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Abstract: Fibroblast growth factor receptors (FGFRs) play diverse roles in control of cell proliferation, cell differentiation, angiogenesis, and development. Activating mutations of FGFRs in the germline have long been known to cause a variety of skeletal developmental disorders, but it is only recently that a similar spectrum of somatic FGFR mutations has been associated with human cancers. Many of these somatic mutations are gain-of-function and oncogenic and create dependencies in tumor cell lines harboring such mutations. A combination of knock-down studies and pharmaceutical inhibition in preclinical models has further substantiated genomically-altered FGFR as a therapeutic target in cancer, and the oncology community is responding with clinical trials evaluating multi-kinase inhibitors with anti-FGFR activity and a new generation of specific pan-FGFR inhibitors.
Targeting Mutant Fibroblast Growth Factor Receptors in Cancer

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Fibroblast growth factor receptors (FGFRs) play diverse roles in control of cell proliferation, cell differentiation, angiogenesis, and development. Activating mutations of FGFRs in the germline have long been known to cause a variety of skeletal developmental disorders, but it is only recently that a similar spectrum of somatic FGFR mutations has been associated with human cancers. Many of these somatic mutations are gain-of-function and oncogenic and create dependencies in tumor cell lines harboring such mutations. A combination of knock-down studies and pharmaceutical inhibition in preclinical models has further substantiated genomically-altered FGFR as a therapeutic target in cancer, and the oncology community is responding with clinical trials evaluating multi-kinase inhibitors with anti-FGFR activity and a new generation of specific pan-FGFR inhibitors.

Physiological FGFR activation

Members of the fibroblast growth factor receptor (FGFR) receptor tyrosine kinase (RTK) family, FGFR1-4, are differentially activated by binding to a subset of 18 fibroblast growth factors (FGFs) in conjunction with heparan sulfate proteoglycan, which stabilizes and sequesters FGFs [1]. Ligand specificity of FGFR1-3 is, in part, controlled by an alternative splicing event that affects the third immunoglobulin (Ig) loop (IgIII) in the ligand-binding domain, resulting in a "IIIb" isoform preferentially expressed in epithelial cells and a "IIIc" isoform preferentially expressed in mesenchymal cells. FGF3, FGF7, FGF10, and FGF22 exclusively bind the IIIb isoform; FGF1 binds both the IIIb and IIIc isoforms; and the remaining 13 FGF family ligands for which FGFR-stimulatory activity has been demonstrated preferentially bind the IIIc isoform [2]. Importantly, ligand expression is controlled in a cell-specific manner such that physiological
receptor stimulation tends to occur in a paracrine rather than autocrine manner; indeed, a switch to autocrine regulation can promote tumorigenesis [3].

Binding of cognate ligands induces FGFR dimerization, trans-autophosphorylation, and kinase activation [4]. Downstream signaling events are summarized in Box 1. Most of the original work dissecting the biochemistry of FGFR signal transduction involved primarily FGFR1; surprisingly little is known of the similarities and differences between the four family members. However, activating somatic mutations in all four FGFR genes have now been found in diverse human cancers, indicating the potential of FGFR inhibition as a powerful new approach to targeted cancer therapy.

**FGFR3 mutations in cancer**

*FGFR3* was the first FGFR family member reported to be somatically mutated in cancer, specifically in multiple myeloma [5] (Table 1). Recurring translocations between the immunoglobulin heavy chain (IGH) locus and *FGFR3* were identified in 25% of patient samples and cell lines tested, frequently resulting in elevated expression levels of *FGFR3* [5, 6]. Translocation only roughly correlates with increased FGFR3 protein expression [5, 7], and there is currently insufficient functional data to conclude that translocation-driven increases in wild-type protein expression are sufficient for tumorigenesis, perhaps implicating the consistently-overexpressed reciprocal translocation partner, multiple myeloma SET domain protein (MMSET) in these cases instead [8]. However, sequence analysis showed that about 10% of *FGFR3* translocations harbor recurring somatic mutations of *FGFR3*, including alleles encoding Y373C, K650E, and K650M, correlating more accurately with increased FGFR3 expression[5]. Interestingly, these alleles and the subsequently-identified allele encoding R248C [9] have been
reported in three sporadic skeletal dysplasias that result from germline mutation of \textit{FGFR3}: R248C and Y373C in thanatophoric dysplasia type I, K650E in thanatophoric dysplasia type II, and K650M in severe achondroplasia with developmental delay and acanthosis (SADDAN) syndrome [10-12], thus establishing a paradigm that would repeatedly apply to FGFR family mutation in cancer.

Studied in the context of thanatophoric dysplasia and SADDAN syndrome, ectopically-expressed Y373C, K650E, and K650M FGFR3 displayed constitutively elevated kinase activity[11, 13, 14]. The K650E substitution is located in the activation loop of the FGFR3 kinase domain and presumably directly affects the active site conformation of the mutant protein [15]. In contrast, the Y373C and R248C substitutions located in the extracellular region of FGFR3 introduce an unpaired cysteine which results in the formation of intermolecular disulfide bonds, leading to constitutive receptor dimerization and therefore constitutive kinase activation [13]. The activated alleles were also tested in cell-based transformation assays. Whereas expression of the wild-type \textit{FGFR3} had no effect on colony formation in soft agar, both the Y373C and K650E-encoding alleles supported anchorage-independent growth of NIH-3T3 cells, indicating that these alleles are in fact oncogenic [16]. Ectopic expression of \textit{FGFR3} K650E in Ba/F3 cells additionally conferred interleukin-3 (IL-3)-independent proliferation, a phenotype often associated with expression of oncogenic RTKs.

Recurring activating mutations of \textit{FGFR3} were subsequently detected in additional tumor types (Table 1), including 35\% of urothelial cell (bladder) carcinomas and 25\% of cervical carcinomas, as well as 39\% of benign seborrheic keratoses [17, 18]. In addition to the mutations described above, the S249C and G370C substitutions that introduce an unpaired cysteine, resulting in constitutive dimer formation, were found in bladder carcinomas as well as
thanatophoric dysplasia type I patients [10, 13, 17, 19, 20]. In contrast to transformed phenotypes observed in NIH-3T3 cell assays, neither FGFR3 S249C nor Y373C supported anchorage-independent growth of immortalized normal human urothelial cells, which are assumed to be more physiologically relevant to bladder cancer than NIH-3T3 cells, with the caveat that no positive control data was presented for this assay [21]. These two mutants did, however, confer increased cell saturation density [21].

FGFR3 mutations in urothelial cell carcinomas correlate with lower tumor grade. However, within the stratum of low-grade non-muscle invasive tumors, FGFR3 mutation correlates with higher risk of recurrence compared to tumors without FGFR3 mutation [22, 23]. Therefore, although FGFR3 mutations are found primarily in low-grade tumors, treatment targeted to FGFR3 mutations might still benefit a subset of bladder cancer patients.

Seborrheic keratosis is a common benign skin tumor originating from keratinocytes of the epidermis, the prevalence of which increases with age [18]. A spectrum of somatic alleles similar to that found in multiple myeloma and urothelial cell carcinoma are also found in seborrheic keratosis (Table 1), with the addition of S371C, another substitution observed in patients with thanatophoric dysplasia type I that results in receptor dimerization [12, 13, 18].

More recently, the FGFR3 K650E substitution was identified in spermatocytic seminomas, rare testicular malignancies that occur in aging patients. An observed clonal expansion of activated K650 mutants in sperm (but not blood) that correlated with increased donor age indicates that positive selection of sperm harboring activated FGFR3 K650E mutants underlies both the sporadic incidence of spermatocytic seminoma and the germline transmission of thanatophoric dysplasia type II [24].
Recurring oncogenic mutations of *FGFR3* have thus been identified in several tumor types. It is reasonable to expect that additional somatic alleles of *FGFR3* previously associated with similar germline skeletal dysplasia syndromes will be oncogenic as well. However, for novel *FGFR3* mutations, detailed functional studies will be required to distinguish “driver” mutations that contribute to tumorigenesis from “passenger” mutations that provide no fitness benefit for the tumor.

**FGFR2 mutations in cancer**

The next most significant discovery of FGFR family mutation in cancer was the identification of *FGFR2* mutations in endometrial carcinoma [25, 26] (Table 1). Sequence analysis of primary tumors and cell lines uncovered somatic extracellular and kinase domain mutations in about 12% of samples tested, with particularly high recurrence of mutations encoding S252W, P253R, and N550K, as well as several different substitutions of K660 [25, 26]. Analogous to the situation with *FGFR3*, the S252W and P253R–encoding alleles of *FGFR2* are also found as autosomal dominant mutations associated with the congenital developmental disorder Apert Syndrome [27]. Mutations that alter N550 and K660 are associated with similar craniosynostosis disorders [27], and are paralogous to FGFR3 N540K and K650E (Figure 2). Substitution of these two residues activates kinase activity by disengaging a “molecular brake” that maintains FGFR2 in an inactive conformation through a network of hydrogen bonds that inhibits movement of the N lobe toward the C lobe and is required for alignment of catalytic residues [15].

A subset of *FGFR2* mutations found in endometrial carcinoma have been tested for oncogenic potential in cell-based transformation assays, and most have scored positive (Table 1),
further suggesting a causative role in cancer for many of these alleles [25, 28]. The S252W and P253R substitutions, in particular, activate FGFR2 in a manner that is unique in oncology to the FGFR family. These altered residues lie in the ligand binding region and confer a gain in ligand binding promiscuity, such that ligands expressed by the epithelial cells that normally only bind the mesenchymally expressed “c” isoform of FGFR2 can now bind the S252W “b” isoform and establish an autocrine loop [29].

FGFR2 somatic mutations have also been identified in other cancers, but it is difficult to impute causation in the absence of functional data. Nevertheless, based on our experience to date, it is reasonable to expect that somatic alleles found in cancer would contribute to tumor formation if they are also observed in the germline of craniosynostosis patients. For example, the W290C-encoding allele found in lung squamous cell carcinoma [30] and the S267P-encoding allele found in gastric cancer [31] are also found in craniosynostosis syndromes [32-34], and thus might play a role in tumor development. In fact, a W290G mutant studied in the context of Crouzon syndrome indirectly caused intermolecular disulfide bond formation, constitutive dimerization, elevated kinase activity, and oncogenic transformation of NIH-3T3 cells, indicating a possible mechanism of action for other oncogenic substitutions at this residue [35].

There are to our knowledge only two reports of patients harboring germline mutations of an FGFR gene who develop cancer. Specifically, two Apert Syndrome patients systemically expressing FGFR2 P253R, 4 and 13 years of age, were reported to develop bladder cancer and ovarian dysgerminoma, respectively [36, 37]. In the absence of a systematic analysis, made difficult by the low number of cases, it remains unclear if craniosynostosis patients harboring germline FGFR mutations have an increased risk of cancer. Another set of germline events, single nucleotide polymorphisms (SNPs) with high minor allele frequencies (38-39%) present in
the second intron of FGFR2 were found to be associated with increased risk of breast cancer in two genome-wide association studies [38, 39]. SNPs in this region were also associated with increased FGFR2 expression [40], suggesting that, under certain circumstances, overexpression of wild-type FGFR2 can also affect cancer incidence.

Although many FGFR mutations found in cancer have been demonstrated or are reasonably hypothesized to confer gain-of-function, FGFR2 mutations identified in 10% of melanoma cell lines and patient samples, including nonsense and frameshift mutations, appear to be loss-of-function mutations [41]. The biological consequences of these mutations for melanoma development remain unclear, but raise the possibility of context-specific functions of FGFRs in tumorigenesis.

**FGFR1 mutations in cancer**

Reminiscent of FGFR3 in multiple myeloma, FGFR1 was shown to be recurrently translocated in the 8p11 myeloproliferative syndrome (EMS), also known as stem cell leukemia/lymphoma (SCLL) [42]. However, whereas translocation of FGFR3 to the IGH locus results primarily in overexpression and occasional subsequent missense mutation of FGFR3, FGFR1 translocations typically result in the production of a fusion transcript, replacing 5’ coding sequences of FGFR1 with coding sequences from one of several possible fusion partners. Many observed translocation products, including zinc finger protein 198 (ZNF198)-FGFR1, 110 kDa centrosomal protein (CEP110)-FGFR1, and breakpoint cluster region protein (BCR)-FGFR1, constitutively dimerize when ectopically expressed or synthesized in vitro, with dimerization mediated by the fusion partner at least in some cases [43-45]. Although these fusion proteins exhibit constitutive kinase activity and transform Ba/F3 cells to IL-3 independence, poorly-
controlled or conflicting data in the literature make it difficult to determine whether these data truly indicate a gain of function over the wild-type FGFR1 protein; it therefore remains unclear what fitness benefit tumors derive from these hybrid proteins.

In contrast to FGFR3, no point mutations in the translocated FGFR1 sequences found in EMS/SCLL have been reported. In fact, FGFR1 has not been shown to be a frequent target of somatic missense mutation in cancer sequencing studies, and the mutations that have been reported in primary tumor samples generally have not been recurrent. However, two FGFR1 mutations found in glioblastoma patients, encoding N546K and K656E [46, 47], are paralogous to FGFR3 germline alleles found in skeletal dysplasias and support morphological transformation of Rat-1 cells and increased focus formation in NIH-3T3 cells, respectively, suggesting that they may be driving tumorigenesis [12, 48-50].

Although few functionally validated FGFR1 mutations have been reported, it is possible that FGFR1 contributes to tumorigenesis primarily through gene amplification and overexpression. In fact, FGFR1 is a statistically significant target of focal amplification across cancers and in lung cancer in particular [51, 52]. Others have suggested that FGFR1 is a target of amplification in breast cancer [53]. Evidence for FGFR1 as a therapeutic target in tumor cells that harbor translocated, mutated, or amplified FGFR1 will be considered below.

**FGFR4 mutations in cancer**

FGFR4 is perhaps the least well-studied of the FGFR family members, and it is only recently that its contributions to cancer have begun to be uncovered. Recurring somatic kinase domain mutations of FGFR4 have recently been described in 8% of rhabdomyosarcoma patients [54]. An FGFR4 N535K-encoding allele, paralogous to the FGFR2 N550K-encoding allele
found in endometrial carcinoma, supported oncogenic transformation of NIH-3T3 cells, as did a V550E-encoding allele. These FGFR4 mutants were also associated with metastatic phenotypes, as ectopic expression of these mutants in the rhabdomyosarcoma cell line RMS772 enhanced the ability of the cells to colonize the lung after intravenous injection [54].

Among other isolated reports of FGFR4 mutation in cancer, likely gain of function can be logically predicted only for the Y367C mutant found in a breast cancer cell line [55]. This residue is paralogous with other mutants found in the germline of patients with skeletal dysplasia or craniosynostosis syndromes and somatically in patients with diverse cancers, including FGFR2 Y375C and FGFR3 Y373C. Ectopic expression of the FGFR4 Y367C-encoding allele in HEK293 cells resulted in elevated levels of endogenous extracellular signal-regulated kinase (ERK) phosphorylation, but no transformed phenotypes were observed in NIH-3T3 cells expressing the Y367C mutant [56]. Definitive characterization of the effects of this substitution on protein function requires further study.

Although not found as a somatic mutation, a SNP in FGFR4 is associated with increased disease aggressiveness. The Gly388–encoding allele (G388) of FGFR4 has been associated with a shorter time to progression after initial surgery and adjuvant therapy in breast cancer patients, and also correlated with lymph node metastasis and disease recurrence in prostate cancer patients [57, 58]. Although no phenotype was observed in unperturbed FGFR4 Arg385knock-in mice (murine equivalent to human FGFR4 Arg388), crosses with mice conditionally overexpressing transforming growth factor α in breast epithelia resulted in mice with increased mass of mammary tumors and increased number of metastases [59]. Biochemically, FGFR4 Arg388 increases protein stability and prolongs protein phosphorylation in response to ligand stimulation [60]. FGFR4 Arg388 appears to affect only tumor progression and not initiation; this more subtle
effect is consistent with the substantial prevalence of this allele in the germline of the Caucasian population, with over 50% of individuals carrying one or two copies of this allele [57].

These data suggest that FGFR4 also plays a role in tumorigenesis for several tumor types. It should be noted that FGFR4 was found to be significantly mutated in lung adenocarcinoma [61]. However, some of these mutations are frameshifting deletions which likely lead to loss of protein function, reminiscent of the situation with loss-of-function FGFR2 mutations in melanoma. Perhaps in these cases the gene expression state of the tissue of origin influences whether a particular gene acts as an oncogene or tumor suppressor. Functional experiments are required to determine the biochemical nature of these FGFR4 mutations and whether they play a role in lung tumorigenesis or are merely passenger mutations, providing no fitness benefit for the tumor.

Validation of FGFRs as therapeutic targets

If FGFR family members mutated or amplified in human cancers are to serve as therapeutic targets, tumor cells harboring such genomic lesions must depend on FGFR activity for survival. Such dependencies have been investigated using either RNA interference techniques or treatment with a commercially-available kinase inhibitor, PD173074, which demonstrates high specificity toward FGFRs [62] but is not suitable for clinical use. Additional experiments have been performed using multi-target kinase inhibitors that have progressed to Phase II/III trials but exhibit a range of activities against FGFRs and other kinases.

The potential clinical utility of inhibiting FGFRs was first shown in multiple myeloma cell lines harboring oncogenic FGFR3 mutations. Treatment of KMS11 cells, harboring an allele encoding FGFR3 Y373C, with the pan-FGFR inhibitor PD173074 induced apoptotic cell
death as evidenced by annexin V staining [63]. Treatment with the clinically-relevant, albeit less specific, kinase inhibitor CHR258/TKI258 caused a reduction in tumor growth and increased survival in KMS11 subcutaneous xenograft models [64], and similarly decreased tumor growth and increased survival in a KMS11 orthotopic xenograft model [65].

In addition to kinase inhibitors, neutralizing antibodies directed against FGFR3 have also shown preclinical efficacy in vivo in multiple myeloma. A neutralizing antibody directed towards FGFR3 that blocks ligand binding in vitro and prevents receptor dimerization associated with constitutively activating extracellular mutations, R3Mab, exerted a potent anti-tumor effect in KMS11 subcutaneous xenografts primarily due the induction of antibody-dependent cell-mediated cytotoxicity (ADCC) [66].

Following the identification of FGFR3 activating mutations in bladder cancer, preclinical in vitro studies showed that knockdown of FGFR3 or inhibition with the SU5402 pan-FGFR kinase inhibitor resulted in a reduction in proliferation and soft agar colony formation of MGH-U3 and 97-7 bladder cancer cells expressing the activated Y373C and S249C mutants, respectively, without inducing apoptosis [67, 68]. Tumor growth inhibition was furthermore observed with UM-UC-14, expressing FGFR3 S249C, and MGH-U3 bladder cancer cell lines when engrafted subcutaneously into SCID mice and treated with PD173074 [69].

More progress has been made in demonstrating that endometrial cancer cells are dependent on mutant FGFR2 for cell survival. Because the most common FGFR2 mutant observed in endometrial tumors, S252W, is located in the extracellular domain and confers only ligand-dependent, albeit ligand-promiscuous activation, it was not known whether cells expressing this variant would depend on mutant FGFR2 for survival, given that the majority of mutated kinases for which oncogene dependence has been reported are constitutively activated in
a ligand-independent manner. Nevertheless, knockdown of FGFR2 in cell lines with both kinase domain mutations and ligand binding domain mutations inhibited transformation and induced cell cycle arrest and cell death [25, 70]. Endometrial cancer cell lines harboring activating mutations in FGFR2 were selectively sensitive to the pan-FGFR inhibitor, PD173074, in both monolayer growth and soft agar colony formation assays [25, 70]. Notably, MAP kinase/ERK kinase 1 and 2 (MEK1 and MEK2) inhibition ameliorates the craniosynostosis phenotype of knock-in mice harboring an FGFR2 S252W–encoding germline allele [71], raising the possibility that inhibition of downstream signaling proteins activated by mutant FGFR2 could also provide additional therapeutic options in cancer.

Data supporting FGFR1 fusion proteins as therapeutic targets in EMS/SCLL are more limited. Because EMS/SCLL frequently transforms into acute myeloid leukemia (AML) [42], the AML cell line KG-1, harboring a translocation encoding an FGFR1OP2-FGFR1 fusion product, was used to study dependence of FGFR1-driven hematopoietic malignancies on FGFR1 function. Treatment of KG-1 cells with FGFR1-specific small interfering RNA (siRNA) caused inhibition of cell growth and induction of apoptosis [72]. The multi-kinase inhibitor TKI-258 similarly inhibited survival of KG-1 cells, as well as primary cells from EMS/SCLL patients harboring FGFR1 translocations, but not from EMS/SCLL patients without FGFR1 translocations [73].

FGFR inhibitors have also shown efficacy in vitro and in vivo in tumor types with amplification of FGFR1 and FGFR2, including gastric, breast, and lung cancers. Treatment of the KATO-III and OCUM-2M gastric cancer cell lines carrying FGFR2 amplifications with the anti-VEGFR/FGFR kinase inhibitor AZD2171 showed an inhibition of proliferation in vitro as well as in subcutaneous xenografts implanted in nude mice [74]. Furthermore, antibodies
specific for the IIIb isoform of FGFR2 decrease FGFR2 phosphorylation in gastric cells which overexpress FGFR2 and display potent anti-tumor activity in xenograft models of gastric cancer [75]. The response of breast cancer cell lines harboring FGFR1 amplifications to FGFR inhibition is less convincing. siRNA knockdown of FGFR1 or treatment with PD173074 had no effect on cell proliferation under two-dimensional culture conditions. However, PD173074 treatment blocked soft agar colony formation by the CAL120 cell line, a breast cancer cell line capable of anchorage-independent growth [53]. In contrast, in the breast cancer cell line MFM223, in which FGFR2 is amplified, treatment with PD173074 resulted in induction of cell death [76]. Lung cancer cell lines harboring focal FGFR1 amplifications similarly exhibited a cytotoxic response to PD173074 treatment [52].

Overall, the data described above support dependence of tumor cell lines harboring FGFR alterations on FGFR activity with the exception of oncogenic FGFR4 mutations, which suffer from a lack of appropriate rhabdomyosarcoma cell lines in which to study such lesions. However, data regarding whether FGFR inhibition results in cell cycle arrest or induction of cell death in cell line models with amplification and/or mutational activation of FGFRs are inconclusive. Whether the apparent disparities reflect true differences in tissue or tumor type dependencies of FGFR signaling or the vagaries of a limited number of cell line models is currently unknown. It should be noted that little data has been published on the molecular mechanism of cell death induced by FGFR inhibition in these different cellular contexts, a necessary piece of the puzzle in understanding the exact mechanism of oncogene addiction that exists in these different cell types and exploiting this oncogene dependency for therapeutic gain.
Agents with anti-FGFR activity in clinical trials

Several small molecules with activity against FGFRs are currently in preclinical or clinical development. The majority of these are multi-kinase inhibitors often designed primarily as anti-angiogenic inhibitors with activity against vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs) and, as such, often don’t demonstrate sufficiently potent anti-FGFR activity. However, at least two second-generation pan-FGFR inhibitors that have increased specificity for the FGFR family have entered Phase I trials and several more are in preclinical development, further supporting the emerging role of FGFRs as therapeutic targets in oncology (Table 2).

TKI-258 (Novartis) is the multi-target kinase inhibitor with the best evidence for physiologically-relevant efficacy against activated FGFRs. TKI-258 is an orally administered, multi-targeted growth factor receptor inhibitor that has activity against FGFRs, VEGFRs, PDGFR, KIT, and FLT3 [77]. In a move that reflects current clinical trial design with new targeted agents, TKI-258 is being evaluated in bladder cancer patients, with the inclusion criterion of availability of archival tumor tissue for FGFR3 mutation testing to enable correlative studies (NCT00790426). Additionally, it is being evaluated in advanced breast cancer patients with and without FGFR1 amplification (NCT00958971), and relapsed multiple myeloma cases with and without the t(4;14) translocation often associated with FGFR3 amplification (NCT01058434). Of note, a more specific pan-FGFR inhibitor, BGJ398 (Novartis), is currently being evaluated in a phase I study in advanced solid malignancies restricted to patients with advanced solid tumors demonstrating either FGFR1 or FGFR2 amplification or FGFR3 mutation for whom no further effective treatment exists (NCT01004224).
Brivanib (Bristol Myers Squibb) is a dual tyrosine kinase inhibitor of VEGFR and FGFR signaling which has shown activity against metastatic solid tumors refractory to standard therapy in Phase I clinical trials and is currently being evaluated in a large number of trials primarily as an anti-angiogenic agent [78]. Based on its additional anti-FGFR activity, it is also being tested in endometrial cancer (NCT00888173); however, in contrast to TKI258, no data has been published regarding whether brivanib shows *in vitro* or *in vivo* tumor growth inhibition in cell line models driven by mutated or amplified FGFRs.

E7080 (Eisai) has activity against VEGFRs, FGFRs, PDGFRs and KIT [79] and is currently in phase II clinical trials for a wide range of solid malignancies. Based on a partial response in the Phase I trial, a Phase II trial was opened to examine the efficacy of E7080 in patients with metastatic endometrial cancer (NCT01111461). Several additional molecules being developed as anti-angiogenic compounds are being trialed in endometrial cancer (AZ2171, NCT01132820; BIBF1120, NCT01225887). Should there be any partial or complete responses in these trials it will be interesting to correlate these with the mutation status of FGFR2.

AZD4547 (AstraZeneca) is a more specific pan-FGFR inhibitor that is currently in Phase I trials for patients with solid malignancies (NCT00979134) and in a Phase I/II trial in breast cancer in combination with an aromatase inhibitor where the Phase II arm includes examination of FGFR1 amplification as a biomarker of response (NCT01202591). No data have yet been published regarding the efficacy of this compound in preclinical models driven by activated FGFRs.

FP-1039 (FivePrime), an FGFR1c:Fc decoy receptor which acts as a broad FGF ligand trap is in Phase I trials in solid malignancies (NCT00687505). A small Phase II trial testing FP1039 in endometrial cancer patients preselected for somatic alleles encoding the
S252W or P253R mutants, and thus presumably dependent on ligands sequestered by this decoy receptor, has opened (NCT01244438). This is the first trial to specifically select patients based on FGFR mutation status alone and the results are eagerly anticipated. Several companies have presented pre-clinical data at meetings reporting additional pan-FGFR inhibitors with increased specificity; we expect to hear more about these in the future.

Concluding remarks

The field of anti-FGFR therapy is in its infancy; therefore it is too soon to predict whether treatments targeting FGFRs will be as successful as those targeting tumors demonstrating oncogene dependence on other activated kinases, such as the BCR-ABL fusion protein in chronic myelogenous leukemia, mutant KIT and PDGFRA in gastrointestinal stromal tumors, and mutant epidermal growth factor receptor (EGFR) in lung adenocarcinoma [80-83]. Although many trials are underway in FGFR-dependent tumor types with multi-target inhibitors, results from Phase I trials of more specific second-generation FGFR inhibitors are eagerly awaited. Should these multi-kinase and/or pan-FGFR inhibitors result in clinically-significant response rates, an emerging area of research will be to identify the main mechanisms of resistance to FGFR inhibition so that combination therapies with chemotherapies or targeted agents that inhibit parallel or downstream signaling pathways can be identified that delay or minimize the likelihood of acquired resistance to anti-FGFR therapy.

It is possible that other tumor types, in addition to those considered here, might respond to FGFR inhibitor therapy. Ongoing massively-parallel next-generation cancer sequencing experiments will exhaustively characterize the cancer genomes of many tumor types, and will facilitate identification of additional diseases in which somatic FGFR mutation or amplification
plays a major role. Furthermore, large scale studies that correlate cancer cell genotype with inhibitor sensitivity, such as the Center for Molecular Therapeutics 1000 (Sanger Institute and Massachusetts General Hospital) and the Cancer Cell Line Encyclopedia project (Broad Institute and Novartis) could reveal novel tumor types sensitive to FGFR inhibition.

Studies of the functional effects of somatic FGFR mutations in cancer have been greatly assisted by the prior characterization of germline mutations in skeletal malformation syndromes. However, FGFR signaling in cancer might also exhibit context dependence, exemplified by the selection for FGFR2 gain-of-function mutations in endometrial carcinoma and loss-of-function mutations in melanoma. In addition, many somatic mutations of FGFR family members, especially those that are not found to be recurrent, or more precisely, those that are not mutated at rates statistically significantly higher than the background mutation rate for the gene in question, could be passenger mutations that do not provide any fitness benefit for the tumor. As in all somatic mutation studies, experimental determination of which mutations play a causative role in tumorigenesis and how mutations affect protein function is currently the rate-limiting step to fully understanding the clinical implications of our genomic data.
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Physiological FGFR signaling

Ligand-stimulated FGFRs phosphorylate the FGFR-associated cytosolic docking protein, FGFR substrate 2 (FRS2), which mediates activation of the RAS/MAPK (mitogen-activated protein kinase) pathway by binding the growth factor receptor-bound 2 (GRB2) - son of sevenless (SOS1) complex and Src homology region 2 domain phosphatase (SHP2). GRB2 also forms a complex with GRB2-associated binding protein 1 (GAB1), facilitating activation of the PI3K (phosphoinositide 3-kinase) pathway. GRB2 can additionally recruit the ubiquitin ligase CBL (casitas B-lineage lymphoma proto-oncogene) to FRS2, resulting in negative regulation of FGFR signaling. Phospholipase C-γ (PLCγ) directly binds the C-terminal tails of FGFRs when phosphorylated, to Tyr 766 in the case of FGFR1, but the significance of PLCγ binding remains unclear.

Box 1, Figure 1. Schematic diagram of signal transduction pathway activated by ligand-stimulated FGFRs.
Glossary Box

Apert syndrome: Autosomal dominant disorder characterized by craniosynostosis (premature fusion of skull bones) and syndactyly (digit fusion) of the hands and feet.

Crouzon syndrome: Autosomal dominant disorder characterized by craniosynostosis.

SADDAN syndrome: Severe achondroplasia (short-limbed dwarfism) with developmental delay and acanthosis nigricans (skin disorder characterized by thick, dark skin); bone growth disorder presumed to be autosomal dominant.

Seborrheic keratosis: Common benign skin growth occurring primarily in older adults.

Spermatocytic seminoma: Rare germ cell tumor of the testis.

Thanatophoric dysplasia: Severe skeletal disorder presumed to be autosomal dominant, characterized by short limbs.
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**Figure Legends**

**Box 1, Figure 1.** Schematic diagram of signal transduction pathways activated by ligand-stimulated FGFRs.

Ligand specificity is determined in part by an alternative splicing event in the C-terminal half of the third immunoglobulin domain (bright blue) in which either exon 8 or exon 9 can be used, producing the IIIb and IIIc isoforms, respectively. Ligand binding results in receptor autophosphorylation in the kinase domain region and phosphorylation of FRS2, which initiates a signaling cascade resulting in activation of the AKT and ERK downstream signaling pathways.

**Figure 2.** Alignment of altered amino acids encoded by somatic mutations found in FGFRs that are known or likely to be oncogenic (Table 1).

Unique amino acid substitutions are represented by green circles. Amino acid numbers are derived from the following reference transcripts: FGFR1, NM_000604; FGFR2, NM_022970; FGFR3, NM_000142; FGFR4, NM_002011. Yellow, Ig-like domains; red, kinase domains.
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<th>Gene</th>
<th>Encoded Substitution</th>
<th>Cancer</th>
<th>Germline Disease</th>
<th>Oncogenic</th>
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<td>FGFR1</td>
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<td>[23, 94, 95]</td>
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</table>
Table 1. Somatic mutations of *FGFR* family genes in cancer with known or likely oncogenic effects.

\[ a \] Amino acid numbers are derived from the following reference transcripts: FGFR1, NM_000604; FGFR2, NM_022970; FGFR3, NM_000142; FGFR4, NM_002011.

\[ b \] MM, multiple myeloma

\[ c \] SK seborrheic keratosis

\[ d \] SS, spermatocytic seminoma

\[ e \] Pfeiffer syndrome

\[ f \] hypochondroplasia

\[ g \] thanatophoric dysplasia, type II

\[ h \] Apert syndrome

\[ i \] Crouzon syndrome

\[ j \] unclassified or unspecified craniosynostosis

\[ k \] uniconoral non-syndromic craniosynostosis

\[ l \] Beare-Stevensen Cutis Gyrata Syndrome

\[ m \] SADDAN syndrome

\[ n \] thanatophoric dysplasia, type I

\[ o \] achondroplasia

\[ p \] acanthosis nigricans

\[ q \] as defined by a positive result in a cell-based transformation assay or animal model of cancer
Table 2. Multi-kinase and pan-FGFR inhibitors in clinical development

<table>
<thead>
<tr>
<th>Pharmaceutical company</th>
<th>Chemical name Drug name</th>
<th>Multi-kinase activity</th>
<th>Clinical Trials</th>
<th>Ref.</th>
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<td>Novartis</td>
<td>TKI258 Dovotinib</td>
<td>FLT3, FGFRs, VEGFRs, PDGFRs, KIT, CSFR</td>
<td>Phase II (Breast, Bladder, Myeloma)</td>
<td>[77]</td>
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<td>Bristol Myers Squib</td>
<td>BMS582664 Brivanib</td>
<td>VEGFRs, FGFRs,</td>
<td>Phase II (Endometrial, Gastric, Bladder)</td>
<td>[103]</td>
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<tr>
<td>Astra Zeneca</td>
<td>AZ2171 Cediranib</td>
<td>VEGFRs, FGFRs, KIT</td>
<td>Phase I (Gastric, Breast)</td>
<td>Phase II (Endometrial)</td>
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<tr>
<td>Eisai</td>
<td>E7080</td>
<td>VEGFR, FGFR, PDGFRs</td>
<td>Phase II (Endometrial ca.)</td>
<td>Phase II – solid malignancies</td>
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<td>Astra Zeneca</td>
<td>AZD4547</td>
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<td>Phase I – solid malignacies</td>
<td>Phase I/II – Breast in combination</td>
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<td>Novartis</td>
<td>BGJ398</td>
<td>Pan-FGFR</td>
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<td>Five Prime Therapeutics</td>
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<td>FGFR1c:Fc ligand trap</td>
<td>Phase I – solid malignancies</td>
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