percent of prostate, breast, or colon cancer cases, but only in trace cell populations [33–36]. Nevertheless, EGFR^{vIII} expression in tumor types other than glioblastoma remains controversial and needs to be unequivocally assessed, as many contradictory data have been published so far [27, 37–41]. Such inconsistencies are particularly associated with technical limitations of applied methodological approaches. Data collected from several research centers indicate that results of EGFR^{vIII}-related analyses tend to be even completely inconsistent. As an example, a research conducted by Moscatello et al. (1995) demonstrated EGFR^{vIII} expression in

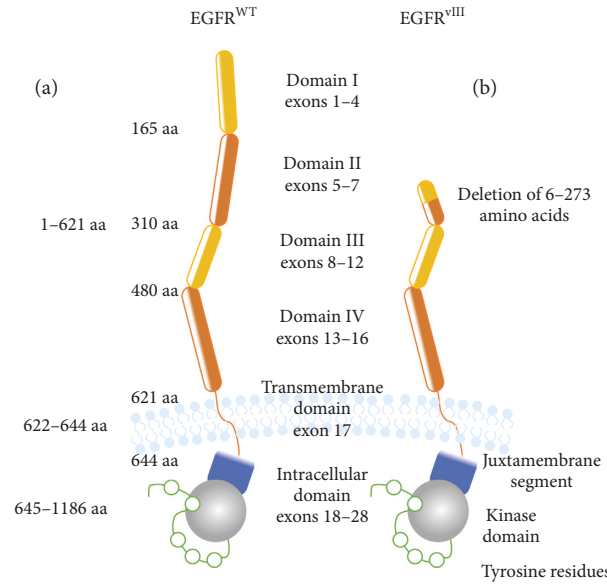

Figure 1: Schematic structure of EGFR^{WT} (a) and EGFR^{vIII} (b). Both receptors are composed of extracellular (I–IV), transmembrane, and intracellular domains, and the major difference is deletion of exons 2–7 encoding extracellular domains I and II in mutated receptor. As a result of deletion, EGFR^{vIII} is unable to bind known ligands and shows enhanced stability in cell membrane.

73% of ovarian cancer samples (Western blot analysis) and was completely contradictory to independent analysis, utilizing other methods, that indicated lack of this oncogene expression at both mRNA and protein levels in analyzed tumor samples, as well as cell lines [34, 42–44]. Similar inconsistencies were detected in case of colon or bladder cancer [43, 45–47] and most interestingly, in breast cancer, in which case EGFR^{vIII} expression is in some reports estimated to be 20–78%, while in others not to exceed 0–4% [34, 36, 43, 48–50]. Nevertheless, various agents acting on EGFR^{WT} or EGFR^{vIII} are extensively studied in different types of cancers (summarized in Table 1).

From a therapeutic point of view, glioblastoma seems to be the most important tumor type in terms of EGFR^{vIII} because of the relatively high expression and frequency of occurrence of this oncogene and, most importantly, continuous lack of effective therapy for GB patients. Due to the deletion of 801 bp encoding *N*-terminal, domains I and II are lost and the mutated receptor becomes unable to bind ligands [8, 25]. Mechanisms leading to the formation of nucleotide sequence encoding EGFR^{vIII} have not been completely elucidated yet; however, it seems plausible that deletion of receptor part is the result of recombination between Alu sequences flanking junctions in introns 1 and 7 of EGFR-encoding gene [117]. As EGFR^{vIII} usually acts as an amplified gene, it may be suggested that increase in the number of gene copies will translate into increased mRNA levels of this oncogenic variant, but no such obvious dependence has been found, even in relation to EGFR^{WT} levels [118, 119]. It may be associated with the fact that the main role of gene amplification in this case is not to provide additional gene copies that will increase mRNA levels of amplified gene. With the current focus on the field of

Table 1: Agents acting specifically on $EGFR^{vIII}$ or on both $EGFR^{vIII}$ and $EGFR^{WT}$, based on the analysis of different cancer types.

		Specificity	Examined cancers	Activity	Stage of research	References
Agents acting only on $EGFR^{vIII}$						
Immunotherapy						
ADC	AMG-595	$EGFR^{vIII}$	Glioblastoma	Potentially active	Phase I	[51]
CARs	CAR-T	e.g., $EGFR^{vIII}$	Glioblastoma	Potentially active	Phase I	[52, 53]
			Lung cancer	Potentially active	Preclinical	[54]
BiTE	bscEGFR^{vIII} × CD3	e.g., $EGFR^{vIII}$	Glioma	Potentially active	Phase I	[55, 56, 57]
Vaccine	Rindopepimut	$EGFR^{vIII}$	Glioblastoma	Inactive	Phase III	[58]
RNA interference						
Ribozymes		e.g., $EGFR^{vIII}$	Breast cancer	Potentially active	Preclinical	[59]
			Glioblastoma	Potentially active		[60]
Antisense oligonucleotides		e.g., $EGFR^{vIII}$	Glioblastoma	Potentially active	Preclinical	[61, 62]
siRNA		e.g., $EGFR^{vIII}$	Glioblastoma	Potentially active	Preclinical	[61, 63]
Agents acting on $EGFR^{vIII}$ and $EGFR^{WT}$						
Tyrosine kinase inhibitors						
First generation	Gefitinib	EGFR/HER1	High-grade gliomas	Limited activity	Phase II	[64, 65]
			Non-small-cell lung cancer	Active	Clinical use	[66, 67]
			Salivary gland cancer	Potentially active	Phase II	[68]
			Breast cancer	Potentially active	Phase II	[69]
			Ovarian, peritoneal, or fallopian tube cancer	Potentially active	Phase I/II	[70]
			Liver cancer	Potentially active	Phase II	[71]
	Lapatinib	EGFR/HER1/ HER2	Glioblastoma	Inactive	Phase I/II	[72, 73]
			Breast cancer	Active	Clinical use	[74]
			Gastric cancer	Limited activity	Phase II	[75]
			Colorectal cancer	Potentially active	Phase II	[76]
			Gliomas	Limited activity	Phase II	[77, 78]
	Erlotinib	EGFR/HER1	Vulvar cancer	Potentially active	Phase II	[79]
			Non–small-cell lung cancer	Active	Clinical use	[80, 81]
			Pancreatic cancer	Active	Clinical use	[82]
			Head and neck cancer	Limited activity	Phase II	[83, 84]
Second generation	Afatinib	EGFR/HER1/ HER2/HER4	Non-small-cell lung cancer	Active	Clinical use	[85]
			Squamous cell carcinoma of the lung	Active	Clinical use	[85]
			Head and neck cancer	Potentially active	Phase III	[86]
			Glioblastoma	Limited activity	Phase I/II	[87, 88]
			Breast cancer	Potentially active	Phase II	[89]
			Colorectal cancer	Potentially active	Phase II	[90]
Immunotherapy						
Antibodies	Cetuximab	EGFR/HER1/ HER2	Head and neck cancer	Active	Clinical use	[91]
			Glioblastoma	Potentially active	Phase II	[92, 93, 94]
			Colorectal cancer	Active	Clinical use	[95]
			Esophageal and gastric cancer	Limited activity	Phase II	[96]
			Non-small-cell lung cancer	Potentially active	Phase II	[97]
			Breast cancer	Limited activity	Phase II	[98]
			Prostate cancer	Inactive	Phase II	[99]
			Cervical cancer	Inactive	Phase II	[100]
	Panitumumab	EGFR/HER1	Colorectal cancer	Active	Clinical use	[101, 102]
			Biliary tract cancer	Potentially active	Phase II	[103]
			Head and neck cancer	Inactive	Phase II	[104, 105]
			Glioblastoma	Potentially active	Phase II	[106, 107]
			Breast cancer	Potentially active	Phase II	[108]
	Nimotuzumab	EGFR/HER1	Glioblastoma	Orphan status in Europe and USA	Clinical use	[109, 110]
			Head and neck cancer	Active	Phase II	[111, 112]
			Pancreatic cancer	Orphan status in Europe	Clinical use	[110, 113]
ADC	ABT-414	EGFR/$EGFR^{vIII}$	Glioblastoma	Limited activity	Phase I	[114, 115]
			Breast cancer	Limited activity	Phase I/II	[116]

extracellular vesicles (EVs), the fact that extrachromosomal amplicons may be transported between cells is gaining importance [120]. Therefore, in such a context, the role of amplicons is not to increase oncogene mRNA levels, but rather to enable more flexible regulation of gene expression as well as transfer of mutated gene to cells initially lacking such alteration. Moreover, detection of $EGFR^{vIII}$ in extrachromosomal amplicons derived from cerebrospinal fluid may constitute a highly specific and less invasive approach, to molecularly diagnose GB patients and make them candidates for currently developed anti-$EGFR^{vIII}$-targeted therapies [118].

3. $EGFR^{vIII}$ Mechanism of Action

Compared to $EGFR^{WT}$ (normal EGFR protein), $EGFR^{vIII}$ signaling is considered to be elevated, due to its ability to dimerize in a ligand-independent manner. However, as it is not clear whether EGF is indeed crucial for $EGFR^{WT}$ dimerization or required only for a dimer to switch from inactive to active state, the role of $EGFR^{vIII}$ dimerization is becoming less evident [3, 4]. Loss of large extracellular receptor fragment makes it difficult to determine whether $EGFR^{vIII}$ dimerizes in tethered or untethered conformation or if such conformation resembles active or rather inactive $EGFR^{WT}$ [16]. Importantly, it has to be emphasized that $EGFR^{vIII}$ exhibits constitutive activity [121, 122]. Our team demonstrated that mutant phosphorylation is elevated when compared to nonstimulated $EGFR^{WT}$, while data obtained by other research teams indicate that constitutive $EGFR^{vIII}$ signaling corresponds to low level of signal intensity induced by ligand-activated $EGFR^{WT}$ [17, 123–125]. Such data indicate that $EGFR^{vIII}$ dimer conformation resembles inactive dimers of $EGFR^{WT}$, thus suggesting that impact of this oncogenic receptor on cell biology is not a result of some specific, dimerization-related kinase activity, but rather a consequence of unique membrane stability [121, 122]. Therefore, constitutive activity of $EGFR^{vIII}$ is not particularly high, but when combined with high membrane stability, it may enable triggering of some significant biological effects by this oncogenic receptor [17, 123–125].

Despite the fact that $EGFR^{vIII}$ stability seems to be more important than its kinase activity, the latter feature is still required to fully exhibit the oncogenic potential of this receptor. Such potential is dependent on signal transduction induced by phosphorylated tyrosines (at least in a model explaining $EGFR^{vIII}$ oncogenicity as a membrane receptor), especially as $EGFR^{vIII}$ was demonstrated to undergo constant phosphorylation and dephosphorylation cycles [123]. Additionally, our data indicate that $EGFR^{vIII}$ signaling may not be associated with slightly elevated kinase activity, but rather its minimally lower sensitivity to phosphatase activity, when compared to wild-type receptor [123]. Moreover, we indicated that enhanced phosphorylation of tyrosine 1045 did not result in $EGFR^{vIII}$ degradation [123], suggesting that the previous model of impaired $EGFR^{vIII}$ degradation requires an update [121]. Nevertheless, without membrane stability, $EGFR^{vIII}$ signaling will not be strong enough to induce a biological effect. So far, the reasons behind the unique $EGFR^{vIII}$ stability have not been fully elucidated. Initially, it was suggested that it is a result of different phosphorylation of tyrosine responsible for interaction with Cbl protein in mutated and wild-type receptors [121]. Additionally, the involvement of FHL2 in $EGFR^{WT}$ and $EGFR^{vIII}$ stabilization was suggested [126]. Stabilization and activity of $EGFR^{vIII}$ are mostly determined by the quaternary structure of the receptor. Long noncoding RNA (lncRNA) EGFR-AS1, an antisense transcript of EGFR, was suggested to be involved in EGFR folding [127], but it is still unknown whether this structure may have a different impact on mutated than wild-type receptor. Nevertheless, gene encoding this lncRNA is located on the same amplicon as EGFR and thus also undergoes amplification [127]. Actually, it is still unknown why $EGFR^{vIII}$ is much more stable than $EGFR^{WT}$; however, such feature of this oncogenic receptor may be considered crucial, as the ability to trigger $EGFR^{vIII}$ degradation, considering its low kinase activity, may deprive this variant of oncogenic properties. Intriguingly, the impact of the dimerization process alone was suggested to be associated with increased $EGFR^{vIII}$ stability, especially since the involvement of the so-called "crypto" domains was described to have an impact on $EGFR^{WT}$ stabilization [128–132].

According to the majority of analyses, $EGFR^{vIII}$ is able to form both hetero- and homodimers [123, 133–140], and in the latter case, covalent and noncovalent dimers can be observed [17, 123]. Our analyses indicate that the most of $EGFR^{vIII}$ monomers are a part of covalent homodimers with covalent bonds formed with the involvement of free cysteine in position 16 of amino acid chain (Figure 2(a)) [123]. Nevertheless, some authors undermine the constitutive activity of the mutant, suggesting that $EGFR^{vIII}$ activity is mostly due to EGF-activated $EGFR^{WT}$ forming a heterodimer with $EGFR^{vIII}$ (Figure 2(b)) [138]. Additionally, it was suggested that only EGF-bound $EGFR^{WT}$ is able to phosphorylate $EGFR^{vIII}$ in a heterodimer, but not vice versa [138]. The way these receptors tend to dimerize is substantial, as molecules inhibiting dimerization may be plausibly used in anticancer therapy. Intriguingly, the possibility of $EGFR^{vIII}$ *cis*-autophosphorylation is very rarely discussed in the literature [135, 141]. Cross-activation of $EGFR^{WT}$ kinases is well recognized as the mechanism crucial for their activation, clearly explaining why receptor dimerization is actually required. Nevertheless, *a priori* rejection of the hypothesis stating that some part of $EGFR^{vIII}$ *cis*-autophosphorylates may be too hasty.

$EGFR^{vIII}$ is also suggested to dimerize with monomers of other inactive receptors (hepatocyte growth factor receptor, HGFR; platelet-derived growth factor receptor, PDGFR) or to have an indirect impact on their function (Figure 2(c)) [142, 143]. Regulation of other receptors may potentially constitute an additional mechanism for $EGFR^{vIII}$-mediated activation of signal transduction pathways, especially in the absence of ligands. This hypothesis was more profoundly tested by Greenall et al., who demonstrated that $EGFR^{vIII}$ is able to activate HGFR via focal adhesion kinase (FAK); however, it was not clearly defined how such activation takes place on a FAK protein platform [139].

All doubts concerning the mechanism of $EGFR^{vIII}$ encourage researchers to search for mechanisms of oncogenic action of this protein that are independent of its membrane

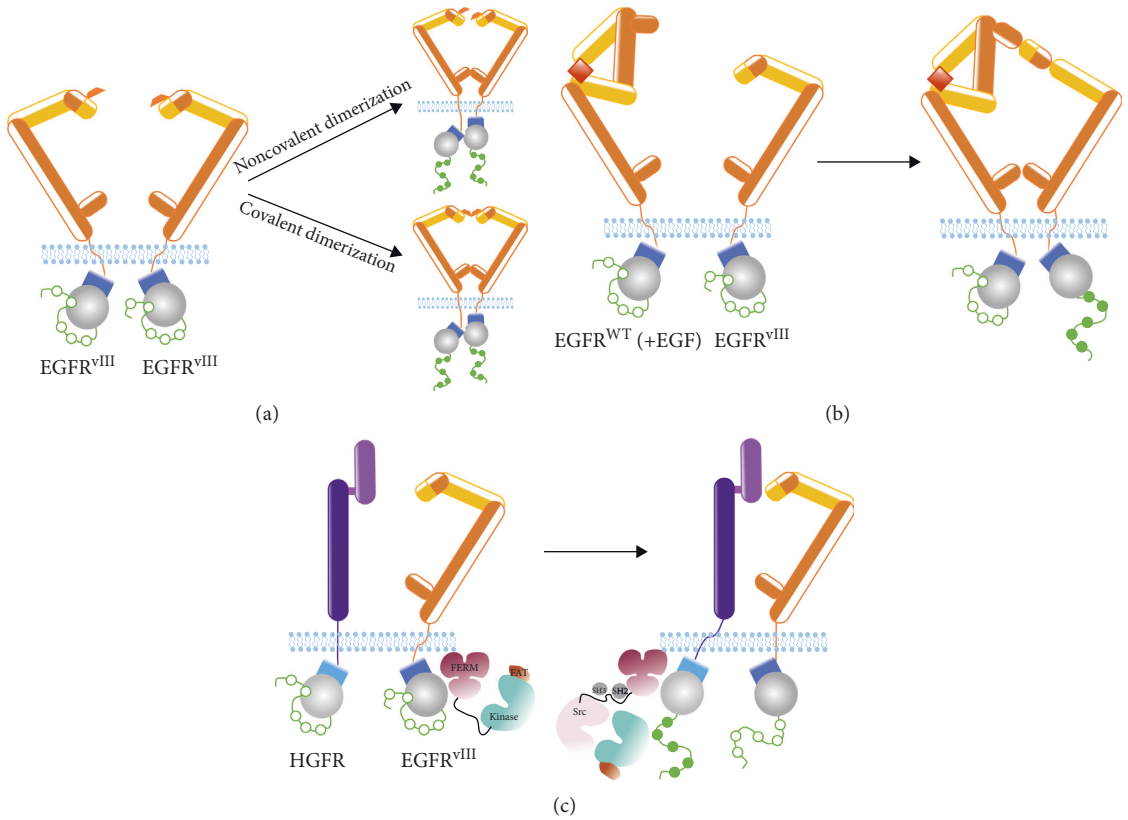

Figure 2: Currently proposed models of EGFR^{vIII} dimerization. (a) Covalently or noncovalently linked EGFR^{vIII} homodimers. In both cases, phosphorylation of tyrosine residues of both monomers can be observed. (b) Heterodimerization of EGFR^{vIII} with ligand-activated (e.g., EGF-activated) EGFR^{WT}. Only EGFR^{vIII} phosphorylation is observed in such a case. (c) EGFR^{vIII} dimers with monomers of other inactive receptors. Example of FAK-mediated EGFR^{vIII} dimerization with HGFR, resulting in phosphorylation of HGFR tyrosine residues.

receptor activities. One of the most interesting analyses is focused on the nuclear role of EGFR^{vIII}, as this function is suggested to be very relevant [144, 145]. Therefore, EGFR^{vIII} interaction with oncostatin M receptor (OSMR) can be considered interesting, as it may be possible to design molecules inhibiting such interaction for therapeutic purposes [146]. In general, results of research conducted so far indicate that the majority of EGFR^{vIII} activity is exhibited outside the nucleus, while its low kinase activity may be compensated by uniquely high stability [17, 121, 123–125, 147–149].

4. Biological Role of EGFR^{vIII}

Despite the fact that cells with high expression of mutated receptor are unable to bind EGFR ligands, these cells are still characterized by increased invasiveness and enhanced proliferation rate when compared to cells with low EGFR^{vIII} expression or EGFR^{vIII}-negative ones [147]. Therefore, it is not only the mechanism of EGFR^{vIII} action, but also biological changes triggered within EGFR^{vIII}-positive cells, as well as the role such cells play in tumor as a whole, that are important when considering the impact of this mutated receptor. This aspect can be especially important, since EGFR^{vIII} expression is not observed in all cells comprising the tumor [33]. Our data suggested that EGFR^{vIII} acts as a classical oncogene, stimulating proliferation and inhibiting apoptosis of glioblastoma cells [147], while other studies indicated much more complicated influence of this oncogene. Considering the impact of EGFR^{vIII} on cells, both autocrine and paracrine effects were investigated. As an example, EGFR^{vIII}-positive cells may secrete leukemia inhibitory factor (LIF) and IL-6 that activate IL-6R/gp130 receptors present on the surface of EGFR^{WT}-positive cells, promoting their proliferation. Moreover, by activation of NF-κB pathway and stimulation of survivin expression, IL-6 may make cells more resistant to apoptosis [150, 151].

EGFR^{vIII} amplicons are present only in part of glioblastoma cells derived from patients and in stable DK-MG cell line (intratumoral heterogeneity). Moreover, only part of these amplicons is active (not epigenetically silenced) and enables expression of the mutated gene [31]. EGFR^{vIII} expression alone is epigenetically controlled, as it was demonstrated that inhibition of histone deacetylation leads to decrease in expression of this oncogenic receptor. It may be explained by the fact that there is a relatively low EGFR^{vIII} expression in tumor parts where high amplification level of this mutated gene is detected [31, 152].

Some researchers suggest that EGFR^{vIII} expression may be present on the surface of brain cancer stem-like cells (bCSCs) that share some similarity with normal neural stem cells (NSCs) [153, 154]. The former cells are characterized by self-renewal potential as well as expression of markers

characteristic for stem cells [155–157]. EGFR^{vIII} is coexpressed with marker characteristic for nondifferentiated cells (CD133 and SOX2) [158, 159], and it is even indicated that this oncogenic receptor may be used to define CSC populations [158]. One can speculate that low kinase activity together with high stability of EGFR^{vIII} is enough to inhibit cell differentiation. Interestingly, it can be also assumed that EGFR^{vIII} epigenetically reprograms cells, depriving them of differentiation potential and, hence, following such process, this mutated receptor may be no longer needed. Brain CSCs are involved in initiation and progression stages of GB, mostly due to their impact on angiogenesis and treatment response [160, 161]. Moreover, presence of bCSCs may hinder long-term maintenance of therapeutic effect, as currently used compounds do not affect these cells, mostly due to very efficient DNA damage repair mechanisms [160, 161]. However, it was demonstrated that usage of bispecific antibodies directed against EGFR^{vIII} and CD133 (CSCs marker) has a cytotoxic effect on bCSCs and impairs their self-renewal abilities [158]. Some researchers suggest that *in vivo* CSCs, but not other cancer cells, are mostly responsible for the process of tumor formation in SCID mice as well as for the propagation of intratumoral heterogeneity [162]. Our results clearly demonstrated SOX2 expression in high percentage of GB cells that, in our opinion, undermines the presence of only a minor stem cell population in glioblastoma tumors [163].

5. Intratumoral Heterogeneity of Glioblastoma in terms of EGFR^{vIII} Expression

The fact that EGFR^{vIII} is not present in all GB cells in tumor mass may complicate the perception of this receptor as a perfect therapeutic target. However, if cells expressing EGFR^{vIII} are cancer stem cells [164] or EGFR^{vIII}-negative cells are somehow dependent on EGFR^{vIII}-positive ones, then discussed targeted therapy may turn out to be effective (Figure 3(a)). Our research indicates that EGFR^{vIII}-negative cells may be indeed dependent on EGFR^{vIII}-positive population. It is supported by the fact that we were unable to establish a subline of DK-MG cell line completely deprived of cell expressing this mutated oncogene, as at least small percentage of EGFR^{vIII}-positive cells was necessary in order to maintain survival and proliferation [33, 147]. On the other hand, at least in 30% of cases, EGFR^{vIII} expression is spontaneously lost in recurrent GB tumors, even when the treatment was not directed against the mutated receptor (Figure 3(b)) [119, 165]. Remarkably, there were also some cases in which EGFR^{vIII} expression was detected only in recurrent GB tumors (Figure 3(c)) [119, 165]. Such observations are of utmost importance, as these enable to evaluate the relevance of EGFR^{vIII} and indirectly cells expressing this mutated receptor, as therapeutic targets. If EGFR^{vIII} is lost (not detected) in recurrent tumors due to the fact that it is present only in a small part of cells and EGFR^{vIII}-negative cells are independent of the activity of this oncogenic variant, it undermines the validity of EGFR^{vIII}-targeting therapies, for example, those based on CAR-T technology [166]. It may be associated with the fact that the expression of some oncogenes, including EGFR^{vIII}, is crucial at earlier stages of neoplastic transformation, but not further during advanced cancer progression. Opinions on the role of EGFR^{vIII} as well as EGFR^{vIII}-positive cells are extremely different, as this oncogene is suggested either to play an insignificant role at the later stages of carcinogenesis, or, on the contrary, to be a marker of GB stem cells (Figures 3(a)–3(c)). Our analyses do not confirm the hypothesis stating that EGFR^{vIII} is irrelevant in fully differentiated GB cells, as DK-MG cells deprived of this oncogene expression lose their proliferation abilities are more prone to apoptosis and unable to give rise to tumors in SCID mice models [147].

Recently, a lot of attention is focused on the ability to transfer extrachromosomal vesicles containing various structures (including DNA amplicons) between cells. Obviously, extrusion of amplicons or decrease in their number during mitoses may lead to generation of cells without amplicons [120]. Simultaneously, amplicons may be transferred to cells initially lacking such structures. Derivation of amplicon-deprived cells from cells with amplicons, as well as "infection" of cells lacking amplicons with these elements of extrachromosomal DNA, is in favor of hypothesis stating that EGFR^{vIII}-positive cells may, in a certain sense, play a role of precursor cells. It clearly emphasizes the biological role of EGFR^{vIII} not at the protein, but DNA level, and it may partially explain why the expression of this mutated protein is in particular cases very low, almost at the detection level of protein analysis methods such as western blot. However, it should not be confused with the role played by so-called cancer stem cells.

The fact that intratumoral heterogeneity may constitute one of the mechanisms responsible for resistance of cancer cells to targeted therapies (including TKIs) was first demonstrated by Nathanson et al. and further confirmed by other research teams. Such a specific adaptation via changing cell phenotypes is mainly focused on achievement of an optimal balance for the unaltered proliferation of the overall population and is mostly due to dynamic regulation of extrachromosomal DNA encoding mutated EGFR [167–169].

6. Targeted Therapies Based on Tyrosine Kinase Inhibitors (TKI), Directed against EGFR^{WT} and EGFR^{vIII}

A wide variety of factors contribute to the fact that glioblastoma is one of the most difficult tumors from a clinical perspective and that effective therapies for patients diagnosed with this tumor type are still lacking. So far, many therapeutic approaches were developed to treat patients with EGFR^{vIII}-positive glioblastoma (Table 1). Generally, average survival rate of GB patients does not exceed 12–14 months from the moment of diagnosis and there has been actually no improvement for many years [170]. Ideal drug directed against GB cells should be well tolerated by the patients, able to cross the blood-brain barrier, and specifically induce tumor cell death. Classical therapeutic regimen in case of GB consists of surgical resection with adjuvant radio- and chemotherapy with alkylating agent temozolomide [171].

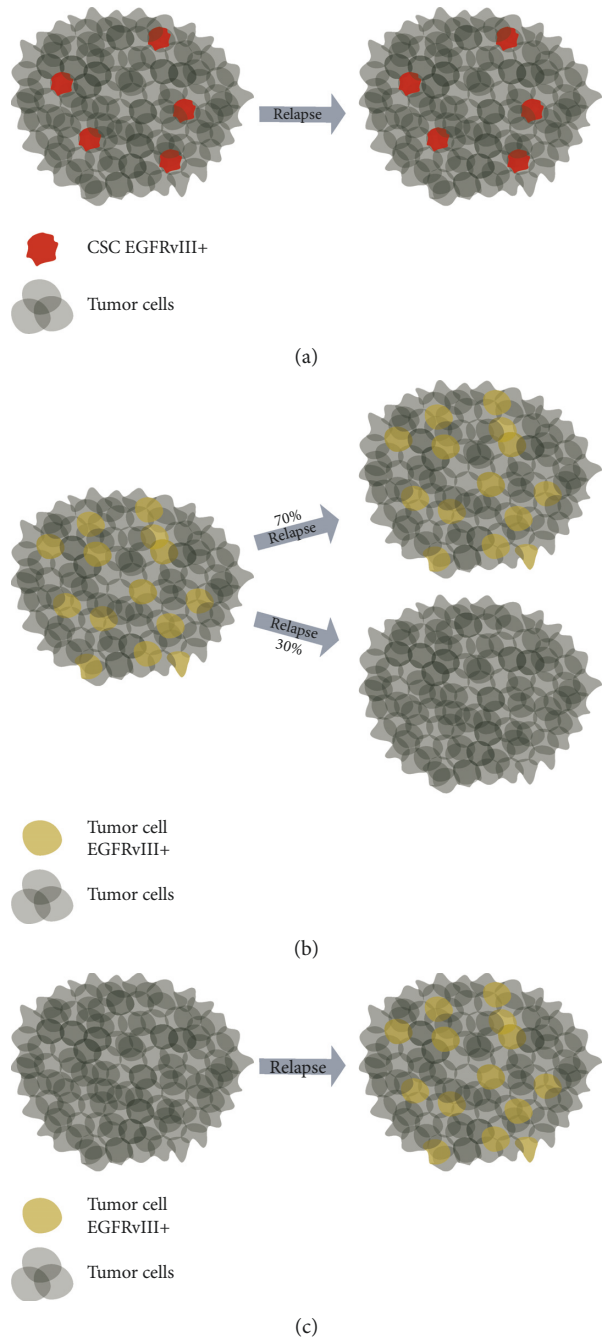

Figure 3: Hypotheses concerning the presence and role of EGFR^{vIII}-positive cells in tumors, on the example of glioblastoma. (a) One of the hypotheses states that EGFR^{vIII} is expressed on the surface of cancer stem cells (CSCs). In such a case, EGFR^{vIII}-positive CSCs should be also detected in recurrent GB tumors [164]. Nevertheless, failure to detect such cells may be due to the exposure of primary tumor to therapeutic compounds. (b) Another hypothesis states that EGFR^{vIII}-positive cells are only crucial during the early stages of carcinogenesis. It is supported by reports demonstrating loss of expression of this mutated oncogene in approx. 30% of patients with EGFR^{vIII}-positive primary tumors [119, 165]. (c) Cells expressing EGFR^{vIII} are also reported in recurrent tumors when primary GB was EGFR^{vIII}-negative [119, 165].

Current clinical and preclinical trials concerning anti-EGFR/EGFR^{vIII} therapies include small molecule tyrosine kinase inhibitors, antibodies, vaccines, as well as therapies based on RNA interference. As silencing of a single gene in a particular signaling pathway may not be sufficient to provide a therapeutic effect in GB patients, there is a need for a complex approach, focusing on several signal transduction pathways [172].

There have been several attempts to experimentally apply EGFR tyrosine kinase inhibitors, also inhibiting EGFR^{vIII}, in glioblastoma therapy, as significant differences between kinase domains of mutated and wild-type receptor have not been described so far. A broad spectrum of anti-EGFR TKIs was developed, with the first-generation inhibitors (gefitinib, lapatinib, and erlotinib) binding reversibly and the second-generation inhibitors (afatinib and dacomitinib) binding

covalently to the receptor [173–175]. Inhibitors of the third generation (rociletinib and osimertinib) covalently bind to ATP-binding site in cells with T790M EGFR mutation, conferring resistance to inhibitors of previous generations [176]. Second-phase clinical trial studies demonstrated that gefitinib, lapatinib, and erlotinib administered to patients with primary or recurrent GB tumors resulted in only marginal therapeutic response, when administered either in monotherapy or in combination [72, 77, 177]. Although osimertinib may be recognized as especially important in terms of $EGFR^{vIII}$, as it was suggested to be efficiently delivered to cancer cells in brain [176], the activity of this compound against $EGFR^{vIII}$-positive cells was lower when compared to afatinib. Since the kinase domain of this splice variant is structurally close to EGFR wild type, this was not unexpected [176].

In case of TKI-based therapy, alterations downstream to $EGFR^{vIII}$, including PTEN mutations, should be taken into account. Despite the fact that inactivating mutations of PTEN have an impact on only one of EGFR-regulated pathways (AKT), it was demonstrated that such mutation is able to hinder the impact of erlotinib on GB cells [178]. Considering this aspect, immunotherapies may possibly outperform small molecule-based approaches. Despite the wide availability of TKIs clinically approved in oncological treatment, none of these inhibitors is used as a standard approach in GB treatment [64, 72, 77, 179]. As $EGFR^{vIII}$ is a key oncogene with kinase activity-dependent function, it seems reasonable to consider whether the efficacy of TKI-based therapies should not be greater, especially since it has been postulated that the blood-brain barrier in advanced GB is disrupted and thus should not enable for crossing of small molecules [180]. It is well established that EGFR-targeting TKIs improve the progression-free survival of patients with EGFR-mutated non-small-cell lung cancers (NSCLCs) [181, 182]. Therefore, the verification whether glioblastoma patients with high frequency of EGFR mutations respond to TKIs is completely justified, even despite different EGFR mutational spectrum. This becomes even more important since Orellana et al. showed that ectodomain EGFR mutations including those leading to $EGFR^{vIII}$ may sensitize tumor cells to tyrosine kinase inhibitors [183]. Reports from *in vitro* studies conducted on $EGFR^{vIII}$-expressing cell lines tend to be contradictory. Some results indicate $EGFR^{vIII}$ sensitivity, while the others demonstrate that $EGFR^{vIII}$, in contrast to $EGFR^{WT}$, appears to be relatively resistant to EGFR-TKIs [87]. By now, several TKI-involving clinical trials on glioblastoma were completed or terminated, however still without any significant patients' benefits [65, 72, 77, 88, 178, 184–188]. Moreover, it was demonstrated that although cetuximab binds to $EGFR^{vIII}$ and decreases expression and leads to overall downregulation of this mutated receptor, it does not inhibit the proliferation of $EGFR^{vIII}$-expressing GB cells and is not effective in GB clinical trials [189–191]. Therefore, it seems that so far neither EGFR-TKIs nor monoclonal antibodies such as cetuximab are effective therapeutic options in glioblastoma patients, irrespective of $EGFR^{vIII}$ occurrence in tumor [177, 188]. Thus, it is difficult to speculate whether $EGFR^{vIII}$ affects EGFR-targeted treatment, as no treatment approach was truly effective in patients, in spite of quite encouraging results from *in vitro* studies. As $EGFR^{vIII}$ presence in other tumor types is highly debatable, there were no clinical trials to investigate the issue of $EGFR^{vIII}$-modulated TKI treatment response in tumors other than glioblastoma. Hence, conclusions from studies on EGFR-TKIs/immunotherapies can only be drawn concerning this particular tumor. Finally, as Orellana et al. recently suggested the high probability that mutated ectodomain of $EGFR^{vIII}$ induces structural changes in the intracellular kinase domain [183], further research focused on detailed understanding of molecular aspects of $EGFR^{vIII}$ should be expected. On the other hand, considering current standard therapeutic GB regimen, $EGFR^{vIII}$ is associated with prolonged survival of GB patients treated with surgery and radio/chemotherapy [192]. It was clearly shown that cases of MGMT-methylated GB with endogenous $EGFR^{vIII}$ expression are significantly more sensitive to temozolomide, than their isogenic $EGFR^{vIII}$ -negative counterparts [193].

7. Immunotherapy in $EGFR^{vIII}$-Positive Tumors

Apart from TKIs, antibodies constitute the most extensively analyzed group of EGFR-targeting compounds; however, their evident efficacy in GB has not been demonstrated so far. High molecular weight may be one of the factors limiting their applicability in treatment of this tumor type [194], but the integrity of blood-brain barrier may be compromised in case of tumors with high level of malignancy [180]. Cetuximab is a chimeric monoclonal IgG1 antibody directed against extracellular domain of EGFR that in clinical studies was demonstrated to exert anticancer effect and increase tumor cell sensitivity to radiotherapy in GB [92]. This molecule was approved by the Food and Drug Administration for the treatment of patients with head and neck cancer and advanced colon cancer. Interestingly, it may be used in case of increased expression of both $EGFR^{WT}$ and $EGFR^{vIII}$ [195, 196], as it was demonstrated that cetuximab may bind to domain III (L2) of $EGFR^{vIII}$ and reduce autophosphorylation of this mutated receptor [197]. Preclinical analyses indicate that following $EGFR^{vIII}$ binding cetuximab induces receptor internalization, resulting in 50% reduction of its active form [197]. Nevertheless, there is a lack of clinical studies evaluating the impact of cetuximab monotherapy on patients with primary glioblastoma [198, 199]. When tested *in vitro* on GB cell lines with EGFR overexpression or using *in vivo* GB models, cetuximab leads to decrease in proliferation rate and enhancement of apoptosis. Additionally, in the latter model, this antibody is able to significantly inhibit tumor growth and increase median survival rate [93]. During analyses conducted using stable cell lines as well as neurospheres, magnetic iron oxide particles (IONPs) were used to increase therapeutic availability of cetuximab and resulted in more effective binding of antibody to GB cells when compared to cetuximab alone, as evaluated by the inhibition of EGFR signaling pathway and increased receptor internalization [200]. There is also an ongoing research on the use of other antibodies in GB therapy, for example, panitumumab (humanized monoclonal IgG2 antibody) or nimotuzumab (humanized monoclonal IgG1 antibody), that are functionally similar to

cetuximab [109, 201]. These antibodies also bind to L2 domain, preventing ligand binding and receptor dimerization [202]. Randomized phase III clinical trials demonstrated that nimotuzumab administration in adult GB patients increases overall survival when compared to standard treatment [109].

In order to achieve higher therapeutic response, it is also possible to conjugate antibodies with other drugs (antibody drug conjugates, ADC). So far, ABT-414 and AMG-595 were developed [114, 203] and the former conjugate was demonstrated to selectively induce apoptosis in cells with $EGFR^{WT}$ overexpression or $EGFR^{vIII}$ expression both *in vitro* and *in vivo* using xenograft models. ABT-414 conjugate consists of ABT-806 monoclonal antibody directed against EGFR and inhibitor of microtubule polymerization—monomethyl auristatin F. Despite the fact that ABT-806 was initially developed to specifically interact with $EGFR^{vIII}$, it also binds to wild-type receptor, however, to a lesser extent [204]. Using xenograft GB models, it was demonstrated that combination of ABT-414 with standard chemo- and radiotherapy resulted in a significant decrease in cell proliferation and overall decrease in tumor growth [114]. Currently, there are ongoing phase I/II clinical trials aimed at evaluating the efficacy of ABT-414 administration in patients with newly diagnosed (NCT02573324) or recurring GB (NCT02343406). Analyses on an orthotopic mouse GB model showed that ind-111-labeled ABT-806 antibody can specifically recognize cancer cells [205].

Nowadays, one of the most promising immunotherapy-based approaches in GB treatment is the usage of autologous T lymphocytes with chimeric antigen receptor—CAR-T cells. These are T lymphocytes that have been modified *ex vivo* and able to recognize their molecular target irrespective of antigen presentation by the molecules of major histocompatibility complex [52, 206]. Structure of CAR-T cells makes them able to exhibit both activity of antibodies and toxicity of T lymphocytes [207]. CAR-T is a technology of interest in research on many cancer types; however, the prerequisite for its efficacy and lack of side effects is antigen expression specifically on cancer cells [208]. As $EGFR^{vIII}$ meets this requirement, it is possible to develop CAR-T recognizing mutated form of the receptor by antigen-specific, humanized single chain of variable fragment of antibody, conjugated with transmembrane and intracellular domains of T lymphocytes and NK cells. Similarly to modified T lymphocytes, NK cells with introduced CAR are able to exhibit cytotoxic activity *in vitro* [209]. CAR-T cells directed against $EGFR^{vIII}$ were able to effectively infiltrate tumor cells in brain in *in vivo* model [53]. Notably, $EGFR^{vIII}$-targeting CAR-T therapies have currently reached phase I of clinical trials to treat GB patients (NCT03283631, NCT02209376). On the other hand, administration of CAR-T therapy in phase I and I/II clinical trials directed against antigens present on both normal and cancer cells (ErbB2, CD19) led to severe side effects and even patients death [210, 211]. It is still not clear whether antibodies designed to recognize $EGFR^{vIII}$ also detect wild-type EGFR [43, 205, 212, 213], but if so, it may lead to some serious side effects following administration of various immunotherapies, including CAR-T approach. Moreover, as glioblastoma tumor is highly heterogeneous, not all GB cells may respond to CAR-T therapy directed against $EGFR^{vIII}$.

Apart from CAR-T technology, there is also an ongoing research on application of another immunotherapy-based approach in GB treatment in a form of bispecific antibodies activating T lymphocytes—bispecific T-cell engagers (BiTEs). BiTEs are recombined immunoglobulins composed of a single chain of variable fragments of two antibodies: one directed against antigen expressed on the surface of T lymphocytes and the second one against the antigen present on target cells [214, 215]. Heavy and light chains of variable antibody fragments are connected with a short, elastic linker, rich in glycine and serine residues. Extracellular $EGFR^{vIII}$ domain is small; hence, it may be efficiently bound by BiTEs [216], and specificity and cytotoxic activity of these molecules against this mutated receptor were demonstrated using *in vitro* and *in vivo* models. Properly designed bispecific antibodies are characterized with very high specificity, resulting in a minimal risk of induction of cross reactions in normal cells [55]. Using BiTEs, it was demonstrated that stimulated regulatory T lymphocytes secrete elevated levels of granzymes and perforins and that their activity is directed against $EGFR^{vIII}$-positive cells [217, 218]. Currently, there is an ongoing phase I clinical trial on administration of AMG 596, drug containing BiTEs directed against $EGFR^{VIII}$ and CD3 surface protein in GB patients (NCT03296696).

It is worth to emphasize that results of our analyses, supported by the data gathered by other research teams, indicate that additional mutations within $EGFR^{vIII}$ that may have an impact on efficacy of antibodies or small molecules directed against $EGFR^{vIII}$-characteristic protein fragments are rarely occurring, but if so, these are distant from $EGFR^{vIII}$-specific parts [219, 220].

Vaccines constitute another therapeutic approach taking advantage of patient's immunological system to destroy $EGFR^{vIII}$-positive cells. So far, only one peptide vaccine, rindopepimut, has been developed to induce humoral response leading to elimination of GB cells expressing mutated EGF receptor [221]. Rindopepimut (CDX-110) is based on 13-amino acid $EGFR^{vIII}$-specific sequence conjugated with keyhole limpet hemocyanin (KLH; hemocyanin neoantigen) adjuvant [222]. *In vivo* preclinical analyses demonstrated that tumor volume significantly decreased in 70% of animals with subcutaneously injected cancer cells following CDX-110 administration, when compared to the control group. It was suggested that antibodies reactive against $EGFR^{vIII}$-KLH are involved in triggering of antibody-dependent cell cytotoxicity (ADCC), regardless of antigen-specific T lymphocytes activity [223]. Median survival rate of GB patients treated with CDX-110 after surgical resection and chemotherapy was prolonged to 24 months, as demonstrated in 3 independent phase II clinical trials (ACTIVATE, ACT II, and ACT III). Moreover, $EGFR^{vIII}$-expressing cells were not detected in 67% of patients receiving CDX-110 treatment for at least 3 months [222]. Nevertheless, phase III clinical trial (ACT IV), comparing the efficacy of temozolomide alone and temozolomide in combination with CDX-

110 in GB, was terminated before the scheduled date, as despite premises from the previous stages of clinical trials, it failed to indicate the significant increase in patient survival (median survival for CDX-110-treated patients was 20.1 months, while in control group 20 months). Still, the researchers emphasized the relevance of research focused on determination of the type of immunological response induced by CDX-110 and highlighted the problem of the selection of the appropriate molecular target for immunotherapy approaches [58].

8. Anti-EGFR^{vIII} Therapy Based on RNA Interference

Concerning the regulation of EGFR^{vIII} expression, emphasis should be also put on noncoding RNAs, especially microRNAs (miRNAs). Aberrant expression of miRNAs has been implicated in various tumor types, including glioblastoma, and demonstrated to impact cancer cell proliferation, EGFR downstream signaling, as well as efficacy of several anti-EGFR-targeting therapeutic approaches. Unfortunately, the majority of data were focused on wild-type receptor and we can only speculate that similar mechanisms apply to EGFR^{vIII}. Decrease in miR-137 level in glioblastoma tissue samples was found to be associated with poor prognosis and, consequently, overexpression of this miRNA in GB models resulted in elevated apoptosis and inhibition of tumor cell growth. It was suggested that miR-137 may act by decreasing translation of EGFR protein, hence decreasing proproliferative activity of this receptor in tumor cells [224]. Similarly, miR-615, miR-1231, or miR-133, also downregulated in glioblastoma, were found to inhibit EGFR levels [225–227]. On the other hand, upregulation of miR-21, often found in glioblastoma patients, promotes EGFR activity and supports tumor growth [228]. Yin et al. showed that miR-34a was often deleted in glioblastoma showing EGFR amplification; however, they did not evaluate the expression of EGFR^{vIII} within analyzed samples. Nevertheless, considering the typical percentage of EGFR^{vIII}-positive GB cases with EGFR amplification, it is very likely that miR-34a deletion coexists with EGFR^{vIII}. Notably, Yin et al. indicated shorter mean survival rate of patients diagnosed with GB with EGFR amplification and miR-34a deletion compared to patients with only one of these alterations [229]. Moreover, EGFR^{vIII}-mediated downstream signaling was found to be associated with inhibition of miR-9 expression, further promoting tumorigenicity in FOXP1-dependent manner [230]. Intriguingly, lncRNA EGFR-AS1 was found to act via miR-133b in regulation of glioblastoma cell migration, invasion, and apoptosis and knockout of this noncoding RNA negatively influenced tumor growth [231].

Besides protein-based therapeutic approaches, research focuses on targeting EGFR^{vIII} at the mRNA level. RNA interference-based therapy relies on usage of ribozymes, antisense oligonucleotides, or siRNA molecules complementary to regions that silencing is beneficial from a clinical point of view [61]. Taking advantage of this technology enables to inhibit activity of EGFR signaling pathways, with relatively low toxicity and maintained high specificity against EGFR^{vIII} [63, 232, 233]. As promising results were obtained in preclinical analyses with antisense oligonucleotides for the treatment of non-small-cell lung carcinoma and prostate cancer [234, 235], possibility to silence EGFR and EGFR^{vIII} gained more attention. Sequence of mRNA nucleotides in junction site between introns 1 and 7 in EGFR^{vIII} is highly specific and absent in any other human genes. Nevertheless, the majority of current literature data concerning siRNA is focused on EGFR in general, without distinguishing normal receptor from mutated one. Constructs with proper antisense RNA sequence were demonstrated to silence expression of mRNA encoding EGFR^{WT}, both *in vitro* on GB cells with EGFR^{WT} expression and *in vivo* on rat GB model. In the former model, significant decrease in level of EGFR mRNA and protein, decrease in proliferation rate, and induction of apoptosis were observed in cells with the expression of introduced construct, while in the latter model all rats with introduced antisense RNA were characterized with prolonged survival rate, when compared to animals with empty construct [236]. Comparison of the construct with antisense RNA complementary to 3′ end and to the whole EGFR^{WT} mRNA encoding region demonstrated that inhibition is more effective in the first case, possibly as delivery of shorter construct may be much easier and efficient [236, 237]. First reports indicate that siRNA complementary to exon 1 and 8 junction site is able to inhibit EGFR^{vIII} expression in human glioma cells, leading to decrease in AKT phosphorylation and inhibition of cell cycle in G2/M [238].

Gene therapy using ribozymes is based on the ability of antisense RNA to catalytically digest mRNA substrate within the specific nucleotide sequence [239]. Low-molecular hairpin-type ribozymes were able to specifically inhibit EGFR expression, as well as proliferation and clonogenicity of GB cells *in vitro* [60, 239]. In terms of gene-editing approaches, it is worth to mention that CRISPR-based technologies have only little chance of being successfully applied in case of EGFR^{vIII}, as deletions within EGFR leading to the formation of this oncogenic variant are quite extensive and tend to differ between patients [240]. It is worth to mention that RNA interference can be achieved by miRNA upregulation. Moreover, one of the miRNAs, miRNA-34a, was demonstrated to enhance the antiproliferative effect of erlotinib [241].

9. In Vitro Models for EGFR^{vIII} Analyses

One of the additional and still unresolved problems regarding development of an effective anti-EGFR^{vIII} therapy is lack of the appropriate *ex vivo*/*in vitro* models reflecting heterogeneity of GB cell genotype and phenotype. Results obtained under *in vitro* conditions often tend to differ significantly from those obtained in clinical trials, as exemplified by results presented above. In primary GB cultures, EGFR^{vIII} expression is quite stable in neurospheres, while in adherent cultures it tends to be lost as soon as after several passages. Analyses of SOX2 expression (marker of neural stem cells and factor crucial to maintain proliferation of GB cells) indicate that neurospheres and adherent cells

Table 2: Issues addressed in the article (except therapies in Table 1).

$EGFR^{vIII}$ issue/process	Mechanism/way to address	Selected references
$EGFR^{vIII}$ presence in tumors/cancers	GB in about 40%, rarely in HNCSCC, lung prostate, colorectal cancer, breast cancer	[27, 33–41, 43]
$EGFR^{vIII}$ mechanism of mutation	Deletion of EGFR exons 2–7	[26–29, 43]
$EGFR^{vIII}$ mechanism of action	Several models: (1) Heterodimerization with $EGFR^{WT}$ (2) Homodimerization (3) $EGFR^{vIII}$ and MET cooperation, FAK involved (4) OSMR mechanism Resistant to degradation important for all models	[16, 17, 121–126, 142, 143]
$EGFR^{vIII}$ biological role	Extreme opinions: from lack of important role at advanced cancer (tumor) stages, to role in self-renewal, survival, and proliferation of cancer stem cells	[33, 147, 150, 152–158, 164, 165]
$EGFR^{vIII}$ cell culture models	3D primary cell cancer cell models, DK-MG model, genetically modified cancer cell lines	[21, 150, 153, 167, 242–244, 246–248]

differ in the state of differentiation—adherent cells gradually lose SOX2, while in spheroids expression of this marker remains at relatively constant level [242]. It is worth to emphasise that the majority of GB cells are SOX2-positive, as it is in contrary to the assumptions that cancer stem cells constitute only so-called side population or, it is possible that SOX2 is a marker not characteristic solely for stem cells [243, 244]. Apart from SOX2, glioblastoma cells also express GFAP, which can be considered quite surprising as GFAP for many years has been considered a marker of mature astrocytes. Nevertheless, GFAP-positive neural stem cells have been described in the literature [245] and these, similar to GB cells, were demonstrated to coexpress many other markers [163, 245].

Despite the fact that spheroid cultures maintain original phenotype of GB cells for a longer period (there is no stable $GFAP^+/SOX2^+$ adherent cell line), this approach is associated with various methodological difficulties. First of all, certain assays on 3D structure may be difficult to be performed. Moreover, cells maintained in medium containing serum are more resistant to the exposure to cytotoxic molecules than neurospheres cultures in serum-free media. Finally, not all primary GB cells are able to form spheroid structures [246]. Basically, *in vitro* culturing should promote survival and proliferation of cancer cells; however, it may lead to spontaneous senescence, mitotic catastrophe, or apoptosis. The occurrence of *in vitro* senescence described to play both pro- and antineoplastic role *in vivo* in primary GB cultures can be plausibly associated with failure in their stabilization [247]. Stable glioblastoma line may not only fail to reflect the heterogeneous nature of tumor cells observed *in vivo*, but also lack extrachromosomal amplicons encoding $EGFR^{vIII}$ [21, 150, 167]. However, the limited amount of tumor material derived from patients and its low stability force scientists to conduct research on commercially available stable cell lines with exogenously introduced $EGFR^{vIII}$-encoding gene [153, 248]. Analyses on such models may be unreliable, as introduction of $EGFR^{vIII}$ cDNA via cell engineering methods may give biased results regarding such aspects as clonality (different results obtained depending on the analyzed clone) or neglect the dynamic regulation of amplicons released from EVs. Additionally, exogenously introduced $EGFR^{vIII}$ may not have an impact on the biology of already fully defined cancer cells, such as U87-MG cell line [147]. Therefore, biological differences observed between U87-MG clones may be easily confused and taken as the effect of $EGFR^{vIII}$ action. Hence, there is an ongoing search for the most appropriate model, reflecting nature of GB cells as precisely as possible.

10. Summary

Table 2 presents most important issues addressed in the article (except therapies in Table 1). $EGFR^{vIII}$ protein may be considered a suitable target in 28–30% of GB cases, as it is selectively expressed on cancer cells and structurally differs from wild-type receptor. Nevertheless, opinions on the role of $EGFR^{vIII}$ in GB biology are contradictory. This mutated receptor seems to play a key role in tumor cells, enhancing their proliferation, inhibiting apoptosis, or being considered a marker of CSCs. On the other hand, it is suggested that $EGFR^{vIII}$ is unnecessary for GB cells, especially at advanced stages of tumorigenesis, that may be considered a drawback in terms of therapeutic approaches directed against this mutated receptor. Despite many years of extensive research, $EGFR^{vIII}$-specific inhibitors have not been developed yet. There are also many controversies regarding antibodies designed to specifically detect this oncogenic variant, which in turn may be negatively correlated with the efficacy of CAR-T and other immunotherapy-based approaches. Many factors hinder glioblastoma treatment, including heterogeneity of $EGFR^{WT}/EGFR^{vIII}$ expression, the impact of receptor signaling on various cellular processes, mechanisms of cells resistance to treatment, or the presence of cancer stem cell populations. Undoubtedly, anti-$EGFR^{vIII}$ therapies constitute the important area of research, but the structure, mechanism of action, and the biological role of $EGFR^{vIII}$ need to be determined for their proper development. In particular, it is crucial to resolve whether $EGFR^{vIII}$-negative glioblastoma cells are dependent on $EGFR^{vIII}$-positive population or not.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

[1] E. Tzahar, H. Waterman, X. Chen et al., "A hierarchical network of interreceptor interactions determines signal transduction by neu differentiation factor/neuregulin and epidermal growth factor," *Molecular and Cellular Biology*, vol. 16, no. 10, pp. 5276–5287, 1996.

[2] I. R. Weingaertner, S. Koutnik, and H. Ammer, "Chronic morphine treatment attenuates cell growth of human BT474 breast cancer cells by rearrangement of the ErbB signalling network," *PLoS One*, vol. 8, no. 1, Article ID e53510, 2013.

[3] R. Harris, E. Chung, and R. J. Coffey, "EGF receptor ligands," *Experimental Cell Research*, vol. 284, no. 1, pp. 2–13, 2003.

[4] X. Yu, K. D. Sharma, T. Takahashi, R. Iwamoto, and E. Mekada, "Ligand-independent dimer formation of epidermal growth factor receptor (EGFR) is a step separable from ligand-induced EGFR signaling," *Molecular Biology of the Cell*, vol. 13, no. 7, pp. 2547–2557, 2002.

[5] N. J. Bessman, A. Bagchi, K. M. Ferguson, and M. A. Lemmon, "Complex relationship between ligand binding and dimerization in the epidermal growth factor receptor," *Cell Reports*, vol. 9, no. 4, pp. 1306–1317, 2014.

[6] M. C. Mendoza, E. E. Er, and J. Blenis, "The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation," *Trends in Biochemical Sciences*, vol. 36, no. 6, pp. 320–328, 2011.

[7] S. Jones and J. Z. Rappoport, "Interdependent epidermal growth factor receptor signalling and trafficking," *The International Journal of Biochemistry & Cell Biology*, vol. 51, pp. 23–28, 2014.

[8] C. Treda, M. Popeda, M. Ksiazkiewicz et al., "EGFR activation leads to cell death independent of PI3K/AKT/mTOR in an AD293 cell line," *PLoS One*, vol. 11, no. 5, Article ID e0155230, 2016.

[9] J. A. J. M. van de Water, T. Bagci-Onder, A. S. Agarwal et al., "Therapeutic stem cells expressing variants of EGFR-specific nanobodies have antitumor effects," *Proceedings of the National Academy of Sciences*, vol. 109, no. 41, pp. 16642–16647, 2012.

[10] S. Sigismund, E. Argenzio, D. Tosoni, E. Cavallaro, S. Polo, and P. P. Di Fiore, "Clathrin-mediated internalization is essential for sustained EGFR signaling but dispensable for degradation," *Developmental Cell*, vol. 15, no. 2, pp. 209–219, 2008.

[11] J. U. Kang, "Characterization of amplification patterns and target genes on the short arm of chromosome 7 in early-stage lung adenocarcinoma," *Molecular Medicine Reports*, vol. 8, no. 5, pp. 1373–1378, 2013.

[12] K.-I. Sato, "Cellular functions regulated by phosphorylation of EGFR on Tyr845," *International Journal of Molecular Sciences*, vol. 14, no. 6, pp. 10761–10790, 2013.

[13] K. Omidfar and Z. Shirvani, "Single domain antibodies: a new concept for epidermal growth factor receptor and $EGFR^{vIII}$ targeting," *DNA and Cell Biology*, vol. 31, no. 6, pp. 1015–1026, 2012.

[14] R. A. Bradshaw, R. J. Chalkley, J. Biarc, and A. L. Burlingame, "Receptor tyrosine kinase signaling mechanisms: devolving TrkA responses with phosphoproteomics," *Advances in Biological Regulation*, vol. 53, no. 1, pp. 87–96, 2013.

[15] J. Tong, P. Taylor, E. Jovceva et al., "Tandem immunoprecipitation of phosphotyrosine-mass spectrometry (TIPY-MS) indicates C19ORF19 becomes tyrosine-phosphorylated and associated with activated epidermal growth factor receptor," *Journal of Proteome Research*, vol. 7, no. 3, pp. 1067–1077, 2008.

[16] E. Purba, E.-I. Saita, and I. Maruyama, "Activation of the EGF receptor by ligand binding and oncogenic mutations: the "rotation model"," *Cells*, vol. 6, no. 2, p. 13, 2017.

[17] H.-J. S. Huang, M. Nagane, C. K. Klingbeil et al., "The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling," *Journal of Biological Chemistry*, vol. 272, no. 5, pp. 2927–2935, 1997.

[18] K. Shtiegman, B. S. Kochupurakkal, Y. Zwang et al., "Defective ubiquitinylation of EGFR mutants of lung cancer confers prolonged signaling," *Oncogene*, vol. 26, no. 49, pp. 6968–6978, 2007.

[19] V. Sangar, C. C. Funk, U. Kusebauch, D. S. Campbell, R. L. Moritz, and N. D. Price, "Quantitative proteomic analysis reveals effects of Epidermal Growth Factor Receptor (EGFR) on invasion-promoting proteins secreted by glioblastoma cells," *Molecular & Cellular Proteomics*, vol. 13, no. 10, pp. 2618–2631, 2014.

[20] M. Nagane, F. Coufal, H. Lin, O. Bögler, W. K. Cavenee, and H. J. S. Huang, "A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis," *Cancer Research*, vol. 56, pp. 5079–5086, 1996.

[21] J. M. Francis, C.-Z. Zhang, C. L. Maire et al., "EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing," *Cancer Discovery*, vol. 4, no. 8, pp. 956–971, 2014.

[22] A. J. Wong, J. M. Ruppert, S. H. Bigner et al., "Structural alterations of the epidermal growth factor receptor gene in human gliomas," *Proceedings of the National Academy of Sciences*, vol. 89, no. 7, pp. 2965–2969, 1992.

[23] A. Guillaudeau, K. Durand, B. Bessette et al., "EGFR soluble isoforms and their transcripts are expressed in meningiomas," *PLoS One*, vol. 7, no. 5, Article ID e37204, 2012.

[24] J. Cho, S. Pastorino, Q. Zeng et al., "Glioblastoma-derived epidermal growth factor receptor carboxyl-terminal deletion mutants are transforming and are sensitive to EGFR-directed therapies," *Cancer Research*, vol. 71, no. 24, pp. 7587–7596, 2011.

[25] I. Okamoto, L. C. Kenyon, D. R. Emlet et al., "Expression of constitutively activated $EGFR^{vIII}$ in non-small cell lung cancer," *Cancer Science*, vol. 94, no. 1, pp. 50–56, 2003.

[26] B. R. Voldborg, L. Damstrup, M. Spang-Thomsen, and H. S. Poulsen, "Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials," *Annals of Oncology*, vol. 8, no. 12, pp. 1197–1206, 1997.

[27] C. J. Wikstrand, L. P. Hale, S. K. Batra et al., "Monoclonal antibodies against $EGFR^{vIII}$ are tumor specific and react with breast and lung carcinomas and malignant gliomas," *Cancer Research*, vol. 55, pp. 3140–3148, 1995.

[28] H. Yamazaki, Y. Ohba, N. Tamaoki, and M. Shibuya, "A deletion mutation within the ligand binding domain is responsible for activation of epidermal growth factor receptor gene in human brain tumors," *Japanese Journal of Cancer Research*, vol. 81, no. 8, pp. 773–779, 1990.

[29] P. A. Humphrey, A. J. Wong, B. Vogelstein et al., "Amplification and expression of the epidermal growth factor receptor gene in human glioma xenografts," *Cancer Research*, vol. 48, pp. 2231–2238, 1988.

[30] C. Abou-Fayçal, A. S. Hatat, S. Gazzeri, and B. Eymin, "Splice variants of the RTK family: their role in tumour progression and response to targeted therapy," *International Journal of Molecular Sciences*, vol. 18, no. 2, 2017.

[31] C. A. Del Vecchio, C. P. Giacomini, H. Vogel et al., "EGFR^{vIII} gene rearrangement is an early event in glioblastoma tumorigenesis and expression defines a hierarchy modulated by epigenetic mechanisms," *Oncogene*, vol. 32, no. 21, pp. 2670–2681, 2013.

[32] M. Snuderl, L. Fazlollahi, L. P. Le et al., "Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma," *Cancer Cell*, vol. 20, no. 6, pp. 810–817, 2011.

[33] J. Peciak, W. J. Stec, C. Treda et al., "Low incidence along with low mRNA levels of EGFR^{vIII} in prostate and colorectal cancers compared to glioblastoma," *Journal of Cancer*, vol. 8, no. 1, pp. 146–151, 2017.

[34] D. K. Moscatello, M. Holgado-Madruga, A. K. Godwin et al., "Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors," *Cancer Research*, vol. 55, pp. 5536–5539, 1995.

[35] E. O. Olapade-Olaopa, D. K. Moscatello, E. H. MacKay et al., "Evidence for the differential expression of a variant EGF receptor protein in human prostate cancer," *British Journal of Cancer*, vol. 82, no. 1, pp. 186–194, 2000.

[36] J. M. Rae, J. O. Scheys, K. M. Clark, R. B. Chadwick, M. C. Kiefer, and M. E. Lippman, "EGFR and EGFR^{vIII} expression in primary breast cancer and cell lines," *Breast Cancer Research and Treatment*, vol. 87, no. 1, pp. 87–95, 2004.

[37] J. C. Sok, F. M. Coppelli, S. M. Thomas et al., "Mutant epidermal growth factor receptor (EGFR^{vIII}) contributes to head and neck cancer growth and resistance to EGFR targeting," *Clinical Cancer Research*, vol. 12, no. 17, pp. 5064–5073, 2006.

[38] N. G. Chau, B. Perez-Ordonez, K. Zhang et al., "The association between EGFR variant III, HPV, p16, c-MET, EGFR gene copy number and response to EGFR inhibitors in patients with recurrent or metastatic squamous cell carcinoma of the head and neck," *Head & Neck Oncology*, vol. 3, no. 1, p. 11, 2011.

[39] H. Ohgaki and P. Kleihues, "Genetic pathways to primary and secondary glioblastoma," *The American Journal of Pathology*, vol. 170, no. 5, pp. 1445–1453, 2007.

[40] S. Saikali, T. Avril, B. Collet et al., "Expression of nine tumour antigens in a series of human glioblastoma multiforme: interest of EGFR^{vIII}, IL-13Rα2, gp100 and TRP-2 for immunotherapy," *Journal of Neuro-Oncology*, vol. 81, no. 2, pp. 139–148, 2006.

[41] M. Azuma, K. D. Danenberg, S. Iqbal et al., "Epidermal growth factor receptor and epidermal growth factor receptor variant III gene expression in metastatic colorectal cancer," *Clinical Colorectal Cancer*, vol. 6, no. 3, pp. 214–218, 2006.

[42] K. D. Steffensen, M. Waldstrom, D. A. Olsen et al., "Mutant epidermal growth factor receptor in benign, borderline, and malignant ovarian tumors," *Clinical Cancer Research*, vol. 14, no. 11, pp. 3278–3282, 2008.

[43] A. A. Jungbluth, E. Stockert, H. J. S. Huang et al., "A monoclonal antibody recognizing human cancers with amplification/overexpression of the human epidermal growth factor receptor," *Proceedings of the National Academy of Sciences*, vol. 100, no. 2, pp. 639–644, 2003.

[44] P. de Graeff, A. P. G. Crijns, K. A. Ten Hoor et al., "The ErbB signalling pathway: protein expression and prognostic value in epithelial ovarian cancer," *British Journal of Cancer*, vol. 99, no. 2, pp. 341–349, 2008.

[45] K.-L. G. Spindler, D. A. Olsen, J. N. Nielsen et al., "Lack of the type III epidermal growth factor receptor mutation in colorectal cancer," *Anticancer Research*, vol. 26, pp. 4889–4893, 2006.

[46] M. P. Cunningham, S. Essapen, H. Thomas et al., "Coexpression, prognostic significance and predictive value of EGFR, EGFR^{vIII} and phosphorylated EGFR in colorectal cancer," *International Journal of Oncology*, vol. 27, pp. 317–325, 2005.

[47] K. N. Blehm, P. E. Spiess, J. E. Bondaruk et al., "Mutations within the kinase domain and truncations of the epidermal growth factor receptor are rare events in bladder cancer: implications for therapy," *Clinical Cancer Research*, vol. 12, no. 15, pp. 4671–4677, 2006.

[48] H. Ge, X. Gong, and C. K. Tang, "Evidence of high incidence of EGFR^{vIII} expression and coexpression with EGFR in human invasive breast cancer by laser capture microdissection and immunohistochemical analysis," *International Journal of Cancer*, vol. 98, no. 3, pp. 357–361, 2002.

[49] Y. Nieto, F. Nawaz, R. B. Jones, E. J. Shpall, and S. Nawaz, "Prognostic significance of overexpression and phosphorylation of epidermal growth factor receptor (EGFR) and the presence of truncated EGFR^{vIII} in locoregionally advanced breast cancer," *Journal of Clinical Oncology*, vol. 25, no. 28, pp. 4405–4413, 2007.

[50] N. Tidow, A. Boecker, H. Schmidt et al., "Distinct amplification of an untranslated regulatory sequence in the EGFR gene contributes to early steps in breast cancer development," *Cancer Research*, vol. 63, pp. 1172–1178, 2003.

[51] M. Rosenthal, R. Curry, D. A. Reardon et al., "Safety, tolerability, and pharmacokinetics of anti-EGFR^{vIII} antibody-drug conjugate AMG 595 in patients with recurrent malignant glioma expressing EGFR^{vIII}," *Cancer Chemotherapy and Pharmacology*, vol. 84, no. 2, pp. 327–336, 2019.

[52] D. M. O'Rourke, M. P. Nasrallah, A. Desai et al., "A single dose of peripherally infused EGFR^{vIII}-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma," *Science Translational Medicine*, vol. 9, no. 399, 2017.

[53] H. Miao, B. D. Choi, C. M. Suryadevara et al., "EGFR^{vIII}-specific chimeric antigen receptor T cells migrate to and kill tumor deposits infiltrating the brain parenchyma in an invasive xenograft model of glioblastoma," *PLoS One*, vol. 9, no. 4, Article ID e94281, 2014.

[54] Z. Zhang, J. Jiang, X. Wu et al., "Chimeric antigen receptor T cell targeting EGFR^{vIII} for metastatic lung cancer therapy," *Frontiers of Medicine*, vol. 13, no. 1, pp. 57–68, 2019.

[55] B. D. Choi, C.-T. Kuan, M. Cai et al., "Systemic administration of a bispecific antibody targeting EGFR^{vIII} successfully treats intracerebral glioma," *Proceedings of the National Academy of Sciences*, vol. 110, no. 1, pp. 270–275, 2013.

[56] K. Ellwanger, U. Reusch, I. Fucek et al., "Highly specific and effective targeting of EGFR^{vIII}-positive tumors with TandAb antibodies," *Frontiers in Oncology*, vol. 7, p. 100, 2017.

[57] Study of AMG 596 in patients with EGFR^{vIII} positive glioblastoma-full text view-ClinicalTrials.gov, 2017, https://clinicaltrials.gov/ct2/show/NCT03296696.

[58] M. Weller, N. Butowski, D. D. Tran et al., "Rindopepimut with temozolomide for patients with newly diagnosed, EGFR^{vIII}-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial," *The Lancet Oncology*, vol. 18, pp. 1373–1385, 2017.

[59] X. Luo, X. Gong, and C. K. Tang, "Suppression of EGFR^{vIII}-mediated proliferation and tumorigenesis of breast cancer cells by ribozyme," *International Journal of Cancer*, vol. 104, no. 6, pp. 716–721, 2003.

[60] M.-E. Halatsch, U. Schmidt, I. C. Bötefür, J. F. Holland, and T. Ohnuma, "Marked inhibition of glioblastoma target cell tumorigenicity in vitro by retrovirus-mediated transfer of a hairpin ribozyme against deletion-mutant epidermal growth factor messenger RNA," *Journal of Neurosurgery*, vol. 92, no. 2, pp. 297–305, 2000.

[61] C.-S. Kang, Z.-Y. Zhang, Z.-F. Jia et al., "Suppression of EGFR expression by antisense or small interference RNA inhibits U251 glioma cell growth in vitro and in vivo," *Cancer Gene Therapy*, vol. 13, no. 5, pp. 530–538, 2006.

[62] A. Shir and A. Levitzki, "Inhibition of glioma growth by tumor-specific activation of double-stranded RNA-dependent protein kinase PKR," *Nature Biotechnology*, vol. 20, no. 9, pp. 895–900, 2002.

[63] F. Yamoutpour, V. Bodempudi, S. E. Park et al., "Gene silencing for epidermal growth factor receptor variant III induces cell-specific cytotoxicity," *Molecular Cancer Therapeutics*, vol. 7, no. 11, pp. 3586–3597, 2008.

[64] E. Franceschi, G. Cavallo, S. Lonardi et al., "Gefitinib in patients with progressive high-grade gliomas: a multicentre phase II study by Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO)," *British Journal of Cancer*, vol. 96, no. 7, pp. 1047–1051, 2007.

[65] J. N. Rich, D. A. Reardon, T. Peery et al., "Phase II trial of gefitinib in recurrent glioblastoma," *Journal of Clinical Oncology*, vol. 22, no. 1, pp. 133–142, 2004.

[66] E. H. A. Sim, I. A. Yang, R. Wood-Baker, R. V. Bowman, and K. M. Fong, "Gefitinib for advanced non-small cell lung cancer," *Cochrane Database of Systematic Reviews*, vol. 2018, 2018.

[67] H. Zhao, Y. Fan, S. Ma et al., "Final overall survival results from a phase III, randomized, placebo-controlled, parallel-group study of gefitinib versus placebo as maintenance therapy in patients with locally advanced or metastatic non-small-cell lung cancer (INFORM; C-TONG 0804)," *Journal of Thoracic Oncology*, vol. 10, no. 4, pp. 655–664, 2015.

[68] J. A. Jakob, M. S. Kies, B. S. Glisson et al., "Phase II study of gefitinib in patients with advanced salivary gland cancers," *Head & Neck*, vol. 37, no. 5, pp. 644–649, 2015.

[69] A. Kalykaki, S. Agelaki, G. Kallergi, A. Xyrafas, D. Mavroudis, and V. Georgoulias, "Elimination of EGFR-expressing circulating tumor cells in patients with metastatic breast cancer treated with gefitinib," *Cancer Chemotherapy and Pharmacology*, vol. 73, no. 4, pp. 685–693, 2014.

[70] Topotecan and gefitinib (iressa) for ovarian, peritoneal, or fallopian tube cancer-full text view-ClinicalTrials.gov, 2019, https://clinicaltrials.gov/ct2/show/NCT00317772?recrs=abdef&cond=gefitinib&draw=2&rank=21.

[71] Adjuvant therapy of gefitinib (iressa, ZD1839) in patients with resectable hepatocellular carcinoma-full text view-ClinicalTrials.gov, 2019, https://clinicaltrials.gov/ct2/show/NCT00282100?recrs=abdef&cond=gefitinib&draw=2&rank=70.

[72] B. Thiessen, C. Stewart, M. Tsao et al., "A phase I/II trial of GW572016 (lapatinib) in recurrent glioblastoma multiforme: clinical outcomes, pharmacokinetics and molecular correlation," *Cancer Chemotherapy and Pharmacology*, vol. 65, no. 2, pp. 353–361, 2010.

[73] Lapatinib with temozolomide and regional radiation therapy for patients with newly-diagnosed glioblastoma multiforme-full text view-ClinicalTrials.gov, 2019, https://clinicaltrials.gov/ct2/show/NCT01591577?term=lapatinib&cond=glioblastoma&rank=1.

[74] Q. Ryan, A. Ibrahim, M. H. Cohen et al., "FDA drug approval summary: lapatinib in combination with capecitabine for previously treated metastatic breast cancer that overexpresses HER-2," *The Oncologist*, vol. 13, no. 10, pp. 1114–1119, 2008.

[75] S. Iqbal, B. Goldman, C. M. Fenoglio-Preiser et al., "Southwest Oncology Group study S0413: a phase II trial of lapatinib (GW572016) as first-line therapy in patients with advanced or metastatic gastric cancer," *Annals of Oncology*, vol. 22, no. 12, pp. 2610–2615, 2011.

[76] Study of trastuzumab-emtansine in patients with HER2-positive metastatic colorectal cancer progressing after trastuzumab and lapatinib.-full text view-ClinicalTrials.gov, 2019, https://clinicaltrials.gov/ct2/show/study/NCT03418558?recrs=abdfh&cond=lapatinib&draw=4.

[77] M. J. van den Bent, A. A. Brandes, R. Rampling et al., "Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034," *Journal of Clinical Oncology*, vol. 27, no. 8, pp. 1268–1274, 2009.

[78] J. J. Raizer, L. E. Abrey, A. B. Lassman et al., "A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy," *Neuro-Oncology*, vol. 12, no. 1, pp. 95–103, 2010.

[79] N. S. Horowitz, A. B. Olawaiye, D. R. Borger et al., "Phase II trial of erlotinib in women with squamous cell carcinoma of the vulva," *Gynecologic Oncology*, vol. 127, no. 1, pp. 141–146, 2012.

[80] Y. Wang, G. Schmid-Bindert, and C. Zhou, "Erlotinib in the treatment of advanced non-small cell lung cancer: an update for clinicians," *Therapeutic Advances in Medical Oncology*, vol. 4, no. 1, pp. 19–29, 2012.

[81] S. Cicènas, S. L. Geater, P. Petrov et al., "Maintenance erlotinib versus erlotinib at disease progression in patients with advanced non-small-cell lung cancer who have not progressed following platinum-based chemotherapy (IUNO study)," *Lung Cancer*, vol. 102, pp. 30–37, 2016.

[82] M. J. Moore, D. Goldstein, J. Hamm et al., "Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group," *Journal of Clinical Oncology*, vol. 25, no. 15, pp. 1960–1966, 2007.

[83] W. N. William, A. S. Tsao, L. Feng et al., "Single arm, phase II study of cisplatin, docetaxel, and erlotinib in patients with recurrent and/or metastatic head and neck squamous cell carcinomas," *The Oncologist*, vol. 23, no. 5, pp. 526–e49, 2018.

[84] J. E. Bauman, U. Duvvuri, W. E. Gooding et al., "Randomized, placebo-controlled window trial of EGFR, Src, or

combined blockade in head and neck cancer," *JCI Insight*, vol. 2, 2017.

[85] FDA Broadens Afatinib Indication to Previously Untreated, Metastatic NSCLC with Other Non-resistant EGFR Mutations, 2019, https://www.fda.gov/drugs/resources-information-approved-drugs/fda-broadens-afatinib-indication-previously-untreated-metastatic-nsclc-other-non-resistant-egfr.

[86] E. E. W. Cohen, L. F. Licitra, B. Burtness et al., "Biomarkers predict enhanced clinical outcomes with afatinib versus methotrexate in patients with second-line recurrent and/or metastatic head and neck cancer," *Annals of Oncology*, vol. 28, no. 10, pp. 2526–2532, 2017.

[87] R. Vengoji, M. A. Macha, R. K. Nimmakayala et al., "Afatinib and temozolomide combination inhibits tumorigenesis by targeting $EGFR^{vIII}$-cMet signaling in glioblastoma cells," *Journal of Experimental & Clinical Cancer Research*, vol. 38, no. 1, 2019.

[88] D. A. Reardon, L. B. Nabors, W. P. Mason et al., "Phase I/randomized phase II study of afatinib, an irreversible ErbB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma," *Neuro Oncol*, vol. 17, pp. 430–439, 2015.

[89] G. Goh, R. Schmid, K. Guiver et al., "Clonal evolutionary analysis during HER2 blockade in HER2-positive inflammatory breast cancer: a phase II open-label clinical trial of afatinib ± vinorelbine," *PLoS Medicine*, vol. 13, 2016.

[90] T. Hickish, J. Cassidy, D. Propper et al., "A randomised, open-label phase II trial of afatinib versus cetuximab in patients with metastatic colorectal cancer," *European Journal of Cancer*, vol. 50, no. 18, pp. 3136–3144, 2014.

[91] J. A. Bonner, P. M. Harari, J. Giralt et al., "Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck," *New England Journal of Medicine*, vol. 354, no. 6, pp. 567–578, 2006.

[92] J. L. Eller, S. L. Longo, M. M. Kyle, D. Bassano, D. J. Hicklin, and G. W. Canute, "Anti-epidermal growth factor receptor monoclonal antibody cetuximab augments radiation effects in glioblastoma multiforme in vitro and in vivo," *Neurosurgery*, vol. 56, no. 1, pp. 155–162, 2005.

[93] J. L. Eller, S. L. Longo, D. J. Hicklin, and G. W. Canute, "Activity of anti-epidermal growth factor receptor monoclonal antibody C225 against glioblastoma multiforme," *Neurosurgery*, vol. 51, pp. 1004-1005, 2002.

[94] B. Hasselbalch, U. Lassen, S. Hansen et al., "Cetuximab, bevacizumab, and irinotecan for patients with primary glioblastoma and progression after radiation therapy and temozolomide: a phase II trial," *Neuro Oncology*, vol. 12, pp. 508–516, 2010.

[95] T. K. Guren, M. Thomsen, E. H. Kure et al., "Cetuximab in treatment of metastatic colorectal cancer: final survival analyses and extended RAS data from the NORDIC-VII study," *British Journal of Cancer*, vol. 116, no. 10, pp. 1271–1278, 2017.

[96] J. A. Chan, L. S. Blaszkowsky, P. C. Enzinger et al., "A multicenter phase II trial of single-agent cetuximab in advanced esophageal and gastric adenocarcinoma," *Annals of Oncology*, vol. 22, no. 6, pp. 1367–1373, 2011.

[97] A. Jatoi, S. E. Schild, N. Foster et al., "A phase II study of cetuximab and radiation in elderly and/or poor performance status patients with locally advanced non-small-cell lung cancer (N0422)," *Annals of Oncology*, vol. 21, no. 10, pp. 2040–2044, 2010.

[98] L. A. Carey, H. S. Rugo, P. K. Marcom et al., "TBCRC 001: randomized phase II study of cetuximab in combination with carboplatin in stage IV triple-negative breast cancer," *Journal of Clinical Oncology*, vol. 30, no. 21, pp. 2615–2623, 2012.

[99] M. T. Fleming, G. Sonpavde, M. Kolodziej et al., "Association of rash with outcomes in a randomized phase II trial evaluating cetuximab in combination with mitoxantrone plus prednisone after docetaxel for metastatic castration-resistant prostate cancer," *Clinical Genitourinary Cancer*, vol. 10, no. 1, pp. 6–14, 2012.

[100] J. Farley, M. W. Sill, M. Birrer et al., "Phase II study of cisplatin plus cetuximab in advanced, recurrent, and previously treated cancers of the cervix and evaluation of epidermal growth factor receptor immunohistochemical expression: a gynecologic oncology group study," *Gynecologic Oncology*, vol. 121, no. 2, pp. 303–308, 2011.

[101] J. Weber and P. L. McCormack, "Panitumumab," *BioDrugs*, vol. 22, no. 6, pp. 403–411, 2008.

[102] N. Asimakopoulou, J. Souglakos, N. Kentepozidis et al., "Efficacy of panitumumab in older patients with metastatic colorectal cancer: a retrospective analysis using the database of the hellenic oncology research group (HORG)," *Journal of Geriatric Oncology*, vol. 10, no. 1, pp. 143–148, 2019.

[103] D. P. S. Sohal, K. Mykulowycz, T. Uehara et al., "A phase II trial of gemcitabine, irinotecan and panitumumab in advanced cholangiocarcinoma," *Annals of Oncology*, vol. 24, no. 12, pp. 3061–3065, 2013.

[104] R. Mesía, M. Henke, A. Fortin et al., "Chemoradiotherapy with or without panitumumab in patients with unresected, locally advanced squamous-cell carcinoma of the head and neck (CONCERT-1): a randomised, controlled, open-label phase 2 trial," *The Lancet Oncology*, vol. 16, no. 2, pp. 208–220, 2015.

[105] D. Rischin, D. R. Spigel, D. Adkins et al., "PRISM: phase 2 trial with panitumumab monotherapy as second-line treatment in patients with recurrent or metastatic squamous cell carcinoma of the head and neck," *Head & Neck*, vol. 38, no. S1, pp. E1756–E1761, 2016.

[106] V. Pillay, L. Allaf, A. L. Wilding et al., "The plasticity of oncogene addiction: implications for targeted therapies directed to receptor tyrosine kinases," *Neoplasia*, vol. 11, no. 5, pp. 448–IN2, 2009.

[107] Panitumumab and Irinotecan for Malignant Gliomas-Full Text View-ClinicalTrials.Gov, 2019, https://clinicaltrials.gov/ct2/show/NCT01017653?cond=panitumumab+glioblastoma-&draw=2&rank=2.

[108] N. Matsuda, X. Wang, B. Lim et al., "Safety and efficacy of panitumumab plus neoadjuvant chemotherapy in patients with primary HER2-negative inflammatory breast cancer," *JAMA Oncology*, vol. 4, no. 9, pp. 1207–1213, 2018.

[109] M. Westphal, O. Heese, J. P. Steinbach et al., "A randomised, open label phase III trial with nimotuzumab, an anti-epidermal growth factor receptor monoclonal antibody in the treatment of newly diagnosed adult glioblastoma," *European Journal of Cancer*, vol. 51, no. 4, pp. 522–532, 2015.

[110] Orphanet: Nimotuzumab, 2019, https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=82913.

[111] X. Wang, J. Gu, C. Shao, K. Han, and J. Meng, "Nimotuzumab plus chemotherapy with docetaxel, cisplatin, 5-fluorouracil for locally advanced head and neck squamous cell carcinoma: a clinical study," *Journal of Cancer Research and Therapeutics*, vol. 15, no. 2, pp. 312–316, 2019.

[112] S. Subramanian, N. Sridharan, V. Balasundaram, and S. Chaudhari, "Efficacy and safety of nimotuzumab in unresectable, recurrent, and/or metastatic squamous cell

carcinoma of the head and neck: a hospital-based retrospective evidence," *South Asian Journal of Cancer*, vol. 7, no. 3, p. 188, 2018.

[113] Comp. committee for orphan medicinal products public summary of positive opinion for orphan designation of nimotuzumab for the treatment of pancreatic cancer, 2008, http://www.emea.europa.eu.

[114] A. C. Phillips, E. R. Boghaert, K. S. Vaidya et al., "ABT-414, an antibody-drug conjugate targeting a tumor-selective EGFR epitope," *Molecular Cancer Therapeutics*, vol. 15, no. 4, pp. 661–669, 2016.

[115] M. van den Bent, H. K. Gan, A. B. Lassman et al., "Efficacy of depatuxizumab mafodotin (ABT-414) monotherapy in patients with EGFR-amplified, recurrent glioblastoma: results from a multi-center, international study," *Cancer Chemotherapy and Pharmacology*, vol. 80, no. 6, pp. 1209–1217, 2017.

[116] G. D. Goss, E. E. Vokes, M. S. Gordon et al., "Efficacy and safety results of depatuxizumab mafodotin (ABT-414) in patients with advanced solid tumors likely to overexpress epidermal growth factor receptor," *Cancer*, vol. 124, no. 10, pp. 2174–2183, 2018.

[117] L. Frederick, G. Eley, Y. Wang, and C. D. James, "Analysis of genomic rearrangements associated with $EGFR^{vIII}$ expression suggests involvement of Alu repeat elements," *Neuro-Oncology*, vol. 2, no. 3, pp. 159–163, 2000.

[118] J. M. Figueroa, J. Skog, J. Akers et al., "Detection of wild-type EGFR amplification and $EGFR^{vIII}$ mutation in CSF-derived extracellular vesicles of glioblastoma patients," *Neuro-Oncology*, vol. 19, no. 11, pp. 1494–1502, 2017.

[119] J. Felsberg, B. Hentschel, K. Kaulich et al., "Epidermal growth factor receptor variant III ($EGFR^{vIII}$) positivity inEGFR-amplified glioblastomas: prognostic role and comparison between primary and recurrent tumors," *Clinical Cancer Research*, vol. 23, no. 22, pp. 6846–6855, 2017.

[120] K. M. Turner, V. Deshpande, D. Beyter et al., "Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity," *Nature*, vol. 543, no. 7643, pp. 122–125, 2017.

[121] M. V. Grandal, R. Zandi, M. W. Pedersen, B. M. Willumsen, B. van Deurs, and H. S. Poulsen, "$EGFR^{vIII}$ escapes down-regulation due to impaired internalization and sorting to lysosomes," *Carcinogenesis*, vol. 28, no. 7, pp. 1408–1417, 2007.

[122] R. Nishikawa, X. D. Ji, R. C. Harmon et al., "A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity," *Proceedings of the National Academy of Sciences*, vol. 91, no. 16, pp. 7727–7731, 1994.

[123] W. Stec, K. Rosiak, C. Treda et al., "Cyclic trans-phosphorylation in a homodimer as the predominant mechanism of $EGFR^{vIII}$ action and regulation," *Oncotarget*, vol. 9, no. 9, pp. 8560–8572, 2018.

[124] S. K. Batra, S. Castelino-Prabhu, C. J. Wikstrand et al., "Epidermal growth factor ligand-independent, unregulated, cell-transforming potential of a naturally occurring human mutant $EGFR^{vIII}$ gene," *Cell Growth & Differentiation*, vol. 6, pp. 1251–1259, 1995.

[125] D. Hills, G. Rowlinson-Busza, and W. J. Gullick, "Specific targeting of a mutant, activated EGF receptor found in glioblastoma using a monoclonal antibody," *International Journal of Cancer*, vol. 63, no. 4, pp. 537–543, 1995.

[126] L. Sun, S. Yu, H. Xu et al., "FHL2 interacts with EGFR to promote glioblastoma growth," *Oncogene*, vol. 37, no. 10, pp. 1386–1398, 2018.

[127] D. S. W. Tan, F. T. Chong, H. S. Leong et al., "Long noncoding RNA EGFR-AS1 mediates epidermal growth factor receptor addiction and modulates treatment response in squamous cell carcinoma," *Nature Medicine*, vol. 23, no. 10, pp. 1167–1175, 2017.

[128] G. Liccardi, J. A. Hartley, and D. Hochhauser, "EGFR nuclear translocation modulates DNA repair following cisplatin and ionizing radiation treatment," *Cancer Research*, vol. 71, no. 3, pp. 1103–1114, 2011.

[129] A. Dowlati, D. Nethery, and J. A. Kern, "Combined inhibition of epidermal growth factor receptor and JAK/STAT pathways results in greater growth inhibition in vitro than single agent therapy," *Molecular Cancer Therapeutics*, vol. 3, pp. 459–463, 2004.

[130] Y. Narita, M. Nagane, K. Mishima, H. J. Huang, F. B. Furnari, and W. K. Cavenee, "Mutant epidermal growth factor receptor signaling down-regulates p27 through activation of the phosphatidylinositol 3-kinase/Akt pathway in glioblastomas," *Cancer Research*, vol. 62, pp. 6764–6769, 2002.

[131] H. Wiley, "Trafficking of the ErbB receptors and its influence on signaling," *Experimental Cell Research*, vol. 284, no. 1, pp. 78–88, 2003.

[132] F. Huang, L. K. Goh, and A. Sorkin, "EGF receptor ubiquitination is not necessary for its internalization," *Proceedings of the National Academy of Sciences*, vol. 104, no. 43, pp. 16904–16909, 2007.

[133] A. S. Gajadhar, E. Bogdanovic, D. M. Munoz, and A. Guha, "In situ analysis of mutant EGFRs prevalent in glioblastoma multiforme reveals aberrant dimerization, activation, and differential response to anti-EGFR targeted therapy," *Molecular Cancer Research*, vol. 10, no. 3, pp. 428–440, 2012.

[134] Y. Hwang, V. Chumbalkar, K. Latha, and O. Bogler, "Forced dimerization increases the activity of $EGFR/EGFR^{vIII}$ and enhances its oncogenicity," *Molecular Cancer Research*, vol. 9, no. 9, pp. 1199–1208, 2011.

[135] R. Kancha, N. von Bubnoff, and J. Duyster, "Asymmetric kinase dimer formation is crucial for the activation of oncogenic $EGFR^{vIII}$ but not for ERBB3 phosphorylation," *Cell Communication and Signaling*, vol. 11, no. 1, p. 39, 2013.

[136] H. Fernandes, S. Cohen, and S. Bishayee, "Glycosylation-induced conformational modification positively regulates receptor-receptor association," *Journal of Biological Chemistry*, vol. 276, no. 7, pp. 5375–5383, 2001.

[137] R. B. Luwor, H.-J. Zhu, F. Walker et al., "The tumor-specific de2–7 epidermal growth factor receptor (EGFR) promotes cells survival and heterodimerizes with the wild-type EGFR," *Oncogene*, vol. 23, no. 36, pp. 6095–6104, 2004.

[138] Q.-W. Fan, C. K. Cheng, W. C. Gustafson et al., "EGFR phosphorylates tumor-derived $EGFR^{vIII}$ driving STAT3/5 and progression in glioblastoma," *Cancer Cell*, vol. 24, no. 4, pp. 438–449, 2013.

[139] S. A. Greenall, J. F. Donoghue, M. Van Sinderen et al., "$EGFR^{vIII}$-mediated transactivation of receptor tyrosine kinases in glioma: mechanism and therapeutic implications," *Oncogene*, vol. 34, no. 41, pp. 5277–5287, 2015.

[140] S. I. Ymer, S. A. Greenall, A. Cvrljevic et al., "Glioma specific extracellular missense mutations in the first cysteine rich region of epidermal growth factor receptor (EGFR) initiate ligand independent activation," *Cancers*, vol. 3, no. 2, pp. 2032–2049, 2011.

[141] Z. Ruan and N. Kannan, "Mechanistic insights into R776H mediated activation of epidermal growth factor receptor kinase," *Biochemistry*, vol. 54, no. 27, pp. 4216–4225, 2015.

[142] D. Chakravarty, A. M. Pedraza, J. Cotari et al., "EGFR and PDGFRA co-expression and heterodimerization in glioblastoma tumor sphere lines," *Scientific Reports*, vol. 7, no. 1, p. 9043, 2017.

[143] J. Garnett, V. Chumbalkar, B. Vaillant et al., "Regulation of HGF expression by ΔEGFR-mediated c-met activation in glioblastoma cells," *Neoplasia*, vol. 15, no. 1, pp. 73–IN21, 2013.

[144] K. Latha, M. Li, V. Chumbalkar et al., "Nuclear EGFR^{vIII}-STAT5b complex contributes to glioblastoma cell survival by direct activation of the Bcl-XL promoter," *International Journal of Cancer*, vol. 132, no. 3, pp. 509–520, 2013.

[145] A. E. Gururaj, L. Gibson, S. Panchabhai et al., "Access to the nucleus and functional association with c-myc is required for the full oncogenic potential of ΔEGFR/EGFR^{vIII}," *Journal of Biological Chemistry*, vol. 288, no. 5, pp. 3428–3438, 2013.

[146] J. P. Newman, G. Y. Wang, K. Arima et al., "Interleukin-13 receptor alpha 2 cooperates with EGFR^{vIII} signaling to promote glioblastoma multiforme," *Nature Communications*, vol. 8, no. 1, p. 1913, 2017.

[147] W. J. Stec, K. Rosiak, P. Siejka et al., "Cell line with endogenous EGFR^{vIII} expression is a suitable model for research and drug development purposes," *Oncotarget*, vol. 7, no. 22, pp. 31907–31925, 2016.

[148] M. H. H. Schmidt, F. B. Furnari, W. K. Cavenee, and O. Bogler, "Epidermal growth factor receptor signaling intensity determines intracellular protein interactions, ubiquitination, and internalization," *Proceedings of the National Academy of Sciences*, vol. 100, no. 11, pp. 6505–6510, 2003.

[149] W. Han, T. Zhang, H. Yu, J. G. Foulke, and C. K. Tang, "Hypophosphorylation of residue Y1045 leads to defective downregulation of EGFR^{vIII}," *Cancer Biology & Therapy*, vol. 5, no. 10, pp. 1361–1368, 2006.

[150] M.-D.-M. Inda, R. Bonavia, A. Mukasa et al., "Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma," *Genes & Development*, vol. 24, no. 16, pp. 1731–1745, 2010.

[151] C. Zanca, G. R. Villa, J. A. Benitez et al., "Glioblastoma cellular cross-talk converges on NF-κB to attenuate EGFR inhibitor sensitivity," *Genes & Development*, vol. 31, no. 12, pp. 1212–1227, 2017.

[152] K. Liffers, K. Kolbe, M. Westphal, K. Lamszus, and A. Schulte, "Histone deacetylase inhibitors resensitize EGFR/EGFR^{vIII}-Overexpressing, erlotinib-resistant glioblastoma cells to tyrosine kinase inhibition," *Targeted Oncology*, vol. 11, no. 1, pp. 29–40, 2016.

[153] M.-T. Stockhausen, K. Kristoffersen, L. Stobbe, and H. S. Poulsen, "Differentiation of glioblastoma multiforme stem-like cells leads to downregulation of EGFR and EGFR^{vIII} and decreased tumorigenic and stem-like cell potential," *Cancer Biology & Therapy*, vol. 15, no. 2, pp. 216–224, 2014.

[154] K. Kristoffersen, M. Villingshøj, H. S. Poulsen, and M.-T. Stockhausen, "Level of Notch activation determines the effect on growth and stem cell-like features in glioblastoma multiforme neurosphere cultures," *Cancer Biology & Therapy*, vol. 14, no. 7, pp. 625–637, 2013.

[155] R. Galli, E. Binda, U. Orfanelli et al., "Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma," *Cancer Research*, vol. 64, no. 19, pp. 7011–7021, 2004.

[156] T. N. Ignatova, V. G. Kukekov, E. D. Laywell, O. N. Suslov, F. D. Vrionis, and D. A. Steindler, "Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers in vitro," *Glia*, vol. 39, no. 3, pp. 193–206, 2002.

[157] X. Yuan, J. Curtin, Y. Xiong et al., "Isolation of cancer stem cells from adult glioblastoma multiforme," *Oncogene*, vol. 23, no. 58, pp. 9392–9400, 2004.

[158] D. R. Emlet, P. Gupta, M. Holgado-Madruga et al., "Targeting a glioblastoma cancer stem-cell population defined by EGF receptor variant III," *Cancer Research*, vol. 74, no. 4, pp. 1238–1249, 2014.

[159] A. Ayuso-Sacido, J. A. Moliterno, S. Kratovac et al., "Activated EGFR signaling increases proliferation, survival, and migration and blocks neuronal differentiation in post-natal neural stem cells," *Journal of Neuro-Oncology*, vol. 97, no. 3, pp. 323–337, 2010.

[160] S. Bao, Q. Wu, S. Sathornsumetee et al., "Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor," *Cancer Research*, vol. 66, no. 16, pp. 7843–7848, 2006.

[161] G. Liu, X. Yuan, Z. Zeng et al., "Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma," *Molecular Cancer*, vol. 5, no. 1, p. 67, 2006.

[162] J. D. Lathia, J. Gallagher, M. J. T. et al., "Direct in vivo evidence for tumor propagation by glioblastoma cancer stem cells," *PLoS One*, vol. 6, no. 9, Article ID e24807, 2011.

[163] P. Rieske, E. Golanska, M. Zakrzewska et al., "Arrested neural and advanced mesenchymal differentiation of glioblastoma cells-comparative study with neural progenitors," *BMC Cancer*, vol. 9, no. 1, p. 54, 2009.

[164] R. Chen, Y. Pan, and D. H. Gutmann, "The power of the few," *Genes & Development*, vol. 31, no. 12, pp. 1177–1179, 2017.

[165] M. J. van den Bent, Y. Gao, M. Kerkhof et al., "Changes in the EGFR amplification and EGFR^{vIII} expression between paired primary and recurrent glioblastomas," *Neuro-Oncology*, vol. 17, no. 7, pp. 935–941, 2015.

[166] P.-P. Ren, M. Li, T.-F. Li, and S.-Y. Han, "Anti-EGFR^{vIII} chimeric antigen receptor-modified T cells for adoptive cell therapy of glioblastoma," *Current Pharmaceutical Design*, vol. 23, no. 14, pp. 2113–2116, 2017.

[167] D. A. Nathanson, B. Gini, J. Mottahedeh et al., "Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA," *Science*, vol. 343, no. 6166, pp. 72–76, 2014.

[168] J. Read, A. Ingram, H. A. Al Saleh et al., "Nuclear transportation of exogenous epidermal growth factor receptor and androgen receptor via extracellular vesicles," *European Journal of Cancer*, vol. 70, pp. 62–74, 2017.

[169] F. Ricklefs, M. Mineo, A. K. Rooj et al., "Extracellular vesicles from high-grade glioma exchange diverse pro-oncogenic signals that maintain intratumoral heterogeneity," *Cancer Research*, vol. 76, no. 10, pp. 2876–2881, 2016.

[170] D. W. Lee, S.-Y. Lee, I. Doh, G. H. Ryu, and D.-H. Nam, "High-Dose compound heat map for 3D-cultured glioblastoma multiforme cells in a micropillar and microwell chip platform," *BioMed Research International*, vol. 2017, Article ID 7218707, 7 pages, 2017.

[171] S. Würstle, F. Schneider, F. Ringel et al., "Temozolomide induces autophagy in primary and established glioblastoma cells in an EGFR independent manner," *Oncology Letters*, vol. 14, no. 1, pp. 322–328, 2017.

[172] S. Kummar, H. X. Chen, J. Wright et al., "Utilizing targeted cancer therapeutic agents in combination: novel approaches and urgent requirements," *Nature Reviews Drug Discovery*, vol. 9, no. 11, pp. 843–856, 2010.

[173] A. Levitzki and E. Mishani, "Tyrphostins and other tyrosine kinase inhibitors," *Annual Review of Biochemistry*, vol. 75, no. 1, pp. 93–109, 2006.

[174] J. H. Park, Y. Liu, M. A. Lemmon, and R. Radhakrishnan, "Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain," *Biochemical Journal*, vol. 448, no. 3, pp. 417–423, 2012.

[175] I. Vivanco, H. I. Robins, D. Rohle et al., "Differential sensitivity of glioma- versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors," *Cancer Discovery*, vol. 2, no. 5, pp. 458–471, 2012.

[176] D. A. E. Cross, S. E. Ashton, S. Ghiorghiu et al., "AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer," *Cancer Discovery*, vol. 4, no. 9, pp. 1046–1061, 2014.

[177] J. N. Rich and D. D. Bigner, "Development of novel targeted therapies in the treatment of malignant glioma," *Nature Reviews Drug Discovery*, vol. 3, no. 5, pp. 430–446, 2004.

[178] I. K. Mellinghoff, M. Y. Wang, I. Vivanco et al., "Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors," *New England Journal of Medicine*, vol. 353, no. 19, pp. 2012–2024, 2005.

[179] J. M. Sepúlveda-Sánchez, M. Á. Vaz, C. Balañá et al., "Phase II trial of dacomitinib, a pan-human EGFR tyrosine kinase inhibitor, in recurrent glioblastoma patients with EGFR amplification," *Neuro-Oncology*, vol. 19, no. 11, pp. 1522–1531, 2017.

[180] J. N. Sarkaria, L. S. Hu, I. F. Parney et al., "Is the blood-brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data," *Neuro-Oncology*, vol. 20, no. 2, pp. 184–191, 2018.

[181] M. Maemondo, A. Inoue, K. Kobayashi et al., "Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR," *New England Journal of Medicine*, vol. 362, no. 25, pp. 2380–2388, 2010.

[182] T. S. Mok, Y.-L. Wu, S. Thongprasert et al., "Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma," *New England Journal of Medicine*, vol. 361, no. 10, pp. 947–957, 2009.

[183] L. Orellana, A. H. Thorne, R. Lema et al., "Oncogenic mutations at the EGFR ectodomain structurally converge to remove a steric hindrance on a kinase-coupled cryptic epitope," *Proceedings of the National Academy of Sciences*, vol. 116, pp. 10009–10018, 2019.

[184] M.-E. Halatsch, E. E. Gehrke, V. I. Vougioukas et al., "Inverse correlation of epidermal growth factor receptor messenger RNA induction and suppression of anchorage-independent growth by OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in glioblastoma multiforme cell lines," *Journal of Neurosurgery*, vol. 100, no. 3, pp. 523–533, 2004.

[185] D. A. Reardon, M. D. Groves, P. Y. Wen et al., "A phase I/II trial of pazopanib in combination with lapatinib in adult patients with relapsed malignant glioma," *Clinical Cancer Research*, vol. 19, no. 4, pp. 900–908, 2013.

[186] P. D. Brown, S. Krishnan, J. N. Sarkaria et al., "Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: north central cancer treatment group study N0177," *Journal of Clinical Oncology*, vol. 26, no. 34, pp. 5603–5609, 2008.

[187] M. D. Prados, S. M. Chang, N. Butowski et al., "Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma," *Journal of Clinical Oncology*, vol. 27, no. 4, pp. 579–584, 2009.

[188] M. E. Hegi, A.-C. Diserens, P. Bady et al., "Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib—A phase II trial," *Molecular Cancer Therapeutics*, vol. 10, no. 6, pp. 1102–1112, 2011.

[189] B. Jutten, L. Dubois, Y. Li et al., "Binding of cetuximab to the $EGFR^{vIII}$ deletion mutant and its biological consequences in malignant glioma cells," *Radiotherapy and Oncology*, vol. 92, no. 3, pp. 393–398, 2009.

[190] J. Fukai, K. Nishio, T. Itakura, and F. Koizumi, "Antitumor activity of cetuximab against malignant glioma cells overexpressing EGFR deletion mutant variant III," *Cancer Science*, vol. 99, pp. 2062–2069, 2008.

[191] B. Neyns, J. Sadones, E. Joosens et al., "Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma," *Annals of Oncology*, vol. 20, no. 9, pp. 1596–1603, 2009.

[192] N. Montano, T. Cenci, M. Martini et al., "Expression of $EGFR^{vIII}$ in glioblastoma: prognostic significance revisited," *Neoplasia*, vol. 13, no. 12, p. 1113, 2011.

[193] N. Struve, T. Brend, L. Stead et al., "TMOD-09. $EGFR^{vIII}$ increases mismatch repair protein expression and is therefore a predictive marker for temozolomide response in O6-methylguanine-DNA methyltransferase negative glioblastoma cells and tumors," *Neuro-Oncology*, vol. 18, no. suppl_6, p. vi208, 2016.

[194] J. F. Poduslo, G. L. Curran, and C. T. Berg, "Macromolecular permeability across the blood-nerve and blood-brain barriers," *Proceedings of the National Academy of Sciences*, vol. 91, no. 12, pp. 5705–5709, 1994.

[195] A. Rabney, K. Baum, D. Pitts et al., "Cetuximab approved by FDA for treatment of head and neck squamous cell cancer," *Cancer Biology & Therapy*, vol. 5, no. 4, pp. 339–348, 2006.

[196] F. Geng, Z. Wang, H. Yin, J. Yu, and B. Cao, "Molecular targeted drugs and treatment of colorectal cancer: recent progress and future perspectives," *Cancer Biotherapy and Radiopharmaceuticals*, vol. 32, no. 5, pp. 149–160, 2017.

[197] D. Patel, A. Lahiji, S. Patel et al., "Monoclonal antibody cetuximab binds to and down-regulates constitutively activated epidermal growth factor receptor vIII on the cell surface," *Anticancer Research*, vol. 27, pp. 3355–3366, 2007.

[198] D. Patel, R. Bassi, A. Hooper, M. Prewett, D. J. Hicklin, and X. Kang, "Anti-epidermal growth factor receptor monoclonal antibody cetuximab inhibits EGFR/HER-2 heterodimerization and activation," *International Journal of Oncology*, vol. 34, pp. 25–32, 2009.

[199] S. Chakraborty, C. G. Filippi, T. Wong et al., "Superselective intraarterial cerebral infusion of cetuximab after osmotic blood/brain barrier disruption for recurrent malignant glioma: phase I study," *Journal of Neuro-Oncology*, vol. 128, no. 3, pp. 405–415, 2016.

[200] M. Kaluzova, A. Bouras, R. Machaidze, and C. G. Hadjipanayis, "Targeted therapy of glioblastoma stem-like cells and tumor non-stem cells using cetuximab-conjugated iron-oxide nanoparticles," *Oncotarget*, vol. 6, no. 11, pp. 8788–8806, 2015.

[201] J. R. Whittle, J. D. Lickliter, H. K. Gan et al., "First in human nanotechnology doxorubicin delivery system to target epidermal growth factor receptors in recurrent glioblastoma," *Journal of Clinical Neuroscience*, vol. 22, no. 12, pp. 1889–1894, 2015.

[202] K. R. Schmitz and K. M. Ferguson, "Interaction of antibodies with ErbB receptor extracellular regions," *Experimental Cell Research*, vol. 315, no. 4, pp. 659–670, 2009.

[203] K. J. Hamblett, C. J. Kozlosky, S. Siu et al., "AMG 595, an anti-EGFR^{vIII} antibody-drug conjugate, induces potent antitumor activity against EGFR^{vIII}-expressing glioblastoma," *Molecular Cancer Therapeutics*, vol. 14, no. 7, pp. 1614–1624, 2015.

[204] T. G. Johns, E. Stockert, G. Ritter et al., "Novel monoclonal antibody specific for the de2–7 epidermal growth factor receptor (EGFR) that also recognizes the EGFR expressed in cells containing amplification of the EGFR gene," *International Journal of Cancer*, vol. 98, no. 3, pp. 398–408, 2002.

[205] E. B. Reilly, A. C. Phillips, F. G. Buchanan et al., "Characterization of ABT-806, a humanized tumor-specific anti-EGFR monoclonal antibody," *Molecular Cancer Therapeutics*, vol. 14, no. 5, pp. 1141–1151, 2015.

[206] M. V. Maus, J. Plotkin, G. Jakka et al., "An MHC-restricted antibody-based chimeric antigen receptor requires TCR-like affinity to maintain antigen specificity," *Molecular Therapy—Oncolytics*, vol. 3, pp. 16023–16029, 2016.

[207] L. A. Johnson, J. Scholler, T. Ohkuri et al., "Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma," *Science Translational Medicine*, vol. 7, no. 275, Article ID 275ra22, 2015.

[208] C. S. M. Yong, V. Dardalhon, C. Devaud, N. Taylor, P. K. Darcy, and M. H. Kershaw, "CAR T-cell therapy of solid tumors," *Immunology and Cell Biology*, vol. 95, no. 4, pp. 356–363, 2017.

[209] J. Han, J. Chu, W. Keung Chan et al., "CAR-engineered NK cells targeting wild-type EGFR and EGFR^{vIII} enhance killing of glioblastoma and patient-derived glioblastoma stem cells," *Scientific Reports*, vol. 5, no. 1, p. 11483, 2015.

[210] R. Brentjens, R. Yeh, Y. Bernal, I. Riviere, and M. Sadelain, "Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial," *Molecular Therapy*, vol. 18, no. 4, pp. 666–668, 2010.

[211] R. A. Morgan, J. C. Yang, M. Kitano, M. E. Dudley, C. M. Laurencot, and S. A. Rosenberg, "Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2," *Molecular Therapy*, vol. 18, no. 4, pp. 843–851, 2010.

[212] T. G. Johns, I. Mellman, G. A. Cartwright et al., "The antitumor monoclonal antibody 806 recognizes a high-mannose form of the EGF receptor that reaches the cell surface when cells over-express the receptor," *The FASEB Journal*, vol. 19, no. 7, pp. 780–782, 2005.

[213] P. Gupta, S.-Y. Han, M. Holgado-Madruga et al., "Development of an EGFR^{vIII} specific recombinant antibody," *BMC Biotechnology*, vol. 10, no. 1, 2010.

[214] M.-R. Wu, T. Zhang, A. T. Gacerez, T. A. Coupet, L. R. DeMars, and C. L. Sentman, "B7H6-Specific bispecific T cell Engagers lead to tumor elimination and host antitumor immunity," *The Journal of Immunology*, vol. 194, no. 11, pp. 5305–5311, 2015.

[215] K. Iwahori, S. Kakarla, M. P. Velasquez et al., "Engager T cells: a new class of antigen-specific T cells that redirect bystander T cells," *Molecular Therapy*, vol. 23, no. 1, pp. 171–178, 2015.

[216] C. Bluemel, S. Hausmann, P. Fluhr et al., "Epitope distance to the target cell membrane and antigen size determine the potency of T cell-mediated lysis by BiTE antibodies specific for a large melanoma surface antigen," *Cancer Immunology, Immunotherapy*, vol. 59, no. 8, pp. 1197–1209, 2010.

[217] B. D. Choi, P. C. Gedeon, J. E. Herndon et al., "Human regulatory T cells kill tumor cells through granzyme-dependent cytotoxicity upon retargeting with a bispecific antibody," *Cancer Immunology Research*, vol. 1, no. 3, pp. 163–167, 2013.

[218] B. D. Choi, P. C. Gedeon, L. Sanchez-Perez, D. D. Bigner, and J. H. Sampson, "Regulatory T cells are redirected to kill glioblastoma by an EGFR^{vIII}-targeted bispecific antibody," *Oncoimmunology*, vol. 2, no. 12, Article ID e26757, 2013.

[219] M. Banaszczyk, E. Stoczynska-Fidelus, M. Winiecka-Klimek et al., "EGFR^{vIII}—a stable target for anti-EGFR^{vIII} therapy," *Anticancer Research*, vol. 33, pp. 5343–5348, 2013.

[220] A. Idbaih, J. Aimard, B. Boisselier et al., "Epidermal growth factor receptor extracellular domain mutations in primary glioblastoma," *Neuropathology and Applied Neurobiology*, vol. 35, no. 2, pp. 208–213, 2009.

[221] D. C. Binder, E. Ladomersky, A. Lenzen et al., "Lessons learned from rindopepimut treatment in patients with EGFR^{vIII}-expressing glioblastoma," *Translational Cancer Research*, vol. 7, no. S4, pp. S510–S513, 2018.

[222] J. Schuster, R. K. Lai, L. D. Recht et al., "A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study," *Neuro-Oncology*, vol. 17, no. 6, pp. 854–861, 2015.

[223] A. B. Heimberger, L. E. Crotty, G. E. Archer et al., "Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors," *Clinical Cancer Research*, vol. 9, pp. 4247–4254, 2003.

[224] Z. Zhang, X. Song, H. Tian et al., "MicroRNA-137 inhibits growth of glioblastoma through EGFR suppression," *American Journal of Translational Research*, vol. 9, no. 3, pp. 1492–1499, 2017.

[225] Y. Ji, Q. Sun, J. Zhang, and H. Hu, "MiR-615 inhibits cell proliferation, migration and invasion by targeting EGFR in human glioblastoma," *Biochemical and Biophysical Research Communications*, vol. 499, no. 3, pp. 719–726, 2018.

[226] F. Xu, F. Li, W. Zhang, and P. Jia, "Growth of glioblastoma is inhibited by miR-133-mediated EGFR suppression," *Tumor Biology*, vol. 36, no. 12, pp. 9553–9558, 2015.

[227] J. Zhang, J. Zhang, W. Qiu et al., "MicroRNA-1231 exerts a tumor suppressor role through regulating the EGFR/PI3K/AKT axis in glioma," *Journal of Neuro-Oncology*, vol. 139, no. 3, pp. 547–562, 2018.

[228] X. Zhou, Y. Ren, L. Moore et al., "Downregulation of miR-21 inhibits EGFR pathway and suppresses the growth of human glioblastoma cells independent of PTEN status," *Laboratory Investigation*, vol. 90, no. 2, pp. 144–155, 2010.

[229] D. Yin, S. Ogawa, N. Kawamata et al., "MiR-34a functions as a tumor suppressor modulating EGFR in glioblastoma multiforme," *Oncogene*, vol. 32, no. 9, pp. 1155–1163, 2013.

[230] G. G. Gomez, S. Volinia, C. M. Croce et al., "Suppression of microRNA-9 by mutant EGFR signaling upregulates FOXP1 to enhance glioblastoma tumorigenicity," *Cancer Research*, vol. 74, no. 5, pp. 1429–1439, 2014.

[231] Z.-Q. Dong, Z.-Y. Guo, and J. Xie, "The lncRNA EGFR-AS1 is linked to migration, invasion and apoptosis in glioma cells by targeting miR-133b/RACK1," *Biomedicine & Pharmacotherapy*, vol. 118, 2019.

[232] M. E. Davis, J. E. Zuckerman, C. H. J. Choi et al., "Evidence of RNAi in humans from systemically administered siRNA via

targeted nanoparticles," *Nature*, vol. 464, no. 7291, pp. 1067–1070, 2010.

[233] Y. Shi, Y. Jiang, J. Cao et al., "Boosting RNAi therapy for orthotopic glioblastoma with nontoxic brain-targeting chimaeric polymersomes," *Journal of Controlled Release*, vol. 292, pp. 163–171, 2018.

[234] J. J. Laskin, G. Nicholas, C. Lee et al., "Phase I/II trial of custirsen (OGX-011), an inhibitor of clusterin, in combination with a gemcitabine and platinum regimen in patients with previously untreated advanced non-small cell lung cancer," *Journal of Thoracic Oncology*, vol. 7, no. 3, pp. 579–586, 2012.

[235] I. F. Tannock, K. Fizazi, S. Ivanov et al., "Aflibercept versus placebo in combination with docetaxel and prednisone for treatment of men with metastatic castration-resistant prostate cancer (VENICE): a phase 3, double-blind randomised trial," *The Lancet Oncology*, vol. 14, no. 8, pp. 760–768, 2013.

[236] P. Pu, X. Liu, A. Liu, J. Cui, and Y. Zhang, "Inhibitory effect of antisense epidermal growth factor receptor RNA on the proliferation of rat C6 glioma cells in vitro and in vivo," *Journal of Neurosurgery*, vol. 92, no. 1, pp. 132–139, 2000.

[237] M. C. Moroni, M. C. Willingham, and L. Beguinot, "EGF-R antisense RNA blocks expression of the epidermal growth factor receptor and suppresses the transforming phenotype of a human carcinoma cell line," *Journal of Biological Chemistry*, vol. 267, pp. 2714–2722, 1992.

[238] Q.-W. Fan and W. A. Weiss, "RNA interference against a glioma-derived allele of EGFR induces blockade at G2M," *Oncogene*, vol. 24, no. 5, pp. 829–837, 2005.

[239] H. Yamazaki, H. Kijima, Y. Abe et al., "Inhibition of tumor growth by ribozyme-mediated suppression of aberrant epidermal growth factor receptor gene expression," *JNCI: Journal of the National Cancer Institute*, vol. 90, no. 8, pp. 581–587, 1998.

[240] T. Koga, B. Li, J. M. Figueroa et al., "Mapping of genomic $EGFR^{vIII}$ deletions in glioblastoma: insight into rearrangement mechanisms and biomarker development," *Neuro-Oncology*, vol. 20, no. 10, pp. 1310–1320, 2018.

[241] C. Stahlhut and F. J. Slack, "Combinatorial action of microRNAs let-7 and miR-34 effectively synergizes with erlotinib to suppress non-small cell lung cancer cell proliferation," *Cell Cycle*, vol. 14, no. 13, pp. 2171–2180, 2015.

[242] M. Witusik-Perkowska, P. Rieske, K. Hułas-Bigoszewska et al., "Glioblastoma-derived spheroid cultures as an experimental model for analysis of EGFR anomalies," *Journal of Neuro-Oncology*, vol. 102, no. 3, pp. 395–407, 2011.

[243] M. Schmitz, A. Temme, V. Senner et al., "Identification of sox2 as a novel glioma-associated antigen and potential target for T cell-based immunotherapy," *British Journal of Cancer*, vol. 96, no. 8, pp. 1293–1301, 2007.

[244] A. D. Berezovsky, L. M. Poisson, D. Cherba et al., "Sox2 promotes malignancy in glioblastoma by regulating plasticity and astrocytic differentiation," *Neoplasia*, vol. 16, no. 3, pp. 193–206, 2014.

[245] P. Rieske, S. A. Azizi, B. Augelli, J. Gaughan, and B. Krynska, "A population of human brain parenchymal cells express markers of glial, neuronal and early neural cells and differentiate into cells of neuronal and glial lineages," *European Journal of Neuroscience*, vol. 25, no. 1, pp. 31–37, 2007.

[246] M. Witusik-Perkowska, M. Zakrzewska, B. Sikorska et al., "Glioblastoma-derived cells in vitro unveil the spectrum of drug resistance capability–comparative study of tumour chemosensitivity in different culture systems," *Biosci Rep*, vol. 37, 2017.

[247] E. Stoczynska-Fidelus, S. Piaskowski, M. Bienkowski et al., "The failure in the stabilization of glioblastoma-derived cell lines: spontaneous in vitro senescence as the main culprit," *PLoS One*, vol. 9, no. 1, Article ID e87136, 2014.

[248] P. H. Huang, E. R. Miraldi, A. M. Xu et al., "Phosphotyrosine signaling analysis of site-specific mutations on $EGFR^{vIII}$ identifies determinants governing glioblastoma cell growth," *Molecular BioSystems*, vol. 6, no. 7, pp. 1227–1237, 2010.