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Genomic aberrations in the FGFR pathway: opportunities for targeted therapies in solid tumors

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The fibroblast growth factor receptor (FGFR) cascade plays crucial roles in tumor cell proliferation, angiogenesis, migration and survival. Accumulating evidence suggests that in some tumor types, *FGFRs* are bona fide oncogenes to which cancer cells are addicted. Because FGFR inhibition can reduce proliferation and induce cell death in a variety of *in vitro* and *in vivo* tumor models harboring *FGFR* aberrations, a growing number of research groups have selected FGFRs as targets for anticancer drug development. Multikinase FGFR/vascular endothelial growth factor receptor (VEGFR) inhibitors have shown promising activity in breast cancer patients with *FGFR1* and/or *FGF3* amplification. Early clinical trials with selective FGFR inhibitors, which may overcome the toxicity constraints raised by multitarget kinase inhibition, are recruiting patients with known *FGFR*(1–4) status based on genomic screens. Preliminary signs of antitumor activity have been demonstrated in some tumor types, including squamous cell lung carcinomas. Rational combination of targeted therapies is expected to further increase the efficacy of selective FGFR inhibitors. Herein, we discuss unsolved questions in the clinical development of these agents and suggest guidelines for management of hyperphosphatemia, a class-specific mechanism-based toxicity. In addition, we propose standardized definitions for *FGFR1* and *FGFR2* gene amplification based on *in situ* hybridization methods. Extended access to next-generation sequencing platforms will facilitate the identification of diseases in which somatic *FGFR*(1–4) mutations, amplifications and fusions are potentially driving cancer cell viability, further strengthening the role of FGFR signaling in cancer biology and providing more possibilities for the therapeutic application of FGFR inhibitors.

Key words: fibroblast growth factor receptor FGFR, amplification, cancer, hyperphosphatemia, oncogene, targeted therapy

introduction

Fibroblast growth factor receptor (FGFR) signaling plays crucial roles in cancer cell proliferation, migration, angiogenesis, and survival. The FGFR pathway was primarily studied in cancer as a direct promoter of endothelial cell proliferation and tumor neoangiogenesis, having complementary and synergistic effects with the vascular endothelial growth factor (VEGF) signaling [1, 2]. Recent studies have uncovered increasing evidence that in addition to its role as an escape mechanism of anti-VEGF therapies, deregulated FGFRs can function as driving oncogenes in certain tumor types, acting in a cell autonomous fashion to maintain the malignant properties of cancer cells [2, 3]. When *FGFRs* are mutated or amplified, aberrant activation of downstream pathways results in mitogenic, mesenchymal, and antiapoptotic responses in cells. The combination of knockdown studies and selective pharmacological inhibition in preclinical models confirms that FGFRs are attractive targets for therapeutic intervention in cancer [2]. In this article, we will focus on the main *FGFR* genomic alterations found in human cancer to date, how they may contribute to specific tumor types, describe the range of treatment strategies currently employed or in development to inhibit deregulated FGFRs and discuss unsolved questions in the clinical development of these agents.

FGFR pathway

The FGFR family includes four receptor tyrosine kinases FGFR (1–4) comprised of an extracellular domain, a transmembrane domain, and a cytoplasmic domain. The extracellular portion contains three immunoglobulin-like (Ig) folds (IgI, IgII, and IgIII) with a stretch of eight consecutive acidic residues between IgI and IgII (the acidic box). While the IgII and IgIII domains are necessary and sufficient for ligand binding, the amino-terminal portion of the receptor containing IgI and the acidic

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box has an auto-inhibitory function. Alternative splicing of the IgIII extracellular fragment of FGFR1, 2, or 3 may generate isoforms that differ in terms of ligand-binding specificity, with IgIIIb and IgIIIc specifically expressed in the epithelium and mesenchyme, respectively. The intracellular region of FGFRs contains a juxta-membrane domain, a split kinase domain with the classical tyrosine kinase motifs, and a carboxy-terminal tail [4].

Fibroblast growth factors (FGFs) are secreted glycoproteins that are readily sequestered by the extracellular matrix and the cell surface by heparan sulfate proteoglycans (HPSGs). Cell-surface HPSGs stabilize the FGF ligand–receptor interaction by protecting FGFs from protease-mediated degradation [2]. In the case of hormone-like FGFs (FGF19, 21, and 23), the FGF–FGFR interaction requires a cell surface co-receptor, klotho or β-klotho, for high-affinity binding and signaling. Upon ligand binding, FGFR substrate 2 (FRS2) functions as a key adaptor protein that associates with the receptor and initiates downstream signaling with activation of mitogen activated protein kinase (MAPK) and the phosphoinositide-3-kinase (PI3K)/AKT pathways. FGFR signaling also couples to phospholipase C-gamma (PLC-γ) in an FRS2-independent manner and stimulates protein kinase C (PKC), which partly reinforces the MAPK pathway activation by phosphorylating RAF. Depending on the cellular context, several other pathways are also activated by FGFRs including the p38 MAPK and Jun N-terminal kinase pathways, signal transducer and activator of transcription signaling and ribosomal protein S6 kinase 2 (RSK2) [2, 4, 5].

The mechanisms of attenuation and negative feedback control of FGFR signaling are poorly understood and are likely to vary depending on the cell type. Downstream signaling can be attenuated through the induction of MAPK phosphatases (MAPK3), Sprouty (SPRY) proteins, and SEF family members that modulate receptor signaling at several points in the signal transduction cascade. In addition, following activation, FGFRs are internalized and then degraded or recycled according to the level of ubiquitination [2, 4, 5].

In cancer, different FGFR pathway aberrations have been identified and include: (i) gene amplification or post-transcriptional regulation giving rise to receptor overexpression; (ii) *FGFR* mutations producing receptors that are either constitutively active or exhibit a reduced dependence on ligand binding for activation; (iii) translocations resulting in expression of FGFR-fusion proteins with constitutive FGFR kinase activity; (iv) alternative splicing of *FGFR* and isoform switching, which substantially alters ligand specificity increasing the range of FGFs that can stimulate tumor cells; and (v) upregulation of FGF expression in cancer or stromal cells and the enhanced release of FGFs from the extracellular matrix, resulting in paracrine/autocrine activation of the pathway. In humans, several gain-of-function germline mutations in the *FGFR* genes result in skeletal dysplasias, with *FGFR2* mutations a common cause of craniosynostosis and *FGFR3* mutations frequent in chondrodysplasia syndromes. Mutations in cancer resemble those seen in hereditary disorders and interestingly, they are not limited to the kinase domain but are spread over the complete length of the gene. Notably, FGFR signaling in cancer exhibits clear context-dependence, with aberrations differing according to tumor type [4–8]. Table 1 summarizes the most frequent *FGFR* genomic deregulations in solid tumors and the details are discussed subsequently.

Table 1. Common FGFR genomic deregulations in solid tumors

Aberration		Tumor	Prevalence (%)
FGFR1	Amplification	Breast (hormone receptor positive)	10
		Lung (squamous cell carcinoma)	10–20
		Lung (small cell)	6
		Head and neck (squamous cell carcinoma)	10–17
		Esophageal (squamous cell carcinoma)	9
		Ovarian	5
		Osteosarcoma	5
FGFR2	Amplification	Breast (triple-negative)	4
		Gastric	5–10
	Mutation	Endometrial	12
FGFR3	Mutation	Bladder (nonmuscle invasive)	50–60
		Bladder (muscle-invasive)	10–15
	Translocation	Bladder (muscle-invasive)	6
		Glioblastoma	3–7
FGFR4	Amplification	Colorectal	5
	Mutation	Rhabdomyosarcoma	8

FGFRs as oncogenic drivers in cancer

breast cancer

FGFR family members are infrequently mutated but frequently overexpressed in breast cancer, and this is often accompanied by increased, or altered, expression of FGF ligands [9]. The 8p11-12 amplicon, which contains *FGFR1*, is observed in about 10% of breast cancer patients, predominantly hormone receptor positive (HR+) disease [9–12]. Importantly, genes other than *FGFR1* in the 8p11-12 amplicon are also likely to contribute to carcinogenesis [13–15]. In addition, it is noteworthy to mention that *FGFR1* is simultaneously amplified with an amplicon containing *CCND1, FGF3, FGF4*, and *FGF19* on chromosome 11q12-14 in one-third of the samples, and *in vitro* studies suggests substantial functional interaction between the genes on 8p11-12 and 11q [16]. The 11q 12-14 amplicon is seen in ~15%–20% of human breast tumors [17, 18], and was shown to correlate with increased invasiveness in node-negative breast carcinoma [17]. FGFR1-overexpressed cancers are more likely to be progesterone receptor negative and present high proliferation, characteristics of the luminal-B subtype [19]. Large series have shown that *FGFR1* amplification is an independent predictor of poor outcome [19, 20] and drives resistance to endocrine therapy [19]. No significant heterogeneity in *FGFR1* amplification status has been observed after matching primary and metastatic carcinoma samples, although this observation is based on a small sample size [12].

Importantly, *in vitro* studies reinforce the potential oncogenic nature of *FGFR1* amplification. Inhibition of FGFR1 kinase activity causes death of breast cancer-derived cell lines that overexpress FGFR1, indicating that these cells are addicted to the pathway for viability [11]. *In vivo* models of *FGFR1*-amplified breast cancer are challenging and still missing.

Additionally, FGFR2 has also been implicated in some cases of breast cancer, with gene amplification in 4% of triple-negative tumors [21]. *FGFR2* amplification appears to promote breast tumorigenicity through maintenance of breast tumor-initiating cells [22]. *In vitro* studies showed that *FGFR2*-amplified cell lines have constitutive activation of the receptor and are highly sensitive to FGFR inhibition, with induction of apoptosis and decrease in the tumor-initiating cells population [21, 22].

Ligand-dependent signaling is also likely to play a key role in breast cancer. Basal-like breast cancer cell lines with epithelial–mesenchymal transition features and triple-negative breast cancers frequently express FGF2. RNA interference targeting of FGF2 in basal-like cell lines significantly reduced growth *in vitro*. Notably, small-molecule tyrosine kinase inhibitors (TKIs) of FGFR induce tumor shrinkage in xenogafts models with an active autocrine FGF2 signaling loop [23]. These experiments highlight the potential driving role of the FGFR pathway in different subtypes of breast cancer. Figure 1 portrays the relative expression of the *FGFR (1-4)* genes according to The Cancer Genome Atlas (TCGA) breast cancer microarray-based dataset, emphasizing the differences across the intrinsic subtypes of breast tumors [9]. The significance of *FGFR4* activation in HER2-positive tumors still needs further investigation but it is noteworthy to mention that it is one of the HER2-enriched specific genes included in the 50-gene intrinsic subtype predictor (PAM50) [24].

lung cancer

FGFR1 is amplified in 10%–20% of squamous non-small-cell lung cancer (NSCLC) [25–28]. In early-stage disease, it appears to correlate with increased cigarette smoking and poor survival [29]. Interestingly, recurrent *FGFR1* amplification is also seen in

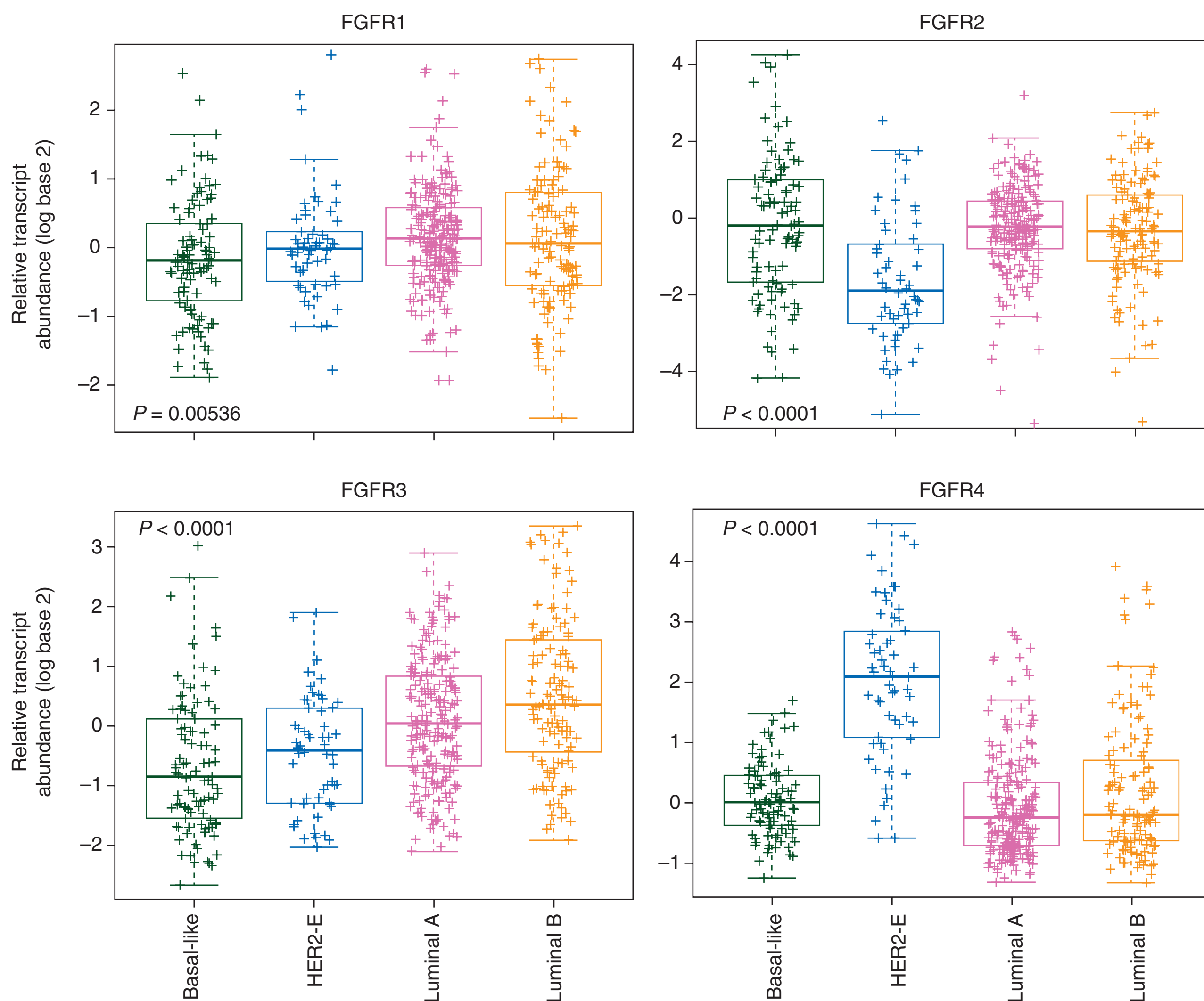

Figure 1. Relative expression of the *FGFR(1–4)* genes across the intrinsic subtypes of breast cancer. Gene expression data and subtype calls have been obtained from The Cancer Genome Atlas (TCGA) breast cancer microarray-based dataset (http://cancergenome.nih.gov/). *P*-values have been obtained by comparing the mean expression across the groups (ANOVA test).

10%–17% of head and neck [30] and 9% of esophageal squamous cell carcinomas [31].

Preclinical studies clearly demonstrate that *FGFR1* amplification confers dependence upon FGFR signaling. Treatment of *FGFR1*-amplified lung cancer cell lines with selective FGFR TKIs resulted in growth inhibition and apoptosis. Moreover, *in vivo* xenograft models derived from both cell lines and patient tumors have also shown increased sensitivity with stasis or regressive effects [25, 32, 33]. Ligand-dependent epithelial–mesenchymal transition in NSCLC cell lines has been shown *in vitro*, and inhibition of FGFR signaling through antisense RNA or anti-FGF2 monoclonal antibodies (mAbs) led to inhibition of cellular proliferation and tumor growth [34].

In small-cell lung cancer (SCLC), exome sequencing identified *FGFR1* locus amplification in 6% of the tumors [35]. There is a clear correlation of high copy number gains in cytogenetic bands encoding FGFR1 and *in vitro*/*in vivo* sensitivity of SCLC models to FGFR inhibition [36, 37]. Paracrine production of FGFs has also been reported and high levels of serum FGF2 in SCLC are associated with a poor prognosis [38].

gastric cancer

FGFR2 amplification is found in 5%–10% of gastric cancers, mainly in the aggressive diffuse subtype [39, 40]. Aberrations are mutually exclusive with other receptor tyrosine kinase amplifications (*ERBB2*, *MET*) [40]. *In vitro*, *FGFR2*-amplified gastric cancer cell lines are selectively sensitive to the growth inhibitory effects of FGFR TKIs [41]. FGFR2 downregulation led to significant inhibition of cell growth and survival that further translated into tumor growth regression *in vivo* [42–44]. Additional evidence of target dependence comes from xenograft models treated with anti-FGFR2 mAbs, which exhibit potent antitumor activity against gastric cancer driven by activated FGFR2 signaling [45, 46].

endometrial cancer

FGFR2 mutations occur in 12% of endometrioid endometrial carcinomas [47–49]. The S252W and P253R substitutions in the extracellular domain, in particular, confer a gain in ligand-binding promiscuity by isoform-switching, thereby establishing an autocrine loop of pathway activation [50]. The N549K and K659E mutations in the kinase domain lead to ligand-independent, constitutively active FGFR2 [48]. Interestingly, *FGFR2* mutations in endometrial cancer show a mutually exclusive pattern with mutations in *KRAS* [51]. In early-stage disease, they are associated with shorter disease-free and overall survival [51]. *FGFR2*-mutant endometrial cancer cell lines and *in vivo* tumor xenograft models are highly sensitive to FGFR TKIs, which reflects oncogenic addiction to the aberrant receptor [43, 44, 47].

urothelial cancer

Up to 80% of low-grade and low-stage tumors express mutant *FGFR3*, but this abnormality is found in less than 20% of invasive high-grade bladder cancer [52–57]. The most common *FGFR3* mutations in urothelial cell carcinoma occur in the extracellular and transmembrane domains (R248C, S249C, G370C, and Y373C) and lead to ligand-independent dimerization and constitutive activation [58]. Several mutations in the kinase domain of *FGFR3* (K650E, K650M), leading to enhanced kinase activity, have also been described [58]. *FGFR3* mutations in bladder cancer are mutually exclusive with mutations in *HRAS* [59]. Multiple preclinical studies including bladder cell culture experiments and mouse models treated with small-molecule inhibitors and anti-FGFR3 mAbs reported decrease in cell proliferation and reduction in tumor growth [43, 60–62]. Activity of an FGFR3-specific antagonistic antibody likely to disrupt dimerization, and hence activation, of the *FGFR3* R248C and S249C mutants, was also extended to wild-type tumors that overexpress the receptor [60].

Fusions of the *FGFR3* gene with transforming acidic coiled-coil (*TACC3*) were recently identified in a subset of invasive bladder tumors (2 of 32 samples) [63]. The FGFR3 component lacks the final exon that includes PLCγ binding site, leading to loss of negative regulation and higher expression of an active receptor. Notably, cell lines were extremely sensitive to selective FGFR inhibition [63].

brain tumors

A small subset of glioblastomas (3%–7%) also harbors oncogenic chromosomal translocations that fuse in-frame the tyrosine kinase coding domains of *FGFR1* or *FGFR3* to *TACC* genes [64, 65]. This aberration is mutually exclusive with epidermal growth factor receptor (*EGFR*), platelet-derived growth factor receptor (*PDGFR*), or *MET* amplifications, known drivers in brain tumors [65]. The fusion, caused by tandem duplication on 4p16.3, leads to loss of the 3′-UTR of *FGFR3*, blocking negative gene regulation and enhancing its expression [65]. In agreement with *in vitro* data showing constitutive kinase activity, *in vivo* models show that administration of an FGFR inhibitor prolongs survival of mice harboring *FGFR3-TACC3*-initiated gliomas [64].

sarcomas

Recurring somatic *FGFR4* kinase domain mutations (K535 and E550) have been described in 8% of rhabdomyosarcoma patients. FGFR4 knockdown in a human rhabdomyosarcoma cell line transplanted into mice reduced tumor growth and metastasis [66]. *FGFR1* amplification was detected in a small subset of osteosarcomas (1 of 17 samples) and predicted sensitivity to a selective FGFR inhibitor *in vitro* [44]. Activation of FGFR signaling pathway as a result of amplification of the *FRS2* adaptor was recently identified in dedifferentiated liposarcomas. Selective FGFR inhibitor was able to inhibit growth of cell lines with FRS2 and FGFR overexpression [67].

colorectal cancer

Colon cancer has been shown to overexpress FGF18 and FGF19, acting through the FGFR3-IIIc and FGFR4 receptors on the tumor cells [5]. An anti-FGF19 mAb that selectively blocks the interaction of FGF19 with FGFR4 inhibited growth of colon tumor xenografts *in vivo*, supporting a role for paracrine/autocrine FGFR activation in cancer maintenance [68].

hepatocellular carcinoma

Ligand-dependent signaling is also likely to play a role in hepatocellular carcinomas (HCCs). FGF2 and FGFs 8, 17, 18, and 19

are upregulated and have been shown to initiate autocrine growth stimulation, cell survival, and neoangiogenesis in liver cancer [5]. Genetic knockdown of *FGF19* inhibits the growth of HCC cell lines carrying the amplicon [69]. Targeting FGF19 with a mAb effectively prevented HCCs in FGF19 transgenic mice [68] and a humanized anti-FGFR4 mAb was reported to inhibit tumor growth in HCC xenograft models [70]. Interestingly, *FGF19* amplification predicts sensitivity to selective FGFR inhibitors *in vitro* in HCC cell lines that express the coreceptor β-klotho, which is essential for high-affinity interactions of FGF19 with FGFR4 [44].

prostate cancer

Extensive correlative studies in human prostate cancer as well as preclinical models indicate that FGFR signaling plays an important role in prostate cancer progression, epithelial–mesenchymal transition and angiogenesis. FGFR1 and FGFR4 are frequently overexpressed, several FGFs are upregulated, while expression of SEF, a negative regulator of the FGFR pathway, is reduced in aggressive cancers [5, 71]. Pathway addiction and a potential role for FGFR targeting in prostate cancer have also been documented. Neutralizing antibody against FGF8 displayed potent antitumor activity in mouse models [72] and FGFR TKI treatment was able to completely inhibit tumor growth and angiogenesis *in vivo* [73].

FGFRs as angiogenic drivers in cancer

FGFs directly promote endothelial cell proliferation and indirectly synergize with the VEGF and PDGF pathways, promoting tumor neoangiogenesis through complementary and overlapping functions. FGFR pathway activation has been shown to mediate resistance to anti-VEGF therapy [1, 3]. In the preclinical setting, tumors progressing to anti-VEGF treatment showed a higher expression of FGF2 and combined VEGFR and FGFR blockade led to increased antitumor activity in the setting of adaptive/evasive resistance [74]. Interestingly, FGF2 is higher in patients with colorectal cancer after the failure of bevacizumab-containing regimens [75] and in glioblastoma patients after treatment with a VEGFR TKI [76].

FGFRs as suppressors in cancer

It is well recognized that context-dependent differences in signaling can lead to either tumor promotion or senescence in response to activated FGFR pathway. FGFR2 signaling is clearly oncogenic in many tumor models. However, several studies support a tumor protective effect of FGFR2 signaling, with FGFR2IIIb expression blocking proliferation *in vitro* and being downregulated on progression of multiple tumor types [2]. Loss-of-function *FGFR2* mutations have been identified in 10% of melanoma tumors and cell lines and are the clearest indication of opposing roles of FGFRs in cancer [77]. These mutations result in receptor loss-of-function through several distinct mechanisms, including loss of ligand binding affinity (R251Q), impaired receptor dimerization (E219K), destabilization of the extracellular domains (G271E), and reduced kinase activity (D530N, I642V, and A648T) [77]. Nevertheless, the mechanisms underlying the tumor suppressive effects of FGFR2 are unknown and oncogene-induced senescence is a possible explanation for the distinct gene functions according to molecular context and tumor type.

resistance to FGFR targeting

Limited preclinical data are available regarding resistance to FGFR inhibitors. Using functional RNA interference screens, loss of *PTEN* was identified as a potential mechanism of resistance that was specific to *FGFR2*-amplified cell lines [78]. Remarkably, ponatinib and the mTOR inhibitor ridaforolimus had a synergistic effect on the *in vitro* growth of endometrial cell lines bearing an activating *FGFR2* mutation, irrespective of *PTEN* status [79]. In contrast, EGFR activation was identified as a resistance mechanism in *FGFR3*-mutant bladder cancer cell lines. Combinations of FGFR and EGFR inhibitors had increased efficacy both *in vitro* and *in vivo* [78]. The concept that activation of the HER family members could compensate for inhibition of FGFRs was also shown in other tumor types, including triple-negative breast cancer [80, 81]. In addition, in xenograft models of FGFR1 and MET co-activation, the combination of selective FGFR and MET inhibitors led to increased antitumor efficacy [80]. It should be noted that few data have been published on the molecular mechanism of cell death induced by FGFR inhibition in different cellular contexts [7]. In order to exploit the oncogene dependency for therapeutic gain, we need to understand the exact mechanism of oncogene addiction that exists in different cell types.

Furthermore, FGFR activation could potentially serve as a mechanism of acquired resistance to targeted therapies possibly related to the extensive cross-talk between FGF and oncogenic pathways. Pathway activation as a result of FGFs released in the tumor microenvironment has been implicated in resistance to gefitinib and erlotinib in NSCLC, cetuximab in *KRAS* wild-type squamous carcinoma cells and to vemurafenib in *BRAF* V600E melanoma cells [82–87]. These data will allow rationale clinical testing of combination of FGFR inhibitors with other targeted agents.

translation to the clinic

The strong evidence of increased sensitivity of multiple FGFR-aberrant cell lines and tumor models to FGFR inhibitors reveals a substantial therapeutic opportunity for selective intervention, validates oncogene addiction for proliferation and/or viability, and provides a rationale for the use of FGFR inhibitors in such instances.

Most FGFR inhibitors currently in development are small molecules kinase inhibitors of the ATP-binding domain. As the catalytic domains of various kinases exhibit significant structural homology, TKIs generally demonstrate activity against more than one kinase. This is a common feature of the first-generation of FGFR inhibitors, which generally have activity against VEGFR and/or PDGFR, two structurally related receptor tyrosine kinases. Multikinase inhibition may increase effectiveness in the treatment of a particular tumor type by disrupting redundant pathways that drive resistance. This might be valid when these agents are used as antiangiogenic therapies. Nevertheless, toxicity is expected to increase significantly and off-targets effects may be detrimental when FGFR is targeted in

the context of the oncogene-addicted tumors discussed above, as side-effects may limit the ability to achieve doses required for effective FGFR inhibition. We are now in a unique position to validate clinically the many hypotheses that have been generated preclinically. In addition to selective FGFR TKIs, mAbs that bind to the extracellular domain of different FGFRs and compete with endogenous ligands (FGFs), thereby blocking FGFR dimerization and downstream activation, are also undergoing clinical testing.

toxicology and pharmacodynamics markers

FGFRs are widely expressed in normal tissues and have a key role in development and physiology, notably the phosphate and vitamin D homeostasis. Preclinical models with highly potent selective FGFR TKIs have caused hyperphosphatemia-mediated tissue calcification owing to blockade of FGF23 release from bone and its signal in the kidney [88]. FGF23 binds FGFR4 and the IIIc isoforms of FGFR1 and FGFR3, but uncertainty remains about the relative contribution of individual FGFR subtypes to hyperphosphatemia [3, 8]. Therefore, increase in serum FGF23, phosphate, and vitamin D levels are potential biomarkers for effective FGFR inhibition. In early clinical trials with FGFR inhibitors, development of hyperphosphatemia as a class-specific toxicity may help in the definition of an optimal biological dose [89].

Additional mechanism-based toxicities observed in toxicology models include skin and other cutaneous events, as well as dose-dependent keratopathy and retinal pigment epithelial detachment. In contrast to multikinase VEGFR/FGFR inhibitors, efficacious doses of potent selective FGFR inhibitors do not induce elevations in blood pressure or proteinuria [90, 91].

multikinase (nonselective) FGFR inhibitors

The most clinically advanced FGFR TKIs have dominant pharmacological activity in other kinases, such as VEGFR, PDGFR, FLT3, RET, KIT, and BCR-ABL. These include brivanib, cediranib, nintedanib, lenvatinib, sulfatinib, dovitinib, ponatinib, and lucitanib. Although *in vitro* kinase and cellular activity of these compounds against FGFR1-3 is variable (IC_{50} ranging from 2 to >500 nM), multiple preclinical models showing increased activity in FGFR-deregulated tumors have been published. *FGFR1* amplification in breast cancer cell lines predicts sensitivity to brivanib, dovitinib, ponatinib, and lucitanib [43, 92–94]. *In vitro* and *in vivo* models of *FGFR1*-amplified lung cancer are highly sensitive to ponatinib and lucitanib as single agents [43, 94]. Cediranib, dovitinib, and ponatinib are very active in *FGFR2*-amplified gastric cancer models [40, 42, 43]. In addition, dovitinib and ponatinib also show significant antitumor activity in *FGFR2*-mutant endometrial and *FGFR3*-mutant bladder cancer xenografts [43, 62, 95].

Several of these molecules are being developed as antiangiogenic agents and trials are underway in a variety of tumor types irrespective of *FGFR* aberrations, such as nintedanib and brivanib in endometrial cancer (clinical trials.gov NCT01225887 and NCT00888173), as well as nintedanib and levantinib in lung cancer (NCT01441297, NCT01529112). Should there be any partial or complete responses in these trials it will be interesting to correlate with the amplification/mutation status of *FGFR1*/*FGFR2*. Levantinib has shown promising efficacy in HCC, but correlation with FGF upregulation when its indication is mainly based on the potent antiangiogenic activity is more challenging [96]. As seen in Table 2, clinical trials with multikinase FGFR inhibitors in patients selected based on FGFR aberrations are also in progress.

The first reported trial, which evaluated the VEGFR/PDGFR/FGFR inhibitor dovitinib in *FGFR1*-amplified and nonamplified metastatic breast cancer, failed to reach its primary end point of improved overall response rate in the genomically selected arm [92]. However, in the subgroup with *FGFR1* amplification, 13% had unconfirmed partial responses. Activity was observed primarily in the subgroup of patients with co-amplification of *FGF3* as measured by quantitative real-time polymerase chain

Table 2. Genomically driven clinical trials of FGFR inhibitors

Agent	Phase	Clinical trials.gov	Description
Multikinase inhibitors			
Dovitinib	Phase II	NCT01379534	*FGFR2*-mutant or wild-type endometrial cancer
	Phase II	NCT01732107	*FGFR3*-mutant or overexpressed BCG refractory urothelial carcinoma
	Phase II	NCT01719549	*FGFR2*-amplified gastric cancer
Lucitanib	Phase I/II	NCT01283945	Expansion cohort in *FGFR1*-amplified tumors
Ponatinib	Phase II/III	NCT01761747	Advanced squamous cell lung cancers with FGFR kinase alterations
Selective FGFR inhibitors			
AZD4547	Phase I	NCT00979134	Expansion cohort in *FGFR1*- or *FGFR2* amplified tumors
	Phase II	NCT01457846	Gastric or lower-esophageal cancer, *FGFR2*-amplified or not, randomized to AZD4547 or paclitaxel
	Phase I/II	NCT01202591	Estrogen receptor + and *FGFR1*-amplified breast cancer, randomized to AZD4547 plus fulvestrant or fulvestrant alone
BGJ398	Phase I	NCT01004224	*FGFR1*- or *FGFR2*-amplified, *FGFR3*-mutant advanced cancer
LY2874455	Phase I	NCT01212107	Advanced cancer with *FGFR* aberrations during dose expansion
JNJ-42756493	Phase I	NCT01703481	Expansion cohort in *FGFR1*-, *FGFR2*-, or *FGFR4*-amplified tumors

reaction (RT-PCR). Pharmacodynamic analysis indicates FGFR and VEGFR inhibition at tolerable doses, with consistent and maintained increase in serum FGF23, VEGF, and PDGF [92, 97].

More recently, the therapeutic potential of FGFR inhibition in breast cancer was uncovered with preliminary results of phase I trial with lucitanib, a potent VEGFR/FGFR inhibitor [98]. Enrollment in the expansion cohort at recommended doses was limited to patients whose tumors harbored *FGFR1* amplification by fluorescent *in situ* hybridization (FISH) or 11q amplification (locus of *FGF3*) as measured by comparative genomic hybridization (CGH) array. Seven of 10 assessable patients had a partial response, 4 with *FGFR1*-amplified breast tumors, and 3 with 11q amplification (one of them without concomitant *FGFR1* amplification). Pharmacodynamic data confirming pathway inhibition is still pending [98]. Interestingly, hyperphosphatemia, an adverse event specific of potent FGFR inhibitors, was not consistently observed with lucitanib or dovitinib [92, 98]. The toxicity profile of these agents is mainly related to VEGFR inhibition, including hypertension, proteinuria, and hypothyroidism, but off-target effects such as gastrointestinal toxicity and asthenia were also dose-limiting [92, 98]. It remains to be proven whether the impressive efficacy of lucitanib is related to FGFR inhibition, VEGFR inhibition or the combination of targets, although prior clinical trials with other multikinase VEGFR inhibitors as single agents in unselected breast cancer patients showed disappointing results [99, 100]. The results of clinical trials with more selective FGFR inhibitors in *FGFR*-deregulated breast cancer will shed light on this issue. A confirmatory phase II trial with lucitanib will start recruitment soon.

selective FGFR inhibitors

Many selective FGFR inhibitors have recently started clinical development. Phase I trials with BGJ398, AZD4547, LY2874455, and JNJ-42756493 are currently recruiting patients. The *in vitro* kinase activity of these compounds against FGFR1, FGFR2, and FGFR3 is very high (IC_{50} < 10 nM), with variable anti-FGFR4 activity. A patient-derived xenograft model of *FGFR1*-amplified lung cancer was highly sensitive to AZD4547 as single agent [32], and BGJ398 also demonstrated potent *in vivo* activity in *FGFR2*-amplified gastric and *FGFR2*-mutant endometrial cancer [44, 95]. Following stringent preclinical data suggesting oncogene addiction, clinical development is centered in *FGFR*-aberrant tumors, as shown in Table 2.

Early results of the ongoing phase I trials with BGJ398 and AZD4547 have been presented. In the BGJ398 study, patients with advanced solid tumors showing *FGFR1* or *FGFR2* amplification or *FGFR3* mutation are still been recruited. Results of the first 29 patients (18 with *FGFR1*-amplified tumors) are available [101]. The most frequently observed adverse events were diarrhea, fatigue, nausea and hyperphosphatemia (about one-third of the patients) and dose-limiting toxicities included grade 3 elevations in transaminase levels and grade 2 corneal events. The incidence of hyperphosphatemia increased at higher doses of BGJ398 but could be managed with phosphate binders and diuretics. One lung cancer patient with *FGFR1* amplification by FISH analysis had a confirmed partial response. Preliminary efficacy data in the breast cancer population with *FGFR* aberrations was disappointing, with no partial responses in the first 13 patients recruited [101]. With regards to AZD4547, dose-limiting toxicities included renal failure (pyelonephritis, dehydration), mucositis, increase in transaminase levels and hyperphosphatemia [102]. In addition to dose-proportional increase in phosphate and vitamin D levels, other potential mechanism-based toxicities included alopecia, nail disorders, dry skin, and asymptomatic retinal pigment epithelial detachment. Investigators did not report soft-tissue calcification. Human exposure at recommended phase II doses was consistent with exposures in preclinical models that induced tumor regressions. Of 20 patients with FGFR pathway aberrations, 5 had clinical benefit as assessed by investigators, 3 of them with significant tumor shrinkage: 1 patient with *FGFR1*-amplified squamous NSCLC had a confirmed partial response; 1 with *FGFR1*-amplified breast cancer patient presented 25% decrease in the size of target lesions; and 1 with *FGFR3*-mutant bladder cancer had 23% reduction in tumor size for more than 6 months. Clinical benefit appeared to be higher in those with high-level *FGFR1* amplification (*FGFR1*/CEP8 ratio >2.8 by FISH) [102]. A phase II study evaluating AZD4547 in combination with endocrine therapy in breast cancer has recently started accrual (NCT01202591).

monoclonal antibodies targeting the FGFR pathway

Therapeutic mAbs can be highly specific for a particular FGF ligand or FGFR isoform, hence displaying a more narrow range of toxicity when compared with pan-FGFR inhibitors. Specific inhibition of a particular oncogenic FGFR molecule, including splice variants that are selectively upregulated in tumor cells, would avoid targeting different FGFR isoforms that have opposing effects in cancer cells. In addition, by recruiting the immune system via antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity, antitumor activity might be increased. Several antibodies targeting the FGFR pathway have been assessed in preclinical studies in a variety of solid tumors, including gastric, bladder, prostate cancer, and HCC, as described above.

The first agent to move into the clinic was FP-1039, a soluble fusion protein consisting of the extracellular domain of human FGFR1 linked to the Fc portion of human IgG1. It was engineered to spare the metabolic hormone FGFs (including FGF23 involved in phosphate and vitamin D metabolism) and to bind tightly to all of the mitogenic FGF ligands [103]. *In vivo* models with genetic aberrations in the FGFR pathway, including *FGFR1*-amplified lung cancer and *FGFR2*-mutated endometrial cancer, were particularly sensitive to FP-1039-mediated tumor inhibition. In addition, it was shown to block FGF- and VEGF-induced angiogenesis *in vivo* [103]. The final report of the phase I trial with FP-1039 is still pending but it does not appear to significantly increase serum calcium and phosphate levels or induce hypertension or proteinuria [103]. Unfortunately, a phase II study testing FP-1039 in *FGFR2* S252W or P253R mutant endometrial cancer patients was not feasible. The original assumption was that at least 5% of patients screened would qualify, but after screening 70 patients, none qualified (NCT01244438).

Another antibody targeting the FGFR pathway tested in the clinic is the human anti-FGFR3 agent MFGR1877S [104]. Based

on preclinical data showing activity of the compound in bladder cancer with FGFR3 overexpression or mutations, the phase I trial was enriched with patients diagnosed with advanced urothelial carcinomas. Five of the 10 bladder cancer patients had stable disease as their best response (4 remained on study for more than three cycles). Dose-limiting toxicity was thrombocytopenia in one patient, and the drug was escalated until effective doses based on preclinical models [104]. Future development of this compound and other mAbs targeting the FGFR pathway is unknown at this time.

challenges in the clinical development of FGFR inhibitors

Despite promising preliminary results with FGFR inhibition in genetically selected tumors, many logistical challenges related to prescreening strategies and biomarker platform selection will need to be overcome for successful clinical development of this class of agents. We need to define precisely the alterations associated with pathway addiction taking into consideration context-dependency of the FGFR signaling. In parallel, optimal molecular diagnostic procedures for FGFR aberrations need to be developed, as companion diagnostics will be required to enroll patients in clinical trials. As an example, the definition of *FGFR1/2* amplification based on *in situ* hybridization techniques varied significantly in the published literature, as shown in Table 3.

Many limitations for a consensus definition of FGFR pathway activation became clear during the last years. First, there is a marked genomic heterogeneity in the FGFR1 amplicon structure in breast (broad) and squamous NSCLC (focal) and the influence of these differences on the degree of FGFR addiction is unknown. Second, *in situ* hybridization analyses showed that tumors might exhibit a focal and heterogenous pattern of amplification with frequent polysomy of CEN8; this could lead to an FGFR1/CEN8 ratio below 2.0 despite an increase in absolute numbers of FGFR1 signals compared with normal tissue. Empiric data will be required to define the exact amplification cut-off that predicts anti-FGFR therapy response. In the meantime, our molecular pathology laboratories have been applying the HER2 FISH CAP/ASCO (College of American Pathologists/American Society of Clinical Oncology) guidelines to score FGFR (amplified is either FGFR/CEP ratio >2.2 or average FGFR gene copy number >6 signals/nucleus). Assays for detecting FGFR fusions will be challenging, as at least some of the variants such as *FGFR3-TACC3* are not amenable to traditional FISH analysis as the genes map too close to each other.

Standardized definition of pathway activation becomes an even more difficult problem for identifying tumors in which paracrine/autocrine signaling is potentially driving tumor cell proliferation. Ligand amplification can be detected by many different techniques, such as RT-PCR, array CGHs, microarrays, and RNA sequencing. Thus far, the inconsistent definitions of FGFs amplifications make their use investigational in nature, although early trials have clearly shown that ligand amplification might help define the population of patients with higher chances of response. Importantly, molecular testing is also evolving, moving from 'one test-one drug' paradigm to multiplex approaches looking for mutations, amplifications, and gene fusions [105, 106]. More robust and reproducible genomic platforms that can screen alterations of multiple FGF-pathway components in a single assay are being gradually incorporated into the prescreening process of FGFR inhibitors trials. With massively parallel sequencing, the number of clinically significant and potentially predictive oncogenic aberrations in the FGFR pathway is expected to increase. This is illustrated by the recent finding of intragenic duplications of the portion of *FGFR1* encoding the tyrosine kinase domain in grade II diffuse childhood gliomas [107], recurrent *FGFR1* hotspot mutations in pediatric pilocytic astrocytomas [108], as well as TKI-sensitive *FGFR2* and *FGFR3* mutations in squamous NSCLC [109]. Newly rare but recurrent *FGFR2* and *FGFR3* fusions were also described across multiple solid tumors, including cholangiocarcinomas and squamous NSCLC [110, 111]. These rearrangements apparently share the mechanism of pathway activation, with different fusion partners mediating oligomerization, which triggers overexpression of the respective FGFR kinase. Patients whose

Table 3. Definition of *FGFR1/2* amplification based on *in situ* hybridization techniques in retrospective studies and or clinical trials

Aberration	Method	Tumor	Threshold	References
FGFR1 amplification	FISH	Squamous lung cancer	Average copy number/nucleus ≥6, FGFR1/CEN8 ratio ≥2, or ≥10% tumor cells with ≥15 FGFR1 signals or large copy number clusters	[27]
			Average copy number/nucleus ≥9	[25, 29]
			FGFR1/CEN8 ratio ≥2.2	[26]
		Breast cancer	Average copy number/nucleus ≥6	[92]
			Average copy number/nucleus ≥6 or FGFR1/CEN8 ≥2.2	[98]
		Any tumor	FGFR1/CEN8 ratio >2 in ≥10% tumor cells	[102]
	CISH	Breast cancer	Average copy number/nucleus ≥5 or large copy number clusters	[19, 20]
			Average copy number/nucleus >6 or large copy number clusters	[12]
FGFR2 amplification	FISH	Gastric cancer	FGFR2/CEN10 ratio >2	[39]
		Any tumor	FGFR2/CEN10 ratio >2 in ≥10% tumor cells	[102]

CEN, centromere; CISH, chromogenic *in situ* hybridization; FISH, fluorescence *in situ* hybridization.

tumors harbor these *FGFR* fusions are being referred to clinical trials with FGFR inhibitors and the preliminary results are eagerly anticipated.

Finally, investigators should keep an open mind during biomarker-driven clinical development of targeted therapies. First, selective FGFR inhibitors might have a more favorable safety profile when compared with multikinase inhibitors and hence could be combined with other targeted agents. Taking into consideration preliminary data showing that the clinical responses of FGFR-amplified tumors to selective FGFR inhibitors have not been impressively high, we envision that rational combination strategies with other targeted agents should be further explored, promising partners being endocrine therapies, anti-EGFR and downstream PI3K pathway or RAF/MEK inhibitors. On the other hand, the clinical activity of selective FGFR inhibitors as single agents in highly addicted *FGFR(1–4)* mutated and *FGFR (2–3)* rearranged tumors is still unknown. Nevertheless, mechanism-based toxicities of FGFR inhibitors may be particularly difficult to control and their long-term consequences are unknown. Table 4 summarizes a specific toxicity management protocol for hyperphosphatemia based on our experience during early clinical development of these agents. Second, multikinase nonselective FGFR inhibitors may show significant antitumor activity in both *FGFR* wild-type and mutant tumors as well as superior efficacy in *FGFR*-aberrant tumors by co-targeting parallel pathways, therefore allowing greater flexibility in patient selection. Correctly designing clinical trials to address these different hypotheses is crucial [112]. Third, antiangiogenic activity of potent VEGFR/FGFR TKIs makes this class of agents suitable in the setting of resistance to prior anti-VEGF therapies, directing clinical investigation of these compounds toward tumor types without FGFR genomic aberrations.

Table 4. Guidelines for management of hyperphoshatemia related to FGFR inhibitors

Serum phosphate ≥5.5–7 mg/dl
Continue FGFR inhibitor
Dietary phosphate intake restriction
Phosphate binder
Sevelamer 1 tablet (800 mg) per meal; i.e. every 8 h
Increase the dose of sevelamer up to 1200 mg every 8 h if needed (phosphate levels still increasing after 1 week)
Serum phosphate >7–9 mg/dl
Continue FGFR inhibitor
If serum phosphate levels continue >7–9 mg/dl despite phosphorus lowering therapy for 2 weeks, the dose of the FGFR inhibitor should be reduced. If serum phosphate levels >7–9 mg/dl despite dose reduction and optimal phosphate lowering therapy for 2 weeks, temporarily discontinue FGFR inhibitor. Restart at reduced dose level when serum phosphate <7 mg/dl.
Dietary phosphate intake restriction
Phosphate binder
Sevelamer 2 tablets (1600 mg) per meal; i.e. every 8 h
Phosphaturic agents
Acetazolamide 1 tablet (250 mg) 2–3 × per day
Serum phosphate >9 mg/dl
Discontinue FGFR inhibitor
Restart at reduced dose level when serum phosphate <7 mg/dl.
Dietary phosphate intake restriction
Phosphate binder
Sevelamer 2 tablets (1600 mg) per meal; i.e. every 8 h
Phosphaturic agents
Acetazolamide 1 tablet (250 mg) 2–3 × per day
Repeated episodes of serum phosphate >9 mg/dl or simultaneous alteration in renal function
Discontinue FGFR inhibitor permanently

conclusion

Targeting FGFRs is a promising therapeutic strategy in a variety of cancers. Early clinical data confirm preclinical studies suggesting that in some tumor types, FGFRs may act as oncogenes to which cancer cells are addicted. Nevertheless, in the clinical scenario, questions such as what class of agents is the most promising (nonselective versus selective FGFR inhibitors) and whether combination therapies are needed in order to obtain meaningful clinical benefit are still unsolved. Successful development will ultimately depend on the selection of tumors in which FGF signaling is driving proliferation and survival. Widespread use of *in situ* hybridization techniques and massively parallel sequencing tests will facilitate the identification of additional diseases in which the therapeutic use of FGFR inhibitors is worth testing. In this context, histology-independent trials ('basket' design) enrolling patients with different FGFR genomic aberrations could be very informative with regard to future directions for clinical investigation of these compounds. *Notably*, not all activating mutations in *FGFR(1–4)* can be effectively targeted by current selective FGFR inhibitors. Therefore, functional experiments designed to determine which aberrations play causative roles in tumorigenesis are required in order to fully understand the clinical implications of genomic data. Eventually, continued translational research in the field will further strengthen the role of FGF signaling in cancer biology and allow personalized use of FGFR inhibitors at the clinic.

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