Challenges and opportunities in the targeting of fibroblast growth factor receptors in breast cancer

Vikram K Jain1 and Nicholas C Turner*2,3

Abstract

Activation of the fibroblast growth factor receptor pathway is a common event in many cancer types. Here we review the role of fibroblast growth factor receptor signalling in breast cancer, from SNPs in FGFR2 that influence breast cancer risk and SNPs in FGFR4 that associate with breast cancer prognosis, and potential therapeutic targets such as receptor amplification and aberrant autocrine and paracrine ligand expression. We discuss the multiple therapeutic strategies in preclinical and clinical development and the current and future challenges to successfully targeting this pathway in cancer.

Introduction

Activation of tyrosine kinase growth factor receptors presents one of the most common oncogenic events in cancer. Targeting these receptors is a proven therapeutic strategy, as exemplified by the efficacy of trastuzumab in HER2 amplified breast cancer. However, in the ~85% of breast cancers that do not have HER2 amplification there has been limited progress with targeting other growth factor receptors. Studies have found potential evidence of efficacy targeting epidermal growth factor receptor (EGFR) in combination with endocrine therapy [1], and insulin-like growth factor 1 receptor in combination with mammalian target of rapamycin inhibitors [2], although none of these approaches have as yet proceeded beyond phase II trials.

Preclinical evidence suggests that activation of fibroblast growth factor receptor (FGFR) signalling is a common event in cancer [3]. Yet the clinical development of therapies targeting the FGFR signalling pathway presents multiple challenges, with diverse mechanisms of pathway activation combined with multiple inhibitors of differing potency and with antibodies in preclinical development. In the present review we discuss the multiple mechanisms through which FGFR signalling contributes to the pathogenesis of breast cancer, and also review the challenges of translating this evidence into clinical trials of therapies targeting the FGFRs.

The fibroblast growth factor signalling system

The fibroblast growth factors (FGFs) and their receptors (FGFRs) play an important role in a wide range of biological functions, controlling developmental events such as brain patterning, morphogenesis and limb development [4,5] with multiple physiological functions in the adult including angiogenesis, wound repair and endocrine functions [6].

The FGF family consists of 18 ligands; FGF ligand nomenclature extends to FGF23 although only 18 FGFs function as ligands, which signal through four high-affinity FGFRs (FGFR1 to FGFR4) [3,6,7]. The majority of FGFs bind to heparan sulphate glycosaminoglycans on the cell surface or in the extracellular matrix, and consequently do not diffuse far from the site of production acting as paracrine or autocrine growth factors – although one FGF ligand family (FGF19, FGF21, FGF23) function as hormones and bind to FGFRs in complex with Klotho proteins [6]. As well as this spatial regulation of ligand–receptor interaction, alternative splicing of the third immunoglobulin domain in the receptor generates two different receptors with highly different ligand specificity (reviewed in [6]).

The majority of FGFs bind receptor in a trimeric complex with heparins, triggering a conformational change in the receptor that leads to activation of the FGFR that results in phosphorylation of multiple sites on the intracellular domain, adapter protein binding and intracellular signalling (reviewed in detail elsewhere [8]). Under physiological conditions, the highly complex FGF signalling pathway is tightly regulated [3]. The deregulation of FGF signalling in cancer results in activation of the pathway without appropriate regulation leading to/ contributing to development of cancer, promoting cancer cell proliferation, survival and migration [9-13].

*Correspondence: nicholas.turner@icr.ac.uk
2The Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK
Full list of author information is available at the end of the article
**FGFR signalling in breast cancer pathogenesis**

The mouse mammary tumour virus was a major cause of mammary tumours in multiple laboratory mouse strains, vertically transmitted from mother to pup. Mouse mammary tumour virus is a retrovirus that is oncogenic through integration into the genome activating the expression of nearby genes, with FGFR3 and FGFR8 being, along with WNT genes, the commonest site of integration [14,15]. The link between FGFR activation and mammary carcinoma was subsequently confirmed by experiments with transgenic mice, with both epithelial FGFR3 overexpression [16] and FGFR1 activation [17] leading to epithelial proliferation and invasive lesions [17].

Genome-wide association studies have subsequently identified SNPs within the second intron of the FGFR2 gene that are associated with increased risk of developing breast cancer [18,19]. The minor, predisposing, allele is present in approximately 40% of western populations, although the associated increased risk is relatively small: 1.26-fold for heterozygotes and 1.63-fold for homozygotes [18]. The minor allele increases the risk of developing oestrogen receptor (ER)-positive breast cancer, with only a minor effect on ER-negative breast cancer [20]. Multiple SNPs in the second intron are in very high linkage disequilibrium, and from genetic data it is not possible to pinpoint the causative SNP(s) – although strong biochemical evidence suggests that rs2981578 may be causative through creation of an OCT1/RUNX2 binding site [21], potentially resulting in increased FGFR2 expression in breast cancers with the minor allele variant [21]. Whether this reflects increased epithelial or stromal expression is less clear [22]. FGFR2 IIIb knockout mice have a gross failure of branching morphogenesis in the breast [23], raising the possibility that increased FGFR2 expression may simply result in nonspecific changes in breast epithelium that predispose to breast cancer. Further research with transgenic models is required to establish how increased FGFR2 expression results in breast cancer predisposition.

A SNP in FGFR4 (G388R, Gly338–Arg338) has been shown to confer a more aggressive behaviour and poor prognosis in multiple cancer types, including breast cancer [24-28]. This SNP may increase invasion and motility through altering receptor internalisation, potentially leading to abnormally sustained signalling [29-31]. Recent data have suggested in addition that the FGFR4 Arg388 allele may be associated with a pathological complete response to chemotherapy [32], although potentially conflicting data have also been reported [27]. Although the SNPs in both FGFR2 and FGFR4 illustrate the potential importance of FGF signalling in breast cancer pathogenesis, there is no current evidence that either SNP presents a therapeutic target in established breast cancer.

**Potential therapeutic targets in breast cancer**

There are multiple mechanisms through which FGFR signalling may be activated in breast cancer, that may present potential therapeutic targets (Figure 1).

**FGFR2 gene amplification**

Amplification of the FGFR2 gene occurs in a small subset of breast cancer, although in these cancers preclinical evidence suggests this gene is potentially an excellent therapeutic target. Breast cancer cell lines with FGFR2 amplification show high sensitivity to FGFR inhibitors in vitro [33-34], and the FGFR2-amplified MFM223 cell line is sensitive in vivo to an FGFR2 targeting antibody [35]. FGFR2 is highly overexpressed in amplified cell lines, along with expression of a C-terminal truncated form that results in impaired receptor internalisation [36], and FGFR2 is constitutively active and ligand independent in the amplified cell lines. FGFR2 amplification is rare in breast cancer, however, present in only 1 to 2% of breast cancer overall [37], although this is enriched to an estimate of ~4% of breast cancers with the aggressive triple-negative breast cancers [38]. FGFR2 amplifications have also been described in approximately 10% of gastric cancers usually associated with the poor-prognosis diffuse-type histology [38].

**FGFR1 gene amplification**

The FGFR1 gene is one of the most commonly amplified genes in cancer [39]. Amplification of the chromosomal region 8p11-12, the genomic location of FGFR1, is seen in approximately 10% of the breast cancers, predominantly in the ER-positive breast cancers [40-44]. The oncogenic driver of 8p11-12 amplifications has been a source of substantial discord in the scientific literature for the last 15 years, although in the last few years clarity has finally emerged.

Prior misunderstandings have arisen in part from attempts to find a single oncogenic driver within the region, a view that follows the paradigm of HER2 and 17q21 amplification. Evidence that this simplified model is incorrect emerged from high-resolution comparative genomic hybridisation analysis of breast cancer suggesting two major cores, or peaks, of amplification (core A1 distal at 36.5 to 37.8 Mb, and core A2 proximal at 38.1 to 38.9 Mb; Genome Build 35) [41]. Although the most common pattern was for amplification of both cores, amplification of either core alone occurred in a minority of cancers. Further evidence supporting the existence of two separate cores, and therefore at least two driver oncogenes, has subsequently come from cross-cancer comparisons. Amplification of 8p11-12 is also found in ~10% of squamous lung cancers but with a different genomic structure, with, at least in the published datasets, a frequent pattern of amplification of the proximal A2 core without amplification of the distal A1 core [13].
Following on from clarity on the genomic structure, pointing to at least two oncogenic drivers, has come further clarity on the likely oncogenic drivers for each amplification core. ZNF703 has been demonstrated, with high likelihood, to be the principle oncogenic driver of the distal A1 core [45,46], resulting in induction of stem-cell-like phenotypes, potentially also suppressing ER and promoting E2F1 transcriptional activity [47]. In contrast, FGFR1 is the likely target of proximal A2 amplifications – although other genes have been implicated, such as the phosphatase PPAPDC1B [47]. FGFR1 promotes the growth of both breast cancer and lung cancer cell lines with FGFR1 amplification [13,43,48], with FGFR1 mRNA overexpression tightly linked to FGFR1 amplification [12,13], although cases of FGFR1 amplification without receptor overexpression have been demonstrated [12].

Amplification of FGFR1 is associated with a marked poor prognosis in breast cancer, specifically in ER-positive breast cancer [11]. We have recently provided evidence that FGFR1 amplification promotes resistance to endocrine therapy [12], potentially through enhanced ligand-dependent signalling in FGFR1 amplified cell lines. FGFR1 signalling promoted cyclin D1 expression and suppressed progesterone receptor expression, and similarly FGFR1 overexpressed cancers were more likely to be progesterone receptor negative and high in proliferation. Up to 25% of luminal-B-type breast cancers potentially have amplification of FGFR1 [12], and in these cancers FGFR1 may present an alternative growth/survival signal to escape the effects of endocrine therapy. An association has been reported between increased FGFR1 expression [49], FGFR1 amplification [43], and lobular breast cancer, although the enrichment for FGFR1 amplification in lobular cancers is relatively weak [11].

Some important questions remain, however, regarding the role of FGFR1 as an oncogene and therapeutic target. In contrast to FGFR2, where an aberrant form of the receptor is expressed, all data currently suggest that
recently demonstrated that a number of triple-negative breast cancer cell lines are sensitive to FGFR inhibitors in vitro [33]. Sensitive cells lines were of the claudin-low subtype, and expressed autocrine FGF2 ligand. Sensitivity was found predominantly in anchorage-independent conditions in vitro, and CAL51 cell line xenografts were also sensitive in vivo [33]. Expression of cytoplasmic FGF2 ligand was also found to be specific to basal-like breast cancers by immunohistochemistry [33]. This raises the possibility that autocrine FGF2 ligand may be a therapeutic target in basal-like breast cancer, although there is uncertainty as to whether this is specific to the subset of basal-like breast cancers with a claudin-low-type expression pattern.

Assessment of the tumour stromal ligand concentration has shown FGF2 ligand to be expressed at high levels in tumour stroma [56]. Indeed, assessment of elevated FGF2 content in nipple aspirates has been suggested to be a potential diagnostic test for breast cancer [57]. Presumably FGF2 is secreted by activated stromal fibroblasts, but there is no direct evidence for the cell of origin and how this relates to cancer biology is unclear. Elevated FGF2 ligand may potentially be a source for signalling by amplifi ed and overexpressed FGFR1. FGF2 is an angiogenic signalling peptide that is also released in an autocrine/paracrine fashion from activated endothelial cells [58]. There is, however, no association between FGF2 ligand concentrations and microvessel density [56], which has been interpreted as evidence that FGF2 does not promote de novo angiogenesis in breast cancer [56]. FGF2 has been shown to cause resistance to VEGFR targeting in vitro [59], although it is unknown whether promotes resistance to bevacizumab in breast cancer.

A potential role for paracrine FGF9/FGFR signalling has also been identified in the oestrogen-mediated expansion of a breast cancer stem-cell-like subpopulation in vitro, potentially through promoting expression of the Tbx3 transcription factor [60]. The full potential role of FGF autocrine and paracrine signalling in breast cancer is therefore yet to be fully elucidated.

Targeting FGFR signalling

The past decade has seen a marked increase in our understanding of the FGF signalling pathway. Given its role in the pathogenesis of various cancers, several pharmaceutical companies have developed agents targeting FGFs or FGFRs, the most common being small-molecule receptor tyrosine kinase inhibitors targeting the FGFR (Table 1).

Tyrosine kinase inhibitors

Multiple FGFR tyrosine kinase inhibitors are currently in early clinical development, although the inhibitors vary substantially in potency (Table 1). The first generation of inhibitors are multi-targeting ATP competitive inhibitors,
with most originally developed as VEGFR inhibitors that also inhibit the FGFRs due to similarity in the ATP binding pocket structure. These inhibitors have varying potency against the FGFRs, and in cellular assays, in particular, have relatively low potency. Consequently, a number of pharmaceutical companies have developed second-generation inhibitors, developing inhibitors that specifically target FGFRs with selectivity over VEGFR and other kinases, with substantially increased potency (Table 1). A number of additional selective FGFR inhibitors are in preclinical development. The kinase domains of FGFR1 to FGFR3 are highly similar and the kinase inhibitors in development inhibit all three members, to a lesser or greater extent. FGFR4 has diverged from the other kinases, and consequently many inhibitors are less potent against FGFR4.

Antibodies
Multiple FGFR antibodies are in preclinical development, with evidence of efficacy for FGFR2 targeting antibodies in FGFR2 amplified breast cancer models [35] and FGFR3 targeting antibodies in FGFR3-driven models [61]. FGFR1 inhibitory antibodies are in preclinical development, but have not proceeded beyond preclinical toxicity testing due to appetite suppression and weight loss, potentially due to FGFR1 targeting in the hypothalamus [62]. A second potential approach is to develop antibodies against specific FGFs, such as FGF2, although none of these antibodies have yet emerged from the early preclinical development. The potential disadvantage of targeting a single FGF is the potential for rescue of any effect by alternative ligands.

Ligand traps
Another approach to targeting ligand-dependent signalling has been to develop ligand traps – such as FP-1039 based on a modified extracellular domain of FGFR1 fused to Fc, which has the potential to sequester multiple ligands including FGF2 [63]. Whether such approaches can work on autocrine ligand production is yet to be fully addressed.

Early clinical trial evidence
The first clinical trial evidence to support FGFR1 as a potential therapeutic target was presented at the 2011 American Society of Clinical Oncology annual meeting. Andre and colleagues presented the results of the phase II (n = 81) multicentre trial of dovitinib, a multi-tyrosine kinase inhibitor that targets FGFR, VEGFR and platelet-derived growth factor receptor in patients with metastatic breast cancer prescreened for FGFR1 amplification [64]. An unconfirmed response was observed in 15% of women with FGFR1 amplified ER-positive breast cancer, with no responses in nonamplified ER-positive breast cancer, although this level of response failed to meet the predefined criteria for a positive study [64]. Many patients withdrew from the study for reasons other than disease progression, with the drug less well tolerated than expected in a very heavily pretreated population [64]. Interestingly this study suggested that co-amplification of the 11q genomic region, encompassing CCND1, FGFR3, FGFR4 and FGFR1, possibly identified sensitive tumours, potentially supporting in vitro evidence of cooperation between CCND1 and FGFR1 in oncogenesis [53,65].
Recently a second multi-targeting inhibitor has reported very preliminary evidence of activity, with responses reported in FGFR1 amplified cancers in the dose escalation study of E3810 [66]

Roadmap for clinical development
The multiple different mechanisms through which FGF signalling can be activated necessitate a complex approach to clinical development. Only a subset of breast cancers are likely to be sensitive to FGFR inhibitors, and screening will be required to specifically identify cancers with amplification, or potentially with FGF2 ligand expression.

Yet this complex approach presents substantial challenges for rare targets such as FGFR2 amplification. One approach is to screen a very large number of patients, as has been done for ELM4–ALK translocations in nonsmall-cell lung cancer leading to the licence of crizotinib [67]. Another approach is to potentially combine different cancer types with the same genetic aberration into a single trial – but this requires the target to be the same in different cancer subtypes. FGFR2 amplification occurs in both breast cancer and gastric cancer, and based on current evidence appears to be a similarly good potential target in both cancers. In contrast, it is not clear that FGFR1 amplification found in breast cancers, squamous lung cancers [13] as well as oral squamous cell carcinomas [68] is similar in the different cancers, as we have discussed previously.

Matching therapeutic approaches to targets
Multiple different therapeutics are in clinical development, so it is important to consider whether different therapeutic approaches lend themselves to specific oncogenic aberrations. Different FGFR tyrosine kinase inhibitors vary substantially in potency against FGFRs. Kinases with constitutive ligand-independent activation, through mutation or amplification, are generally more sensitive to tyrosine kinase inhibitors than wild-type receptors. Consequently, for targeting oncogenic aberrations such as FGFR2 amplification, which results in constitutive activation, it is likely that multi-targeted first-generation inhibitors will be of sufficient potency to induce tumour shrinkage. For most of the multi-targeted inhibitors, however, the maximum tolerated dose is not defined by the side effects of FGFR inhibition, and consequently may be administered at a dose below that required to achieve full wild-type FGFR inhibition. Targets such as FGF2 ligand autocrine expression, and potentially FGFR1 amplification, which signal through a wild-type receptor, may therefore be best approached through antibodies or more potent second-generation inhibitors.

The only first-generation inhibitor that has been shown, at the time of writing, to have inhibitory properties in clinical trials against wild-type FGFR signalling is dottinib/TKI258, which results in a moderate increase in FGFR3 ligand. FGFR3 is secreted in bone, and hormonally regulates phosphate excretion from the kidney [64], and inhibition of FGFR in the kidney is expected to increase FGFR3 levels. Recent data, however, have suggested that FGFR signalling also promotes FGFR3 expression in bone, making interpretation of FGFR3 levels complex [69]. This observation emphasises the importance of assessing further biomarkers in inhibitor development, although at present there are no biomarkers that can be used on clinical tumour material to assess FGFR directly, and this is an area that requires urgent attention to direct future development.

The second-generation inhibitors have potentially different challenges around high potency inhibition of multiple FGFRs, which have important physiological roles such as phosphate excretion (bone-derived FGFR2 hormonally acting on renal FGFR1) [6]. The potential toxicity of pan-FGFR inhibition could therefore be avoided by use of FGFR inhibitory antibodies whose side effects would be limited to those of a single FGFR member, although FGFR1/FGFR2 antibodies have yet to progress beyond preclinical development.

Challenges to study design
Conducting clinical trials in small subsets presents challenges of recruitment in a study that only enrols a small proportion of potentially eligible patients. For example, considering the 10% rate of FGFR1 amplification in breast cancer, nearly 1,000 patients would need to be screened for a 100-patient phase II trial; and an even larger number would be needed for a phase III trial. The complexity of targets such as FGFR1 amplification potentially also requires even larger trials to identify within amplified cancers those cancers that are sensitive to FGFR inhibition. This factor potentially argues for a different approach to clinical development, focused on biomarker analysis – ideally with biopsy at study entry, as biomarkers may alter through prior therapy, paired with biopsy on study completion to confirm target inhibition and to identify potential determinants of sensitivity.

Conclusion
Substantial progress is being made in understanding how FGF signalling may impact breast cancer pathogenesis and progression, but we are only at the beginning of understanding how, and in which cancers, FGF signalling might be targeted for therapeutic benefit. Should FGFR inhibitors be developed in combination with conventional therapies? How does FGFR signalling effect respond to chemotherapy? With everolimus heading towards licensing in metastatic breast cancer [70], how will mammalian target of rapamycin inhibition impact on
FGFR signalling? We look forward to further scientific and clinical research to clarify the potential role of FGFR targeting in breast cancer treatment.

Abbreviations
FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; SNP, single nucleotide polymorphism.

Competing interests
NCT has consulted for and received honoraria from Novartis, and has received research funding from AstraZeneca. VKJ denotes that he has no competing interests.

Acknowledgements
NCT is funded by a Cancer Research UK Clinician Scientist fellowship. The authors acknowledge National Health Service funding from the National Institute for Health Research Biomedical Research Centre.

Author details
1 GI Unit, Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, UK.
2 Breast Unit, Royal Marsden Hospital, Fulham Road, London SW3 6JJ, UK. The Breakthrough Breast Cancer Research Centre, Institute of Cancer Research, 237 Fulham Road, London SW3 6AB, UK.

Published: 19 June 2012

References
1. Osborne CK, Neven P, Dirix LY, Mackey JR, Robert J, Underhill C, Schiff R, Dirix LY, Mackey JR, Robert J, Underhill C, Schiff R, Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, Natrajan R, Marchio C, Iorns E, Mackay A, Gillett C, Grigoriadis A, Tutt A, Reis-Filho JS, Ellis IO, Reis-Filho JS: FGFR2 signalling? We look forward to further scientifi c and clinical research to clarify the potential role of FGFR targeting in breast cancer treatment.

Ashworth A. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 2010, 70:2085-2094.

Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, Ullrich RT, Menon R, Maier S, Soltermann A, Moch H, Wagener P, Fischer F, Heynck S, Koker M, Schottle J, Leenfers P, Gabeler F, Dabow I, Quenning S, Heukamp LC, Balke-Want H, Ansen S, Rauth D, Baessmann I, Altamuller J, Wright G, Russell P, et al.: Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010, 2:22ra93.

Shackelford GM, MacArthur CA, Kwan HC, Varmus HE: Mouse mammary tumor virus infection accelerates mammary carcinogenesis in Wnt-1 transgenic mice by insertional activation of int-2/FGF-3 and hst/FGF-4. Proc Natl Acad Sci USA 1993, 90:740-744.

Theodorou V, Kimm MA, Boer M, Wessels L, Theelen W, Jonkers J, Hilken J: MMTV insertional mutagenesis identifies genes, gene families and pathways involved in mammary cancer. Nat Genet 2007, 39:759-769.

Muller W, Lee FS, Dickson C, Peters G, Patenaille F, Leder P: The int-2 gene product acts as an epithelial growth factor in transgenic mice. EMBO J 1990, 9:907-913.

Welm BE, Freeman KW, Chen M, Contreras A, Spencer DM, Rosen JI: Inducible dimerization of FGFR1: development of a mouse model to analyze progressive transformation of the mammary gland. J Cell Biol 2002, 157:703-714.

Easton DF, Pooley KA, Dunn JK, Pharoah PD, Thompson D, Ballinger DG, Stratton MR, Morrison J, Field H, Luben R, Wareham N, Ahmad S, Healey CS, Bowman R, Meyer RB, Harman AL, Kellow UK, Henderson BE, Le Marchand L, Brenan P, Sangrajragan S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, et al.: Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007, 447:1087-1093.

Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hanksin SE, Wacholder S, Wang Z, Welch R, Hutchinson A, Wang J, Yu K, Chatterjee N, Orr N, Willett WC, Colditz GA, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Hayes RB, Tucker M, Gerhard DS, Fraumeni JF, Jr, Hoover RN, Thomas G, Chanson SJ: A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007, 39:760-784.

Garcia-Closs M, Hall P, Nevanlinna H, Pooley K, Morrison J, Richesson DA, Bresnick J, Rosewell I, Crafton T, Poulsom R, Stamp G, Dickson C: Patterns of somatic mutation in human cancer genomes. Nat Rev Cancer 2011, 11:235-253.

Johnson DE, Williams LT: Structural and functional diversity in the FGFR receptor multigene family. Adv Cancer Res 1993, 60:1-41.

Esvarakumar VP, Laux I, Schlessinger J: Cellular signalling by fibroblast growth factor receptors. Cytokine Growth Factor Rev 2005, 16:139-149.

Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, O'Meara S, Vastrik I, Schmidt EE, Avis T, Barthorpe S, Bhama G, Buck G, Choudhury B, Clemens J, Cole J, Dick E, Forbes S, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, et al.: Patterns of somatic mutation in human cancer genomes. Nature 2007, 446:153-158.

Taylor JG, Cheuk AT, Tang PS, Chung JY, Song YK, Desai K, Yu Y, Chen QR, et al.: FGFR2 expression in human rhabdomyosarcomas that promote metastasis in xenotransplanted models. J Clin Invest 2009, 119:3395-3407.

Elbouamy Elsheikh S, Green AR, Lambros MB, Turner NC, Grainge MJ, Powe D, IO, Reis-Filho JS: FGFR1 amplification in breast carcinomas: a chromogenic in situ hybridisation analysis. Breast Cancer Res 2007, 9:R33.

Turer N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, Natrajan R, Marchio C, Iorns E, Mackay A, Gillett C, Grigoriadis A, Tutt A, Reis-Filho JS, Ellis IO, Reis-Filho JS: FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 2010, 70:2085-2094.

Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, Ullrich RT, Menon R, Maier S, Soltermann A, Moch H, Wagener P, Fischer F, Heynck S, Koker M, Schottle J, Leenfers P, Gabeler F, Dabow I, Quenning S, Heukamp LC, Balke-Want H, Ansen S, Rauth D, Baessmann I, Altamuller J, Wright G, Russell P, et al.: Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010, 2:22ra93.

Shackelford GM, MacArthur CA, Kwan HC, Varmus HE: Mouse mammary tumor virus infection accelerates mammary carcinogenesis in Wnt-1 transgenic mice by insertional activation of int-2/FGF-3 and hst/FGF-4. Proc Natl Acad Sci USA 1993, 90:740-744.

Theodorou V, Kimm MA, Boer M, Wessels L, Theelen W, Jonkers J, Hilken J: MMTV insertional mutagenesis identifies genes, gene families and pathways involved in mammary cancer. Nat Genet 2007, 39:759-769.

Muller W, Lee FS, Dickson C, Peters G, Patenaille F, Leder P: The int-2 gene product acts as an epithelial growth factor in transgenic mice. EMBO J 1990, 9:907-913.

Welm BE, Freeman KW, Chen M, Contreras A, Spencer DM, Rosen JI: Inducible dimerization of FGFR1: development of a mouse model to analyze progressive transformation of the mammary gland. J Cell Biol 2002, 157:703-714.

Easton DF, Pooley KA, Dunn JK, Pharoah PD, Thompson D, Ballinger DG, Stratton MR, Morrison J, Field H, Luben R, Wareham N, Ahmad S, Healey CS, Bowman R, Meyer RB, Harman AL, Kellow UK, Henderson BE, Le Marchand L, Brenan P, Sangrajragan S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, et al.: Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007, 447:1087-1093.

Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hanksin SE, Wacholder S, Wang Z, Welch R, Hutchinson A, Wang J, Yu K, Chatterjee N, Orr N, Willett WC, Colditz GA, Ziegler RG, Berg CD, Buys SS, McCarty CA, Feigelson HS, Calle EE, Thun MJ, Hayes RB, Tucker M, Gerhard DS, Fraumeni JF, Jr, Hoover RN, Thomas G, Chanson SJ: A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 2007, 39:760-784.

Garcia-Closs M, Hall P, Nevanlinna H, Pooley K, Morrison J, Richesson DA, Bresnick J, Rosewell I, Crafton T, Poulsom R, Stamp G, Dickson C: Patterns of somatic mutation in human cancer genomes. Nat Rev Cancer 2011, 11:235-253.
Fibroblast growth factor receptor 4 regulates prostate cancer progression. Neoplasia 2010, 12:847-856.

Sugiyama N, Varjosalo M, Meller P, Lohi J, Chan KM, Zhou Z, Alitalo K, Taipale J, Turner N, Lambros MB, Horlings HM, Pearson A, Sharpe R, Natrajan R, Geyer CJ, Tannheimer SL, Rehemtulla A, Ethier SP, Characterization of the recurrent 8p11-12 amplicon identifies PAPPCDC1, a phosphatase protein, as a potential therapeutic target in breast cancer. Cancer Res 2008, 68:7165-7175.

Sharpe R, Pearson A, Herrera-Abreu MT, Johnson D, Mackay A, Welt JC, Natrajan R, Reynolds AR, Reis-Filho JS, Ashworth A, Turner NC. 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, 17:5275-5286.

Wang J, Yu W, Cai Y, Ren C, Ittmann MM. Altered fibroblast growth factor receptor 4 stability promotes prostate cancer progression. Neoplasia 2010, 12:847-856.

Sugiyama N, Varjosalo M, Meller P, Lohi J, Chan KM, Zhou Z, Alitalo K, Taipale J, Keski-Oja J, Lehti K. FGFR receptor-4 (FGFR4) polymorphism acts as a switch activity of a membrane type 1 matrix metalloproteinase-FGFR4 complex. Proc Natl Acad Sci U S A 2010, 107:15786-15791.

Setzer N, Mayr T, Streit S, Ullrich A. A single nucleotide change in the mouse genome accelerates breast cancer progression. Cancer Res 2010, 70:802-812.

Marne F, Werft W, Benner A, Bunwinkel B, Sinn P, Sohn C, Lichter P, Hahn M, Scheweiss A. FGFR4 Arg388Gln genotype is associated with pathological complete response to neoadjuvant chemotherapy for primary breast cancer. Ann Oncol 2010, 21:1636-1642.

Turner N, Lambros MB, Hafletics HM, Pearson A, Sharpe R, Natrajan R, Geyer CJ, van Kouwenhove M, Kreike B, Mackay A, Ashworth A, van de Vlier M, Reis-Filho JS. Integrative molecular profiling of triple negative breast cancers identifies ampiclon drivers and potential therapeutic targets. Oncogene 2010, 29:2013-2023.

Tannheimer SL, Rehemtulla A, Ethier SP. Characterization of fibroblast growth factor receptor 2 overexpression in the human breast cancer cell line SUM-52PE. Breast Cancer Res 2000, 2:211-217.

A bail Meetei K, Vo NY, Kollipara S, Maza EA, Winston WM, Weiler S, Poling LL, Chen T, Ismail NS, 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.

Cha JY, Maddess S, Minn N, Harden TK, Der CJ. Altered fibroblast growth factor receptor internalization and enhanced FR52-dependent signaling contribute to the transformation activity of the fibroblast growth factor receptor 2 IIIb C3 isoform. J Biol Chem 2009, 284:6227-6240.

Heiskanen M, Kononen J, Barlund M, Torhorst J, Sauter G, Kallioniemi A, Lutterbach B. Mapping of DNA amplifications at 15 chromosomal loci across human cancers. Proc Natl Acad Sci U S A 2008, 105:2360-2365.

Kuni K, Davis L, Gorenstein J, Hatch H, Yashiro M, DiBacco A, Elbi C, Lutterbach B. FGFR2-amplified gastric cancer cell lines require FGFR2 and FR52 for their tumorigenicity and enhance oncoprotein translation in the mouse mammary tumor virus-Wnt1 mouse model of breast cancer. Cancer Res 2010, 70:4868-4879.

Bade LK, Goldberg JE, Dehut HA, Hall MK, Schwartfeger KL. Mammary tumorigenesis induced by fibroblast growth factor receptor 1 requires activation of the epidural growth factor receptor. J Cell Sci 2011, 124(Pt 18):3106-3117.

Kang S, Song S, Gu TL, Guo A, Chen MS, Lonal S, Khoury HJ, Fabbro D, Gilliland DG, Bergsgal PG, Tauntton J, Polakiewicz RD, Chen J. FGFR3 activates RSK2 to mediate hematopoietic transformation through tyrosine phosphorylation of RSK2 and activation of the MEK/ERK pathway. Cancer Cell 2007, 12:201-214.

Kwek SS, Roy R, Zhou H, Climent J, Martinez-Climent JA, Fridlyand J, Albertson DG. Co-amplified genes at 8p11-12 and 1q11-13 in breast tumors cooperate with two major pathways in oncogenesis. Oncogene 2009, 28:1892-1903.

Dutt A, Salvesen HB, Chen TH, Onofrio RC, Hatton C, Nicoletti R, Winckler W, Grewal R, Hanna M, Wyhs N, Ziaugra L, Richter DJ, Trovik J, Engelsen IB, Stefansson IM, Fennell T, Cibulskis K, Zody MC, Akslen LA, Gabriel S, Wong KK, Sellers WR, Meyerson M, Greulich H. Drug-sensitive FGFR2 mutations in endometrial carcinoma. Proc Natl Acad Sci U S A 2008, 105:18715-18720.

Cappellen D, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, Chapelin D, Thiery JP, Radvanyi F. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet 1999, 23:18-20.

Reff M, Leleuene S, Scott PA, Fox S, Smith K, Level R, Moghadam A, Whitehouse R, Bicknell R, Hains AL. Expression of the angiogenic factor vascular endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 1997, 57:963-969.

Liu Y, Wang JL, Chang H, Barisky SH, Fabbro D, Gilliland DG, Bergsgal PG, Tauntton J, Polakiewicz RD, Chen J. FGFR3 activates RSK2 to mediate hematopoietic transformation through tyrosine phosphorylation of RSK2 and activation of the MEK/ERK pathway. Cancer Cell 2007, 12:201-214.

Kwek SS, Roy R, Zhou H, Climent J, Martinez-Climent JA, Fridlyand J, Albertson DG. Co-amplified genes at 8p11-12 and 1q11-13 in breast tumors cooperate with two major pathways in oncogenesis. Oncogene 2009, 28:1892-1903.

Dutt A, Salvesen HB, Chen TH, Onofrio RC, Hatton C, Nicoletti R, Winckler W, Grewal R, Hanna M, Wyhs N, Ziaugra L, Richter DJ, Trovik J, Engelsen IB, Stefansson IM, Fennell T, Cibulskis K, Zody MC, Akslen LA, Gabriel S, Wong KK, Sellers WR, Meyerson M, Greulich H. Drug-sensitive FGFR2 mutations in endometrial carcinoma. Proc Natl Acad Sci U S A 2008, 105:18715-18720.

Cappellen D, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, Chapelin D, Thiery JP, Radvanyi F. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet 1999, 23:18-20.

Reff M, Leleuene S, Scott PA, Fox S, Smith K, Level R, Moghadam A, Whitehouse R, Bicknell R, Hains AL. Expression of the angiogenic factor vascular endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res 1997, 57:963-969.

Liu Y, Wang JL, Chang H, Barisky SH, Fabbro D, Gilliland DG, Bergsgal PG, Tauntton J, Polakiewicz RD, Chen J. FGFR3 activates RSK2 to mediate hematopoietic transformation through tyrosine phosphorylation of RSK2 and activation of the MEK/ERK pathway. Cancer Cell 2007, 12:201-214.

Kwek SS, Roy R, Zhou H, Climent J, Martinez-Climent JA, Fridlyand J, Albertson DG. Co-amplified genes at 8p11-12 and 1q11-13 in breast tumors cooperate with two major pathways in oncogenesis. Oncogene 2009, 28:1892-1903.

Dutt A, Salvesen HB, Chen TH, Onofrio RC, Hatton C, Nicoletti R, Winckler W, Grewal R, Hanna M, Wyhs N, Ziaugra L, Richter DJ, Trovik J, Engelsen IB, Stefansson IM, Fennell T, Cibulskis K, Zody MC, Akslen LA, Gabriel S, Wong KK, Sellers WR, Meyerson M, Greulich H. Drug-sensitive FGFR2 mutations in endometrial carcinoma. Proc Natl Acad Sci U S A 2008, 105:18715-18720.

Cappellen D, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, Chapelin D, Thiery JP, Radvanyi F. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet 1999, 23:18-20.
62. Sun HD, Malabunga M, Tonra JR, DiRenzo R, Carrick FE, Zheng H, Berthoud HR, McGuinness OP, Shen J, Bohlen P, Leibl RL, Kusse P: Monoclonal antibody antagonists of hypothalamic FGF1 cause potent but reversible hypophagia and weight loss in rodents and monkeys. *Am J Physiol Endocrinol Metab* 2007, 292:E964-E976.

63. Marshall ME, Hinz TK, Kono SA, Singleton KR, Bichon B, Ware KE, Marek I, Frederick BA, Raben D, Heasley LE: Fibroblast growth factor receptors are components of autocrine signaling networks in head and neck squamous cell carcinoma cells. *Clin Cancer Res* 2011, 17:5016-5025.

64. Andre F, Bachelot TD, Campone M, Dalenc F, Perez-Garcia JM, Hurvitz SA, Tashiro E, Maruki H, Minato Y, Doki Y, Weinstein IB, Imoto M: 

65. basalga J, burris HA, 3rd, rugo hs, sahmoud t, singer y, chang h, liang sb, yayon a: everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 2010, 363:1693-1703.

66. Freier K, Schwaenzen C, Stich C, Flentzenmacher C, Muhling J, Hofele C, Radlwimmer B, Lichter P, Joos S: Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010, 363:1693-1703.

67. Wohlrte S, Bonny O, Beluch N, Gaulis S, Stamm C, Scheibler M, Muller M, Kinzel B, Thuery A, Brueggen J, Hynes NE, Sellers WR, Hofmann F, Graus-Porta D: FGF receptors control vitamin D and phosphate homeostasis by mediating renal FGF-23 signaling and regulating FGF-23 expression in bone. *J Bone Miner Res* 2011, 26:2486-2497.

68. Baselga J, Campone M, Piccart M, Burris HA, 3rd, Rugo HS, Sahmoud T, Noguchi S, Gr antes M, Pritchard KL, Lebrun F, Beck JT, Ito Y, Yardley D, Deleu I, Perez A, Bachelot T, Vittori L, Xu Z, Mukhopadhyay P, L ebohwil D, Hortobagyi GN: Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. *N Engl J Med* 2012, 366:520-529.

69. Ratain MJ, Schwartz GN, Ozawa AM, Ruxin CM, Kaye SB, De Jonge MJ, Khayat D, Awada A, Sawyer MB, Obel JC, Medironi I, Evans-Totp, De Greve J, Soetekouw PM, Baurain J, O’Dwyer PJ, Hartman C, Poulart V, Walters IB: Brivanib (BMS-827864) in advanced solid tumors (AST): results of a phase II randomized discontinuation trial (RDT) [abstract 3079]. *J Clin Oncol* 2011, 29(Suppl).

70. Ratain MJ, Schwartz GK, Oza AM, Rudin CM, Kaye SB, De Jonge MJ, Christensen JG, Haber DA, Wilner K, Shreeve SM, Ratain MJ, Settleman J, McGuinness OP, Shen J, Bohlen P, Leibel RL, Kusse P: FGFR inhibitor, in patients with advanced solid tumors. *Clin Cancer Res* 2011, 17:5016-5025.

71. Ratain MJ, Schwartz GK, Oza AM, Rudin CM, Kaye SB, De Jonge MJ, Khayat D, Awada A, Sawyer MB, Obel JC, Medironi I, Evans-Totp, De Greve J, Soetekouw PM, Baurain J, O’Dwyer PJ, Hartman C, Poulart V, Walters IB: Brivanib (BMS-827864) in advanced solid tumors (AST): results of a phase II randomized discontinuation trial (RDT) [abstract 3079]. *J Clin Oncol* 2011, 29(Suppl).

72. Bouché O, Druceux M, Liegoed G, André T, Mairaudt-Goebel F; Stopper F; OumHamed Z, Chadmja M, de Gamont A: A phase II trial of weekly alternating sequential administration of BEB-1120 and BIBW2992 in patients with advanced colorectal cancer [abstract 13001]. *J Clin Oncol* 2008, 26(Suppl).

73. Talpaz M, Shah NP, Deininger MW, Mauro MJ, Finn IW, Lustgarten S, Lindmark W, Gozgit JM, Clackson T, Turner CD, Haluska FG, Kantnjian H, Cortes JE: Ponatinib in patients with acute myeloid leukemia (AML): preliminary findings from a phase I study in hematologic malignancies [abstract 6518]. *J Clin Oncol* 2011, 29(Suppl).

74. An Open Label Phase I Dose Escalation Study of E7080 Administered to Patients with Solid Tumors [http://clinicaltrials.gov/ct2/show/ NCT00280397?term=e7080&rank=4]

75. Study of Oral E-3810, a Dual VEGFR-FGFR Tyrosine Kinase Inhibitor, in Patients with Solid Tumors [http://clinicaltrials.gov/ct2/show/ NCT01283945?term=e3810&rank=5]

76. Xu J, Wang Y, Ye CY, Huang C, Zhang XH, Sui Y, Shen L, MU H: Phase I study on safety and pharmacokinetics of sunitinib, a selective VEGFR/FGFR dual inhibitor, in Chinese patients with advanced solid tumors [abstract e13558]. *J Clin Oncol* 2011, 29(Suppl).

77. Xie L, Su X, Zhang D, Tang L, Xu J, Wang M, Yin L, Zhang J, Ye K, Wang Z, Kilgour E, Qunsheng J: AZD4547, a potent and selective inhibitor of FGFR-receptor tyrosine kinases 1, 2 and 3, inhibits the growth of FGFR-receptor 2 driven gastric cancer models in vitro and in vivo [abstract 1643]. In Proceedings of the 102nd Annual Meeting of the American Association for Cancer Research, 2–6 April 2011; Orlando, FL. Philadelphia, PA: AACR, 2011.

78. A Randomised Open-Label Phase IIa Study to Assess the Efficacy and Safety of AZD4547 Monotherapy versus PacliTaxel in Patients with Advanced Gastric or Gastro-oesophageal Junction Cancer with FGFR2 Polysomy or Gene Amplification (SHINE Study) [http://clinicaltrials.gov/ct2/ show/NCT01457846?term=AZD4547&rank=2]

79. A Phase I, Open-label, Multi-center, Dose Escalation Study of Oral BGJ398, a Pan FGF-R Kinase Inhibitor, in Adult Patients with Advanced Solid Malignancies [http://clinicaltrials.gov/ct2/show/NCT01004224]

80. Trudel S, Stewart AK, Rom E, Wei E, Li ZH, Kotzer S, Chumakov I, Singer Y, Chang H, Liang SB, Yayon A: The inhibitory anti-FGFR2 antibody, PRO-001, is cytotoxic to t(4;14) multiple myeloma cells. *Blood* 2006, 107:4039-4046.

81. A Phase I, Open-label, Dose-finding Study Evaluating the Safety and Pharmacokinetics of FP-1039 in Subjects with Metastatic or Locally Advanced Unresectable Solid Tumors [http://clinicaltrials.gov/ct2/show/ NCT00687505?term=fp-1039&rank=2]

Cite this article as: Jain VK, Turner NC. Challenges and opportunities in the targeting of fibroblast growth factor receptors in breast cancer. *Breast Cancer Research* 2012, 14:208.