Title: Current Status of Fibroblast Growth Factor Receptors Targeted Therapies in Breast Cancer

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Abstract

Breast cancer (BC) is the most common malignancy and second only to lung cancer in terms of mortality in women. Despite the incredible progress made in this field, the metastatic breast cancer leaves a poor prognosis. In an era of personalized medicine, there is an urgent need for a better knowledge of the biology leading to the disease, which can lead to the design of always more accurate drugs against patients' specific molecular aberrations. Among one of the actionable targets is the Fibroblast Growth Factor Receptor (FGFR) pathway, triggered by specific ligands. The FGFRs/FGFs axis offers interesting molecular targets to be pursued in clinical development. This mini-review will focus on the current knowledge of the FGFRs mutations leading to tumour formation and summarizes the state-of-the-art of therapeutic strategies for targeted treatments against the FGFRs/FGFs axis in the context of BC.

Keywords

Fibroblast Growth Factor; Fibroblast Growth Factor Receptor; Targeted Treatments; Breast Cancer.

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Introduction

The biochemical structure of the receptor

Breast Cancer (BC) is the most common malignancy and second only to lung cancer in terms of mortality in women worldwide, with an estimated 268,670 new diagnosis and 41,400 deaths in 2018 in the USA for both men and women [1]. With the advancement of personalized medicine, patients have been stratified on the bases of expression of actionable molecular targets. Among such actionable targets in BC is the fibroblast growth factor receptor (FGFR).

The FGFR family is characterised by four receptors, binding to 18 ligands called fibroblast growth factors (FGFs), employing heparin as a co-factor [2,3]. These receptors have pivotal roles in embryogenesis and metabolism [4] and play a critical role in the development of the skeletal system [5]. Fibroblast growth factors (FGFs) are secreted glycoproteins that are promptly sequestered by the extracellular matrix and at cell membrane by heparan sulfate proteoglycans (HPSGs) which, in turn, make the FGF ligand-receptor interaction stable by safeguarding FGFs from protease-mediated degradation [6]. Each ligand tether FGFRs with different specificity; some are promiscuous, such as FGF1, and bind to multiple receptors, while others, such as FGF7, tether only one receptor isoform [7]. FGFRs are a class of receptor tyrosine kinase (RTK) and are single-pass membrane proteins made of N-terminal extracellular (EC) domains with three immunoglobulin-like subdomains (D1, D2 and D3), a transmembrane (TM) domain with a single α-helix, and an intracellular (IC) region including tyrosine kinase motifs, a juxta-membrane domain and a carboxyl-terminal tail [8,9]. There is a total of seven signalling receptors, encoded by four FGFR genes, FGFR1-4 [10]. Furthermore, immunoglobulin-like subdomains D2 and D3 are necessary and sufficient for ligand binding

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whereas the aminoterminal part of the receptor including D1 has an auto-inhibitory function. In addition, alternative splicing of the D3 extracellular fragment of FGFR1, 2 or 3 might encode isoforms that differ in relation to the specificity of ligand binding [11].

FGFR signalling

Since the discovery of RTKs around fifty years ago, the most widely accepted model of RTKs transduction is the "diffusion-based", also known as the "canonical" model. This model states that RTKs are monomers in need of a ligand in order to dimerize and cross-phosphorylate and therefore activate each other. After its activation, FGFRs transmit biochemical signals with lateral dimerization within the plasma membrane. The dimerization of the receptor is a necessary step as it shortens the distance between the two tyrosine kinase domains, allowing them to cross-phosphorylate on tyrosine residues at the activation chain of the receptors [12]. These kinases triggering process tethers adaptors and phosphorylates proteins within the cytoplasm, triggering downstream signalling cascades [13]. Noteworthy, among such adaptors is FGFR substrate 2 (FRS2), which upon ligand binding and its association with the receptor, triggers the downstream signalling with the activation of mitogen-activated protein kinase (MAPK) and the phosphoinositide-3-kinase (PI3K)/AKT pathways. Of note, FGFR signalling has been also found connected to phospholipase C-gamma (PLC-γ) in an FRS2-unrelated mode and activates protein kinase C (PKC), which partially strengthen the MAPK pathway activation by phosphorylating RAF. However, in relation to the cellular context, many other pathways might be activated by FGFRs involving signal transducer and activator of transcription signalling and ribosomal protein S6 kinase 2 (RSK2), the p38 MAPK and Jun N-terminal kinase pathways [6,9,14]. Interestingly, all such related pathways are captivating

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targets to be explored in the context of clinical development of anti-cancer agents against the FGFs/FGFRs axis (Figure 1).

The control of FGFR signalling

Regulation of FGF signalling is critical in order to ensure a balanced response to receptor stimulation but, unfortunately, the mechanism of attenuation is poorly understood and it is likely to vary depending on the cell type. Nevertheless, the current understanding is that it takes place largely via a negative feedback mechanism, involving receptor internalisation through ubiquitination [15] and induction of negative regulators, such as SEF, SPRY, SPRED 1 and 2 [16,17]. An additional level of control takes place in the form of receptor auto-inhibition [18]; in addition, the electrostatic bonding between the acid box and the HS-binding site creates an autoinhibited and closed conformation [19]. This process of autoinhibition sustains FGF binding specificity to receptors.

FGFRs as oncogenic drivers

A long series of evidence are pointing toward the direction that deregulated FGFRs can work as driving oncogenes in several tumour types [6,20]. When FGF receptor is deregulated, aberrant activation of downstream signalling results in mesenchymal, antiapoptotic and mitogen responses in cells. To date, several and different FGFR pathways aberrations have been uncovered in cancer and include: (i) translocations of FGFR-fusion proteins with constitutive FGFR kinase activity; (ii) gene amplification or post-transcriptional regulation resulting in high expression levels of the receptor protein; (iii) upregulation of FGF in cancer, stromal cells and/or extracellular matrix, showing paracrine/autocrine activation of the

pathway; (iv) FGFR alternative splicing and isoform switching, are alterations that modify ligand specificity rising the range of FGFs that can stimulate proliferation; (v) FGFR mutations result in receptors that are constitutively active; in this regard, accordingly to Sarabipour et al [21], FGFR dimers exist also in the lack of ligand at physiological concentrations and these unliganded dimers are stabilized via contacts between the TM domains and the IC domains. Furthermore, unliganded FGFR dimers are phosphorylated, giving an explanation of the fact that the overexpression of FGFR leads to cancer [22–24]. However, structural changes induced by the ligand occur in FGFR dimers in the plasma membrane and the ligand can control the structure of the TM domain causing a switch to a specific conformation. Such a resulting TM dimers' configuration regulates the receptor activity. Ultimately, the structural transformations in response to FGF1 and FGF2 are quite different, leading to different distances between IC domains and the different level of phosphorylation of FGF1 - and FGF2 - bound dimers. For this reason, there are several resulting active ligand-bound states for the FGF receptors [21]. In humans, many gain-of-function germline mutations in the FGFR genes bring to skeletal dysplasias and mutation in cancer are similar to those seen in hereditary disease and intriguingly, they are not limited to the kinase domain but can cover the full length of the gene. Especially, FGFR signalling in cancer shows a clear dependence with the context, resulting in aberrations differing according to tumour type [14,25–28]. For the purpose of this mini-review we will focus in the next chapter on FGFR abnormalities that have been identified in breast cancer.

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FGFR genetic alterations in breast cancer

The first documentation for the amplifications of the *FGFR*s genes in human breast cancer relates back to the early 1990s [29]. A large series of studies since then have both confirmed the initial observation of the oncogenic potential of FGFRs and significantly expanded upon mechanisms by which FGFs/FGFRs axis contributes to breast cancer formation. In addition to gene amplification, higher expression of ligands and receptors, mutations and single nucleotide polymorphisms have been identified in FGFRs in BC patients' samples, suggesting that more than one mechanism is involved in the aberrant activation of FGFRs.

The FGF/FGFR signalling pathway is frequently deregulated in human cancers. In breast cancer, FGFR1 amplification is the most frequent genomic aberration, whereas the *FGFR2-4* genes amplifications and FGFR activating mutations are uncommon.

Amplification of *FGFRs*

About 14% of breast cancer patients bear mutations in the 8p11-12 chromosomal region, which is a site harbouring the *FGFR1* gene locus [24,30,31]. Always in the context of BC, amplification of *FGFR1*- and/or 11q12-14, which is a chromosomal region containing *CCND1*, *FGF3*, *FGF4*, and *FGF19*, have been detected in 23% of hormone receptor-positive (HR+) BC, 27% HER2-positive BC, and 7% TNBC and it has been shown to be a prognostic indicator for early relapses and poor patients' outcome [32–36]. As shown by *in vitro* studies, the expression of *FGFR1* is required for the survival of FGFR1-amplified BC cell lines, supporting the oncogenic role of *FGFR1* amplification. Using a couple of cell lines with either *FGFR1* (MDA-MB-134) or *FGFR2* amplified (SUM52) Andrè et al showed how the proliferation and tumour growth were both reduced after treatment with anti-FGFR1 dovitinib (TKI1258)

therapy. The IC_{50} for cell growth inhibition was of 190 nmol/L and 180 nmol/L. In negative controls, that did not express neither *FGFR1* nor *FGFR2*, the IC_{50} values were more than 2000 nmol/L. Moreover, the authors showed through an *in vivo* mouse model with *FGFR1*-amplified BC primary xenograft (HBCx-3) that tumour regressed after treatment with 50 mg/kg of dovitinib, compared to mice treated with just a vehicle control ($p<0.001$) [37]. Additionally, *FGFR1* amplification has been shown to drive resistance to endocrine therapy. As a matter of fact Turner et al, through a viability assay, showed that *FGFR1*- overexpressing breast cancer cell-lines MDA-MB-134 and SUM44 were resistant to 4-hydroxytamoxifen (4-OHT). Such resistance was reversed when the cells were treated with siRNA against *FGFR1 (siFGFR1)*, suggesting therefore that FGFR1 drives sensitivity to this type of therapy. Another proof of concept that such a mechanism of sensitivity to the drug was attributable to *FGFR1* comes from the fact that the addition of FGFR2 to siFGFR1-treated cell lines did not reverse the resistance to 4-OHT. Moreover, the authors proved that treatment with an FGFR1 inhibitor (PD173074) caused a loss in the cell lines of their colony-formation ability, suggesting a role that FGFR1 has in those cancer cells in making them capable to grow colonies and therefore confirming its tumorigenic function in BC [24].

Accordingly to several studies, FGFR2 amplifications - shown to occur in 4% of TNBC- as well as activating mutations of the receptor, have been associated with high sensitivity to FGFR inhibitors [37–39] and maintenance of tumour-initiating cells [38].

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FGFRs activating mutations

In the context of FGFR-driven tumour formation, although its gene amplifications are the most common type of alterations leading to BC, there are some evidences showing that activating mutations have also an oncogenic role in this type of cancer. Activating FGFRs mutations may result in aberrant FGFR signalling through different mechanisms, including: i) constitutive dimerization of the receptors; ii) enhancement of the kinase domain's activity; iii) alteration in affinity for FGF ligands. In fact the most commonly occurring oncogenic FGFRs aberrations in BC are the following ones: FGFR1 translocation [40]; FGFR1 amplification (10-15%) [24,32–37], which has shown transforming potential in several *in vivo* models, conferring also sensitivity to FGFR inhibitors [35] and ability to drive resistance to endocrine therapy [24] as previously described; FGFR2 translocation, which in preclinical models exhibited transforming potential and sensitivity to FGFR inhibitors [40]; FGFR2 amplifications (4%, [38]), which in preclinical models conferred resistance to FGFR inhibitors [37–39]. In different solid tumors, including breast cancer, various studies in literature have described FGFRs activating mutations:

FGFR1: two point mutations (K656E and N546) have observed *in vitro* affecting the intracellular domain of the receptor and therefore acting as activating mutations [41,42].

FGFR2: in the COSMIC database 12 mutations have been reported, but only 7 of them are activating mutations (missense mutations) of the extracellular domain, with the most common ones being P253R, N549K and S253R [30].

FGFR3: in the COSMIC database 13 different point mutations have been described, with S249C being the most common one. The most frequent activating mutations of FGFR3 affect either the trans-membrane (A391E, G370C, G380R, Y373C, S371C) or the extracellular (R248C,

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S249C) domains of the protein. Rarer mutations are those in the kinase domain (N540S, K650E, K650M, K650N, K650Q, and K650T) [30,43].

FGFR4: there are only 4 FGFR4 activating mutations occurring in the kinase domain. Two of them (E550 and K535) cause auto-phosphorylation and thereby constitutive activation of the receptor [30,44].

Some of these mutations have been associated with an increased risk in developing breast cancer. Genome-Wide-Association-Studies (GWAS) from several independent research groups showed how SNPs on intron 2 of FGFR2 is a risk factor associated with disease. Easton et al in their GWAS composed of 4398 BC cases and 4316 controls investigated commonly known SNPs to find risk factors associated with the disease. The authors identified SNPs in five new loci that exhibited strong and consistent association with breast cancer ($p<10^{-7}$). Among these loci there was FGFR2 whose oncogenic role in BC had already been consolidated in literature [45]. Accordingly, Stacey et al in their GWAS made of 6145 BC cases and 33016 controls identified 2 SNPs (rs4415084 and rs1094179) on 5p12 conferring risk to develop BC especially in ER+ BC ($p=1.3 \times 10^{-17}$) [46]. By the use of gene expression microarray data, Meyer et al showed that there is a trend of increasing FGFR2 expression in rare homozygotes. The authors proved by RT-PCR that there is a different trend between FGFR2 rare and common homozygotes (Wilcox p-value of 0.028) and proved that Oct-1/Runx2 binding site is probably the dominant determinant for such a differential expression [47]. Accordingly to Easton et al and Stacey et al, the GWAS of Hunter et al identified alleles in FGFR2 associated with a higher risk of sporadic post-menopausal BC. Their study investigated 528173 SNPs in 1145 postmenopausal women of European ancestry with invasive BC and 1142 controls. The authors identified several genomic locations as potentially associated with BC and 4 of the ones with the most significant p-values – r1219648, rs2420946, rs11200014 and rs2981579- were located on the intron 2 of FGFR2 [45].

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Although GWAS from several groups confirmed that germ-line polymorphism in intron 2 of FGFR2 has been associated with BC susceptibility [45–48], emphasizing the relevance of FGFR2 in BC development, little is known about the mechanism by which FGFR2 functions as a risk factor leading to BC. A plausible explanation comes from the work of Kim et al showing that FGFR2 promotes breast cancer tumorigenicity by maintaining tumour-initiating cells (TICs). As a matter of fact, the authors revealed in their model of BC TICs –isolated through flow-cytometer with $CD29^{high}$ $CD24^{+}$ markers –several markedly upregulated genes compared to non-TICs. The genes that exhibited significantly higher mRNA expression levels were GABRA4, FGFR2 and FOXA1. The group then proved that also the FGFR2 protein levels were higher in TICs. Furthermore, their *in vivo* results proved that down-regulation of FGFR2 by shRNA substantially reduced (64% to 70%) the TICs subpopulation ($CD29^{high}$ $CD24^{+}$). Intriguingly, shFGFR2 significantly increased (65 to 67%) the non-TIC cells ($CD29^{low}$ $CD24^{-}$). These results suggest that FGFR2 causes a decrease of TICs and an increase of non-TICs. Moreover, the same research group also proved that ectopic expression of FGFR2 in shFGFR2-treated mice resulted in a considerable increase of bipotent precursor-like cells (K18+K14+), suggesting that FGFR2 rescued the bipotent capacity driven by the *FGFR2* knockdown. Therefore inhibiting FGFR2 could be a valid strategy to destroy those TICs populations in BC. Kim et al in an *in vivo* mice model proved that treatment with FGFR2-inhibitor (TKI258) suppressed tumour growth. Such a growth inhibition was accompanied by a significantly reduced phosphorylation of FGFR2 and Erk1/2, suggesting that such an inhibition was dependent on FGFR2 activation and its targets [38]. Guagnano et al screened 541 cancer cell lines, including BC cell lines, for “FGFR genetic alterations” and investigated the sensitivity of the cells to an anti-FGFR inhibitor (NVP-BGJ398). They considered 9 distinct types of FGFR genetic alterations already established in literature: FGFR1-4 copy number gains; FGFR1-3

activating mutations; FGFR1 or FGFR2 chromosomal translocations. Their compound NVP-BGJ398 proved to be a strong multi-kinase inhibitor targeting FGFR1-4 and VEGFR2. Finally the research group showed that such FGFR genetic alterations were considered as a top predictor of response to NVP-BGJ398 [39]. Reis-Filho et al demonstrated through comprehensive molecular analysis in a small study made of 13 lobular breast carcinomas that high level of gains were detected at the chromosomal position 8p12-p11.2 in 6 of their primary cases. Furthermore, through siRNA and a small-molecule inhibitor of FGFR1 (SU5402) they proved that inhibition of FGFR1 was capable of blocking survival of the ductal carcinoma cell line MDA-MB-134 [35]. Therefore, the analysis of different research groups are supportive of the fact that inhibition of the FGFR/FGF axis could be a valid approach to further investigate in large and randomized clinical trials. Accordingly to more recent studies based on NGS the levels of FGF3 and FGFR4 were very low in BC. In fact, Helsten et al NGS study made of 4853 solid tumors – including 522 BC- proved that the amplification of the FGFR3 and FGFR4 is expressed in less than 1% and 2% of BC patients, respectively [30]. On the other hand, in a much older study based on RT-PCR and made of 10 tumor cell lines and 103 breast-tumour samples, FGFR4 was expressed in up to 32% of patients with BC while FGFR3 was not detected [49].

Gene fusions of FGFRs

Gene fusion consists in the joining of two different genes, either a translocation or an inversion. It represent 8% of FGFR aberrations [30]. There are at least 11 fusion partners identified for FGFR1. Such fusions include ZNF198, BCR and FOP. The most commonly occurring FGFR genes with this kind of alteration are the FGFR2 and the FGFR3. The majority of gene fusions have been identified in patients with myeloproliferative disorder stem cell leukaemia/lymohoma syndrome. Gene fusions with TACC3 gene, leading to an FGFR3-TACC fusion protein, lead to a constitutive activation of the receptor [50,51]. As to breast cancer, FGFR1-3 gene fusions have been observed to occur with multiple partners (i.e. TACC1, TACC2, TACC3, BAIAP2L1, BICC1, NPM1, PPAPDC1A, AFF3, SLC45A3 and AHCYL1) [30,40,52].

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Anti-FGFRs therapies

The relevance of the FGFR/FGF pathway in the development and progression of BC justifies the growing interest in developing new targeted therapies for this pathway [53]. Small inhibitors of FGFR tyrosine kinase under clinical evaluation mainly at early stages trials and efforts are being made to increase the selectivity to the intracellular ATP-binding domain of the receptor, to minimize the toxicity [14]. BGJ398 (infigratinib), a pan-FGFR inhibitor, is currently under evaluation as single agent to establish the MTD (NCT01004224). Moreover, another phase I trial (NCT01928459) was conducted in order to determine MTD for BGJ398 with BYL719 for the treatment of solid tumors bearing *FGFR 1-3* alterations and *PIK3CA* mutations. AZD4547 is another TKI that has shown strong activity against FGFR1, FGFR2 and FGFR3, while yet weaker activity against FGFR4; its safety and effectiveness is under evaluation in ER+ patients harboring *FGFR1* polisomy or gene amplification after progression to endocrine-based therapies (NCT01791985). Another phase I study (NCT03238196) has been conducted for ER+ HER2- metastatic breast cancer (MBC) patients in order to evaluate Erdafitinib, which is an orally administered FGFR inhibitor, in combination with anti-CDK4/6 palbociclib and anti-HR fulvestrant. Moreover, there are tyrosine kinase inhibitors with multiple targets: TKI258 (dovitinib) is effective against VEGFR1-3, FGFR1-3, and PDGFR and is under evaluation in combination with fulvestrant for the treatment of HER2 negative metastatic breast cancer.

The development of non-selective TKI targeting FGFRs has been recently shown to be very fruitful in preclinical studies [54]. Some of these inhibitors have passed phase I trial with encouraging results as to safety and tolerability. E3810 (lucitanib), a drug that inhibits VEGFR1, VEGFR2, VEGFR3, FGFR1 and colony stimulating factor 1 receptor (CSF1R), and FGFR2 has been administered as single agent two phase II trial in MBC patients with or

without FGFR1 amplification: one phase II study (NCT02202746), is ongoing. A phase I study is currently evaluating safety and tolerability of the combination of letrozole and nindetanib, a triple kinase inhibitor (VEGFR, PDGFR, FGFR), in postmenopausal women with ER+ MBC (NCT02619162).

Other strategies used to inhibit FGFR/FGF axis are under investigation [55]. Similarly to the development of antibodies against HER2+ isoforms, antibodies against FGFR isoforms represent a valid therapeutic strategy to intervene in BC. As a matter of fact, GP369 recognizes FGFR-IIIb isoform and has exhibited good results in blocking breast cancer cell lines proliferation [56]. Such preliminary results warrant further research. Lastly, another approach against the FGFR/FGF axis consists in the use of inhibitors of FGF ligands. Long pentraxin-3 (PTX3) is an inhibitor of various FGFR ligands, among them FGF2 and FGF8b, which are both found implicated in breast cancer development [57]. FP-1039 is a ligand-trap recently developed in which a ligand-binding domain of FGFR1 is fused to an Ig-Fc domain. This compound showed promising activity *in vitro* passed a phase I clinical trial (NCT00687505) for solid tumours, including breast cancer [58]. Of note, FGFR might play a role in the development of resistance to anti-VEGFR therapy. Therefore a proposed strategy is the use of small molecules targeting both receptors [59]. Additionally, several studies have suggested that inhibition of FGFR activation may lead to a synergic activity with endocrine-based therapies and anti-ErbB therapies. For this reasons it would be interesting considering the combination of anti-FGFRs therapies with other already established treatments for breast cancer targeting other pathway in order to obtain a higher effect while developing more powerful molecules to better treat this disease [60]. Table 1 summarizes ongoing clinical trials testing anti-FGFRs therapies in breast cancer. These results are certainly relevant but a deeper understanding of the FGFR action in the promotion of breast cancer and its connection with other already established pathways are surely needed.

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Discussions

FGFR is an already established BC oncogenic driver involved in various mechanisms leading to formation of vessels, tumour growth and avoidance of apoptosis. Various FGFRs genetic alterations have been found associated with BC and therapeutic strategies have been implemented in order to inhibit FGFRs. As a matter of fact, several anti-FGF/FGFR therapies have been tested at phase 1 and 2 clinical trials. Among them are FGFR inhibitors erdafitinib and nindetanib, a pan-FGFR inhibitor infigratinib, FGFR1-3 inhibitors AZD4547 and dovitinib. It is worth noting that *FGFR1* amplification is the most frequent genomic aberration, whereas the *FGFR2-4* gene amplifications and FGFR activating mutations are uncommon. Therefore, in line of future therapeutic strategies involving FGFRs in BC the FGFR1 should be considered as a primary target to be predominantly pursued as it is the most commonly FGFR altered gene found up-to-date in this context. The role of anti-FGFR therapies should be tested in combination with other molecules targeting downstream molecules of the same pathway, FGFs and of other tyrosine kinase cell membrane receptors -like EGF, PDGF, VEGF, CCK, AXL, ROS, RET, RYK, TIE, LMR and HGF. Through stratification of patients in groups on the basis of specific molecular alterations and through the evaluation of always-more-accurate predictive biomarkers, it becomes easier to choose which combination of therapies could be most beneficial for the patients. Moreover, in an era where immunotherapy is at the front-line of innovation, it would be interesting testing combinations of anti-FGFR or anti-FGF therapies with specific immune stimulating molecules – like with checkpoint inhibitors- in order to improve survival and quality of life of BC patients with novel and always more accurate therapeutic strategies.

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Authors Contributions:

Sobhani N. contributed to the implementation of the work. He also contributed in reviewing the literature and editing the manuscript; Ianza A. contributed to reviewing the literature, writing and editing the manuscript; D'Angelo A. contributed to reviewing the literature, writing and editing the manuscript; Generali D. contributed to the original idea of the work. By giving his expert opinion he also contributed in the writing and editing of the manuscript.

Conflicts of interest:

The authors declare no competing interests.

Figure 1. Current status of fibroblast growth factor receptor therapeutic strategies in breast cancer

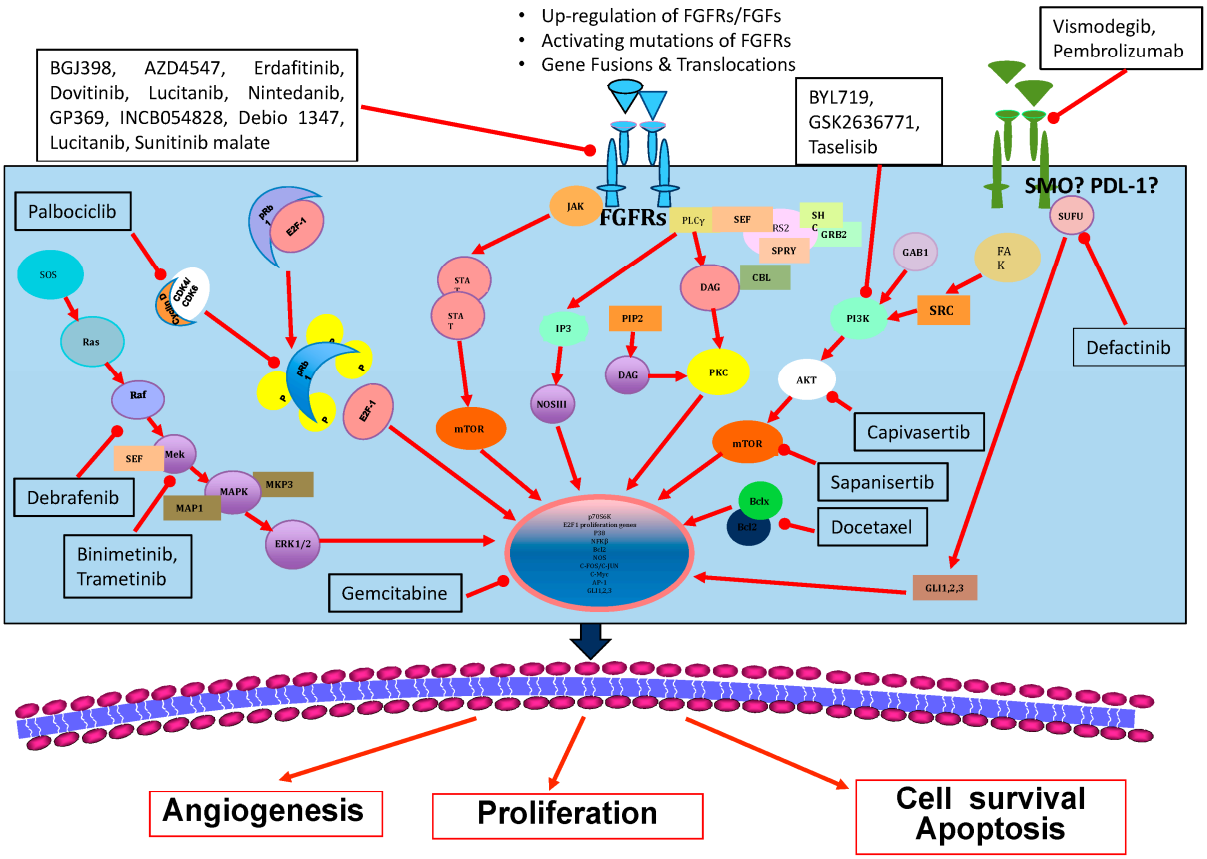

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Table 1. Selected ongoing trials with FGFRs inhibitors in breast cancer.

Clinical trial identifier	Study Design	Intervention/s	Setting	Primary endpoint	Phase	Status
NCT03238196	32 Participants, Non-Randomized, Open label	Fulvestrant + palbociclib + erdafitinib as an escalation (Arm A: 4-8mg once daily for erdafitinib, 125mg once every 21 days followed by 1 week of rest [without taking the drug] and 500mg once daily for erdafitinib) or the same combination of drugs as an expansion (Arm A: 4-8mg once daily for erdafitinib, 125mg once every 21 days followed by 1 week of rest [without taking the drug] and 500mg once daily for erdafitinib).	Second line	Safety and Tolerability	1	Recruiting
NCT02465060	6452 participants, Non-Randomized, Parallel assignment, Open Label	Adavosertib, afatinib, binimetinib, capivasertib, crizotinib, dabrafenib, dasatinib, defactinib, AZD4547, larotrectinib, nivolumab, osimertinib, palbociclib, pertuzumab, GSK2636771, sapanisertib, sunitinib malate, taselisib, trametinib,	Second line	OR	2	Recruiting

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		trastuzumab, trastuzumab emtansine, vismodegib				
NCT02202746	178 participants, Parallel Assignment, Open label	Lucitanib in patients with FGFR1-amplified or 11q-amplified (Arm A: 10 mg once daily), and in patients with FGFR1- non amplified and 11q non-amplified (Arm B: 10 mg once daily)	Second Line	PFS	2	Active, not recruiting
NCT01004224	208 participants, Single group assignment, Non-Randomized, Open label	BGJ398 (dose escalation)	Second line	MTD	1	Active, not recruiting
NCT01791985	56 participants, Single group assignment, Open label	Anastrazole (1 mg daily), letrozole (2.5 mg once daily) and AZD4547 (80 mg twice daily)	Second line	Safety and Tolerability	1 & 2	Active, not recruiting
NCT02619162	22 participants, Single group assignment, Open label	Letrozole (2.5 mg) with nintedanib (100-150 mg)	Second line	DLT	1	Recruiting
NCT03344536	55 participants, Single group assignment, Open label	Fulvestrant (500 mg 1, 15, 29 and every 28 days [+/- 3 days] thereafter) and Debio 1347 (dose escalation, administered once daily).	Second line maximum for phase 2; phase 1 could have received more than one prior treatment	DLT	1 & 2	Recruiting
NCT02393248	280 participants, Single group assignment, Open label	Combination therapy: Gemcitabine + Cisplatin + INCB054828; Pembrolizumab +	Second line	MTD	1 & 2	Recruiting

		INCB054828; Docetaxel + INCB054828; Trastuzumab + INCB054828.				

Abbreviations: Progression Free Survival, PFS; Objective Response, OR; Dose Limiting Toxicity, DLT; Maximum Tolerated Dose, MTD.

References

1. Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer statistics, 2018. *CA. Cancer J. Clin.* **2018**, *68*, 7–30, doi:10.3322/caac.21442.
2. Ornitz, D. M.; Itoh, N. Fibroblast growth factors. *Genome Biol.* **2001**, *2*, REVIEWS3005.
3. Eswarakumar, V. P.; Lax, I.; Schlessinger, J. Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev.* **2005**, *16*, 139–149, doi:10.1016/j.cytogfr.2005.01.001.
4. Belov, A. A.; Mohammadi, M. Molecular mechanisms of fibroblast growth factor signaling in physiology and pathology. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a015958, doi:10.1101/cshperspect.a015958.
5. Deng, C.; Wynshaw-Boris, A.; Zhou, F.; Kuo, A.; Leder, P. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. *Cell* **1996**, *84*, 911–21.
6. Turner, N.; Grose, R. Fibroblast growth factor signalling: from development to cancer. *Nat. Rev. Cancer* **2010**, *10*, 116–129, doi:10.1038/nrc2780.
7. 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*, 15292–7.
8. Linggi, B.; Carpenter, G. ErbB receptors: new insights on mechanisms and biology. *Trends Cell Biol.* **2006**, *16*, 649–56, doi:10.1016/j.tcb.2006.10.008.
9. Knights, V.; Cook, S. J. De-regulated FGF receptors as therapeutic targets in cancer. *Pharmacol. Ther.* **2010**, *125*, 105–17, doi:10.1016/j.pharmthera.2009.10.001.

10. Johnson, D. E.; Williams, L. T. Structural and functional diversity in the FGF receptor multigene family. *Adv. Cancer Res.* **1993**, *60*, 1–41.

11. Dienstmann, R.; Rodon, J.; Prat, A.; Perez-Garcia, J.; Adamo, B.; Felip, E.; Cortes, J.; Iafrate, A. J.; Nuciforo, P.; Tabernero, J. Genomic aberrations in the FGFR pathway: opportunities for targeted therapies in solid tumors. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **2014**, *25*, 552–63, doi:10.1093/annonc/mdt419.

12. Schlessinger, J. Common and distinct elements in cellular signaling via EGF and FGF receptors. *Science* **2004**, *306*, 1506–7, doi:10.1126/science.1105396.

13. Mohammadi, M.; Dikic, I.; Sorokin, A.; Burgess, W. H.; Jaye, M.; Schlessinger, J. Identification of six novel autophosphorylation sites on fibroblast growth factor receptor 1 and elucidation of their importance in receptor activation and signal transduction. *Mol. Cell. Biol.* **1996**, *16*, 977–89.

14. Wesche, J.; Haglund, K.; Haugsten, E. M. Fibroblast growth factors and their receptors in cancer. *Biochem. J.* **2011**, *437*, 199–213, doi:10.1042/BJ20101603.

15. Wang, L.; Rudert, W. A.; Loutaev, I.; Roginskaya, V.; Corey, S. J. Repression of c-Cbl leads to enhanced G-CSF Jak-STAT signaling without increased cell proliferation. *Oncogene* **2002**, *21*, 5346–55, doi:10.1038/sj.onc.1205670.

16. Kovalenko, D.; Yang, X.; Nadeau, R. J.; Harkins, L. K.; Friesel, R. Sef inhibits fibroblast growth factor signaling by inhibiting FGFR1 tyrosine phosphorylation and subsequent ERK activation. *J. Biol. Chem.* **2003**, *278*, 14087–91, doi:10.1074/jbc.C200606200.

17. Torii, S.; Kusakabe, M.; Yamamoto, T.; Maekawa, M.; Nishida, E. Sef Is a Spatial Regulator for Ras/MAP Kinase Signaling. *Dev. Cell* **2004**, *7*, 33–44, doi:10.1016/j.devcel.2004.05.019.

18. Stauber, D. J.; DiGabriele, A. D.; Hendrickson, W. A. Structural interactions of fibroblast growth factor receptor with its ligands. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 49–54.

19. Kalinina, J.; Dutta, K.; Ilghari, D.; Beenken, A.; Goetz, R.; Eliseenkova, A. V.; Cowburn, D.; Mohammadi, M. The Alternatively Spliced Acid Box Region Plays a Key Role in FGF Receptor Autoinhibition. *Structure* **2012**, *20*, 77–88, doi:10.1016/j.str.2011.10.022.

20. Crawford, Y.; Ferrara, N. Tumor and stromal pathways mediating refractoriness/resistance to anti-angiogenic therapies. *Trends Pharmacol. Sci.* **2009**, *30*, 624–630, doi:10.1016/j.tips.2009.09.004.

21. Sarabipour, S.; Hristova, K. Mechanism of FGF receptor dimerization and activation. *Nat. Commun.* **2016**, *7*, 10262, doi:10.1038/ncomms10262.

22. Seo, A. N.; Lee, H. J.; Kim, E. J.; Kim, H. J.; Jang, M. H.; Lee, H. E.; Kim, Y. J.; Kim, J. H.; Park, S. Y. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. *Br. J. Cancer* **2013**, *109*, 2705–13, doi:10.1038/bjc.2013.634.

23. Xie, L.; Su, X.; Zhang, L.; Yin, X.; Tang, L.; Zhang, X.; Xu, Y.; Gao, Z.; Liu, K.; Zhou, M.; Gao, B.; Shen, D.; Zhang, L.; Ji, J.; Gavine, P. R.; Zhang, J.; Kilgour, E.; Zhang, X.; Ji, Q. FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547. *Clin. Cancer Res.* **2013**, *19*, 2572–83, doi:10.1158/1078-0432.CCR-12-3898.

24. Turner, N.; Pearson, A.; Sharpe, R.; Lambros, M.; Geyer, F.; Lopez-Garcia, M. A.; Natrajan, R.; Marchio, C.; Iorns, E.; Mackay, A.; Gillett, C.; Grigoriadis, A.; Tutt, A.; Reis-Filho, J. S.; Ashworth, A. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res.* **2010**, *70*, 2085–94, doi:10.1158/0008-5472.CAN-09-3746.

25. Knights, V.; Cook, S. J. De-regulated FGF receptors as therapeutic targets in cancer. *Pharmacol. Ther.* **2010**, *125*, 105–117, doi:10.1016/j.pharmthera.2009.10.001.

26. Heinzle, C.; Sutterlüty, H.; Grusch, M.; Grasl-Kraupp, B.; Berger, W.; Marian, B. Targeting fibroblast-growth-factor-receptor-dependent signaling for cancer therapy. *Expert Opin.*

Ther. Targets **2011**, *15*, 829–846, doi:10.1517/14728222.2011.566217.

27. Greulich, H.; Pollock, P. M. Targeting mutant fibroblast growth factor receptors in cancer. *Trends Mol. Med.* **2011**, *17*, 283–292, doi:10.1016/j.molmed.2011.01.012.
28. Brooks, A. N.; Kilgour, E.; Smith, P. D. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. *Clin. Cancer Res.* **2012**, *18*, 1855–62, doi:10.1158/1078-0432.CCR-11-0699.
29. Adnane, J.; Gaudray, P.; Dionne, C. A.; Crumley, G.; Jaye, M.; Schlessinger, J.; Jeanteur, P.; Birnbaum, 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*, 659–63.
30. 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, doi:10.1158/1078-0432.CCR-14-3212.
31. Elbauomy Elsheikh, S.; Green, A. R.; Lambros, M. B.; Turner, N. C.; Grainge, M. J.; Powe, 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, doi:10.1186/bcr1665.
32. Massard, C.; Michiels, S.; Ferté, C.; Le Deley, M.-C.; Lacroix, L.; Hollebecque, A.; Verlingue, L.; Ileana, E.; Rosellini, S.; Ammari, S.; Ngo-Camus, M.; Bahleda, R.; Gazzah, A.; Varga, A.; Postel-Vinay, S.; Loriot, Y.; Even, C.; Breuskin, I.; Auger, N.; Job, B.; De Baere, T.; Deschamps, F.; Vielh, P.; Scoazec, J.-Y.; Lazar, V.; Richon, C.; Ribrag, V.; Deutsch, E.; Angevin, E.; Vassal, G.; Eggermont, A.; André, F.; Soria, J.-C. High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: Results of the MOSCATO 01 Trial. *Cancer Discov.* **2017**, *7*, 586–595, doi:10.1158/2159-8290.CD-16-1396.
33. André, F.; Bachelot, T.; Commo, F.; Campone, M.; Arnedos, M.; Dieras, V.; Lacroix-Triki, M.; Lacroix, L.; Cohen, P.; Gentien, D.; Adélaide, J.; Dalenc, F.; Goncalves, A.; Levy, C.; Ferrero, J.-M.; Bonneterre, J.; Lefeuvre, C.; Jimenez, M.; Filleron, T.; Bonnefoi, H.

Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). *Lancet Oncol.* **2014**, *15*, 267–274, doi:10.1016/S1470-2045(13)70611-9.

34. Brunello, E.; Brunelli, M.; Bogina, G.; Caliò, A.; Manfrin, E.; Nottegar, A.; Vergine, M.; Molino, A.; Bria, E.; Massari, F.; Tortora, G.; Cingarlini, S.; Pedron, S.; Chilosi, M.; Zamboni, G.; Miller, K.; Martignoni, G.; Bonetti, F. FGFR-1 amplification in metastatic lymph-nodal and haematogenous lobular breast carcinoma. *J. Exp. Clin. Cancer Res.* **2012**, *31*, 103, doi:10.1186/1756-9966-31-103.

35. Reis-Filho, J. S.; Simpson, P. T.; Turner, N. C.; Lambros, M. B.; Jones, C.; Mackay, A.; Grigoriadis, A.; Sarrio, D.; Savage, K.; Dexter, T.; Iravani, M.; Fenwick, K.; Weber, B.; Hardisson, D.; Schmitt, F. C.; Palacios, J.; Lakhani, S. R.; Ashworth, A. FGFR1 Emerges as a Potential Therapeutic Target for Lobular Breast Carcinomas. *Clin. Cancer Res.* **2006**, *12*, 6652–6662, doi:10.1158/1078-0432.CCR-06-1164.

36. 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*, 4360–7.

37. Andre, F.; Bachelot, T.; Campone, M.; Dalenc, F.; Perez-Garcia, J. M.; Hurvitz, S. A.; Turner, N.; Rugo, H.; Smith, J. W.; Deudon, S.; Shi, M.; Zhang, Y.; Kay, A.; Graus Porta, D.; Yovine, A.; Baselga, J. Targeting FGFR with Dovitinib (TKI258): Preclinical and Clinical Data in Breast Cancer. *Clin. Cancer Res.* **2013**, *19*, 3693–3702, doi:10.1158/1078-0432.CCR-13-0190.

38. 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.; Reddy, V. A. FGFR2 promotes breast tumorigenicity through maintenance of breast tumor-initiating cells. *PLoS One*

2013, *8*, e51671, doi:10.1371/journal.pone.0051671.

39. Guagnano, V.; Kauffmann, A.; Wohrle, S.; Stamm, C.; Ito, M.; Barys, L.; Pornon, A.; Yao, Y.; Li, F.; Zhang, Y.; Chen, Z.; Wilson, C. J.; Bordas, V.; Le Douget, M.; Gaither, L. A.; Borawski, J.; Monahan, J. E.; Venkatesan, K.; Brummendorf, T.; Thomas, D. M.; Garcia-Echeverria, C.; Hofmann, F.; Sellers, W. R.; Graus-Porta, D. FGFR Genetic Alterations Predict for Sensitivity to NVP-BGJ398, a Selective Pan-FGFR Inhibitor. *Cancer Discov.* **2012**, *2*, 1118–1133, doi:10.1158/2159-8290.CD-12-0210.
40. Wu, Y.-M.; Su, F.; Kalyana-Sundaram, S.; Khazanov, N.; Ateeq, B.; Cao, X.; Lonigro, R. J.; Vats, P.; Wang, R.; Lin, S.-F.; Cheng, A.-J.; Kunju, L. P.; Siddiqui, J.; Tomlins, S. A.; Wyngaard, P.; Sadis, S.; Roychowdhury, S.; Hussain, M. H.; Feng, F. Y.; Zalupski, M. M.; Talpaz, M.; Pienta, K. J.; Rhodes, D. R.; Robinson, D. R.; Chinnaiyan, A. M. Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov.* **2013**, *3*, 636–47, doi:10.1158/2159-8290.CD-13-0050.
41. 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, doi:10.1038/sj.onc.1203650.
42. 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-ra6, doi:10.1126/scisignal.2000021.
43. 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, doi:10.1016/j.urolonc.2010.07.014.
44. Sun, X.; Guo, W.; Shen, J. K.; Mankin, H. J.; Hornicek, F. J.; Duan, Z. Rhabdomyosarcoma: Advances in Molecular and Cellular Biology. *Sarcoma* **2015**, *2015*, 1–14, doi:10.1155/2015/232010.

45. Easton, D. F.; Pooley, K. A.; Dunning, A. M.; Pharoah, P. D. P.; Thompson, D.; Ballinger, D. G.; Struewing, J. P.; Morrison, J.; Field, H.; Luben, R.; Wareham, N.; Ahmed, S.; Healey, C. S.; Bowman, R.; Luccarini, C.; Conroy, D.; Shah, M.; Munday, H.; Jordan, C.; Perkins, B.; West, J.; Redman, K.; Driver, K.; Meyer, K. B.; Haiman, C. A.; Kolonel, L. K.; Henderson, B. E.; Le Marchand, L.; Brennan, P.; Sangrajrang, S.; Gaborieau, V.; Odefrey, F.; Shen, C.-Y.; Wu, P.-E.; Wang, H.-C.; Eccles, D.; Evans, D. G.; Peto, J.; Fletcher, O.; Johnson, N.; Seal, S.; Stratton, M. R.; Rahman, N.; Chenevix-Trench, G.; Bojesen, S. E.; Nordestgaard, B. G.; Axelsson, C. K.; Garcia-Closas, M.; Brinton, L.; Chanock, S.; Lissowska, J.; Peplonska, B.; Nevanlinna, H.; Fagerholm, R.; Eerola, H.; Kang, D.; Yoo, K.-Y.; Noh, D.-Y.; Ahn, S.-H.; Hunter, D. J.; Hankinson, S. E.; Cox, D. G.; Hall, P.; Wedren, S.; Liu, J.; Low, Y.-L.; Bogdanova, N.; Schürmann, P.; Dörk, T.; Tollenaar, R. A. E. M.; Jacobi, C. E.; Devilee, P.; Klijn, J. G. M.; Sigurdson, A. J.; Doody, M. M.; Alexander, B. H.; Zhang, J.; Cox, A.; Brock, I. W.; MacPherson, G.; Reed, M. W. R.; Couch, F. J.; Goode, E. L.; Olson, J. E.; Meijers-Heijboer, H.; van den Ouweland, A.; Uitterlinden, A.; Rivadeneira, F.; Milne, R. L.; Ribas, G.; Gonzalez-Neira, A.; Benitez, J.; Hopper, J. L.; McCredie, M.; Southey, M.; Giles, G. G.; Schroen, C.; Justenhoven, C.; Brauch, H.; Hamann, U.; Ko, Y.-D.; Spurdle, A. B.; Beesley, J.; Chen, X.; Aghmesheh, M.; Amor, D.; Andrews, L.; Antill, Y.; Armes, J.; Armitage, S.; Arnold, L.; Balleine, R.; Begley, G.; Beilby, J.; Bennett, I.; Bennett, B.; Berry, G.; Blackburn, A.; Brennan, M.; Brown, M.; Buckley, M.; Burke, J.; Butow, P.; Byron, K.; Callen, D.; Campbell, I.; Chenevix-Trench, G.; Clarke, C.; Colley, A.; Cotton, D.; Cui, J.; Culling, B.; Cummings, M.; Dawson, S.-J.; Dixon, J.; Dobrovic, A.; Dudding, T.; Edkins, T.; Eisenbruch, M.; Farshid, G.; Fawcett, S.; Field, M.; Firgaira, F.; Fleming, J.; Forbes, J.; Friedlander, M.; Gaff, C.; Gardner, M.; Gattas, M.; George, P.; Giles, G.; Gill, G.; Goldblatt, J.; Greening, S.; Grist, S.; Haan, E.; Harris, M.; Hart, S.; Hayward, N.; Hopper, J.; Humphrey, E.; Jenkins, M.; Jones, A.; Kefford, R.; Kirk, J.; Kollias, J.; Kovalenko, S.; Lakhani, S.; Leary, J.; Lim, J.; Lindeman, G.; Lipton, L.;

Lobb, L.; Maclurcan, M.; Mann, G.; Marsh, D.; McCredie, M.; McKay, M.; Anne McLachlan, S.; Meiser, B.; Milne, R.; Mitchell, G.; Newman, B.; O'Loughlin, I.; Osborne, R.; Peters, L.; Phillips, K.; Price, M.; Reeve, J.; Reeve, T.; Richards, R.; Rinehart, G.; Robinson, B.; Rudzki, B.; Salisbury, E.; Sambrook, J.; Saunders, C.; Scott, C.; Scott, E.; Scott, R.; Seshadri, R.; Shelling, A.; Southey, M.; Spurdle, A.; Suthers, G.; Taylor, D.; Tennant, C.; Thorne, H.; Townshend, S.; Tucker, K.; Tyler, J.; Venter, D.; Visvader, J.; Walpole, I.; Ward, R.; Waring, P.; Warner, B.; Warren, G.; Watson, E.; Williams, R.; Wilson, J.; Winship, I.; Young, M. A.; Bowtell, D.; Green, A.; deFazio, A.; Chenevix-Trench, G.; Gertig, D.; Webb, P.; Mannermaa, A.; Kosma, V.-M.; Kataja, V.; Hartikainen, J.; Day, N. E.; Cox, D. R.; Ponder, B. A. J. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* **2007**, *447*, 1087–1093, doi:10.1038/nature05887.

46. Stacey, S. N.; Manolescu, A.; Sulem, P.; Thorlacius, S.; Gudjonsson, S. A.; Jonsson, G. F.; Jakobsdottir, M.; Bergthorsson, J. T.; Gudmundsson, J.; Aben, K. K.; Strobbe, L. J.; Swinkels, D. W.; van Engelenburg, K. C. A.; Henderson, B. E.; Kolonel, L. N.; Le Marchand, L.; Millastre, E.; Andres, R.; Saez, B.; Lambea, J.; Godino, J.; Polo, E.; Tres, A.; Picelli, S.; Rantala, J.; Margolin, S.; Jonsson, T.; Sigurdsson, H.; Jonsdottir, T.; Hrafnkelsson, J.; Johannsson, J.; Sveinsson, T.; Myrdal, G.; Grimsson, H. N.; Sveinsdottir, S. G.; Alexiusdottir, K.; Saemundsdottir, J.; Sigurdsson, A.; Kostic, J.; Gudmundsson, L.; Kristjansson, K.; Masson, G.; Fackenthal, J. D.; Adebamowo, C.; Ogundiran, T.; Olopade, O. I.; Haiman, C. A.; Lindblom, A.; Mayordomo, J. I.; Kiemeney, L. A.; Gulcher, J. R.; Rafnar, T.; Thorsteinsdottir, U.; Johannsson, O. T.; Kong, A.; Stefansson, K. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor–positive breast cancer. *Nat. Genet.* **2008**, *40*, 703–706, doi:10.1038/ng.131.

47. 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, doi:10.1371/journal.pbio.0060108.

48. Hunter, D. J.; Kraft, P.; Jacobs, K. B.; Cox, D. G.; Yeager, M.; Hankinson, S. E.; Wacholder, S.; Wang, Z.; Welch, R.; Hutchinson, A.; Wang, J.; Yu, K.; Chatterjee, N.; Orr, N.; Willett, W. C.; Colditz, G. A.; Ziegler, R. G.; Berg, C. D.; Buys, S. S.; McCarty, C. A.; Feigelson, H. S.; Calle, E. E.; Thun, M. J.; Hayes, R. B.; Tucker, M.; Gerhard, D. S.; Fraumeni, J. F.; Hoover, R. N.; Thomas, G.; Chanock, S. J. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat. Genet.* **2007**, *39*, 870–874, doi:10.1038/ng2075.

49. Penault-Llorca, F.; Bertucci, F.; Adélaïde, J.; Parc, P.; Coulier, F.; Jacquemier, J.; Birnbaum, D.; deLapeyrière, O. Expression of FGF and FGF receptor genes in human breast cancer. *Int. J. cancer* **1995**, *61*, 170–6.

50. 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.

51. 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, doi:10.1158/1541-7786.MCR-15-0497.

52. Stransky, N.; Cerami, E.; Schalm, S.; Kim, J. L.; Lengauer, C. The landscape of kinase fusions in cancer. *Nat. Commun.* **2014**, *5*, 4846, doi:10.1038/ncomms5846.

53. Bedussi, F.; Bottini, A.; Memo, M.; Fox, S. B.; Sigala, S.; Generali, D. Targeting fibroblast growth factor receptor in breast cancer: a promise or a pitfall? *Expert Opin. Ther. Targets* **2014**, *18*, 665–678, doi:10.1517/14728222.2014.898064.

54. Bello, E.; Taraboletti, G.; Colella, G.; Zucchetti, M.; Forestieri, D.; Licandro, S. A.; Berndt, A.; Richter, P.; D'Incalci, M.; Cavalletti, E.; Giavazzi, R.; Camboni, G.; Damia, G. The Tyrosine Kinase Inhibitor E-3810 Combined with Paclitaxel Inhibits the Growth of Advanced-Stage Triple-Negative Breast Cancer Xenografts. *Mol. Cancer Ther.* **2013**, *12*,

131–140, doi:10.1158/1535-7163.MCT-12-0275-T.

55. Brady, N. J.; Chuntova, P.; Bade, L. K.; Schwertfeger, K. L. The FGF/FGF receptor axis as a therapeutic target in breast cancer. *Expert Rev. Endocrinol. Metab.* **2013**, *8*, 391–402, doi:10.1586/17446651.2013.811910.

56. Bai, A.; Meetze, K.; Vo, N. Y.; Kollipara, S.; Mazsa, E. K.; Winston, W. M.; Weiler, S.; Poling, L. L.; Chen, T.; Ismail, N. S.; Jiang, J.; Lerner, L.; Gyuris, J.; Weng, Z. GP369, an FGFR2-IIIb-Specific Antibody, Exhibits Potent Antitumor Activity against Human Cancers Driven by Activated FGFR2 Signaling. *Cancer Res.* **2010**, *70*, 7630–7639, doi:10.1158/0008-5472.CAN-10-1489.

57. Sharpe, R.; Pearson, A.; Herrera-Abreu, M. T.; Johnson, D.; Mackay, A.; Welti, J. C.; Natrajan, R.; Reynolds, A. R.; Reis-Filho, J. S.; Ashworth, A.; Turner, N. C. FGFR signaling promotes the growth of triple-negative and basal-like breast cancer cell lines both in vitro and in vivo. *Clin. Cancer Res.* **2011**, *17*, 5275–86, doi:10.1158/1078-0432.CCR-10-2727.

58. Tolcher, A. W.; Papadopoulos, K. P.; Patnaik, A.; Wilson, K.; Thayer, S.; Zanghi, J.; Gemo, A. T.; Kavanaugh, W. M.; Keer, H. N.; LoRusso, P. M. A phase I, first in human study of FP-1039 (GSK3052230), a novel FGF ligand trap, in patients with advanced solid tumors. *Ann. Oncol.* **2016**, *27*, 526–532, doi:10.1093/annonc/mdv591.

59. Semrad, T. J.; Kim, E. J.; Tanaka, M. S.; Sands, J.; Roberts, C.; Burich, R. A.; Li, Y.; Gandara, D. R.; Lara, P.; Mack, P. C. Phase II study of dovitinib in patients progressing on anti-vascular endothelial growth factor therapy. *Cancer Treat. Res. Commun.* **2017**, *10*, 21–26, doi:10.1016/j.ctarc.2016.12.002.

60. Issa, A.; Gill, J. W.; Heideman, M. R.; Sahin, O.; Wiemann, S.; Dey, J. H.; Hynes, N. E. Combinatorial targeting of FGF and ErbB receptors blocks growth and metastatic spread of breast cancer models. *Breast Cancer Res.* **2013**, *15*, R8, doi:10.1186/bcr3379.