Advances and challenges in targeting FGFR signalling in cancer

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Abstract

Fibroblast growth factors (FGFs) and their receptors (FGFRs) regulate numerous cellular processes. Deregulation of FGFR signalling is frequently observed in many cancers, making activated FGF receptors a highly promising potential therapeutic target supported by multiple pre-clinical studies. However, early phase clinical trials have produced mixed results with FGFR-targeted cancer therapies, revealing substantial complexity to targeting aberrant FGFR signalling. In this Review, we discuss the increasing understanding of the differences between diverse mechanisms of oncogenic activation of FGFR, and the factors that determine response and resistance to FGFR targeting.

Introduction

Fibroblast growth factor receptors (FGFRs) form a family of four highly conserved trans-membrane receptor tyrosine kinases (FGFR1-4), and one receptor that has the ability to bind fibroblast growth factor (FGF) ligands but lacks an intracellular kinase domain (FGFR5, also known as FGFRL1)[1]. Receptor activation by FGFs initiates a cascade of intracellular events that activate major survival and proliferative signalling pathways[2]. FGFs mediate crucial physiological mechanisms, such as tissue and metabolism homeostasis, endocrine functions and wound repair[2].

Deregulation of the FGF signalling axis has been implicated in oncogenesis, tumour progression and resistance to anti-cancer therapy across many tumour types[3]. Although multiple studies have proposed aberrant FGFR signalling pathway as a potential therapeutic target in various tumour types, the efficacy of anti-FGFR therapy in the clinic has been variable[4]. Responses to therapy have been reported in early phase clinical trials for patients who harbour *FGFR2* amplification in gastric cancer[5], and *FGFR2* and *FGFR3* translocations in cholangiocarcinoma and urothelial

cancers[6] respectively, although results from later phase studies are awaited. Disappointingly, modest levels of clinical activity have been reported for patients with other aberrations such as *FGFR1* amplification[7] or *FGFR2* mutation in advanced-stage endometrial cancer[8].

Nevertheless, as more data emerge as a result of clinical trials and functional studies, we begin to discern which FGFR aberrations are oncogenic drivers and would benefit from monotherapy. Likewise, certain passenger FGFR anomalies are being identified as potential targets for combination therapy in select patient populations, particularly with respect to FGFR-mediated drug resistance. In this Review we address the diverse mechanisms of oncogenic FGFR signalling, focussing on success and limitations of the use of FGFR inhibitors in the clinic, and discuss the recent scientific findings that provide insight into the variable therapeutic effects of anti-FGFR therapy.

[H1] Oncogenic FGFR signalling

Enhanced FGFR signalling in oncogenesis is mediated by genetic alterations (receptor amplification, mutations and chromosomal translocations); autocrine and paracrine signalling, angiogenesis and epithelial-mesenchymal transition (EMT) (Figure 1).

[H3] FGFR amplification

Amplification of *FGFR1* (8q12 locus) is found in approximately 17% of squamous non-small cell lung carcinoma (NSCLC)[9,10] and ~6% of small cell lung carcinoma[11], and is an independent adverse prognostic marker in early stage NSCLC[12]. *FGFR1* amplification is also prevalent in breast cancer and was reported in nearly 15% of hormone-receptor positive and in around 5% of the more aggressive, triple-negative

breast cancers[13-15]. Response to FGFR inhibition has been observed *in vitro* in *FGFR1*-amplified lung cancer models, of both squamous and non-squamous types, although response to FGFR inhibition in xenografts has been variable[10,16]. *In vitro* inhibition of FGFR1 through small interfering RNA (siRNA) or a selective FGFR inhibitor PD173074 modestly reduced the growth of breast cancer cell lines that overexpressed *FGFR1* or in which *FGFR1* was amplified[17,18]. However, FGFR inhibition can reverse resistance to endocrine therapy promoted by FGFR signalling[31]. Large-scale kinase profiling of 117 cell lines of several cancer types revealed increased sensitivity of *FGFR1*- and *FGFR2*-amplified osteosarcoma cell lines to several FGFR inhibitors[19], which was confirmed in a study of 500 cell lines, in response to FGFR inhibitor NVP-BGJ398[20].

Amplification of *FGFR2* is less frequent than amplification of *FGFR1* across cancer types, and has only been described in 5-10% of gastric cancer, particularly of the aggressive diffuse subtype [21], and in 2% of breast cancer overall, with approximately 4% of triple negative breast cancer harbouring *FGFR2* amplification[22]. Amplified *FGFR2* in some cancers, such as diffuse gastric cancer, is accompanied by deletion of the C-terminal exon, which results in preferential expression of a truncated form of the receptor potentially promoting oncogenesis through impaired internalisation and subsequent degradation of the active receptor[23]. Gastric[24], rectal[25] and breast cancer[19] cell lines with high levels of amplification of *FGFR2* are highly sensitive to selective FGFR inhibitor in vitro and in vivo, which suggests that *FGFR2* amplification in these cancers could signify addiction to the FGFR pathway for growth.

Differences in apparent addiction to *FGFR1* and *FGFR2* amplification are in part explained by the amplicon: the *FGFR2* amplicon is frequently narrow and centred on *FGFR2* with few other genes co-amplified, whereas the *FGFR1* amplicon is usually broad, with co-amplification of several genes potentially contributing to

carcinogenesis. The amplification is frequently broader in oestrogen receptor (ER)-positive breast cancer than in NSCLC, with strong evidence pointing at *ZNF703* oncogene as a further driver in the *FGFR1* amplicon[26,27], which may also predict resistance to tamoxifen[28]. Amplification of *FGFR3* and *FGFR4* is not frequently reported, and oncogenic activation of these receptors is often linked to a mutation, or ligand amplification. For example, FGFR3 protein overexpression is recurrent in bladder cancer but it is not linked to *FGFR3* gene amplification[29,30].

[H3] Activating mutations

In contrast to activating mutations in EGFR, mutations in FGFRs are frequently observed outside the kinase domain (Figure 2). Somatic activating mutations of *FGFR1* are rarely observed in cancer, and are more common in *FGFR2* and *FGFR3.*

FGFR2 mutations are found in 10-12% of endometrial carcinomas[31,32], nearly 4% of NSCLC and gastric cancer[33], as well as in around 2% of urothelial cancer[34]. Mutations in the extracellular IgII and IgIII loops (Figure 2), as well as in their linker domain, may provide gain of function either through increasing receptor-ligand binding affinity and interaction[35,36] (for example the S252W mutation in FGFR2, with an identical mechanism described for the P252 residue in FGFR1 and P250 in FGFR3[35]) or through generation of aberrant disulphide bridges that result in constitutive receptor dimerization (S373C and Y376C in the FGFR2-IIIc isoform and analogous mutations in FGFR3-IIIc, G370C and Y373C[37]). Similarly, *FGFR2* insertion mutation A266_S267ins and deletion 290_291WI>C, where amino acid residues WI are replaced by a Cysteine (C), have recently been described to have oncogenic potential via increased dimer formation in a ligand-independent manner[38]. Mutations in *FGFR3* are very frequent in non-muscle invasive urothelial cell carcinomas (75%), also occurring in around 15% of high-grade invasive urothelial

cancer[32,39] and around 5% of cervical cancer[39,40]. The most common mutations in FGFR3 also occur in the extracellular (R248, S249) and transmembrane (G370, Y373) domains of the receptor, resulting in increased receptor dimerization and ligand-independent signalling, analogous to FGFR2 mutations in those regions[41]. Although it is likely that enhanced dimerization directly leads to upregulation of FGFR kinase activity, this has not been established yet and additional factors might be required.

Mutations in the kinase domain of FGFR1 and FGFR2 (most frequently N546K and N549H/K, respectively) constitutively activate the receptors and transform cell lines[42,43], although these mutations are rare, with the FGFR2 N549 mutations found in around 1.4% endometrial and <1% invasive breast cancers[34]. FGFR4 kinase mutations K535 and E550 have been recorded in rhabdomyosarcoma[44] and knockdown of FGFR4 with inducible short hairpin RNA (shRNA) in a human rhabdomyosarcoma cell line reduced tumour growth *in vivo*[44].

In addition to the somatic activating mutations in the FGFRs, germline single nucleotide polymorphisms (SNPs) have been reported to associate with cancer incidence. A non-coding SNP in the second intron of *FGFR2* (rs2981582), which contains putative transcription factor binding sites, has been linked to predisposition to breast cancer in postmenopausal women[45-47]. A SNP in *FGFR4* (rs351855), which results in G388R substitution, is linked to poor survival in several cancer types, such as breast, colorectal and lung, among others[48], and has been shown to increase breast cancer cell motility *in vitro*[49]. This genetic association of the rs351855 SNP with cancer aggressiveness can at least in part be explained by increased association of FGFR4 harbouring the SNP with signal transducer and activator of transcription 3 (STAT3)[50]. The G388R substitution results in a conformational change of the receptor, thereby exposing a membrane-proximal STAT3 binding site, with

expression of the FGFR4_G388R variant significantly enhanced STAT3 signalling in knock-in mice and transgenic mouse models for breast and lung cancers[50].

[H3] Oncogenic fusions

More recently, activating gene fusions in the FGFRs have been discovered in a number of cancers, typically at low incidence[51,52] (Table 1). The majority of FGFR fusion partners contain dimerization domains, which induce ligand-independent receptor dimerization and oncogenic effects. *FGFR3* fusions are relatively common in glioblastoma and bladder cancer, with rare reports in lung cancer[52]. Many *FGFR3* gene fusions are with transforming acidic coiled-coil containing protein 3 (*TACC3*), in which the coiled coil domain is involved in protein oligomerisation and protein-protein interactions[53,54]. In the FGFR3-TACC3 fusion protein, the final exon at the C-terminus of FGFR3 is replaced with TACC3, which results in oncogenic constitutive kinase activity, localisation of the fused protein to spindle poles and subsequent chromosomal segregation defects and aneuploidy[51]. The fused protein can activate MAPK/ERK and JAK/STAT signalling pathways, but not PKC, due to the loss of phospholipase C (PLCγ) binding site[51,55].

TACC3 is frequently *FGFR3* 3' fusion partner whereas *FGFR2* has several reported fusion partners. *FGFR2* fusions are found in roughly 15% of intrahepatic cholangiocarcinoma[56,57], and rarely in lung, thyroid and prostate cancers[52]. Many fusion proteins contain protein-binding domains (citron Rho-interacting kinase (CIT), coiled-coil domain-containing protein 6 (CCDC6), cell cycle and apoptosis regulator protein 2 (CCAR2, also known as KIAA1967), oral-facial-digital syndrome 1 protein (OFD1), BicC family RNA-binding protein 1 (BICC1)) fused to the cytoplasmic tail of FGFR2, deleting the C-terminal exon of FGFR2[52], similar to the deletion of this exon in some cancers with amplified *FGFR2*. The fusion partners likely mediate increased

fusion-receptor dimerization and ligand-independent signalling[52]. Interestingly, N-terminal fusions of other proteins with FGFRs have also been reported. A fusion of the prohibitin -containing protein ER lipid raft associated 2 (*ERLIN2*) with *FGFR1* has been described in breast cancer, and *SLC45A3-FGFR2* gene fusion was identified in a patient with prostate cancer[52]. Although the most probable consequences of the described N-terminal fusions are increased receptor dimerization and increased kinase activation, the *SLC45A3-FGFR2* gene fusion represents a unique pathogenic mechanism, in which the entire open reading frame of *FGFR2* falls under the promoter of an androgen-regulated *SLC45A3,* resulting in overexpression of FGFR2[52].

Overexpression of FGFR2-BICC1, FGFR3-brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1 (BAIAP2L1), and FGFR3-TACC3 in 293T cells enhanced cancer cell proliferation *in vitro*, as well as increased susceptibility to FGFR inhibitors *in vitro* and *in vivo*[52]. Furthermore, stable expression of FGFR3-BAIAP2L1, FGFR3-TACC3, and FGFR2-CCDC6 fusion proteins in a benign telomerase reverse transcriptase (hTERT) – human mammary epithelial (HME) mammary gland cell line promoted cell proliferation via increased MAPK/ERK and JAK/STAT pathway activation, highlighting a role for FGFRs in oncogenic transformation[52]. As more FGFR fusions emerge, their individual oncogenic potential will need to be investigated, particularly in the case of out-of-frame fusions.

[H3] FGF ligand signalling, EMT and angiogenesis

Deregulation of FGF expression and secretion in cancer or stromal cells may also contribute to or drive carcinogenesis. Most evidence for abnormal autocrine and paracrine FGF loops comes from xenograft and cell line models, particularly in prostate cancer. Multiple FGFs have been implicated in development and

progression of prostate cancer, including FGF1, FGF2, FGF6, FGF8[58-63] and more recently, endocrine FGF19[62,63] and FGF23[64,65] (Box 1). Amplification of the 11q13 locus, including cyclin D1 (*CCND1*) and *FGF3*, *FGF4* and *FGF19,* is frequent in many cancers. Although amplified FGFs are not expressed in many *11q13*-amplified cancers[66-68], in the 15% of hepatocellular carcinomas (HCC) with amplification of the 11q13 locus, FGF19 is expressed and contributes to cancer pathogenesis[69]. Furthermore, transgenic mice with overexpression of FGF19 at an ectopic site (skeletal muscle) developed HCC by 10 months of age[70], confirming the endocrine-like oncogenic effects of FGF19 on hepatocytes. Pre-clinical studies showed that FGF19 stimulates tumour progression via activation of STAT3[71], and RNAi-mediated knockdown[69] and neutralising antibodies against FGF19[71,72] had a profound anti-proliferative effect in HCC *in vitro* and *in vivo* models.

FGF2, FGF8 and FGF9 are capable of facilitating EMT in cancer by inducing mesenchymal characteristics in of epithelial cells[73-77], similar to their established roles during embryogenesis. High levels of FGF2 are expressed and secreted in triple-negative breast cancer cell lines[78], specifically of mesenchymal phenotype[79]. In patients, increased FGF2 levels in plasma are observed in many cancers, such as leukaemia, lung and breast cancers, particularly in metastatic disease[80,81], likely reflecting increased release of FGF2 bound to heparan sulfate proteoglycans in the extracellular matrix by invading cancer cells, although this FGF2 release is of uncertain pathogenic relevance.

Switching from an FGFR-IIIb isoform (with higher affinity for FGF1, FGF3, FGF7 and FGF10), which is enriched in epithelia, to a ‘mesenchymal’ IIIc isoform (with higher affinity for FGF1, FGF2, and FGF9)[74,82,83] may facilitate EMT with enhanced FGF signalling via increased affinity for oncogenic FGFs secreted by the tumour or the surrounding stroma. The switch from FGFR2-IIIb to FGFR2-IIIc is associated with

increased invasiveness of bladder and prostate[84], pancreatic[85] and colon cancer cell lines [86].

FGF2 has a key role in wound healing [87,88] and angiogenesis by promoting proliferation and migration of endothelial cells [89,90] in murine models, particularly in combination with vascular endothelial growth factor (VEGF)[91,92]..Increased FGF2 levels were reported in patients who were resistant to anti-angiogenic agents[93], indicating a possible role for FGFs in mediating resistance to anti-VEGF therapy (Box 2). Indeed, dual inhibition of FGF and VEGF inhibited tumour growth and angiogenesis in mouse pancreatic neuroendocrine tumours that were resistant to VEGF inhibition[94].

[H3] Signal transducers

Differential expression of key signal transducing proteins may shape the signal transduction pathways activated by FGFR signalling. *FRS2* amplification and protein overexpression — which may promote MAPK/ERK signalling — were reported in undifferentiated high-grade pleomorphic sarcoma and ovarian cancer, and *FRS2* silencing reduced cell proliferation of liposarcoma and ovarian cancer cell lines[95,96]. Growth factor receptor-bound protein 2 (GRB2) and PLCγ compete for a mutual binding site on FGFR2, and reduced GRB2 levels translate into PLCγ-mediated cancer cell migration and invasion[97]. A combination of increased PLCγ and low GRB2 expression levels correlate with poor clinical prognosis in ovarian[98] and lung[99] cancers.

[H1] Targeting FGFR in the clinic

The contribution of aberrant FGFR signalling to tumourigenesis has led to the development of a plethora of therapies targeting the FGFR pathway, many of which

were promising in pre-clinical studies of various tumour types harbouring FGFR aberrations. Although there are no FGFR-targeted therapies approved for the treatment of cancer at present, the results of a large number of early phase therapeutic trials have revealed important information on targeting FGFR in the clinic, with therapies including small molecule tyrosine kinase inhibitors (TKIs) that target the ATP-binding cleft of the kinase domains of several growth factor receptors (multi-targeting TKIs), TKIs that selectively target the kinase domain of FGFRs (selective TKIs), monoclonal antibodies anti-FGFR and FGF ligand traps (Table 2).

[H3] Multi-targeting TKIs

The kinase domains of FGFR, VEGFR and platelet-derived growth factor receptor (PDGFR) families are phylogenetically related, and several non-selective TKIs originally developed to inhibit the VEGFRs also inhibit FGFR. Dovitinib (TKI258) is a non-selective TKI that targets VEGFR1-3, FGFR1-3 and PDGFRb at nanomolar concentrations[7]. Dovitinib demonstrated prominent anti-tumour activity in a phase I study in patients with renal cell carcinoma (RCC) [100], although reduced efficacy was observed in a phase II study in metastatic RCC patients[101]. A subsequent randomised phase III study of 570 patients for third line treatment for RCC demonstrated no difference in efficacy outcomes between dovitinib and sorafenib, another multi-targeting VEGFR inhibitor that does not appreciably inhibit FGFRs [101]. Baseline levels of FGF2 did not predict for relative benefit, and were also not different between sorafenib and dovitinib when measured during treatment. These data questioned whether all efficacy of dovitinib in patients was through inhibition of VEGFR. In a separate phase II trial, treatment with dovitinib induced relatively infrequent partial responses in patients with *FGFR1* or *11q13*-amplified ER+ breast cancer, compared with no response in patients who harboured no amplifications [7],

potentially suggesting an oncogenic role for FGFRs in patients in whom *FGFR1* or *11q13* was amplified.

Lucitanib (E3810) is another multi-TKI that targets FGFR1-2 and VEGFR1-3 among other tyrosine kinase receptors. A phase I/IIa study assessing lucitanib in solid tumours demonstrated clinical benefit in patients harbouring FGFR aberrations, with 6 out of 12 patients achieving RECIST partial response [102]. Additional non-selective TKI with anti-FGFR activity include nintedanib (BIBF1120) and ponatinib (AP24534), which so far demonstrated modest anti-tumour activities in advanced solid tumours[103] and leukaemia[104].

There is general uncertainty over whether these multi-targeting TKIs sufficiently inhibit FGFRs in the clinic. Dosing is limited by hypertension through VEGFR inhibition, and by non-specific toxicity[102], with adverse effects specific to the selective FGFR inhibitors frequently not observed. Stratification of patients on the basis of their FGFR expression/mutation profile identified partial responses in breast cancers with *FGFR1 (8q12)* and/or *11q13* amplifications[7]. However it remains uncertain how much of the activity of these TKIs is through multi-targeted inhibition of VEGFR and other non-FGFR kinases.

[H3] Selective inhibitors

In order to facilitate on-target FGFR inhibition in patients who harbour FGFR abnormalities, and also reduce toxic effects associated with multi-TKIs, selective inhibitors of the FGFRs have been developed (Table 2). The kinase domains of FGFR1-3 show high structural similarity[105], and most selective inhibitors inhibit all three FGFRs to varying degrees. The FGFR4 kinase domain is structurally distinct and is therefore not appreciably inhibited by most inhibitors[106].

A retrospective analysis of the early selective inhibitor trials has revealed substantial variability in response rates between genetic aberrations. *FGFR1*-amplified cancers responded infrequently to selective FGFR inhibition. In a phase I study of AZD4547, an FGFR1-3 catalytic inhibitor, only one patient with *FGFR1*-amplified squamous NSCLC had a confirmed RECIST partial response (32% reduction in target lesions), from a total of 20 patients enrolled in the study[107]. In a phase I study of 132 patients with FGFR1-3 genetic aberrations, NVP-BGJ398 — a further FGFR1-3 selective inhibitor — demonstrated partial responses in four patients with *FGFR1*-amplified NSCLC, and stable disease in 14 patients[108]. Regarding breast cancer, in a phase II multicentre proof-of-concept study evaluating AZD4547, one out of eight patients with *FGFR1*-amplified breast cancer responded[5] to the inhibitor. Similarly, only one patient with *FGFR1*-amplified breast cancer had a tumour regression when treated with NVP-BGJ398 in the phase I study[108].

The response rate in *FGFR3*-aberrant urothelial cancer is also uncertain. Two out of twenty patients with *FGFR3*-mutated bladder cancer achieved stable disease in response to AZD4547 in a phase I study[107], although partial responses were also reported in *FGFR3*-mutated bladder cancer in a phase I trial of NVP-BGJ398[108]. Additional clinical data in patients harbouring FGFR mutations are required to reliably assess the potential of distinct individual FGFR mutations to predict response to targeted agents.

By contrast, there have been high rates of response reported for *FGFR2* amplification. In a phase II trial evaluating AZD4547, three out of nine patients with *FGFR2*-amplified gastric cancer had a response to AZD4547 that lasted for 27–45 weeks[5]. However, a separate phase II study showed no statistically-significant advantage of AZD4547 versus paclitaxel in patients with FGFR2-amplified advanced-stage gastric cancer (41 patients assigned to the AZD4547 arm versus 30 patients in

paclitaxel arm)[109], with evidence that response was limited by intra-tumoural heterogeneity, as discussed later in the Review.

Tumours with FGFR fusions seem to have a high response rate to FGFR inhibition. Tumour shrinkage was observed in one cholangiocarcinoma and one HCC patient with *FGFR2-BICC1* gene fusions in response to NVP-BGJ398 in a phase I study[108]. Consequently, this drug is now being investigated in phase II studies in advanced-stage cholangiocarcinoma[110], advanced-stage gastrointestinal stromal tumours (GIST)[111] and other solid and haematologic malignancies[112]. Patients with urothelial tumours harbouring either FGFR2 truncation or FGFR3-TACC3 fusion also demonstrated clinical responses in a phase I dose-escalation study of JNJ-42756493[6], which is now being assessed in a phase II study in unresectable urothelial cancers with FGFR genomic aberrations[113]. Two more inhibitors, LY2874455[114] and TAS120[112], are currently in phase I trials.

Collectively, early trials of selective TKIs proved highly successful in targeting FGFR fusions and selected patients with *FGFR2* amplification, although only marginal success was seen when targeting other FGFR aberrations.

[H3] Monoclonal antibodies targeting FGF and FGFR

Although several monoclonal antibodies (mAbs) against FGFRs have been developed, limited clinical data are currently available. MGFR1877S is an anti-FGFR3 mAb that was evaluated in a phase I dose-escalation trial in patients with advanced-stage solid tumours[115]. Stable disease was reported to be the best response in patients with urothelial cell carcinoma (5 out of 10 patients), with thrombocytopenia, fatigue and nausea reported as predominant adverse effects[116]. Following promising *in vitro* findings, pre-clinical evaluation of an isoform-specific

mAb against FGFR1-IIIc, named IMC-A1, was shown to induce severe anorexia in animal models[117], and thus was never translated into the clinic. The FGFR2-IIIb blocking mAb FPA144 inhibited growth of *FGFR2*-amplified gastric cancer xenografts by 72% to 100%[118] and recently entered a phase I trial[119]. Data from 13 patients enrolled to date in this trial showed no dose-limiting toxic effects associated with FPA144 administration, with upper respiratory infection, alopecia and fatigue reported as adverse events in more than one patient[120].

FP-1039 is a FGF ligand trap; a soluble fusion protein that contains the extracellular domain of FGFR1-IIIc splice isoform and demonstrated anti-angiogenic and anti-proliferative properties in multiple cancer cell line models via selective sequestration of non-hormonal FGFs[121]. Recently, a first in-human phase I study evaluating FP-1039 in patients with metastatic or locally advanced-stage solid tumours has been completed[122]. In an unselected patient population, the best response was recorded to be stable disease (41.7%) and major adverse effects observed were diarrhoea (43.6%), fatigue (43.6%), and nausea (25.6%)[122]. No apparent relationship was reported between tumour response and FGF pathway aberrations in the 39 patients enrolled.

[H1] Challenges and opportunities

[H3] Challenges of patient selection

Prospective selection of patients with specific *FGFR* aberrations is one of the major challenges in clinical trials. The overall infrequency of individual *FGFR* aberrations complicates identification of the best target population for each selective inhibitor. Further complicating early phase clinical trials have been basket trials, an approach that includes all patients with any FGFR aberration. As it has become clear that different FGFR aberrations have highly variable sensitivity to drugs, studies have

focused on individual aberrations[123]. Tumour biopsy material is often limited, and increasing evidence supports the potential to screen for FGFR aberration in plasma on circulating tumour DNA[24]. Non-invasive and inexpensive approaches like this could aid broader capture of genetic landscape of a tumour, and studies investigating detection of FGFR genetic aberrations in plasma are currently ongoing.

Additional challenges have emerged in selecting patients with *FGFR* amplification, with ambiguity over the criteria for amplification and the importance of clonality in determining response. Early-phase trials of FGFR inhibitors selected patients on the basis of criteria used to define *HER2* amplification (gene to centromere ratio >2), yet evidence suggests only tumours with higher FGFR copy number (gene to centromere ratio >4/5) are likely to respond to FGFR inhibition[24]. FGFR1 protein is frequently not overexpressed in cancers with lower levels of *FGFR1* amplification[124-126], and mRNA levels of *FGFR1* in those cases may be more reliable. Moreover, intra-tumour heterogeneity presents a major selection challenge. *FGFR2* amplification in gastric cancer is frequently sub-clonal[109], with response observed only in cancers with clonal amplification[24]. The importance of clonality in response to mutations and fusions has yet to be explored. In general, oncogenic fusions are early truncal events in cancer, frequently occurring in genomically stable tumours, reinforcing the potential for therapeutic targeting of FGFR fusions.

[H3] Variable addiction to FGFR amplification

Increasing evidence suggests that only a fraction of cancers with *FGFR* aberrations are addicted to FGFR signalling. Differential activation of signal transduction pathways by different FGFRs and by distinct oncogenic events is likely critical in determining whether tumours depend on FGFR signalling to grow, which in turn may predict effectiveness of anti-FGFR therapy. FGF-mediated activation and regulation

of MAPK/ERK signalling is particularly important during organogenesis[127,128]; FGFRs have been shown to signal primarily through ERK1/2 during development, and FGF, FGFR, and ERK1/2 loss-of-function phenotypes are very similar[129]. In cancer, the MAPK/ERK signalling pathway is also most strongly activated by FGFR signalling across diverse aberrations, such as mutation or overexpression of the receptor molecules.. In many cellular contexts, this dominant signalling through the MAPK/ERK pathway is insufficient to drive addiction to FGFR signalling. Although FGFR signalling may contribute to oncogenesis and FGFR inhibition may result in reduced proliferation in cancer cell lines, this has not translated into single agent efficacy in the clinic.

In vitro studies identified a moderate correlation between *FGFR1* locus *8q12* amplification and sensitivity to FGFR inhibitors in NSCLC[10] and breast cancer[130]. Yet mouse models question whether *FGFR1* amplification and overexpression induces oncogene addiction. Exogenous overexpression of FGFR1 in animal models does not result in malignant transformation, and induced dimerization of FGFR1 is required to trigger invasive properties in normal breast epithelial cell lines[131] and transgenic mouse models of progressive mammary gland tumourigenesis[132]. In *FGFR1*-amplified cell lines, FGFR inhibition frequently results in inhibition of MAPK/ERK signalling, but without substantially affecting other signal transduction pathways such as PI3K/AKT signalling. Co-aberrant genes in *FGFR1*-amplified cancers may also result in reduced addiction to the FGFR pathway, including *PIK3CA* activating mutations and amplification of *CCND1*[32]. Although FGFR1 may contribute to aspects of tumour progression, such as endocrine resistance in breast cancer (Box 2), FGFR1 is not a dominant oncogene.

By contrast, *FGFR2*-amplified models seem to be highly addicted to FGFR signalling, and this is confirmed by an apoptotic response to FGFR inhibition, suggesting a

wider control of signal transduction and mTOR activity by FGFR2 signalling[24]. *FGFR2* amplified at very high levels results in supra-physiological FGFR2 expression, signalling and oncogene addiction, with a partial crosstalk between FGFR2 and other receptor tyrosine kinases, including ERBB3 (also known as HER3) and insulin growth factor receptor 1 (IGF1R)[24].

The mechanisms through which FGFR fusion proteins mediate addiction to FGFR signalling remain to be elucidated, and is likely to be cancer type-dependent. Overexpression of FGFR3 fusion proteins transformed 293T cells[52] and Rat1A fibroblasts[51], and enhanced cell proliferation compared with overexpression of wild-type receptors[52]. Bladder cancer cell lines and xenograft models expressing fused FGFR3 proteins were sensitised to the FGFR inhibition[51,55], although not by expression of FGFR3 containing hotspot mutations[52,55].

HCC harbouring *FGF19* amplification may also represent a subset of cancers strongly addicted to the FGFR pathway. *FGF19* amplification, and consequent ligand overexpression and FGFR4 activation, contribute to HCC development[70]. Pre-clinical data show that a blocking anti-FGFR4 monoclonal antibody (LD1) significantly reduced HCC xenograft growth[133]. A small-molecule inhibitor of FGFR4, BLU9931, with high selectivity against the other FGFR family members, suppressed tumour growth in HCC xenograft models with *FGF19* amplification[134]. The pan-FGFR inhibitor JNJ-42756493, which inhibits FGFR4 at doses similar to those used to inhibit the other FGFR receptors, is currently being investigated in patients with advanced-stage HCC[135] (Table 2).

Collectively, these data have led to a growing understanding of the importance of identifying cancers strongly addicted to FGFR signalling. Although the potential of screening for mutations in *FGFR2* and *FGFR3* as biomarkers of response has been

demonstrated in xenograft models of NSCLC and head and neck squamous cell carcinoma (HNSCC), and in one patient[136], many cancers with mutations in *FGFR2* and *FGFR3* display limited FGFR-dependent signalling. Select cancers with high levels of *FGF19* and *FGFR2* amplification and FGFR fusions present putative biomarkers of FGFR addiction and confer sensitivity to targeted agents, unlike low-level *FGFR1* amplification.

Combination therapeutic approaches may overcome the limitations of single agent FGFR inhibition in *FGFR1*-amplified cancers. Inhibitors of the PI3K-mTOR pathway are synergistic with FGFR inhibition, in part as mTOR activity is frequently only weakly inhibited by targeting FGFR, with synergy described both *in vitro* and *in vivo* in HNSCC cell lines[16], endometrial cancer models[137], gastric adenocarcinoma[24] and HCC[138]. Despite these observations, combined individual toxic effects of these inhibitors will likely become a limiting factor in implementing this combination in the clinic.

[H3] Toxicity limits pan-FGFR inhibition in the clinic

On-target toxicity from pan-FGFR1-3 inhibition — including hyperphosphatemia , skin and eye dryness, keratopathy and asymptomatic retinal pigment detachment — limits dosing [139,140]. Higher specificity with antibodies, or a next generation of selective inhibitors against a single FGFR, could minimise the appearance of adverse effects. Blockade of FGFs with the ligand binding trap FP-1039 reduced growth of *FGFR1*-amplified lung cancer cell lines, xenografts[121] and in a phase I study[122], with no effect on serum calcium or phosphate levels. Similarly, a small-molecule ligand trap derived from long pentraxin 3 protein (NSC12) demonstrated potent anti-tumour action in FGFR-dependent xenograft models without systemic toxicity in the treated animals[141].

[H3] Mechanisms of acquired resistance to FGFR inhibitors

As with the majority of targeted treatments, a growing challenge of FGFR inhibition efficacy is development of drug resistance. *In vitro* studies have identified 'gatekeeper' mutations in the FGFRs, and bypass activation of downstream signalling via alternate receptor tyrosine kinase, as frequent mechanisms of acquired or intrinsic resistance to targeted therapies (Figure 3).

'Gatekeeper' mutations in the ATP binding cleft that induce resistance to FGFR inhibition have been identified pre-clinically. A 'gatekeeper' mutation FGFR3_V555M, along with comparable residues FGFR1_V561 and FGFR2_V564, induces resistance to multiple FGFR inhibitors *in vitro*[142-144]. Protein modelling studies suggest that these 'gatekeeper' mutations in the ATP cleft strengthen the hydrophobic spine of the kinase and may create a steric conflict to hinder drug-binding efficiency[143]. The substitution of V561 for a 'bulky' Met amino acid resulted in complete disruption of FGFR1 binding to PD173074[144]. Although these studies demonstrate emergence of mutants resistant to FGFR inhibitors as mechanisms of acquired resistance pre-clinically, they are yet to be confirmed in samples of patient who have experienced clinical progression. In light of emergence of 'gatekeeper' mutations in FGFRs, irreversible covalent FGFR inhibitors that bind such FGFRs have been developed with the aim to overcome resistance to selective FGFR inhibitors[145].

Activation of alternative receptor tyrosine kinases, in particular the ERBB receptor family has been described as an escape mechanism in FGFR-resistant tumours. FGFR3-dependent bladder cancer cell lines developed rapid resistance to the FGFR inhibitor NVP-BGJ398 via switching to signalling through either ERBB2 (also known as HER2) or ERBB3 in a reversible manner, and correlated with an increased production of ERBB ligands, such as neuregulins 1, 2, 4 and betacellulin[146].

Furthermore, dual inhibition of FGFR3 and EGFR activity in FGFR3-mutant bladder cancer cell lines resulted in increased cell death[147]. In *FGFR1*-amplified NSCLC cell lines resistant to FGFR therapy, PDGFRA and HER2 were reported to be co-activated[126]. Use of novel approaches to allow detection of alternatively activated tyrosine kinase receptors or signalling pathways may augment selection of cancers for which FGFR inhibition is effective.

[H1] Conclusion

The great diversity of FGFR activating mechanisms has challenged the clinical translation of FGFR inhibitors, and the importance of considering individual aberrations is now clear from pre- and clinical evidence. Although some FGFR abnormalities are potential targets for monotherapy, such as high level and clonal amplification of *FGFR2* or *FGFR2/3* fusions, others do not seem to be biomarkers of response and need to be carefully evaluated in individual cancers against other potential oncogenic drivers. Taken together, preclinical and early clinical data demonstrate that targeting the FGFR signalling pathway can be a promising therapeutic strategy as monotherapy and in combination with other agents.

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Box 1: Fibroblast growth factor receptor (FGFR) pathway signalling.

The four fibroblast growth factor (FGF) receptors have extracellular, trans-membrane and cytoplasmic domains and can be detected in all adult tissues at varying levels[148]. The extracellular immunoglobulin-like loops bind FGFs, and in FGFR1-3, alternative splicing of the third loop, the IgIII domain, yields two isoforms (IIIb and IIIc) that vary in ligand-binding specificity, thus diversifying signalling patterns (Fig 2)[2].

FGF ligands are a family of 18 glycoproteins (FGFs 1-10 and 16-23) that influence organ development, wound repair and angiogenesis, via directly activating FGFRs. An additional four FGFs (FGF11-14) are not FGFR ligands and have unrelated intracellular functions[2,149]. The 15 canonical FGF ligands predominantly act in an autocrine and paracrine fashion binding to FGFRs in complex with heparan sulphate proteoglycans (HSPGs), which protect FGFs from degradation and stabilise FGF-FGFR interaction[150]. The three endocrine FGFs (FGF19, 21 and 23) act as hormones and lack affinity for HSPG binding, which allows their diffusion from the site of production into the circulation[151]. They play a crucial part in bile acid, glucose and lipid metabolism, as well as control of vitamin D and phosphate levels, thereby maintaining whole-body homeostasis (reviewed in [152]).

FGF ligands induce dimerization and cross-phosphorylation of the kinase domains of cognate receptors, thus recruiting various downstream effector molecules. FGFR

substrate 2 (FRS2) is a key transducer of FGFR signalling[2,153](Fig. 1). Upon dimerization of FGFRs, FRS2 binds to the juxtamembrane region of FGFRs and is consequently phosphorylated at several residues, which act as a docking site for son of sevenless (SOS) and growth factor receptor-bound 2 (GRB2). This complex in turn activates RAS-MAPK-ERK pathway.

FGFR signalling can be diversified via recruitment of GRB2-associated binding protein 1 (GAB1) to FRS2 complex, thus activating PI3K/AKT signalling pathway. Another FGFR binding partner is phospholipase C γ (PLCγ), which binds at the C-terminal tail upon autophosphorylation of FGFR, stimulating the release of intracellular calcium and consequent activation of the protein kinase C (PKC) family of proteins, thus resulting in cell migration, proliferation and cell differentiation[154]. In addition, FGFRs have the ability to activate JAK/STAT signalling pathway in a context-dependent manner[43]. Physiological negative regulation of FGFR signalling can be mediated by Cbl-regulated endocytosis and ubiquitination; MAPK phosphatases, which de-phosphorylate activated MAPK molecules; and SPROUTY and SPROUTY-related EVH1 domain-containing (SPRED) proteins, which bind to growth factor receptor-bound protein 2 (GRB2), thus attenuating downstream signaling[155-157].

Box 2. Resistance to therapy mediated by FGFR signalling.

FGFR signalling may promote resistance to a variety of anti-cancer therapies. *FGFR1* amplification has been implicated in driving endocrine resistance in breast cancer cell lines, in patient samples[17,158], and associated with poor response to neo-adjuvant chemotherapy in osteosarcoma[159]. There are ongoing studies evaluating a pan-FGFR inhibitor AZD4547 in combination with endocrine therapies fulvestrant[160] or letrozole and analstrozole[161] in patients with oestrogen receptor (ER) positive

breast cancer. Combination short hairpin RNA (shRNA) screening identified *FGFR1* — but not other FGF receptors — as mediator of acquired resistance to the MEK inhibitor trametinib in KRAS-mutant lung cancer *in vitro,* and synergistic effects of trametinib with FGFR inhibitors were described in KRAS-mutant pancreatic xenografts and patient derived xenograft (PDX) models of lung cancer[162]. Elevated expression of *FGFR2* and *FGFR3* was described in NSCLC cell lines in response to gefinitib treatment[163], suggesting the potential for combination therapy of FGFR and EGFR inhibitors in NSCLC. In addition, FGFR3 upregulation has been described to be an escape mechanism in vemurafenib-resistant BRAF-mutant melanoma[164] and gastric cancer cell lines resistant to MET-targeted therapy[165].

Cancer evolution may lead to selection of *FGFR* activating mutations in some tumours. Hotspot mutations in FGFR1[166] and *FGFR2*[167] were shown to be acquired during chemotherapy and endocrine therapy in breast cancer, and a novel driver *FGFR3* mutation was described in PDX models of EGFR tyrosine kinase inhibitor-resistant lung cancer [168]. Similarly, FGF ligands may also mediate resistance to targeted therapies. Activation of FGF2-FGFR1 autocrine loop has been described to be a mechanism of acquired resistance in gefitinib-resistant[169,170], as well as afatinib-resistant NSCLC cell lines[171], in which selective FGFR inhibitors re-sensitised cells to EGFR therapies. Patients who progressed on anti-VEGF therapy exhibited elevated FGF2 levels in plasma[93],possibly due to overlapping roles of FGF2, VEGF and PDGF in angiogenesis. Additionally, *in vivo* studies proposed that targeting FGFR could restore sensitivity to anti-VEGF therapies[94]. In a cervical cancer xenograft model, treatment with a PDGFR inhibitor imatinib resulted in upregulation of FGF2 and FGF7 by the stroma, thus promoting tumour proliferation and angiogenesis[172]. More recently, higher levels of FGF2 were described in biopsy samples from patients with imatinib-resistant gastrointestinal stromal tumours (GIST) compared with specimens from patients who had not been treated with imatinib [173]. Therefore, these findings

strongly suggest that FGFR inhibition may revert acquired resistance to anti-cancer therapies.

Figure 1. Mechanisms of oncogenic FGFR signalling.

FGFR signalling contributes to oncogenesis in several ligand-dependent and –independent mechanisms. **1.** FGFR gene amplification often translates into protein overexpression, leading to elevated receptor accumulation and activation of the downstream signalling pathways. **2.** Activating mutations often result in increased dimerization of the receptors in the absence of ligand, or constitutive activation of the kinase domain. **3.** As a result of chromosomal translocations, parts of FGF receptors may become fused with genes encoding other proteins at either C- or N-termini, thereby either increasing dimerization of the receptors (purple fusion), or falling under the promoter regions of a different protein (blue fusion), resulting in receptor hyper-activation in a ligand-independent manner. **4.** FGFRs can be over-stimulated by their ligands in autocrine fashion, in which FGFs are produced by the tumour cells (light blue); or via paracrine signalling, where FGFs are secreted by the stromal compartment (dark blue). In response to a stimulus, or due to gene amplification, the third IgIII loop can also be alternatively spliced from IIIb to IIIc isoform, which alters the receptors' ligand specificity and affinity, resulting in altered autocrine signalling. **5.** FGFs secreted by the tumour cells, or tumour-associated stromal cells, may also contribute to angiogenesis or **6**, epithelial-mesenchymal transition (EMT), which are implicated in tumour progression. **7**. Deregulation of FGFR binding partners FRS2 and PLCγ due to their gene amplification or protein overexpression can lead to hyper-activation of the FGFR downstream signalling pathways.

Figure 2. Structure of FGFR and frequency of the receptors' somatic mutations with their relative locations.

FGF receptors consist of an extracellular domain encompassing three Ig-like domains (IgI-III), followed by a transmembrane domain and two tyrosine kinase sub-domains, TK I and TK II. An acidic box, which is a stretch of acidic amino acids responsible to FGFR interaction with partners other than FGFs, is located between IgI and IgII, and a heparan sulfate proteoglycan-binding domain, which helps stabilise FGF-FGFR interaction, is found on IgII. IgIII can be alternatively spliced to yield IIIb or IIIc isoforms.
The second part of the figure shows the frequency of FGFR somatic mutations reported in patients with cancer and their relative location on the proteins. Residue locations correspond to various regions on the receptors, using FGFR1 molecule as a reference. Mutations in FGFR1 and FGFR4 are not frequently reported, but mutations in FGFR2 and FGFR3 are common and occur predominantly in the ligand-binding and transmembrane domains of the receptors, with fewer mutations reported in the kinase domains. Graphs were created using raw data extracted from COSMIC, GRCh37[174], using the following filters: Tumour source= tumour sample; mutation type= insertions/deletions (both frameshift and in-frame), missense; mutation type= pathogenic, as determined by the Functional Analysis through Hidden Markov Models algorithm, where scores are ≥ 0.7[175].

Figure 3. Mechanisms of resistance to FGFR inhibitors.

Mechanisms of resistance to targeted anti-FGFR therapies are beginning to emerge, although predominantly from *in vitro* functional studies. (**a.**) Prolonged treatment of cell lines with selective FGFR inhibitors can result in emergence of point mutations in FGFR kinase domains, contributing to the conformational changes preventing adequate drug binding in select models. Alternatively, other RTKs, such as IGF1R or ERBB family members, may become upregulated in response to FGFR therapy, thereby serving as a bypass mechanism for activation of cell survival and proliferative pathways. (**b.**) PI3K signalling pathway is frequently implicated in mediating resistance to FGFR inhibitors, by either directly affecting cell proliferation, or via activation of mTOR and consequent alteration in cell metabolism and anti-apoptotic signals. KRAS activating mutations or amplification can in turn stimulate MAPK/ERK signalling pathway when FGFR signalling is unavailable.

Table 1. FGFR fusion partners[51,52,56].

Cancer Type	5' gene	3' gene	Cases Reported	Frame	Fusion Type
Bladder Cancer	***FGFR3***	*TACC3*	3/ 121	In-frame	Short
Breast cancer	***FGFR1***	*ADAM18*	1/ 1019	Out-of-frame	Middle
	RHOT1	***FGFR1***	1/ 1019	CDS-5UTR	InterChr
	WHSC1L1	***FGFR1***	2/ 1019	In-frame	Short
	FGFR2	*CCDC6*	1/ 1019	In-frame	Long
Glioblastoma	***FGFR3***	*TACC3*	6/ 158	In-frame	Short
Head and Neck Squamous Cell Carcinoma	***FGFR3***	*TACC3*	2/ 300	In-frame	Short
	FGFR3	*TPRG1*	1/ 300	Out-of-frame	InterChr
Intrahepatic Cholangiocarcinoma	***FGFR2***	*AHCYL1*	7/ 66	In-frame	InterChr
	FGFR2	*BICC1*	2/ 66	In-frame	InterChr
Low-grade Glioma	***FGFR3***	*ELAVL3*	1/ 266	In-frame	InterChr
	FGFR3	*TACC3*	1/ 266	In-frame	Short
Lung Adenocrcinoma	***FGFR1***	*SLC20A2*	1/ 487	CDS-5UTR	Middle
Lung Squamous Cell Carcinoma	*BAG4*	***FGFR1***	1/ 220	In-frame	Short
	FGFR2	*KIAA1967*	1/ 220	In-frame	InterChr
	KIAA1967	***FGFR2***	1/ 220	5UTR-CDS	InterChr
	FGFR3	*TACC3*	5/ 220	In-frame	Short
Ovarian Cancer	***FGFR2***	*USP10*	1/ 400	In-frame	InterChr
Prostate Adenocarcinoma	*SLC45A3*	***FGFR2***	1/ 84	CDS-5UTR	InterChr
	FGFR3	*AES*	1/ 178	In-frame	InterChr
Thyroid Carcinoma	***FGFR2***	*OFD1*	1/ 494	In-frame	InterChr
	VCL	***FGFR2***	1/ 494	In-frame	Long

Abbreviations: CDS-5UTR, the 5' untranslated region of a protein coding sequence; In-frame, a fusion transcript without a frame shift, resulting in transcription of both genes; Out-of-frame, a fusion transcript that causes a frame shift in one of the genes. InterChr, inter-chromosomal fusion.

Table 2. Summary of FGFR inhibitors currently investigated in clinical trials: IC50 and their progress.

	Company	Target	IC50	Drug	Clinical trial ID	Phase I	Phase II	Phase III
Non-Selective Inhibitors	Novartis Pharmaceuticals	FLT3 c-Kit FGFR1 VEGFR3/FLT4 FGFR3 VEGFR1/FLT1	1 nM 2 nM 8 nM 8 nM 9 nM 10 nM	**Dovitinib**	NCT01719549 NCT01732107 NCT01223027		FGFR2-amplified gastric cancer FGFR3-mutated or -overexpressed urothelial cancer	Metastatic renal cell cancer
	ARIAD Pharmaceuticals Inc.	Abl PDGFRa VEGFR2 FGFR1 c-Src	<1 nM 1.1 nM 1.5 nM 2.2 nM 5.4 nM	**Ponatinib**	NCT02265341 NCT02272998		FGFR2-fusions in biliary cancer FGFR genetically-aberrant advanced cancers	
	Clovis Oncology	VEGFR1 VEGFR3 FGFR1 VEGFR2 FGFR2	7 nM 10 nM 18 nM 25 nM 83 nM	**Lucitanib**	NCT01283945 NCT02202746 NCT02053636 NCT02109016	FGFR1-amplified advanced solid tumours	FGFR genetically-aberrant metastatic breast cancer FGFR1/11q-amplified ER+ metastatic breast cancer Any FGF-related aberration in advanced/metastatic lung cancer	
Selective Inhibitors	AstraZeneca	FGFR1 FGFR3 FGFR2 VEGFR2	<1 nM 1.8 nM 2.5 nM 24 nM	**AZD4547**	NCT02664935 NCT02117167 NCT02154490		FGFR genetically-aberrant non-small cell lung cancer FGFR genetically-aberrant metastatic non-small cell lung cancer FGFR genetically-aberrant squamous cell lung cancer	
	Novartis Pharmaceuticals	FGFR1 FGFR3 FGFR2 FGFR3 (K650E) FGFR4	<1 nM 1 nM 1.4 nM 4.9 nM 60 nM	**BGJ398**	NCT01004224 NCT01697605 NCT01928459 NCT01975701 NCT02160041 NCT02150967	FGFR1-3 genetically aberrant solid tumours; FGFR1-amplified squamous lung cancer; FGFR3-mutated or fused bladder cancer FGFR genetically-aberrant advanced solid tumours in Asian population FGFR genetically-aberrant advanced solid tumours with PIK3CA mutations	Glioma subtypes with FGFR1-TACC1, FGFR3-TACC3 fusion and/or activating mutation in FGFR1-3. FGFR genetically-aberrant solid and/or hematologic cancers FGFR genetically-aberrant advanced or metastatic cholangiocarcinoma	
	Janssen Research & Development	FGFR1 FGFR2 FGFR4 FGFR3 FGFR3 (G697C)	<1 nM <1 nM <1 nM 1.05 nM 1.9 nM	**JNJ-42756493**	NCT02421185 NCT02365597 NCT02699606	FGF19-amplified advanced hepatocellular carcinoma	FGFR genetically-aberrant advanced/metastatic urothelial cancer Asian participants with non-small-cell lung cancer, gastric cancer, urothelial cancer, esophageal cancer and cholangiocarcinoma.	
	Eli Lilly and Company	FGFR1 FGFR2 FGFR3 FGFR4 VEGFR2	2.8 nM 2.6 nM 6.4 nM 6 nM 7nM	**LY2874455**	NCT01212107	Advanced cancer		
	Taiho Oncology, Inc.	FGFR1 FGFR2 FGFR3 FGFR4	3.9 nM 1.3 nM 1.6 nM 8.3 nM	**TAS120**	NCT02052778	FGFR genetically-aberrant advanced solid tumors or multiple myeloma		
	Debiopharm International	FGFR2 FGFR1 FGFR3	7.6 nM 9.3 nM 22 nM	**Debio-1347**	NCT01948297	FGFR1-3 genetically aberrant solid tumours		
Ligand Trap & Antibodies	GSK	FGF2	0.023 µg/ml	**FP-1039**	NCT01868022	FGFR genetically-aberrant solid malignancies in combination with paclitaxel and carboplatin/docetaxel		
	Five Prime Therapeutics	FGFR2_IIb		**FPA114**	NCT02318329	Advanced solid tumours		
	Genentech, Inc.	FGFR3		**MFGR1877S**	NCT01363024	Advanced solid tumours		

* Values listed were obtained in cell-free assays[121,139,140,176-182], except #median reported IC50, obtained using cell lines