Targeting Aberrant FGFR Signaling to Overcome CDK4/6 Inhibitor Resistance in Breast Cancer

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**Abstract:** Breast cancer (BC) is the most common cause of cancer-related death in women worldwide. Therapies targeting molecular pathways altered in BC had significantly enhanced treatment options for BC over the last decades, which ultimately improved the lives of millions of women worldwide. Among various molecular pathways accruing substantial interest for the development of targeted therapies are cyclin-dependent kinases (CDKs)—in particular, the two closely related members CDK4 and CDK6. CDK4/6 inhibitors indirectly trigger the dephosphorylation of retinoblastoma tumor suppressor protein by blocking CDK4/6, thereby blocking the cell cycle transition from the G1 to S phase. Although the CDK4/6 inhibitors abemaciclib, palbociclib, and ribociclib gained FDA approval for the treatment of hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative BC as they significantly improved progression-free survival (PFS) in randomized clinical trials, regrettably, some patients showed resistance to these therapies. Though multiple molecular pathways could be mechanistically responsible for CDK4/6 inhibitor therapy resistance, one of the most predominant ones seems to be the fibroblast growth factor receptor (FGFR) pathway. FGFRs are involved in many aspects of cancer formation, such as cell proliferation, differentiation, and growth. Importantly, FGFRs are frequently mutated in BC, and their overexpression and/or hyperactivation correlates with CDK4/6 inhibitor resistance and shortened PFS in BC. Intriguingly, the inhibition of aberrant FGFR activity is capable of reversing the resistance to CDK4/6 inhibitors. This review summarizes the molecular background of FGFR signaling and discusses the role of aberrant FGFR signaling during cancer development in general and during the development of CDK4/6 inhibitor resistance in BC in particular, together with other possible mechanisms for resistance to CDK4/6 inhibitors. Subsequently, future directions on novel therapeutic strategies targeting FGFR signaling to overcome such resistance during BC treatment will be further debated.

**Keywords:** breast cancer; FGFR; CDK4; CDK6; inhibitor; therapy resistance

1. Introduction

Breast cancer (BC) is the most common cancer among females worldwide, with an estimated incidence rate of 279,100 new cases per year just in the United States, and the leading cause of cancer death in women worldwide [1,2]. In spite of substantial improvements in diagnosis and treatment, breast cancer remains a significant global health burden. Ten percent of newly diagnosed patients have locally advanced or metastatic disease. Additionally, up to 30% of patients diagnosed with early breast cancer eventually relapse...
and develop metastatic disease [3]. Approximately 70% of breast cancers express the estrogen receptor (ER), and estrogen signaling drives breast cancer cell growth and progression [2]. Endocrine therapies are commonly used to treat ER+ breast cancer, including tamoxifen, aromatase inhibitors, and the selective estrogen receptor degrader fulvestrant. Although these endocrine therapies improved the survival of ER+ breast cancer patients, a large proportion of patients with metastatic BC eventually acquire resistance to endocrine therapy [4].

Recently, a combination of inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6)—palbociclib, ribociclib, and abemaciclib—with aromatase inhibitors or the ER inhibitor fulvestrant resulted in a markedly improved progression-free survival (PFS) compared to endocrine therapy alone in patients with advanced ER+ breast cancer [5]. Even though the majority of patients with advanced disease treated with CDK4/6 inhibitors and antiestrogens benefit from this combination, virtually all patients eventually display disease progression, underscoring the need to discover mechanisms of resistance to this new standard of care. Preclinical evidence evinced various mechanisms contributing to the intrinsic or acquired resistance to CDK4/6 inhibitors, and predominantly, among these are alterations in the fibroblast growth factor receptor (FGFR) signaling pathway. For these reasons, the focus of this review is to discuss the role of FGFR alterations during the development of resistance to CDK4/6 inhibitors and how targeting the FGFR pathway could be utilized therapeutically to overcome CDK4/6 inhibitor therapy resistance in BC will be further debated.

2. Biology of FGFR Signaling

Fibroblast growth factor receptors constitute a family of four single-pass transmembrane receptors (FGFR1–4) with an intracellular tyrosine kinase domain. They become activated upon the extracellular binding of their cognate ligands from the fibroblast growth factor (FGF) family. To this end, their extracellular parts contain three immunoglobulin (Ig)-like domains (IgI-IgIII), as well as an acidic region between IgI and IgII. While IgI and the acidic region seem to play an inhibitory role, the IgII and IgIII domains cooperate in ligand binding [6]. Interestingly, alternative splicing of the third Ig-like domain (IgIII) in FGFR1–3 yields two additional isoforms, namely IIIb and IIIc, which are mainly expressed in epithelial and mesenchymal tissues, respectively [7,8]. Similarly, the functional relevance of distinct family members is tissue-specific to some extent, with FGFR1 and FGFR3 being more important in mesenchymal tissues and FGFR2 being more relevant in epithelial tissues [9]. The diversity of receptor structures yields receptors with different binding affinities for the plethora of FGF ligands [10,11]. In addition, a fifth FGFR-like protein has been identified (FGFR5/FGFRL1), which can also bind FGF ligands but lacks the tyrosine kinase domain essential for intracellular signaling [12]. Nevertheless, FGFR5 may be involved in FGFR signaling by functioning as a coreceptor for FGFR1 [13].

While the nature of the ligand, as well as tissue-specific expression and splicing of the four FGFR receptor tyrosine kinases determine the precise cellular outcome of FGFR signaling, the activation of intracellular signaling cascades frequently impinge on the pathways involved in proliferation, cellular survival, and differentiation. Thereby, FGF-FGFR signaling mediates multiple physiological processes such as organ development, tissue homeostasis, metabolism, wound repair, and angiogenesis.

Since most of the specificity of FGF-FGFR signaling can be attributed to the nature of the ligand, it is not surprising that the FGF family comprises a large and functionally diverse group of proteins. In humans, this family consists of 22 members and can be divided into three major groups. First, the group of 15 canonical (or paracrine) FGF ligands acts in an autocrine or paracrine fashion by binding to FGFRs in complex with extracellular heparan sulfate proteoglycans (HSPGs). HSPGs are important for this signaling, since they protect FGFs from degradation and stabilize the interaction between FGFs and FGFRs. Canonical FGF ligands can be further divided into five subfamilies, i.e., the FGF1/2, FGF4/5/6, FGF3/7/10/22, FGF8/17/18, and FGF9/16/20 subfamilies. Second, the group
of endocrine FGF ligands (FGF19/21/23) also binds to FGFRs but utilizes Klotho or β-Klotho as coreceptors and does not bind to HS PGs. Therefore, they are capable of diffusing into the bloodstream and act as hormones. Most notably, they regulate bile acid synthesis, glucose, and lipid metabolism, as well as vitamin D and phosphate levels. Fgf15 is also a member of this group but exists only in mice and is a homolog to human FGF19 [14]. Third, the group of intracellular (or intracellular) FGFs (FGF11–14) do not bind to FGFRs. Instead, they perform intracellular functions in the nervous system [15].

FGF ligands activate FGFR signaling by triggering FGFR dimerization and the trans-phosphorylation of intracellular tyrosine kinase domains (Figure 1). Specific phosphotyrosine residues then provide docking sites for multiple adapter proteins that induce downstream signaling cascades. Among them, FGFR substrate 2 (FRS2) is probably one of the most important mediators, although it regulates only a subset of FGFR functions [16]. Upon binding to the juxtamembrane intracellular domain of FGFR, FRS2 (as well as FRS3) becomes phosphorylated at multiple tyrosine residues, thereby recruiting the SHB-SHP2 complex and, subsequently, the GRB2-SOS complex, which, in turn, activates RAS and the well-known ERK1/2 MAPK signaling cascade [17]. In addition, ERK1/2 can become activated in a RAS-independent manner via the recruitment of members of the CRK protein family to activate FGFR [18]. Although the ERK1/2 MAPK cascade is not the only signaling pathway activated by FGFRs, it mediates many FGFR functions in diverse biological contexts, and FGFR signaling is a major driver of ERK1/2 activation during development [9]. Depending on the cellular context and the nature of the ligand, FGFRs can also activate a number of additional pathways. For instance, the adapter protein FRS2 not only activates ERK1/2 but, also, induces the P3K-AKT signaling pathway via the recruitment of GRB2-associated protein 1 (GAB1) [19], e.g., during neuronal development or in lens cells [9]. Furthermore, FGFRs can directly recruit phospholipase C γ (PLCγ) to their autophosphorylated carboxy terminus, leading to PLCγ phosphorylation, thereby activating protein kinase C (PKC) [20]. Moreover, FGFRs were shown to activate the JAK-STAT signaling pathway. For instance, FGF1-FGFR3 recruits and activates STAT1 to inhibit proliferation in chondrocytes [21]. Intriguingly, this pathway is also exploited in cancer. In this regard, the amplification of FGFR1 in cancer cells was shown to trigger the recruitment and activation of STAT3 [22]. Similarly, a single-nucleotide polymorphism (SNP) in FGFR4 (rs351855, causing G388R substitution) is associated with many types of cancers and increases the recruitment and activation of STAT3, thereby enhancing cancer progression [23]. Finally, other MAPK signaling pathways can become activated upon FGF signaling. In hepatocytes, for instance, FGF19-FGFR4 induces activation of the JNK signaling pathway, which results in reduced bile acid synthesis via repression of the critical enzyme CYP7A1 [24]. Furthermore, in chondrocytes, FGF1 and FGF18 were shown to activate p38 MAPK signaling [25,26].

Another aspect of FGFR signaling is the mechanisms involved in the termination and negative regulation of this signaling cascade. In this regard, the ubiquitin ligase CBL plays an important role. Upon the activation of FGFRs, FRS2 and GRB2 participate in recruiting CBL. CBL then ubiquitinates FGFRs and FRS2, thereby triggering endocytosis and the proteolytic degradation of FGFRs [27]. Furthermore, CBL is also involved in recruiting Sprouty proteins to activated FGFRs, leading to the inhibition of downstream ERK1/2 signaling [28]. In addition, GRB14 was suggested to inhibit FGFR signaling through PLCγ [29].

In summary, FGFR signaling employs a variety of distinct downstream signaling pathways depending on the biological context—most notably, the ERK1/2 MAPK signaling cascade. Of note, it has been suggested that FGFR1 may employ multiple effector pathways (e.g., FRS2, CRK, and PLCγ) converging on the activation of ERK1/2 in multiple cellular contexts (called “additive” signaling), whereas FGFR3 utilizes different pathways (e.g., STAT1 and ERK1/2) to elicit separate cellular responses (called “differential” signaling) [9].
Figure 1. Fibroblast growth factor-FGF receptor (FGF-FGFR) and cyclin D-cyclin-dependent kinase (CDK)4/6 pathways in breast cancer. The molecular components of these signaling pathways and their connections are indicated, together with the available inhibitors that target FGF, FGFR, or CDK4/6.

3. Oncogenic FGFR Signaling

In the context of tumorigenesis, in recent years, it has been shown that FGFs play crucial roles in regulating excessive cell growth and angiogenesis. As a matter of fact, they promote hepatocellular carcinoma progression [30], and their serum levels have been suggested to be indicative of recurrent Hepatocellular Carcinoma (HCC) [31] and an independent indicator of lymphatic invasion in patients undergoing the surgical removal of colorectal cancer [32]. Somatic genetic alterations and different patterns of FGFR expressions have been observed in many different types of cancers [33,34]. For instance, Helsten et al. demonstrated the presence of FGFR alterations in a large cohort of 4853 tumors using next-generation sequencing: FGFR mutations were present in 7.1% of malignancies, and these mutations could be categorized as gene amplifications (66%), point mutations (26%), or genomic rearrangements (8%) [35]. In particular, the FGFR1 gene (located on chromosome 8p11-12) is mutated in many cancers, while the other receptors are not frequently mutated. In breast cancer, for example, FGFR1 is mutated in ~15% of patients [35,36]. Over the last years, five types of alterations of FGFR signaling have been discovered that promote tumorigenesis: (a) the overexpression of FGFR genes (e.g., due to FGFR gene amplification) and post-transcriptional alterations that ultimately lead to an increase in FGFR protein levels, (b) the overexpression of FGF ligands in stromal and/or tumor cells, triggering autocrine and/or paracrine pathway activation, (c) FGFR point mutations leading to constitutive receptor activation, (d) the expression of FGFR fusion proteins due to genomic translocations causing constitutive activation of the FGFR kinase domain, and (e) the alternative splicing of FGFR genes, thereby modifying the FGFR isoform and ligand specificity and, hence, the range of FGF ligands capable to bind [6]. As a result of deregulated FGFR signaling, the cells display enhanced proliferation, elevated epithelial-to-mesenchymal transition (EMT), and reduced apoptosis [37].

The amplification of genes encoding for FGFRs were first discovered in BC patients in 1991 [38]. In a study involving 1875 breast cancer patients, amplification of the FGFR1
gene was observed in 10.5% of patients [39]. In addition to FGFR overexpression, elevated levels of FGFs can also trigger angiogenesis. In fact, FGF2 increases the expression of other proangiogenic molecules, such as VEGF and angioprotein-2 [40,41]. Both in vitro and in vivo studies indicated a crosstalk between FGF2 and VEGF that affects blood vessel formation and tumor formation [40,42,43]. Furthermore, FGF2 synergizes with platelet-derived growth factor (PDGF)-BB in the promotion of tumor angiogenesis and the formation of scaffolds through the recruitment of pericytes and vascular smooth muscle cells [44]. FGF2 was also shown to upregulate the expression of PDGFR, thereby enhancing the responsiveness to PDGF-BB. On the other hand, PDGF-BB-treated vascular smooth muscle cells upregulate FGFR1, which enhances their responsiveness to FGF2 responsiveness [44]. Of note, the combined inhibition of FGFR and VEGFR reduced angiogenesis and delayed tumor growth in a mouse model of pancreatic cancer, suggesting a potential therapeutic potential of this combination in cancer treatment [45]. Another study showed that blocking PDGFR-β suppressed FGF2 expression, leading to a reduction of cell proliferation and angiogenesis [46].

Furthermore, constitutively activating mutations of FGFR have been shown to lead to cancer or Crouzon syndrome [47–49]. Constitutive activating point mutations trigger conformational changes that cause the receptors to dimerize without the need for ligand binding, followed by trans-phosphorylation of the receptors and activation of the downstream signaling cascade [50]. The activity of the receptors is directed by the transmembrane conformation of the receptor dimers [50]. For instance, it has been reported that FGF1 and FGF2 ligands induce different conformational changes of their receptor, thereby modifying the distance between their intracellular domains and, hence, the number of dimers capable of trans-phosphorylation. This fine-tuning of FGFR signaling can lead to a varying degree of proliferation, apoptosis, EMT, and angiogenesis and may ultimately promote cancer formation [50]. It is important to notice that FGFR mutations are not only limited to the kinase domain [35]. Furthermore, in different tissues and different tumor types, distinct FGFR mutations have been found. The most well-known activating FGFR mutations discovered so far are: (1) N656E and N546K in FGFR1 [51]; (2) N549K, S252W, and P253R in FGFR2 [35,52]; (3) S249C, R248C, G370C, Y373C, R399C, and K650E in FGFR3 [35,53,54]; and (4) N535D, N535K, V550E, and V550L in FGFR4 [35,55].

FGFRs fusion proteins constitute yet another mechanism of oncogenic alterations of FGFR signaling. They account for 8% of FGFR signaling aberrations [35]. Gene fusions leading to FGFR fusion proteins have been discovered most frequently for the family members FGFR2 and FGFR3. For instance, the FGFR3-TACC3 gene fusion constitutes the receptor [56]. Furthermore, FGFR1 gene fusions have been detected with 11 other genes, such as BCR, FOP, and ZNF3 [57]. Apart from these examples, many other gene fusions have been discovered for FGFR family genes [35,58,59].

Interestingly, SNPs in FGFRs can be used to predict the development of premenopausal BC, as evinced by several genome-wide association studies [60–63]. For instance, Easton et al. showed that FGFR2 was among the five loci with SNPs significantly associated with breast cancer [60]. Similarly, Stacey et al. described that rs4415084 and rs1094179 were associated with an increased risk of developing BC [61]. In addition, Hunter et al. discovered that four SNPs in FGFR2 (rs2420946, rs1219648, rs2981579, and rs11200014) significantly correlated with malignancy in BC [63]. It has been suggested that such a correlation is due to the presence of a population of tumor-initiating cells, which express high levels of FGFR2 together with GABRA4 and FOXA1. In addition, when FGFR2 was downregulated using shRNAs, the population of tumor-initiating cells was also significantly downregulated [64].

Several studies have tested FGFR-targeting drugs at various stages of clinical development, and alterations of FGFRs can be a predictor for the efficacy of these drugs, as shown for NCP-BGJ398 [65]. For detailed analyses specific on the FGF/FGFR axis in breast cancer, refer to our previous publications [66,67]. Since the focus of this review is on the role of FGFR alterations during CDK4/6 inhibitor resistance, the following sections will focus
almost exclusively on CDK4/6 inhibition and the biological role of FGFR alterations in conferring resistance to CDK4/6-targeting drugs.

4. Inhibition of CDK4/6 Proteins for Treatment of Breast Cancer

The retinoblastoma protein (RB) is an important tumor suppressor that binds to and inhibits the family of E2F transcription factors to prevent unscheduled and excessive cell growth [68]. When the cell is ready for division, RB is phosphorylated and, thereby, inactivated. In several cancers, the growth-inhibitory function of RB is compromised. During transition from the G1 to the S phase of the cell cycle, CDK4 or CDK6 associates with cyclin D family proteins (cyclin D1, D2, or D3). The resulting cyclin D-CDK4/6 complex phosphorylates RB, which, in turn, dissociates from E2F, resulting in cell cycle progression [69]. There are various factors that influence the activity of cyclin D-CDK4/6 to hyperphosphorylate RB, leading to uncontrolled cell proliferation. During the past decade, CDK4/6 inhibitors have gained interest for the treatment of cancer. Currently, there are three CDK4/6 inhibitors in clinical development: palbociclib, abemaciclib, and ribociclib [70]. Their antitumor efficacy, in combination with endocrine therapies, led to their first FDA approval for the treatment of advanced BC in 2015 [71]. In fact, a first-line treatment with palbociclib, abemaciclib, or ribociclib together with aromatase inhibitors significantly prolonged the PFS of patients (from ~15 months to ~25 months) in comparison to aromatase inhibitors alone, as shown in the clinical trials PALOMA-2, MONARCH-3, and MONALEESA-2, respectively [72–74]. Moreover, combining palbociclib, abemaciclib, or ribociclib with fulvestrant leads to substantially prolonged PFS compared to fulvestrant alone (as shown in trials PALOMA-3, MONARCH-2, and MONALEESA-3, respectively) when used as a second-line therapy after progression occurs with aromatase inhibitors [75–77].

5. FGFR Overexpression Correlates with Resistance to CDK4/6 Inhibitors

For a long time, the loss of RB (encoded by RB1) has been considered as the most evident mechanism leading to a resistance to CDK4/6 inhibitor treatment. However, clinical data from the PALOMA-2 trial does not support this hypothesis, because only a small number of patients resistant to CDK4/6 inhibitors were RB-negative (n = 51) [72]. FGFRs are involved in many key mechanisms leading to cancer, such as proliferation, differentiation, and cell survival [33]. In addition, as mentioned previously, FGFRs are often mutated in BC. Therefore, it is not surprising that the expression or mutation of FGFRs may be associated with disease progression or therapy resistance in BC. For instance, FGFR1 and FGFR2 are not only associated with endocrine resistance but, also, with resistance to CDK4/6 inhibitors [78]. A large genome-scale functional screen regarding the genes involved in endocrine resistance showed that the FGFR, ERBB, insulin receptor, and MAPK pathways are key players in the phenomenon of endocrine resistance. Further comparisons of pretreatment and post-resistance samples revealed that FGFR/FGF axis alterations were acquired under the selective pressure of one ER-directed therapy (tamoxifen, AI, SERDs) [78]. Moreover, there are two activating FGFR2 mutations (M538I and N550K) that have been identified to be associated with resistance to palbociclib and/or fulvestrant in endocrine-resistant ER+ metastatic breast cancer (MBC) patients [78]. In these cases, alternative signaling pathways could possibly drive cancer growth and compensate for the inactivated cyclin D-CDK4/6-RB pathway. For instance, FGFR1 amplification could lead to the activation of PI3K/AKT and RAS/MEK/ERK signaling pathways in cancer cells that are endocrine therapy-resistant [79]. Clear clinical evidence for the involvement of FGFR signaling in therapy resistance during BC was uncovered from the analysis of data from larger trials. In fact, blood samples of 34 patients from the MONALEESA-2 trial, who progressed after CDK4/6 inhibitor therapy, were further investigated for circulating tumor DNA (ctDNA) using next-generation sequencing [80]. Surprisingly, FGFR1/2 amplifications or mutations were observed in 41% of the patients (14/34), who progressed after the ribociclib plus fulvestrant treatment. In addition, the occurrence of FGFR1 amplification in ctDNA samples from patients of the MONALEESA-2 trial
correlated with significantly shorter PFS compared to patients with wild-type FGFR1 [80]. Patients with FGFR1 amplification treated with ribociclib and letrozole displayed a PFS of 22 months. On the other hand, those patients without FGFR1 amplification treated with ribociclib and letrozole did not reach the median PFS after 32 months of follow-up, implying a significantly better PFS (HR: 95%; CI: 0.56 (0.36–0.87); p = 0.01). To corroborate this, at the ASCO 2019 meeting, O’Leary et al. also showed that FGFR1 amplification and the TP53 mutation were significantly correlated with worse survival in a cohort of 521 ER+ HER2− MBC patients treated either with palbociclib and fulvestrant or a placebo and fulvestrant [81]. These data further support the notion that FGFR1 amplification is associated with accelerated disease progression after treatment with CDK4/6 inhibitors and antiestrogens.

Furthermore, involvement of the FGFR signaling pathway during CDK4/6 inhibitor resistance was indicated by a number of very recent reports. For instance, data from 1503 patients in the MONALEESA-2/3/7 trials were presented at the ASCO 2020 meeting. It showed that potential biomarkers for resistance to ribociclib in ctDNA are FRS2 (altered in >1.87%; p = 0.28), MDM2 (altered in >2%; p = 0.06), PRKCA (altered; p = 0.04), AKT1 (altered; p = 0.03), BRCA1/2 (altered; p = 0.058), and HER2 (altered in 3.5%; p = 0.13) [81]. Therefore, FRS2, HER2, and MDM2 were the most commonly altered biomarkers in relation to ribociclib treatment resistance. With regard to genetic alterations most commonly associated with decreased ribociclib efficacy, the investigators identified alterations in CHD4 (p = 0.0073), CDKN2A/B/C (p = 0.046), and ATM (p = 0.0533) [82]. In addition, Razavi et al. reported an enrichment of alterations in multiple genes among samples from BC patients with CDK4/6 inhibitor resistance, such as PI3K/AKT, RB1, Hippo signaling genes, CDKN2A, and FGFR1 [83].

6. Therapeutically Reversing CDK4/6 Inhibitor Resistance by Targeting FGFR

Based on the prevalence of FGFR alterations in breast cancer and their relevance during resistance to CDK4/6 inhibition, as discussed above, targeting aberrant FGFR activity may hold clinical value for treating therapy-resistant BC. In a preclinical model of breast cancer using an ER+ cell line (MCF-7), the overexpression of FGFR1 causes a resistance to treatment with fulvestrant and a CDK4/6 inhibitor, either palbociclib or ribociclib [80]. Astonishingly, the acquired resistance was reversed when these cancer cells were subsequently treated with the tyrosine kinase inhibitor (TKI) lucitanib, which targets FGFR, as well as other receptor tyrosine kinases. Most importantly, in a patient-derived xenograft model with FGFR1-amplified ER+ breast cancer, the addition of the selective FGFR TKI erdafitinib to palbociclib and fulvestrant led to a complete tumor regression in some of the animals. In fact, the triple combination reduced the tumor size by more than 50% after a three-week treatment regimen, while 30% of the xenografts reached a complete response, which was maintained even after treatment was discontinued. Another interesting observation was discovered upon the NanoString expression analysis after treatment with fulvestrant and palbociclib: The expression of most cell cycle genes were reduced, while FGFR1, FGFR2, FGFR3, and the genes involved in MAPK signaling increased [80]. This suggests that the inhibition of CDK4/6, together with endocrine treatment, pushes cells towards an alternative, CDK4/6-independent mechanism to sustain cellular proliferation, such as through the FGFR-MAPK axis. On the contrary, the immunohistochemistry analysis showed that treatment with fulvestrant and the FGFR inhibitor erdafitinib were able to diminish ERα expression and FGFR1 phosphorylation, respectively [80]. Currently, there is an ongoing phase Ib clinical study evaluating a combination treatment with fulvestrant, palbociclib, and the FGFR inhibitor erdafitinib in endocrine-resistant ER+ HER2− MBC patients with an amplification of FGFR genes (NCT03238196) (Table 1). Notably, both FGFR
signaling and ER transcriptional activity induce the expression of cyclin D1 as a mediator of resistance to endocrine therapies with or without CDK4/6 inhibitors [81–83].

Table 1. Clinical trials using selective fibroblast growth factor receptor (FGFR) inhibitors in breast cancer.

| Identifier       | Phase | Administered Drug and Setting                              | Primary Endpoint                       | Status               |
|------------------|-------|------------------------------------------------------------|----------------------------------------|----------------------|
| NCT03238196      | I     | Erdafitinib; Palbociclib; Fulvestrant                      | AE                                     | Recruiting           |
| NCT04483505      | I     | Rogaratinib + palbociclib + fulvestrant                   | Recommended Phase 2 Dose; AE           | Recruiting           |
| NCT03344536      | I/II  | Fulvestrant; Debio 1347                                    | DLT (phase I); Best ORR (phase II)     | Active, not recruiting|
| NCT04504331      | I     | Infigratinib; Tamoxifen; Omnipaque 350; Iopamidol        | DLT                                    | Recruiting           |
| NCT04024436      | II    | TAS-120; Fulvestrant                                       | ORR, CBR, PFS                          | Recruiting           |
| NCT02299999      | II    | AZD2014; AZD4547; AZD5363; AZD8931; Selumetinib; Vandetanib; Bicalutamide; Olaparib; Anthracyclines; Taxanes; Cyclophosphamide; DNA intercalators; Methotrexate; vinca alkaloids; Platinum based chemotherapies; Bevacizumab; Mitomycin C; Eribulin; MEDI4736 | PFS                                    | Active, not recruiting|
| NCT02465060      | II    | Adavosertib; Afinatinib Dimaleate; Binimetinib; Capivasertib; Copanlisib; Copanlisib Hydrochloride; Crizotinib; Dabrafenib; Dabrafenib Mesylate; Dasatinib; Defactinib; Defactinib Hydrochloride; Erdafitinib; FGFR Inhibitor AZD4547; Ipatasertib; Larotrectinib; Larotrectinib Sulfate; Nivolumab; Osimertinib; Palbociclib; Pertuzumab; GSK2636771; Sapanisertib; Sunitinib Malate; Taselisib; Trametinib; Trastuzumab; Trastuzumab Emtansine; Ulixertinib; Vismodegib | ORR                                    | Recruiting           |
| NCT04125693      | II    | Rogaratinib (BAY1163877); Combination drug                | TEAE                                   | Enrolling by invitation|
| NCT01791985      | Ia    | AZD4547 + anastrozole or letrozole                         | Safety and tolerability; Change in tumor size at 12 weeks | Completed            |
| NCT01202591      | I/II  | AZD4547 + exemestane; AZD4547 + fulvestrant; Placebo + fulvestrant | Safety and Tolerability               | Completed            |

AE, Adverse Events; DLT, Dose-Limiting Toxicity; ORR, Overall Response Rate; CBR, Clinical Benefit Rate; PFS, Progression-free survival; and TEAE, Treatment-emergent adverse events.

Furthermore, Haines et al. showed that, in palbociclib-resistant lung cancer cells (H358 cell line), the expression of G1 cyclins and CDK changed in response to FGFR inhibition [84]. In fact, the treatment with FGFR inhibitor LY2874455 was associated with a decreased expression of CDK6, cyclin D1, and cyclin D3 in these cells. Interestingly, FGFR inhibition exerted the same effect on these cells as MEK and mTOR inhibition. The authors further demonstrated that the secretion of FGF2, also known as basic FGF (bFGF), was capable of promoting resistance to palbociclib and sensitivity to the MEK inhibitor by further enhancing FGFR1 activity. The treatment with FGF2 was shown to be associated with an increase in FGFR1 activation and ERK1/2 phosphorylation, whereas the treatment with an FGF2 neutralizing antibody significantly blocked FGFR1 and ERK1/2 activity. Notably, when these cells were stimulated with FGF2, the cycle profile was not changed by the presence of palbociclib, suggesting that stimulation of the FGF-FGFR axis regulates cell cycle progression irrespective of CDK4/6 activity, and hence, cell cycle is independent from the inhibitory effect of palbociclib [84].
In summary, the data described above strongly suggest that the FGFR pathway is capable of promoting tumor progression even in the presence of CDK4/6 inhibitors and that the addition of FGFR inhibitors is a promising therapeutic avenue to delay or stop tumor progression—in particular, for CDK4/6 inhibitor-resistant BC.

7. Other Mechanisms of CDK4/6 Inhibitor Resistance

While the hyperactivation of FGFR represents an important resistance mechanism to the CDK4/6 blockade in vitro and in vivo, there are several other pathways and alterations that confer resistance to CDK4/6 inhibition, either in concert with FGFR or alone. A recent study by Wander et al. reported the whole-exome sequencing of biopsies from ER+ BC patients with acquired or intrinsic resistance to CDK4/6 inhibitors [85]. Besides amplification and activating mutations in FGFR2, several other genomic alterations likely to mediate resistance were reported. Among them was RB1 loss and the aberrant activation of HER2 (encoded by ERBB2), AKT1, RAS, cyclin E2 (CCNE2), and Aurora kinase A (AURKA) [85]. Although RB1 loss is well-documented in mediating resistance to CDK4/6 inhibition [86–88], it appears to be a rare event in ER+ BC, occurring in only about 5% of patients [89]. In contrast, the hyperactivation of cyclin E-CDK2, another mechanism that has been described early in the search for resistance mechanisms, seems to be a frequent event in patient tumors. Cyclin E-CDK2 mediates G1-S progression of the cell cycle in concert with cyclin D-CDK4/6 [90]. Similar to cyclin D-CDK4/6, cyclin E-CDK2 complexes phosphorylate RB, thereby allowing E2F transcriptional activity and cell cycle progression. As cyclin E-CDK2 acts downstream of cyclin D-CDK4/6, the hyperactivation of cyclin E-CDK2 renders CDK4/6 inhibition ineffective and allows cell cycle progression despite the CDK4/6 blockade. In line with this, an analysis of the data from the PALOMA-3 clinical trial (palbociclib in combination with fulvestrant in patients with advanced ER+ BC) confirmed the important role of cyclin E during the response to CDK4/6 inhibitors. Patients with a high expression of cyclin E1 mRNA displayed a significantly shorter PFS compared to patients with low levels of cyclin E1 mRNA (7.6 months vs. 14.1 months) [91]. The hyperactivation of cyclin E-CDK2 can be mediated by several mechanisms. The amplification of CCNE1 and CCNE2, the genes encoding cyclin E1 and cyclin E2, leads to significantly increased cyclin E protein levels [85,92]. Furthermore, the downregulation of the endogenous CDK2 inhibitor proteins p21CIP1 and p27KIP1 leads to the increased activity of cyclin E-CDK2 complexes and has been shown to occur in CDK4/6 inhibitor-resistant BC cells [93]. A recent study by Costa et al. showed that PTEN loss and the subsequent activation of AKT signaling causes the exclusion of p27KIP1 from the nucleus, resulting in the reduced p27KIP1-mediated inhibition of CDK2 and, hence, enhanced cyclin E-CDK2 activity. Importantly, the cotreatment with a CDK4/6 inhibitor and an inhibitor of AKT signaling restored the sensitivity to CDK4/6 inhibition in vitro and in vivo [94].

Moreover, Jansen et al. demonstrated that increased levels of PDK1 can drive a resistance to CDK4/6 inhibition [95]. In this case, the aberrant activation of downstream AKT signaling results in increased expression of cyclins E, A, and D1, as well as increased activation of CDK2. Importantly, the addition of a PDK1 inhibitor to CDK4/6 inhibitor treatment reversed the resistance in ER+ breast cancer cell lines, and both PDK inhibition, as well as PI3K inhibition, potentiated the effect of a CDK4/6 blockade in xenograft models. The inhibition of AKT signaling therefore represents a promising addition to CDK4/6 inhibition in the clinic to prevent or delay resistance [95].

Another mechanism of CDK4/6 inhibitor resistance is the overexpression of CDK6 [87,96,97]. While, in some cases, this is triggered by CDK6 gene amplification [96], the upregulation of CDK6 was recently shown to be linked to FAT1 loss, causing activation of the HIPPO pathway [87]. In line with this, patients with FAT1-deficient tumors displayed a significantly shortened PFS compared to patients with FAT1-proficient tumors. Furthermore, a study by Cornell et al. reported an increased expression of CDK6 being elicited by suppression of the TGF-β pathway via exosomal miRNA 432-5p [98].
Finally, in triple-negative breast cancer (TNBC), an increased number of tumor cell lysosomes has been shown to mediate the intrinsic resistance to CDK4/6 inhibition [98]. Driven by their weak base properties, CDK4/6 inhibitors are sequestered into the enlarged lysosomal compartment and, therefore, are hindered from reaching their target, i.e., cyclin D-CDK4/6 complexes in the nucleus. Importantly, a cotreatment with lysosomotropic agents such as chloroquine and azithromycin rendered TNBC cells fully sensitive to CDK4/6 inhibition [98].

8. Conclusions

FGFR alterations occur frequently in breast cancer patients. Importantly, aberrant activation of the FGFR/FGF axis is commonly involved in endocrine resistance or resistance to CDK4/6 inhibitors during the treatment of BC. Mechanistically, this may occur through cellular rewiring of ER signaling and the activation of several pathways, such as the ERK1/2 MAPK signaling pathway. Although FGFR signaling induces cyclin D1, it is unlikely that cyclin D1 would be involved in the development of CDK4/6 inhibitor resistance. As a matter of fact, earlier data shed light on this matter, showing that there is a lack of association between cyclin D1 amplification with the survival of patients treated with the CDK4/6 inhibitor palbociclib (in the PALOMA-1/2 trials). Taken together, these data encourage the support for clinical trials using combinations of ER, CDK4/6, and FGFR or ERK antagonists in therapy-resistant BC patients. Since FGFR1 is among the most common mutations in BC patients, a concomitant treatment with the inhibitors of ER, CDK4/6, and FGFR1 could also be a valid first-line treatment alternative to avoiding the emergence of therapy resistance. Additionally, we herein discussed that various other mechanisms were identified that confer CDK4/6 inhibitor resistance. With the advent of molecular precision medicine, it will be both important and feasible to identify the specific resistance mechanism for each patient to select patients that will most likely benefit from an additional line of targeted therapy, such as FGFR inhibition.

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References
1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018, 68, 394–424. [CrossRef] [PubMed]
2. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2020. CA Cancer J. Clin. 2020, 70, 7–30. [CrossRef] [PubMed]
3. O’Shaughnessy, J. Extending survival with chemotherapy in metastatic breast cancer. Oncologist 2005, 10, 20–29. [CrossRef]
4. Hoffmann, J.; Bohlamann, R.; Heinrich, N.; Hofmeister, H.; Kroll, J.; Künzer, H.; Lichtner, R.B.; Nishino, Y.; Parczyk, K.; Sauer, G.; et al. Characterization of new estrogen receptor destabilizing compounds: Effects on estrogen-sensitive and tamoxifen-resistant breast cancer. J. Natl. Cancer Inst. 2004, 96, 210–218. [CrossRef] [PubMed]
5. Sobhani, N.; D’Angelo, A.; Pittacolo, M.; Roviello, G.; Otto, T.; D’Angelo, A.; Pittacolo, M.; Roviello, G.; Miccoli, A.; Corona, S.P.; et al. Alternative splicing of fibroblast growth factor receptor IgIII loops in cancer. J. Nucleic Acids 2012, 2012. [CrossRef]
6. Johnson, D.E.; Lu, J.; Chen, H.; Werner, S.; Williams, L.T. The human fibroblast growth factor receptor 3. Alternative splicing in immunoglobulin-like domain III creates a receptor highly specific for acidic FGF/FGF-1. J. Biol. Chem. 1994, 269, 11620–11627. [CrossRef]
7. Brewer, J.R.; Mazot, P.; Soriano, P. Genetic insights into the mechanisms of Fgf signaling. Genes Dev. 2016, 30, 751–771. [CrossRef]
8. Katoh, M.; Nakagama, H. FGF Receptors: Cancer biology and therapeutics. Med. Res. Rev. 2014, 34, 280–300. [CrossRef]
9. Koc, M.; Friesel, R.E. The role of fibroblast growth factors in tumor growth. Curr. Cancer Drug Targets 2009, 9, 639–651. [CrossRef] [PubMed]
10. Sleeman, M.; Fraser, J.; McDonald, M.; Yuan, S.; White, D.; Grandison, P.; Kumble, K.; Watson, J.D.; Murison, J.G. Identification of a new fibroblast growth factor receptor, FGFR5. Gene 2001, 271, 171–182. [CrossRef]
13. Regeenes, R.; Silva, P.N.; Chang, H.H.; Arany, E.J.; Shukalyuk, A.I.; Audet, J.; Kilkenney, D.M.; Rocheleau, J.V. Fibroblast growth factor receptor 5 (FGFR5) is a co-receptor for FGFR1 that is up-regulated in beta-cells by cytokine-induced inflammation. *J. Biol. Chem.* **2018**, *293*, 17218–17228. [CrossRef] [PubMed]

14. Wright, T.J.; Ladher, R.; McWhirter, J.; Murre, C.; Schoenwolf, G.C.; Mansour, S.L. Mouse FGF15 is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. *Dev. Biol.* **2004**, *269*, 264–275. [CrossRef]

15. Smallwood, P.M.; Munoz-Sanjuan, I.; Tong, P.; Macke, J.P.; Hendry, S.H.C.; Gilbert, D.J.; Copeland, N.G.; Jenkins, N.A.; Nathans, J. Fibroblast growth factor (FGF) homologous factors: New members of the FGF family implicated in nervous system development. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 9830–9837. [CrossRef]

16. Brewer, J.R.; Molotkov, A.; Mazot, P.; Hoch, R.V.; Soriano, P. Fgfr1 regulates development through the combinatorial use of signaling proteins. *Genes Dev.* **2015**, *29*, 1863–1874. [CrossRef]

17. Kouhara, H.; Hadari, Y.R.; Spivak-Kroizman, T.; Schilling, J.; Bar-Sagi, D.; Lax, I.; Schlessinger, J. A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* **1997**, *99*, 693–702. [CrossRef] [PubMed]

18. Larsson, H.; Klint, P.; Landgren, E.; Claesson-Welsh, L. Fibroblast growth factor receptor-1-mediated endothelial cell proliferation is dependent on the Src homology (SH) 2/SH3 domain-containing adaptor protein Crk. *J. Biol. Chem.* **1999**, *274*, 25726–25734. [CrossRef] [PubMed]

19. Ong, S.H.; Hadari, Y.R.; Gotoh, N.; Guy, G.R.; Schlessinger, J.; Lax, I. Stimulation of phosphotyrosylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 6074–6079. [CrossRef] [PubMed]

20. Mohammadi, M.; Honegger, A.M.; Rotin, D.; Fischer, R.; Bellot, F.; Li, W.; Dionne, C.A.; Jaye, M.; Rubinstein, M.; Schlessinger, J. A tyrosine-phosphorylated carboxy-terminal peptide of the fibroblast growth factor receptor (Flg) is a binding site for the SH2 domain of phospholipase C-gamma 1. *Mol. Cell. Biol.* **1991**, *11*, 5068–5078. [CrossRef] [PubMed]

21. Sahni, M.; Ambrosetti, D.C.; Mansukhani, A.; Gertner, R.; Levy, D.; Basilico, C. FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway. *Genes Dev.* **1999**, *13*, 1361–1366. [CrossRef] [PubMed]

22. Dudka, A.A.; Sweet, S.M.M.; Heath, J.K. Signal transducers and activators of transcription-3 binding to the fibroblast growth factor family. *Fibroblast growth factor receptor is activated by receptor amplification. Cancer Res.* **2010**, *70*, 3391–3401. [CrossRef] [PubMed]

23. Ulaganathan, V.K.; Sperl, B.; Rapp, U.R.; Ullrich, A. Germline variant FGFR4 p.G388R exposes a membrane-proximal STAT3 binding site. *Nature* **2015**, *528*, 570–574. [CrossRef] [PubMed]

24. Holt, J.A.; Luo, G.; Billin, A.N.; Bisi, J.; McNeill, Y.Y.; Kozarsky, K.F.; Donahee, M.; Wang, D.Y.; Mansfield, T.A.; Kliewer, S.A.; et al. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev.* **2003**, *17*, 1581–1591. [CrossRef] [PubMed]

25. Raucci, A.; Laplantine, E.; Mansukhani, A.; Basilico, C. Activation of the ERK1/2 and p38 mitogen-activated protein kinase pathways mediates fibroblast growth factor-induced growth arrest of chondrocytes. *J. Biol. Chem.* **2004**, *279*, 1747–1756. [CrossRef] [PubMed]

26. Shimoaka, T.; Ogasawara, T.; Yonamine, A.; Chikazu, D.; Kawano, H.; Nakamura, K.; Itoh, N.; Kawaguchi, H. Regulation of osteoblast, chondrocyte, and osteoclast functions by fibroblast growth factor (FGF)-18 in comparison with FGF-2 and FGF-10. *J. Biol. Chem.* **2002**, *277*, 7493–7500. [CrossRef] [PubMed]

27. Wong, A.; Lamotte, B.; Lee, A.; Schlessinger, J.; Lax, I. IRS2a attenuates FGF receptor signaling by Grb2-mediated recruitment of the ubiquitin ligase Cbl. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 6684–6689. [CrossRef] [PubMed]

28. Fong, C.W.; Leong, H.F.; Wong, E.S.M.; Lim, J.; Yussof, P.; Guy, G.R. Tyrosine phosphorylation of Sprouty2 enhances its interaction with c-Cbl and is crucial for its function. *J. Biol. Chem.* **2003**, *278*, 33456–33464. [CrossRef] [PubMed]

29. Browaeys-Poly, E.; Blanquart, C.; Perdereau, D.; Antoine, A.F.; Goenaga, D.; Luzy, J.P.; Chen, H.; Garbay, C.; Issad, T.; Cailliau, K.; et al. Grb14 inhibits FGF receptor signaling through the regulation of PLCγ recruitment and activation. *FEBS Lett.* **2010**, *584*, 4383–4388. [CrossRef]

30. Itoh, N.; Nakayama, Y.; Konishi, M. Roles of FGFRs as paracrine or endocrine signals in liver development, health, and disease. *Front. Cell Dev. Biol.* **2016**, *4*, 30. [CrossRef]

31. Poon, R.T.P.; Ng, I.O.L.; Lau, C.; Yu, W.C.; Fan, S.T.; Wong, J. Correlation of serum basic fibroblast growth factor levels with c-Cbl and is crucial for its function. *Am. J. Surg.* **2014**, *271*, 22–27. [CrossRef] [PubMed]

32. Turner, N.; Grose, R. Fibroblast growth factor signalling: From development to cancer. *Nat. Rev. Cancer* **2010**, *10*, 116–129. [CrossRef]

33. Ornitz, D.M.; Xu, J.; Colvin, J.S.; McEwen, D.G.; MacArthur, C.A.; Coulier, F.; Gao, G.; Goldfarb, M. Receptor specificity of the fibroblast growth factor family. *J. Biol. Chem.* **1996**, *271*, 15292–15297. [CrossRef] [PubMed]

34. Helsten, T.; Elkin, S.; Arthur, E.; Tomson, B.N.; Carter, J.; Kurzrock, R. The FGFR landscape in cancer: Analysis of 4,853 tumors by next-generation sequencing. *Clin. Cancer Res.* **2016**, *22*, 259–267. [CrossRef] [PubMed]

35. Elbauomy Elsheikh, S.; Green, A.R.; Lambros, M.B.; Turner, N.C.; Grainge, M.J.; P owe, D.; Ellis, I.O.; Reis-Filho, J.S. FGFR1 amplification in breast carcinomas: A chromogenic in situ hybridisation analysis. *Breast Cancer Res.* **2007**, *9*, R23. [CrossRef] [PubMed]

36. Fearon, A.E.; Gould, C.R.; Grose, R.P. FGFR signalling in women’s cancers. *Int. J. Biochem. Cell Biol.* **2013**, *45*, 2832–2842. [CrossRef]
38. Adnane, J.; Gaudray, P.; Dionne, C.A.; Crumley, G.; Jaye, M.; Schlessinger, J.; Jeanteur, P.; Birmbaum, D.; Theillet, C. BEK and FLG, two receptors to members of the FGF family, are amplified in subsets of human breast cancers. Oncogene 1991, 6, 659–663.

39. Courjal, F.; Cuny, M.; Simony-Lafontaine, J.; Louason, G.; Speiser, P.; Zeillinger, R.; Rodriguez, C.; Theillet, C. Mapping of DNA amplifications at 15 chromosomal localizations in 1875 breast tumors: Definition of phenotypic groups. Cancer Res. 1997, 57, 4360–4367.

40. Pepper, M.S.; Ferrara, N.; Orci, L.; Montesano, R. Potent synergism between vascular endothelial growth factor and basic fibroblast growth factor in the induction of angiogenesis in vitro. Biochem. Biophys. Res. Commun. 1992, 189, 824–831. [CrossRef]

41. Fuji, T.; Kuwano, H. Regulation of the expression balance of angiopoietin-1 and angiopoietin-2 by Shh and FGF-2. Vitr. Cell. Dev. Biol. Anim. 2010, 46, 487–491. [CrossRef] [PubMed]

42. Presta, M.; Dell’Era, P.; Mitola, S.; Moroni, E.; Ronca, R.; Rusnati, M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 2005, 16, 159–178. [CrossRef] [PubMed]

43. Courjal, F.; Cuny, M.; Simony-Lafontaine, J.; Louason, G.; Speiser, P.; Zeillinger, R.; Rodriguez, C.; Theillet, C. Mapping of DNA amplifications at 15 chromosomal localizations in 1875 breast tumors: Definition of phenotypic groups. Cancer Res. 1997, 57, 4360–4367.

44. Neilson, L.J.; Cao, R.; Hedlund, E.M.; Wang, Z.; Zhao, X.; Wedderskog, D.; Fun, K.; Bråkenhielm, E.; Cao, Y. Angiogenic factors FGF2 and PDGF-BB synergistically promote murine tumor neovascularization and metastasis. J. Clin. Invest. 2007, 117, 2766–2777. [CrossRef]

45. Allen, E.; Walters, I.B.; Hanahan, D. Brivanib, a dual FGF/VEGF inhibitor, is active both first and second line against mouse pancreatic neuroendocrine tumors developing adaptive/evasive resistance to VEGF inhibition. Clin. Cancer Res. 2011, 17, 5299–5310. [CrossRef]

46. Pietras, K.; Pahler, J.; Bergers, G.; Hanahan, D. Functions of paracrine PDGF signaling in the proangiogenic tumor stroma revealed by pharmacological targeting. PLoS Med. 2008, 5, e19. [CrossRef]

47. Galvin, B.D.; Hart, K.C.; Meyer, A.N.; Webster, M.K.; Donoghue, D.J. Constitutive receptor activation by Crouzon syndrome mutations in fibroblast growth factor receptor (FGFR) 2 and FGFR2/Neu chimeras. Proc. Natl. Acad. Sci. USA 1996, 93, 7894–7899. [CrossRef]

48. Presta, M.; Dell’Era, P.; Mitola, S.; Moroni, E.; Ronca, R.; Rusnati, M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 2005, 16, 159–178. [CrossRef] [PubMed]

49. Neilson, K.M.; Friesel, R.E. Constitutive activation of fibroblast growth factor receptor-2 by a point mutation associated with Crouzon syndrome. J. Biol. Chem. 1995, 270, 26037–26040. [CrossRef]

50. Sarabipour, S.; Hristova, K. Mechanism of FGF receptor dimerization and activation. Nat. Commun. 2016, 7. [CrossRef]

51. Lew, E.D.; Furdui, C.M.; Anderson, K.S.; Schlessinger, J. The precise sequence of FGF receptor autophosphorylation is kinetically driven and is disrupted by oncogenic mutations. Sci. Signal. 2009, 2, ra6. [CrossRef] [PubMed]

52. Dutt, A.; Salvesen, H.B.; Chen, T.H.; Ramos, A.H.; Onofrio, R.C.; Hatton, C.; Nicoletti, R.; Winckler, W.; Grewal, R.; Hanna, M.; et al. Drug-sensitive FGFR2 mutations in endometrial carcinoma. Proc. Natl. Acad. Sci. USA 2008, 105, 8713–8717. [CrossRef] [PubMed]

53. Hart, K.C.; Robertson, S.C.; Kanemitsu, M.Y.; Meyer, A.N.; Tynan, J.A.; Donoghue, D.J. Transformation and Stat activation by derivatives of FGFR1, FGFR3 and FGFR4. Oncogene 2000, 19, 3309–3320. [CrossRef] [PubMed]

54. Pandith, A.A.; Shah, Z.A.; Siddiqi, M.A. Oncogenic role of fibroblast growth factor receptor 3 in tumorigenesis of urinary bladder cancer. Urol. Oncol. Semin. Orig. Investig. 2013, 31, 398–406. [CrossRef] [PubMed]

55. Taylor VI, J.G.; Cheuk, A.T.; Tsang, P.S.; Chung, J.Y.; Song, Y.K.; Desai, K.; Yu, Y.; Chen, Q.R.; Shah, K.; Youngblood, V.; et al. Identification of FGFR4-activating mutations in human rhabdomyosarcomas that promote metastasis in xenotransplanted models. J. Clin. Investig. 2009, 119, 3395–3407. [CrossRef]

56. Nelson, K.N.; Meyer, A.N.; Siari, A.; Campos, A.R.; Motamedchaboki, K.; Donoghue, D.J. Oncogenic gene fusion FGFR3-TACC3 is regulated by tyrosine phosphorylation. Mol. Cancer Res. 2016, 14, 458–469. [CrossRef]

57. Parker, B.C.; Engels, M.; Annala, M.; Zhang, W. Emergence of FGFR family gene fusions as therapeutic targets in a wide spectrum of solid tumours. J. Pathol. 2014, 232, 4–15. [CrossRef]

58. Wu, Y.-M.; Su, F.; Kalyana-Sundaram, S.; Khazanov, N.; Ateeq, B.; Cao, X.; Lonigro, R.J.; Vats, P.; Wang, R.; Lin, S.-F.; et al. Identification of targetable FGFR gene fusions in diverse cancers. Cancer Discov. 2013, 3, 636–647. [CrossRef]

59. Stransky, N.; Cerami, E.; Schalm, S.; Kim, J.L.; Lengauer, C. The landscape of kinase fusions in cancer. Nat. Commun. 2014, 5, 4846. [CrossRef]

60. Easton, D.F.; Pooley, K.A.; Dunning, A.M.; Pharoah, P.D.P.; Thompson, D.; Ballinger, D.G.; Struwing, J.P.; Morrison, J.; Field, H.; Luben, R.; et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007, 447, 1087–1093. [CrossRef]

61. Stacey, S.N.; Manolescu, A.; Sulem, P.; Thorlacius, S.; Gudjonsson, S.A.; Jonsson, G.F.; Jakobsdottir, M.; Berghorsson, J.T.; Gudmundsson, J.; Aben, K.K.; et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat. Genet. 2008, 40, 703–706. [CrossRef] [PubMed]

62. Meyer, K.B.; Maia, A.-T.; O’Reilly, M.; Teschendorff, A.E.; Chin, S.-F.; Caldas, C.; Ponder, B.A.J. Allele-specific up-regulation of FGFR2 increases susceptibility to breast cancer. PLoS Biol. 2008, 6, e108. [CrossRef] [PubMed]
Cells 2021, 10, 293

63. Hunter, D.J.; Kraft, P.; Jacobs, K.B.; Cox, D.G.; Yeager, M.; Hankinson, S.E.; Wacholder, S.; Wang, Z.; Welch, R.; Hutchinson, A.; et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat. Genet.* 2007, 39, 870–874. [CrossRef] [PubMed]

64. Kim, S.; Dubrovska, A.; Salamone, R.J.; Walker, J.R.; Grandinetti, K.B.; Bonamy, G.M.C.; Orth, A.P.; Elliott, J.; Porta, D.G.; Garcia-Echeverria, C.; et al. FGFR2 promotes breast tumorigenicity through maintenance of breast tumor-initiating cells. *PLoS ONE* 2013, 8, e65161. [CrossRef] [PubMed]

65. Guagnano, V.; Kauffmann, A.; Wohrle, S.; Stamm, C.; Ito, M.; Barys, L.; Pornon, A.; Yao, Y.; Li, F.; Zhang, Y.; et al. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. *Cancer Discov.* 2012, 2, 1118–1133. [CrossRef] [PubMed]

66. Sobhani, N.; Ianza, A.; D’Angelo, A.; Roviello, G.; Giudici, F.; Bortul, M.; Zanconati, F.; Bottin, C.; Generali, D.; D’Angelo, A.; et al. Current status of fibroblast growth factor receptor-targeted therapies in breast cancer. *Cells* 2018, 7, 76. [CrossRef]

67. Sobhani, N.; Fan, C.; Flores-Villanueva, P.O.; Generali, D.; Li, Y. The fibroblast growth factor receptors in breast cancer: From oncogenesis to better treatments. *Int. J. Mol. Sci.* 2020, 21, 2011. [CrossRef]

68. Otto, T.; Sicinski, P. Cell cycle proteins as promising targets in cancer therapy. *Nat. Rev. Cancer* 2017, 17, 93–115. [CrossRef]

69. Topacio, B.R.; Zatloukal, E.; Cristea, S.; Xie, S.; Tambo, C.S.; Rubin, S.M.; Sage, J.; Koivomägi, M.; Skotheim, J.M. Cyclin D-Cdk4,6 drives cell-cycle progression via the retinoblastoma protein’s C-terminal helix. *Mol. Cell* 2019, 74, 758–770. [CrossRef]

70. Hamilton, E.; Infante, J.R. Targeting CDK4/6 in patients with cancer. *Cancer Treat. Rev.* 2016, 45, 129–138. [CrossRef]

71. Finn, R.S.; Crown, J.P.; Lang, I.; Boer, K.; Bondarenko, I.M.; Kulyk, S.O.; Ettl, J.; Patel, R.; Pinter, T.; Schmidt, M.; et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): A randomised phase 2 study. *Lancet Oncol.* 2015, 16, 25–35. [CrossRef]

72. Finn, R.S.; Martin, M.; Rugo, H.S.; Jones, S.; Im, S.-A.A.; Gelmon, K.; Harbeck, N.; Lipatov, O.N.; Walshe, J.M.; et al. Palbociclib and letrozole in advanced breast cancer. *N. Engl. J. Med.* 2016, 375, 1925–1936. [CrossRef] [PubMed]

73. Hortobagyi, G.N.; Steimer, S.M.; Burris, H.A.; Yap, Y.-S.; Sonke, G.S.; Paluch-Shimon, S.; Campone, M.; Blackwell, K.L.; André, F.; Winer, E.P.; et al. Ribociclib as first-line therapy for HR-positive, advanced breast cancer. *N. Engl. J. Med.* 2016, 375, 1738–1748. [CrossRef] [PubMed]

74. Goetz, M.P.; Toi, M.; Campone, M.; Trédan, O.; Bourayou, N.; Sohn, J.; Park, I.H.; Paluch-Shimon, S.; Huober, J.; Chen, S.C.; et al. MONARCH 3: Abemacibibl as initial therapy for advanced breast cancer. *J. Clin. Oncol.* 2017, 35, 3638–3646. [CrossRef] [PubMed]

75. Cristofanilli, M.; Turner, N.C.; Bondarenko, I.; Ro, J.; Im, S.-A.A.; Masuda, N.; Colleoni, M.; DeMichele, A.; Loi, S.; Verma, S.; et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): Final analysis of the multicentre, double-blind, phas. *Lancet Oncol.* 2016, 17, 425–439. [CrossRef]

76. Sledge, G.W.; Toi, M.; Neven, P.; Sohn, J.; Inoue, K.; Pivota, V.; Burdaeva, O.; Okera, M.; Masuda, N.; Kaufman, P.A.; et al. MONARCH 2: Abemacibibl in combination with fulvestrant in women with HR+/HER2-advanced breast cancer who had progressed while receiving endocrine therapy. *J. Clin. Oncol.* 2017, 35, 2875–2884. [CrossRef]

77. Slamon, D.J.; Neven, P.; Chia, S.; Fasching, P.A.; De Laurentiis, M.; Im, S.-A.; Petrakova, K.; Bianchi, G.V.; Esteva, F.J.; Martin, M.; et al. Phase III randomized study of ribociclib and fulvestrant in hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: MONALEESA-3. *J. Clin. Oncol.* 2018, 36, 2465–2472. [CrossRef]

78. Mao, P.; Cohen, O.; Kowalski, K.J.; Kusiel, J.G.; Buendia-Buendia, J.E.; Cuoco, M.S.; Exman, P.; Wünder, S.A.; Waks, A.G.; Nayar, U.; et al. Acquired FGFR and FGF alterations confer resistance to Estrogen Receptor (ER) targeted therapy in ER+ metastatic breast cancer. *Clin. Cancer Res.* 2020, 26, 5974–5989. [CrossRef]

79. Turner, N.; Pearson, A.; Sharpe, R.; Lambros, M.; Geyer, F.; Lopez-Garcia, M.A.; Natrajan, R.; Marchio, C.; Iorns, E.; Mackay, A.; et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res.* 2020, 80, 2085–2094. [CrossRef]

80. Formisano, L.; Lu, Y.; Servetto, A.; Hanker, A.B.; Jansen, V.M.; Bauer, J.A.; Sudhan, D.R.; Guerrero-Zotano, A.L.; Crootsmann, S.; Guo, Y.; et al. Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. *Nat. Commun.* 2019, 10, 1373. [CrossRef]

81. O’Leary, B.; Cutts, R.; Huang, X.; Hreibien, S.; Liu, Y.; Garcia-Murillas, I.; Andre, F.; Loi, S.; Loibl, S.; Cristofanilli, M.; et al. Genomic markers of early progression on palbociclib or without palbociclib for ER+ advanced breast cancer in the PALOMA-3 trial. *J. Clin. Oncol.* 2019, 37, 1010. [CrossRef]

82. Andre, F.; Su, F.; Solovieff, N.; Arteaga, C.L.; Hortobagyi, G.N.; Chia, S.K.L.; Neven, P.; Bardia, A.; Tripathy, D.; Lu, Y.-S.; et al. Pooled ctDNA analysis of the MONALEESA (ML) phase III advanced breast cancer (ABC) trials. *J. Clin. Oncol.* 2020, 38, 1009. [CrossRef]

83. Razavi, P.; dos Anjos, C.H.; Brown, D.N.; Ping, L.; Herbert, J.; Colon, J.; Liu, D.; Mao, M.; Norton, L.; et al. Molecular profiling of ER+ metastatic breast cancers to reveal association of genomic alterations with acquired resistance to CDK4/6 inhibitors. *J. Clin. Oncol.* 2019, 37, 1009. [CrossRef]

84. Haines, E.; Chen, T.; Komajosyula, N.; Chen, Z.; Herter-Sprie, G.S.; Cornell, L.; Wong, K.K.; Shapiro, G.I. Palbociclib resistance confers dependence on an FGFR-MAP kinase-mTOR-driven pathway in KRAS-mutant non-small cell lung cancer. *OncoTarget* 2018, 9, 31572–31589. [CrossRef] [PubMed]
85. Wander, S.A.; Cohen, O.; Gong, X.; Johnson, G.N.; Buendia-Buendia, J.E.; Lloyd, M.R.; Kim, D.; Luo, F.; Mao, P.; Helvie, K.; et al. The genomic landscape of intrinsic and acquired resistance to cyclin-dependent kinase 4/6 inhibitors in patients with hormone receptor–positive metastatic breast cancer. Cancer Discov. 2020, 10, 1174–1193. [CrossRef] [PubMed]

86. Condorelli, R.; Spring, L.; O'Shaughnessy, J.; Lacroix, C.; Bailleux, C.; Scott, V.; Dubois, J.; Nagy, R.J.; Lanman, R.B.; Iafrate, A.J.; et al. Polyclonal RB1 mutations and acquired resistance to CDK 4/6 inhibitors in patients with metastatic breast cancer. Ann. Oncol. 2018, 29, 640–645. [CrossRef] [PubMed]

87. Li, Z.; Razavi, P.; Li, Q.; Toy, W.; Liu, B.; Ping, C.; Hsieh, W.; Sanchez-Vega, F.; Brown, D.N.; Da Cruz Paula, A.F.; et al. Loss of the FAT1 Tumor Suppressor Promotes Resistance to CDK4/6 Inhibitors via the Hippo Pathway. Cancer Cell 2018, 34, 893–905. [CrossRef] [PubMed]

88. Dean, J.L.; McClendon, A.K.; Hickey, T.E.; Butler, L.M.; Tilley, W.D.; Witkiewicz, A.K.; Knudsen, E.S. Therapeutic response to CDK4/6 inhibition in breast cancer defined by ex vivo analyses of human tumors. Cell Cycle 2012, 11, 2756–2761. [CrossRef] [PubMed]

89. O’leary, B.; Cutts, R.J.; Liu, Y.; Hrebien, S.; Huang, X.; Fenwick, K.; André, F.; Loibl, S.; Loi, S.; Garcia-Murillas, I.; et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov. 2018, 8, 1390–1403. [CrossRef]

90. Malumbres, M.; Barbacid, M. Cell cycle, CDKs and cancer: A changing paradigm. Nat. Rev. Cancer 2009, 9, 153–166. [CrossRef]

91. Turner, N.C.; Liu, Y.; Zhu, Z.; Loi, S.; Colleoni, M.; Loibl, S.; DeMichele, A.; Harbeck, N.; André, F.; Bayar, M.A.M.; et al. Cyclin E1 expression and palbociclib efficacy in previously treated hormone receptor-positive metastatic breast cancer. J. Clin. Oncol. 2019, 37, 1169–1178. [CrossRef] [PubMed]

92. Herrera-Abreu, M.T.; Palafox, M.; Asghar, U.; Rivas, M.A.; Cutts, R.J.; Garcia-Murillas, I.; Pearson, A.; Guzman, M.; Rodriguez, O.; Grueso, J.; et al. Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. Cancer Res. 2016, 76, 2301–2313. [CrossRef] [PubMed]

93. Dean, J.L.; Thangavel, C.; McClendon, A.K.; Reed, C.A.; Knudsen, E.S. Therapeutic CDK4/6 inhibition in breast cancer: Key mechanisms of response and failure. Oncogene 2010, 29, 4018–4032. [CrossRef] [PubMed]

94. Costa, C.; Ye, W.; Ly, A.; Hosono, Y.; Murchi, E.; Walmsley, C.S.; Huynh, T.; Healy, C.; Peterson, R.; Yanase, S.; et al. Pten loss mediates clinical cross-resistance to CDK4/6 and PI3Kα inhibitors in breast cancer. Cancer Discov. 2020, 10, 72–85. [CrossRef] [PubMed]

95. Jansen, V.M.; Bhola, N.E.; Bauer, J.A.; Formisano, L.; Lee, K.M.; Hutchinson, K.E.; Witkiewicz, A.K.; Moore, P.D.; Estrada, M.V.; Sánchez, V.; et al. Kinome-wide RNA interference screen reveals a role for PDK1 in acquired resistance to CDK4/6 inhibition in ER-positive breast cancer. Cancer Res. 2017, 77, 2488–2499. [CrossRef]

96. Yang, C.; Li, Z.; Bhatt, T.; Dickler, M.; Giri, D.; Scaltriti, M.; Baselga, J.; Rosen, N.; Chandraratnapa, S. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. Oncogene 2017, 36, 2255–2264. [CrossRef]

97. Cornell, L.; Wander, S.A.; Visal, T.; Wagle, N.; Shapiro, G.I. MicroRNA-Mediated Suppression of the TGF-β Pathway Confers Transmissible and Reversible CDK4/6 Inhibitor Resistance. Cell Rep. 2019, 26, 2667–2680. [CrossRef]

98. Fassl, A.; Brain, C.; Abu-Remaileh, M.; Stukan, I.; Butter, D.; Stepien, P.; Feit, A.S.; Bergholz, J.; Michowski, W.; Otto, T.; et al. Increased lysosomal biomass is responsible for the resistance of triple-negative breast cancers to CDK4/6 inhibition. Sci. Adv. 2020, 6. [CrossRef]