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FGFR4: A promising therapeutic target for breast cancer and other solid tumors

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Abstract

The fibroblast growth factor receptor (FGFR) signaling pathway has long been known to cancer researchers because of its role in cell survival, proliferation, migration, and angiogenesis. Dysregulation of FGFR signaling is frequently reported in cancer studies, but most of these studies focus on FGFR1–3. However, there is growing evidence implicating an important and unique role of FGFR4 in oncogenesis, tumor progression, and resistance to anti-tumor therapy in multiple types of cancer. Importantly, there are several novel FGFR4-specific inhibitors in clinical trials, making FGFR4 an attractive target for further research. In this review, we focus on assessing the role of FGFR4 in cancer, with an emphasis on breast cancer. First, the structure, physiological functions and downstream signaling pathways of FGFR4 are introduced. Next, different mechanisms reported to cause aberrant FGFR4 activation and their functions in cancer are discussed, including FGFR4 overexpression, FGF ligand overexpression, FGFR4 somatic hotspot mutations, and the FGFR4 G388R single nucleotide polymorphism. Finally, ongoing and recently completed clinical trials targeting FGFRs in cancer are reviewed, highlighting the therapeutic potential of FGFR4 inhibition for the treatment of breast cancer.

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1. Introduction

Fibroblast growth factor receptor 4 (FGFR4) is a member of a highly conserved tyrosine kinase family, along with FGFR1–3. This family consists of an intracellular tyrosine kinase domain, a single transmembrane domain, and extracellular ligand binding domains. FGFRs have been reported to be involved in many physiological processes, including embryonic and postnatal development, metabolic homeostasis, and tissue maintenance and repair (Itoh & Ornitz, 2011). Aberrant FGFR activation due to genetic alterations has been implicated in the development and progression of multiple cancer types, including breast, liver, lung, gastric, uterine, bladder, and rhabdomyosarcomas (Kato, 2019). One recent report characterized the genetic landscape of FGFR alterations in 4853 tumors, and found FGFR aberrations in 7.1% tumors, of which 66% were amplifications, 26% were mutations, and 8% were rearrangements (Helsten, et al., 2016). Changes were most frequent in FGFR1, with alterations present in 3.5% of patients, followed by FGFR3 (2.0%), FGFR2 (1.5%) and FGFR4 (0.5%).

Possibly due to its relatively low frequency of somatic DNA alterations, FGFR4 has been traditionally understudied relative to the other FGFR family members. However, FGFR4 overexpression at the RNA level, expression of the FGFR4-specific ligand (fibroblast growth factor 19), and an FGFR4 single nucleotide polymorphism (SNP) are frequently observed in a wide range of tumors. In particular, the level of FGFR4 overexpression should not be ignored, as aberrant tyrosine kinase activation mediated by receptor overexpression has been successfully targeted before for the treatment of cancer, including the landmark example of HER2 overexpression for breast cancer (Cobleigh, et al., 1999).

Like the FGFR family as a whole, aberrant FGFR4 activation is associated with cancer progression and resistance to different types of anti-cancer therapy. Additionally, preclinical studies have shown that FGFR4 knockdown or pharmacologic inhibition can inhibit tumor growth and metastasis both *in vitro* and *in vivo*. Importantly, FGFR4 has the least homology among the FGFR family (Kostrzewa & Müller, 1998), enabling the design of FGFR4-specific inhibitors.

Much of the current clinical trials for FGFR4 are focused on hepatocellular carcinoma (HCC), with FGF19 overexpression being a frequent event in this cancer type. However, there is increasing evidence suggesting that FGFR4 may play an important role in breast cancer, specifically indicated by recent DNA and RNA sequencing studies of breast metastases. In this review, we begin with a discussion of FGFR4 structure and function, followed by a focus on the potential clinical utility of FGFR4 inhibition for breast cancer treatment.

2. Structure and ligands of FGFR4

2.1. General structure of FGFRs

The structure of the FGFRs is highly conserved, consisting of an extracellular domain, a single transmembrane domain and an intracellular tyrosine kinase domain (Eswarakumar, et al., 2005; Turner & Grose, 2010) (Fig. 1A). The extracellular domain contains three immunoglobulin (Ig)-like loops (loop I, II, III) and a serine-rich sequence containing 4–8 acidic amino acids, termed the acid box, between loops I and II (Kostrzewa & Müller, 1998). Loop I and the acid box are responsible for the automatic inhibition of FGFRs (F. Wang, et al., 1995). Loops II and III are the binding domains of heparin and fibroblast growth factors (FGFs). The intracellular kinase domain is a prototypical split kinase domain, composed of a smaller N-terminal domain and a larger C-terminal domain (Lesca, et al., 2014).

Phosphorylation sites of the FGFR4 kinase domain that have been reported at least 5 times in the PhosphoSitePlus database (Hornbeck, et al., 2015) are indicated in Fig. 1A, including S573, Y642, Y643 and Y754. FGFR splice variants lacking the transmembrane and intracellular domains have been reported to be soluble and secreted outside of the cell, acting as dominant-negative decoy receptors to trap respective ligands and inhibit endogenous signaling (Duan, et al., 1992; Ezzat, et al., 2001; D. C. Tomlinson, et al., 2005).

2.2. Structural differences between FGFR4 and FGFR1–3

Among the FGFRs, FGFR4 shares the least homology (Kostrzewa & Müller, 1998). While FGFR4 has only one isoform of loop III (loop IIIc), loop III of FGFR1–3 can be alternatively spliced into two different isoforms, loop IIIb and loop IIIc, which can profoundly change their specificity to ligands (Miki, et al., 1992; Yayon, et al., 1992). Loop IIIb is mainly expressed in epithelial tissue and prefers ligands expressed in mesenchymal tissue, while loop IIIc is mainly expressed in mesenchymal tissue and favors ligands expressed in epithelial tissue (Ornitz, et al., 1996; X. Zhang, et al., 2006). The ability to switch ligand binding specificity through alternative splicing of loop III has been reported to facilitate epithelial–mesenchymal transition (EMT) and has been associated with tumor progression (Wesche, et al., 2011). In addition, FGFR4 contains a unique amino acid in the kinase domain compared to FGFR1–3 (Fig. 1B), the C552 residue, which enables the design of FGFR4 specific inhibitors. Four of the existing FGFR4 specific small molecule inhibitors, including BLU-554 (also known as Fisolitinib, a modified version of BLU-9931) (Hagel, et al., 2015), H3B-6527 (Joshi, et al., 2017), FGF401 (also known as Roblinitinib) (Zhou, et al., 2019) and INCB062079 (Ruggeri, et al., 2017) covalently bind to the C552 residue located in the hinge region of the kinase domain (colored red in Fig. 1B) to inhibit the activation of FGFR4.

2.3. FGF ligand specificity

Twenty-two different types of FGF ligands have been reported to be expressed in human tissue, named FGF1–23, with FGF15 not present in humans, but instead functioning in mice as an ortholog to human FGF19 (Ornitz & Itoh, 2015). These 22 FGFs can be divided into 7 subfamilies based on biochemical functions, sequence similarities, and evolutionary relationships (Table 1) (Ornitz & Itoh, 2015). The members of the FGF1, FGF4, FGF7, FGF8, and FGF9 subfamilies are also called paracrine FGFs. They bind to their

corresponding receptors in concert with heparin (Klint & Claesson-Welsh, 1999). These FGFs bind with heparin and FGFRs to form a 2:2:2 FGF-FGFR-heparin complex (Fig. 2), which is essential for the activation of FGFR signaling. The members of the FGF11 subfamily are also called intracrine FGFs, which locate intracellularly. They do not interact with FGFRs and mainly function in regulating the excitability of neurons and other excitable cells (Goldfarb, et al., 2007). Members of the FGF19 family are also called endocrine FGFs, which have low binding affinity to heparin, facilitating their escape from the extracellular matrix, allowing them to function in an endocrine manner. Within this family, FGF19 and FGF21 utilize beta-Klotho and FGF23 utilizes alpha-Klotho to function as cofactors to bind to FGFRs and enhance downstream FGFR signaling (Goetz & Mohammadi, 2013).

Two studies (Ornitz, et al., 1996; X. Zhang, et al., 2006) have previously investigated the specificity of the FGF ligands to FGFR1–4 using a mitogenesis-based assay. FGFR4 can potentially bind to several paracrine FGFs, with highest activity for FGF1, 2, 4, and 8 (Table 1). One limitation of this study is the lack of beta-Klotho co-expression, which makes it difficult to interpret the binding affinity of the FGF19 family. In recent studies, FGF19 is reported to predominantly bind to FGFR4 with the help of beta-Klotho (Beenken & Mohammadi, 2009), which is important for regulating bile acid synthesis, and hepatic protein and glycogen metabolism (Holt, et al., 2003; Kir, Beddow, et al., 2011). Direct binding affinity between FGFs and corresponding receptors has also been measured with surface plasmon resonance technology, which shows comparable results (Beenken, et al., 2012; Olsen, et al., 2004).

3. FGFR4 physiological functions and downstream signaling

3.1. FGFR4 physiological functions

FGFR4 is widely expressed in the embryonic stage, functioning in tissue differentiation and organogenesis (Thisse & Thisse, 2005). After birth, the expression of FGFR4 is restrained to actively growing tissue, such as liver, lung and bone, with functions in regulating bile acid production, metabolism, muscle differentiation, and tissue repair (Powell, et al., 1998; Rappolee, et al., 1998; Stark, et al., 1991).

3.1.1. Role in embryonic and postnatal development—The expression pattern of FGFR4 is significantly different to that of FGFR1–3 (Iseki, et al., 1997; Molteni, et al., 1999; K. Peters, et al., 1993; K. G. Peters, et al., 1992). During mouse embryonic development within E9.5, FGFR4 mRNA is first restricted to the inner cell mass and primitive endoderm (Rappolee, et al., 1998). Later, it is primarily present in the developing lung and gut and cells of mesodermal origin, particularly within precursors of muscle tissue (Korhonen, et al., 1992; Partanen, et al., 1991). After birth, the highest level of FGFR4 transcripts are detected in liver, kidney (Korhonen, et al., 1992), and differentiating myoblasts (S. Yu, et al., 2004). In addition, FGFR4 is expressed in the ovary of pregnant mice, and expressed in ovarian follicles at different stages of follicular development (Puscheck, et al., 1997).

Despite the high FGFR4 expression during embryonic development, FGFR4 deletion does not cause developmental abnormalities (Weinstein, et al., 1998). In contrast, knockout of

FGFR1 or FGFR2 leads to embryonic lethality, blocking mesodermal differentiation and stopping inner cell mass growth, respectively (Arman, et al., 1998; Deng, et al., 1994; Yamaguchi, et al., 1994). Although all four FGFRs are expressed in adult bony tissue, and mutations in FGFR1–3 genes are known to cause skeletal abnormalities (Lazarus, et al., 2007), these deformities have not been linked to FGFR4 gene mutations. Although FGFR4 appears to be dispensible during development, the functional impact of FGFR4 inhibition in the postnatal stage remains to be elucidated.

3.1.2. Role in bile acid production—Although the four FGFRs are all expressed in the adult liver, only FGFR4 is expressed in mature hepatocytes (Kan, et al., 1999). FGFR4 knockout mice have a significant increase of the excreted and total bile acid pool (C. Yu, et al., 2000). This occurs through an FGF15-FGFR4-MST1/2 pathway, which downregulates Cyp7A expression to limit the synthesis of bile acid in the mouse liver (Inagaki, et al., 2005; Ji, et al., 2019) (Fig. 2). FGF19, the human orthologue gene of mouse FGF15, regulates bile acid biosynthesis in human hepatocytes in a similar manner (Holt, et al., 2003). This FGF15-FGFR4-MST1/2 signaling pathway has recently been reported to also be involved in tumorigenesis (Ji, et al., 2019; Turunen, et al., 2019), although the protumorigenic effects appear to be context-dependent (Ji, et al., 2019; Luo, et al., 2010).

3.1.3 Role in metabolic regulation—In addition to bile acid synthesis, FGFR4 knockout mice also have alterations in cholesterol metabolism (C. Yu, et al., 2000). FGF19, an FGFR4-specific ligand, has also been shown to stimulate protein translation machinery and to stimulate glycogen synthase activity through an insulin-independent endocrine pathway (Kir, Beddow, et al., 2011; Kir, Kliever, et al., 2011). Mice lacking FGF15 cannot properly maintain blood glucose concentrations and normal postprandial amounts of liver glycogen, but showed a normal insulin level and normal insulin sensitivity (Kir, Beddow, et al., 2011; Schaap, 2012). Additionally, human FGF19 overexpression transgenic mice show an increased energy expenditure through increased brown adipose tissue and decreased liver expression of acetyl coenzyme A carboxylase, leading to a significant and specific reduction in fat mass (E. Tomlinson, et al., 2002).

3.1.4. Role in muscle differentiation and tissue repair—FGFR4 is highly expressed in adult mouse and chick muscle satellite cells, which are responsible for proliferation and differentiation (Korhonen, et al., 1992; Marics, et al., 2002; P. Zhao, et al., 2006; P. Zhao & Hoffman, 2004). FGFR4 mRNA is normally undetectable in mouse muscle (Korhonen, et al., 1992; P. Zhao & Hoffman, 2004), but when muscle injury occurs, transiently high expression of FGFR4 can be detected in newly formed myotubes (P. Zhao & Hoffman, 2004), through a MyoD- TEAD2- FGFR4 pathway (P. Zhao, et al., 2006). Additionally, PAX3, a critical factor regulating skeletal muscle stem cell behavior, can directly bind to the promoter of FGFR4, promoting myogenesis (Cao, et al., 2010; Lagha, Kormish, et al., 2008; Lagha, Sato, et al., 2008). Inhibition of FGFR4 suppresses differentiation of muscle progenitor cells and leads to a dramatic loss of limb muscles, with altered expression of multiple muscle cell markers (Marics, et al., 2002). FGFR4 knockout mice have no defects in myogenic development (Weinstein, et al., 1998), but show an

impaired muscle regeneration, with the newly formed muscle fibers being smaller and more irregular with areas of calcification and fatty infiltration (P. Zhao, et al., 2006).

Mice lacking FGFR4 have a normal liver regeneration ability following partial hepatectomy. However, they are less resistant to liver injury induced by carbon tetrachloride (CCl₄), with delayed hepatolobular repair (C. Yu, et al., 2002).

3.2. FGFR4 signaling

3.2.1 Canonical FGFR4 downstream signaling pathways—As stated above, canonical FGFR signaling is dependent on FGF ligand binding (Turner & Grose, 2010), through a 2:2:2 FGF-FGFR-heparin structure, leading to tyrosine phosphorylation in the intracellular domain of FGFRs. These phosphorylated tyrosine residues on the receptor function as docking sites for adaptor proteins, leading to the activation of multiple signal transduction pathways (Eswarakumar, et al., 2005; Turner & Grose, 2010) (Fig. 2). Like the other three members of the FGFR family, activated FGFR4 directly phosphorylates two major targets, phospholipase γ (PLC γ) and FGFR substrate 2 (FRS2), which lead to activation of many downstream targets, including protein kinase C, MAPK, and AKT (Burgess, et al., 1990; Eswarakumar, et al., 2005; Gotoh, 2008; Kouhara, et al., 1997).

Additional targets that can be activated by FGF15/19-FGFR4 signaling pathways include Wnt/GSK-3 β /catenin (Pai, et al., 2008; X. Yu, et al., 2019; H. Zhao, et al., 2016) and MST1/2 (Ji, et al., 2019; Turunen, et al., 2019). Other pathways activated by the FGFRs, in which FGFR4 has shown to play a significant role, are the signal transducer and activator of transcription (STAT) signaling pathways, in particular STAT1 and STAT3 (Hart, et al., 2000). Together, canonical FGFR4 signaling leads to a pro-growth and anti-apoptotic state.

3.2.2. Non-canonical FGFR4 signaling pathways—In addition to canonical signaling pathways, recent evidence strongly implicates that FGFR4 can be activated through alternative mechanisms (Fig. 2). Firstly, heparin can activate FGFR4 in the absence of FGF ligands, leading to autophosphorylation and downstream signaling at a level comparable to FGF ligands (G. Gao & Goldfarb, 1995).

Next, FGFR4 has been shown to function in a complex with several cell-cell adhesion molecules, including NCAM and N-cadherin (Murakami, et al., 2008). It has been reported that NCAM can activate FGFR4 signaling through a complex with N-cadherin in β -cells from pancreatic tumors in an FGF-ligand independent manner (Cavallaro, et al., 2001). In this model, NCAM can activate FGFR4 signaling to promote matrix adhesion but not neurite outgrowth, thereby leading to a different downstream output than that mediated by canonical FGFs. Another group demonstrated that membranous FGFR4 can bind with NCAM and N-cadherin, while a pituitary tumor-derived N-terminally truncated cytoplasmic receptor isoform of FGFR4 cannot (Ezzat, et al., 2004). More recently, it has been reported that the acid box between the Ig I and Ig II domains of the FGFRs is the common binding motif for N-cadherin and NCAM (Sanchez-Heras, et al., 2006). N-cadherin has also been shown to stabilize membranous FGFRs following FGF ligand binding, leading to increased downstream signaling (Suyama, et al., 2002).

In addition to cell adhesion molecules, FGFR4 has shown ligand-independent interaction with several other proteins, including ephrin receptor EphA4 and ephexin1. EphA4, also a receptor tyrosine kinase, can bind directly to the juxtamembrane region of FGFRs, including FGFR4, through the N-terminal portion of its protein tyrosine kinase core (Yokote, et al., 2005). The interaction between EphA4 and FGFRs can lead to transactivation, phosphorylation of FRS2, and activation of MAPK (Yokote, et al., 2005). Ephexin1, a guanine nucleotide exchange factor for Rho family GTPases, directly binds to the kinase domain of FGFRs and acts as a downstream target of the EphA4-FGFR complex for roles in cell proliferation, migration and morphological changes (Y. Zhang, et al., 2007).

Additional non-canonical downstream signaling targets of FGFR4 include N-cadherin and NF κ B. In particular, the FGFR4-388R variant can increase N-cadherin expression via STAT3 activation (Quintanal-Villalonga, et al., 2018), and activated FGFR4 can inhibit NF κ B signaling via IKK β phosphorylation (Drafahl, et al., 2010).

Lastly, FGFR4 has also been reported to be able to form a complex with membrane-type-1 matrix metalloproteinase (MT1-MMP), which can be used by tumor cells for invasion and metastasis. Both SNP alleles at position 388, FGFR4-R388 and FGFR4-G388, can form a complex with MT1-MMP and induce MT1-MMP autophosphorylation. However, the effects are opposite, with the risk allele FGFR4-R388 stabilizing MT1-MMP to increase tumor cell invasion ability, and the FGFR4-G388 allele down-regulating MT1-MMP (Sugiyama, et al., 2010).

4. Aberrant FGFR4 activation in cancer

Multiple mechanisms have been reported to cause aberrant activation of FGFR4 signaling in cancer, including FGFR4 overexpression, the upregulation of FGFR4 ligands (FGF19), FGFR4 somatic mutations, and the G388R SNP. FGFR4 oncogenic fusions are rarely reported and thus not discussed here.

4.1. FGFR4 overexpression

4.1.1. FGFR4 overexpression in breast cancer—FGFR4 is overexpressed in multiple types of primary tumors compared to adjacent normal tissue, with breast cancer among the most upregulated cancer types. Fig. 3A shows tumor types with at least a 1.5-fold increase of FGFR4 expression in primary tumors compared to normal tissue, based on the pan-cancer TCGA analysis (data version Jan 28th, 2016) (Cancer Genome Atlas Research, et al., 2013). FGFR4 has long been known to be overexpressed in ~30% of breast tumors compared to normal tissues (Penault-Llorca, et al., 1995).

Even relative to this upregulation in primary tumors, FGFR4 RNA expression is often further increased in breast cancer metastases. Among a panel of 105 breast cancer genes, Cejalvo et al. reported that FGFR4 is the most frequently upregulated gene in paired breast metastases (Cejalvo, et al., 2017). In particular, there was a significant gain of FGFR4 RNA expression in metastases relative to primary luminal A tumors, suggesting a role of FGFR4 in endocrine resistance and/or in metastasis for estrogen receptor positive (ER+) tumors. Our recently reported data also suggests this, showing that FGFR4 is overexpressed in 26/29

(90%) ER+ breast metastases compared to their matched primary tumors, in patients who had received endocrine therapy prior to recurrence (Levine, et al., 2019). Two-thirds of the samples had a >2-fold increase in FGFR4 RNA, with the invasive lobular carcinoma (ILC) tumors having an average gain of 4.8-fold. The role of FGFR4 in endocrine resistance is also supported by *in vitro* models, in which 12/12 endocrine resistant cell lines had an increase in FGFR4 expression (Levine, et al., 2019). Together, these data suggest that FGFR4 may mediate acquired resistance to endocrine therapy.

FGFR4 may also play a role in *de novo* resistance to endocrine therapy. High mRNA levels of FGFR4, but not FGFR1–3, have been reported to be a predictor of poor clinical benefit to tamoxifen treatment (Meijer, et al., 2008). This is true in multivariable models including HER2 expression, an important point to note as FGFR4 and HER2 are often co-expressed in primary tumors and HER2 is a known mediator of endocrine resistance (Schiff, et al., 2004; Shou, et al., 2004).

Although FGFR4 and HER2 are frequently co-expressed in primary tumors, the rate of co-expression in metastatic samples is not clearly defined. In cell line models, FGFR4 RNA overexpression is a frequent outlier event in HER2-gained breast cancer cells (Kothari, et al., 2013). One report for paired breast cancer brain metastases showed that FGFR4 and HER2 expression are often increased in tandem (Priedigkeit, et al., 2017), while another report for matched samples suggested that FGFR4 may be driving a HER2-enriched PAM50 phenotype in the absence of HER2 expression (Cejalvo, et al., 2017). In a HER2-enriched, yet HER2-negative PDX model (WHIM11), inhibition of FGFR4 with a selective inhibitor decreased tumor growth while shifting the PAM50 towards a more luminal phenotype (Garcia Recio, et al., 2019). Additionally, in a mouse model of breast cancer with activated HER2 and N-cadherin expression (MMTV-NeuNT), FGFR protein expression and activation were increased, with an FGFR inhibitor able to decrease cell invasiveness and stemness (Qian, et al., 2014).

FGFR4 expression has also been reported to be associated with breast cancer progression, breast cancer cell line survival, and resistance to chemotherapy. Luo et al. reported that the ablation of FGFR4 in mice delayed the incidence and progression of TGF α induced breast cancer, possibly through changes in major metabolic pathways related to adipocyte function, lipid and glucose metabolism, and mitochondrial functions controlled by FGF21 (Luo, et al., 2013). Tiong et al. identified FGFR4 as a key driver mediating the survival of a subset of breast cancer cell lines co-expressing FGFR4 and FGF19, including MDA-MB 468 and HCC1937, through a shRNA based screening targeting 673 human kinase genes (Tiong, et al., 2016). They showed that depletion of FGFR4 or FGF19 with siRNA or an anti-FGF19 antibody can significantly inhibit cell proliferation and induce cell apoptosis, which is mediated mainly through the PI3K/AKT signaling pathway. FGFR4 has also been reported to mediate resistance to doxorubicin treatment in breast cancer through enhanced glucose metabolism (Xu, et al., 2018) and upregulation of Bcl-xl (Andreas Roidl, et al., 2009), as well as resistance to nab-paclitaxel plus gemcitabine treatment in triple negative breast cancer (Gluz, et al., 2020).

4.1.2. FGFR4 overexpression in other cancers

In addition to the TCGA pan-cancer data presented in Fig. 3A, FGFR4 overexpression has also been reported in individual studies, including rhabdomyosarcoma (RMS) (Khan, et al., 2001) and ovarian cancer (Zaid, et al., 2013). However, the oncogenic functions of FGFR4 likely vary in different tumor types. In some cancers, high FGFR4 expression is associated with shorter survival, increased tumor proliferation, increased invasion, or treatment resistance, and silencing of FGFR4 with siRNA or pharmacologic inhibitors can reverse these phenotypes. Such reports are noted in colorectal (Heinzle, et al., 2012; C. S. Li, et al., 2014), esophageal (Xin, et al., 2018), liver (Cheng, et al., 2011; Ho, et al., 2009), gastric (J. Li, et al., 2016; Murase, et al., 2014), ovarian (Zaid, et al., 2013), RMS (Croese, et al., 2012; Taylor, et al., 2009), and lung (Quintanal-Villalonga, et al., 2019) cancers. However, in other cancer types, high FGFR4 expression is either not associated with poor prognosis or is associated with better survival, including cervical cancer (Choi, et al., 2016), cholangiocarcinoma (Yoo, et al., 2017), pancreatic cancer (Motoda, et al., 2011), and head and neck cancer (HNSC) (Koole, et al., 2015). Although the number of reports is limited, these studies suggest that the functions of FGFR4 overexpression are likely to be context dependent.

4.1.3. Mechanisms of FGFR4 overexpression—Unlike the other FGFRs, FGFR4 amplification is a rare event, and there is limited correlation between FGFR4 DNA and RNA levels (Helsten, et al., 2016; Perou, et al., 2000), suggesting that there are alternative mechanisms of FGFR4 overexpression. There have been several reports investigating the mechanisms of FGFR4 overexpression in different cancer types, but to date, these appear to be tissue specific. In RMS, overexpression of FGFR4 can be caused by a PAX3-FOXO1 fusion (Cao, et al., 2010) or by Sp1 transcription factor binding (S. J. Yu, et al., 2004). In pancreatic cancer, FGFR4 overexpression can be mediated by an intronic enhancer activated by HNF1 α (Shah, et al., 2002). In colorectal cancer, FGFR4 can be upregulated by overexpression of transcription factor FOXC1 (J. Liu, et al., 2018). It remains to be elucidated if these or other transcription factors can explain a significant amount of the FGFR4 overexpression in breast cancer.

4.2. FGF19 overexpression

FGF19 is located on the 11q13 locus, together with FGF3, FGF4, and cyclin D1. This locus is frequently amplified, leading to the overexpression of FGF19 in many cancer types, including breast (14%), liver (15%), head and neck (24%), lung squamous (14%), bladder (10%), and esophageal (34%) cancers (Cerami, et al., 2012; X. Huang, et al., 2002; Sawey, et al., 2011). A recent report suggests that this locus is amplified frequently in metastatic ER + breast cancers (Mao, et al., 2019). Fig. 3B shows the overexpression of FGF19 in primary tumors compared to adjacent normal tissues according to TCGA studies.

FGF19 is co-expressed with FGFR4 in 28% of primary breast tumors (Dallol, et al., 2015). High expression of FGF19 is associated with poor survival outcomes of breast cancer (X. Zhao, et al., 2018). *In vitro*, FGF19 can stimulate FGFR4 and increase breast cancer cell proliferation and invasion, and *in vivo*, genetic knockdown of FGF19 represses breast tumor progression and metastasis (X. Zhao, et al., 2018).

Dysregulation of the FGF19-FGFR4 axis through FGF19 overexpression has been implicated in tumorigenesis and progression of HCC. Liver tumorigenesis in FGF19 transgenic mice is reversible with FGFR4 knockout (French, et al., 2012). In addition, high FGF19 expression can promote HCC cell line proliferation, while knockdown of FGF19 has the opposite effect both *in vitro* and *in vivo* (Hagel, et al., 2015; Miura, et al., 2012). Elevated FGF19-FGFR4 signaling is also associated with increased HCC metastasis, through increasing EMT (H. Zhao, et al., 2016) or upregulation of SRY-related HMG box 18 (SOX 18) (J. Chen, et al., 2019).

In colorectal (Desnoyers, et al., 2007; Pai, et al., 2008), head and neck (L. Gao, et al., 2018), lung (Cui, et al., 2016; X. Zhang, et al., 2017), gastric (S. Wang, et al., 2016), and thyroid cancers (X. Zhang, et al., 2016), high FGF19 expression has also been reported to be associated with a poor survival rate, increased cell proliferation, and metastasis. Further studies are required to validate those findings as well as identify the mechanisms driving those phenotypes.

4.3. FGFR4 somatic mutations

4.3.1. Recurrent FGFR4 mutation sites and distribution in cancers—In the Cosmic database (v87), 439 out of 47935 (0.9%) tumor samples have an FGFR4 mutation (Harsha, et al., 2018). Among those, there are 18 sites with at least 3 reported mutations, accounting for 84 cases, including 31/84 (37.0%) in the extracellular domain, 3/84 (3.6%) in the transmembrane domain, and 50/84 (59.5%) in the intracellular domain (Fig. 4A). We also investigated the distribution of FGFR4 mutations across primary and metastatic cancers in the MSK-IMPACT database for breast cancer (Razavi, et al., 2018), and other cancer types (Zehir, et al., 2017) (Fig. 4B). The mutation rate of FGFR4 varies from 0.5% to 5.5% across different types of cancer, with metastatic melanoma showing the highest mutation rate. Of note, breast cancer is the only one showing a significantly increased FGFR4 mutation rate in metastatic tumors compared to primary tumors (Fisher exact test p-value: 0.0006).

4.3.2. FGFR4 hotspot mutations—Although the Y367C is reported to be a dominant oncogenic mutation of FGFR4 in the breast cancer cell line MDA-MB 453 (A. Roidl, et al., 2009), this mutation is rarely observed in clinical samples. Instead, there are two hotspot mutations of FGFR4, previously identified in studies of RMS, that are prevalent. Approximately 8% of RMS cases contain a missense mutation in amino acids N535 or V550, including N535D/K and V550E/L/M (Shern, et al., 2014; Taylor, et al., 2009). N535 is reported to be a key residue for inhibiting the autophosphorylation of FGFR4 (H. Chen, et al., 2007) and V550 to be a gatekeeper residue that can control the binding of ATP (Lesca, et al., 2014). N535K and V550E mutations have been shown to promote RMS tumor proliferation and metastasis in mouse xenograft models through enhancing FGFR4 autophosphorylation and subsequent activation of STAT3 signaling (S. Q. Li, et al., 2013; Taylor, et al., 2009).

The FGFR4 N535K mutation has been reported to mediate resistance to nonselective tyrosine kinase inhibitors (Z. Huang, et al., 2015) and the V550M and V550L mutations

resistance to the FGFR4-specific inhibitor BLU-554 (Hatlen, et al., 2019). Paralogous mutations at N535 have been reported in FGFR1 (N546D/K) and FGFR2 (N549D/K/H/S/T) (Babina & Turner, 2017; Zehir, et al., 2017), including recently in a metastatic ER+ sample (N549K) (Mao, et al., 2019). Additionally, a paralogous V550M mutation has been reported in FGFR1 (V561M) as a mechanism of resistance for a pan-FGFR inhibitor in lung cancer (Ryan, et al., 2019).

We recently reported that on a pan-cancer scale for adult tumors, the FGFR4 hotspot mutations, N535 (N535K) and V550 (V550L/M), are almost exclusively found within metastatic breast cancer (Levine, et al., 2019) (Fig. 4C). There is an additional enrichment for these hotspot mutations in metastatic ILC (Fig. 4D), suggesting that they may play a particularly important role in endocrine resistance for this histological subtype of breast cancer.

4.4. Single nucleotide polymorphism (SNP)

One SNP G388R (rs351855) at the transmembrane domain of FGFR4 has been frequently reported to be associated with tumorigenesis, progression and prognosis of a variety of cancer types, including breast, colorectal, prostate, lung, soft tissue sarcoma and HNSC (Bange, et al., 2002; Morimoto, et al., 2003). In the EXaC database, the FGFR4-R388 allele has an allele frequency of ~13% in the African population, ~30% in the European population, and ~45% in the Latino and East Asian populations, with homozygote rates matching Hardy-Weinberg expected frequencies in all of these populations (Lek, et al., 2016).

In breast cancer, some studies have shown that the FGFR4-R388 allele is linked with tumorigenesis, cell motility, cancer risk, sensitivity to and side effects of chemotherapy, and immune evasion. For tumorigenesis, knockin of the FGFR4-R385 allele (corresponding to R388 in human FGFR4) in TGF alpha-induced breast cancer mouse models can accelerate tumorigenesis and progression with significant advances in tumor mass, size, and onset of lung metastases (Seitzer, et al., 2010). Regarding cell motility, introducing the FGFR-G388 allele to MDA-MB-231 cell lines, a cell line with very low endogenous FGFR4 expression, has been shown to decrease cell migration, while no such negative effect was found with introducing the FGFR-R388 allele (Bange, et al., 2002; Stadler, et al., 2006). In terms of cancer risk, while some studies show no association (Jezequel, et al., 2004; Naidu, et al., 2009), a recent meta-analysis of 6 studies involving 1492 patients suggested that the FGFR4-R388 allele increases breast cancer risk slightly (Xiong, et al., 2017). Wei et. al. also reported that the FGFR4-R388 allele is associated with increased protein expression of FGFR4, increased lymph node metastasis and poor survival through an analysis of 747 breast cancer patients (Wei, et al., 2018). With regard to treatment effects, the FGFR4-R388 allele has been reported to be associated with poor response to systemic adjuvant therapy, especially chemotherapy in lymph node positive primary breast cancer (Thussbas, et al., 2006). There are also reports showing an association between the FGFR4-R388 allele and a higher incidence of febrile neutropenia during neoadjuvant chemotherapy in HER2-negative breast cancer (Charehbili, et al., 2015). For immune invasion, Kogan et al. reported that the

homozygous FGFR4-R388 allele can promote tumor immune evasion in transgenic mouse models of breast (WAP-Tgfa) and lung cancer (SPC-CRAF-BxB) (Kogan, et al., 2018).

The FGFR4-R388 allele has also been reported to be associated with cancer progression and poor prognosis in prostate, colorectal and lung cancer, as well as soft tissue sarcoma and HNSC (Bange, et al., 2002; Morimoto, et al., 2003; Spinola, et al., 2005; Streit, et al., 2004; J. Wang, et al., 2004). However, there are controversial reports about the effects of the FGFR4-R388 allele on tumor progression and prognosis, as reviewed by Heinzle et al. (Heinzle, et al., 2014).

In terms of mechanism, it has been reported that the FGFR4-R388 allele can change the transmembrane spanning segment and expose a STAT3 binding site in the inner cell membrane, leading to enhanced STAT3 signaling (Ulaganathan, et al., 2015). There is also a report showing that the FGFR4-R388 allele can enhance FGFR4 protein stability, leading to increased downstream signaling (J. Wang, et al., 2008).

5. Therapeutic potential of targeting FGFR4

Multiple strategies of inhibiting FGFR4 in cancer have been developed, including small molecule inhibitors, neutral antibodies targeting FGFR4 or FGF19, and an extracellular protein trap. Most small molecule inhibitors developed for targeting FGFRs are FGFR1–3 selective inhibitors or pan-FGFR inhibitors that have a lower affinity to FGFR4 (Babina & Turner, 2017). Recently, several FGFR4-specific inhibitors have been developed targeting a cysteine residue within the ATP binding pocket (Cys552) that is unique to FGFR4, to increase specificity and reduce toxicity of FGFR targeted therapy. Current clinical trials testing pan-FGFR inhibitors or selective FGFR inhibitors that were ongoing as of May 13, 2020 are listed in Table 2. Of note, this table does not include FGFR2/FGFR3 antibodies, non-selective FGFR inhibitors (e.g., 3D185 that has similar affinity for FGFR1–3 and CSF-1R) (Peng, et al., 2019), or FGFR inhibitors without reported IC50 values (e.g., HMPL-453, ICP-105, ICP-192).

5.1. FGFR1–3 selective inhibition and pan-FGFR inhibition

The first generation of FGFR inhibitors tested in clinical trials have largely included nonselective tyrosine kinase inhibitors (e.g., dovitinib and ponatinib), which in addition to FGFRs, inhibit a wide range of tyrosine kinases including VEGFR1–3, CSF1R, and RET (Katoh, 2019). The nonselective inhibitors have a major toxicity profile related to the inhibition of VEGFR, making it difficult to interpret the full potential of FGFR inhibition (Chae, et al., 2017). More recently, second generation FGFR inhibitors, including selective FGFR1–3 inhibitors (AZD454798, Debio1347, E7090161, HMPL-453, Pemigatinib) and pan-FGFR inhibitors (BGJ398, Erdafitinib, PRN1371, Rogaratinib, TAS120, ASP5878, FIIN2, LY2874455) have been deployed for clinical testing.

In the early phase trials reported thus far, the toxicity profile of FGFR selective inhibitors includes hyperphosphatemia, stomatitis, and decreased appetite (Chae, et al., 2017; Tabernero, et al., 2015). Compared to nonselective tyrosine kinase inhibitors, the adverse effects related to hypertension and cardiovascular were reduced. However, the success of

these trials has generally been limited and have not led to significantly improved outcomes compared to nonselective inhibitors. Katoh summarized the benefits and adverse effects of all completed clinical trials involving FGFR inhibitors by the end of 2018 (Katoh, 2019). Often the response rate is ~5–15%, however, there has been some recent success with the pan-FGFR inhibitor Erdafitinib. This drug became the first FGFR inhibitor approved by the FDA, for use in urothelial carcinoma for patients with specific FGFR2 or FGFR3 alterations (Loriot, et al., 2019). This example demonstrates the substantial need to develop biomarkers for patient selection, including the possibility of mRNA and protein expression (Katoh, 2019).

5.2. FGFR4-specific inhibition

5.2.1. Small molecule inhibitors—Five FGFR4-specific small molecular inhibitors (FGF401, BLU-554, H3B-6527, INCB062079, and ICP-105) are in current or recently completed ([NCT02325739](#)) clinical trials, mostly for the treatment of HCC, where FGFR4 or FGF19 is highly expressed (Table 2). Results of a phase I clinical trial of BLU-554 for treating HCC were published recently (R. D. Kim, et al., 2019). BLU-554 was well tolerated, with the most common grade 3 treatment related adverse events being elevated aspartate aminotransferase (AST, 15%) and alanine aminotransferase (ALT, 11%) levels. Compared with pan-FGFRs inhibitors, treatment did not cause hyperphosphatemia, supporting the lower toxicity of a more selective FGFR4 inhibitor. Across doses, the overall response rate was 17% (11/66) in FGF19-positive patients, with 1 complete response and 10 partial responses, and 0% (0/32) in patients with negative or unknown FGF19 expression. Among 7 responders with pre- and post-treatment circulating tumor DNA available for mutation analysis, 2 developed on-target resistance to BLU-554 caused by FGFR4 mutations at sites V550 and C552. Of note, those mutations are still sensitive to a pan-FGFR inhibitor (LY2874455) demonstrated with *in vitro* or *in vivo* models (Hatlen, et al., 2019). Partial results of another Phase I trial ([NCT02325739](#)), examining FGF401 (Porta, et al., 2017; Weiss, et al., 2019) in HCC patients with FGFR4 and beta-Klotho expression were released, including data up to November 2016 (Chan, et al., 2017). Again, the only grade 3/4 adverse effect observed in >5% patients was elevated aspartate aminotransferase (AST, 16%) and alanine aminotransferase (ALT, 12%), suggesting manageable toxicity of FGFR4-specific inhibition. The overall response rate across doses in patients with HCC (N=53) was 8%, with stable disease seen in 53% of patients with positive or negative FGF19 expression (Chan, et al., 2017). It will be important to examine if transaminase elevation remains a serious clinical issue outside of patients with HCC.

5.2.2. Other strategies for specific FGFR4 inhibition—In addition to small molecule inhibitors, there are several other treatment strategies for specifically targeting FGFR4, including the FGFR4-specific antibodies U3-1784 (Bartz, et al., 2019) and LD1 (French, et al., 2012), an anti-FGF19 antibody (Desnoyers, et al., 2007), and an antisense oligonucleotide for FGFR4 (ISIS-FGFR4RX) (X. X. Yu, et al., 2013). This oligonucleotide has been evaluated in one clinical trial for obesity ([NCT02476019](#)), but its application for cancer treatment is yet to be studied.

5.3. FGFR4 inhibition in breast cancer

5.3.1. Patient selection—Results of recent clinical trials with FGFR inhibitors have been nicely reviewed by Katoh (Katoh, 2019). Noteworthy in this review is the low response rate of FGFR inhibition in patients harboring FGFR genetic alterations, and the fact that some patients without known genetic alterations can respond to FGFR inhibition. For example, in a phase I trial of the FGFR-selective inhibitor LY2874455 in advanced solid tumors ([NCT01212107](#)), 2/51 patients had a radiological partial response, both of whom were lacking an FGFR1 or FGFR2 amplification (Michael, et al., 2017). Therefore, developing predictive biomarkers beyond genomics will likely be key for successful anti-FGFR4 treatment, especially for breast cancer where FGFR4 is commonly overexpressed without corresponding DNA changes. There is precedence for using FGFR RNA and protein expression as a marker for therapy benefit, with one study in lung cancer showing that FGFR1 overexpression, rather than amplification, correlated well with sensitivity to tyrosine kinase inhibition (Wynes, et al., 2014). Ligand expression may also be a useful biomarker. As mentioned above for the clinical trial involving BLU-554, FGF19+ HCC patients had a better response to FGFR4 targeted therapy than FGF19- patients. It has also been reported that in breast cancer cell lines, only cells co-expressing FGFR4 and FGF19 have a good response to FGFR4 inhibition (Tiong, et al., 2016). There are several clinical trials selecting patients based on their mRNA or protein level of FGFRs and FGF19, including [NCT01976741](#), [NCT02508467](#), [NCT02592785](#), [NCT03410693](#) and [NCT03473756](#), the results of which should be informative for biomarker selection.

5.3.2. Combination therapy—For breast cancer, it is likely that combination therapy targeting FGFR4 with ER and/or HER2 will provide better outcomes. As discussed above, FGFR4 is frequently overexpressed in endocrine resistant cell models and breast cancer metastases previously treated with endocrine therapy. A previous clinical trial ([NCT01528345](#)) (Musolino, et al., 2017) evaluated the effect of combination therapy involving a nonselective FGFR inhibitor (Dovitinib) and endocrine therapy (Fulvestrant) vs endocrine therapy alone for ER+ breast cancer, showing a higher response rate of combination therapy vs monotherapy (28% vs 15%). In addition, for patients harboring an FGFR1, FGFR3, or FGF3 amplification, the combination therapy group had longer median disease-free survival vs monotherapy (10.5 months vs 5.5 months). There are several clinical trials investigating the safety and efficacy of FGFR inhibition in metastatic breast cancer as listed in Table 2. Specifically, the results of trials testing Debio1347 in combination with Fulvestrant, Erdafitinib in combination with Palbociclib and Fulvestrant, and TAS-120 in combination with Fulvestrant will provide valuable data for assessing the potential of co-targeting FGFR4 and ER. As mentioned above, FGFR4 and HER2 are often co-expressed in primary tumors and appear to be co-expressed in at least of a subset of metastases (Cejalvo, et al., 2017; Priedigkeit, et al., 2017), supporting the potential opportunity of combination therapy. Co-targeting FGFR4 and HER2 in cell line models of co-expression had additive effects on inhibiting cell growth, supporting the design of future clinical trials (Kothari, et al., 2013).

6. Conclusion

Potential mechanisms for FGFR4 activation include FGFR4 overexpression, FGF19 overexpression, the G388R SNP, and newly appreciated hotspot somatic mutations at positions N535 and V550. Blocking FGFR4 activation via knockdown or small molecule inhibitors can suppress tumor growth and metastasis in both *in vitro* and *in vivo* preclinical studies. Current early phase clinical trials examining specific inhibition of FGFR4 in HCC reveal a manageable toxicity profile and a reasonable response rate, at least within a subset of patients. Based on these early clinical data and outcomes of completed clinical trials with pan-FGFR inhibitors, specific biomarkers for patient selection and possible combination therapies will likely be essential for achieving ideal clinical outcomes with FGFR4 targeted therapy.

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Abbreviations

EMT	epithelial–mesenchymal transition
ER	estrogen receptor
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FRS2	FGFR substrate 2
HCC	hepatocellular carcinoma
HER2	human epidermal growth factor receptor 2
IDC	invasive ductal carcinoma
ILC	invasive lobular carcinoma
MST1/2	mammalian sterile20-like kinases
MT1-MMP	membrane-type-1 matrix metalloproteinase
NCAM	neural cell adhesion molecule
PLCγ	phospholipase γ
RMS	rhabdomyosarcoma
SNP	single nucleotide polymorphism
STAT	signal transducer and activator of transcription
TCGA	The Cancer Genome Atlas

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Figure 1. FGFR4 protein structure (transcript variant 1 NM_002011.4) and unique kinase domain sequences compared to FGFR1–3.

(A) The structure of full length FGFR4 with major domains and their positions annotated. The 4 sites within kinase domain marked in red are the phosphorylation sites that have been reported at least 5 times in the PhosphoSitePlus database (Hornbeck, et al., 2015). SP: signal peptide, TM: transmembrane. (B) Comparison of key kinase domain sequences between FGFR1–4 (Turner & Grose, 2010). C552 in FGFR4 is the unique target for most of the FGFR4-specific inhibitors designed to date.

Figure 2. FGFR4 signaling network. Left: Canonical FGF-dependent FGFR4 signal transduction.

After ligand binding and receptor activation, the active FGFR4 then recruits adaptor proteins, leading to the activation of PLCγ, FRS2, RAS-RAF-MAPK, PI3K-AKT (Eswarakumar, et al., 2005) and STAT-dependent signaling (Hart, et al., 2000). With the help of beta-Klotho, FGF19 binding to FGFR4 leads to phosphorylation of MST1/2, which negatively regulates Cyp7A to limit bile acid synthesis (Inagaki, et al., 2005; Ji, et al., 2019; Turunen, et al., 2019). Furthermore, FGF19-FGFR4 specific activation can modulate Wnt/GSK-3β/β-catenin signaling (Pai, et al., 2008; X. Yu, et al., 2019; H. Zhao, et al., 2016).

Right: Non-Canonical FGF-independent FGFR4 signal transduction. High affinity binding with heparin can activate FGFR4. Additionally, FGFR4 can form complexes with several molecules to participate in different signaling pathways, such as cell-cell adhesion molecules NCAM (Cavallaro, et al., 2001) and N-cadherin (Christensen, et al., 2011), ephrin receptor EphA4 (Yokote, et al., 2005) and membrane-type-1 matrix metalloproteinase (MT1-MMP) (Sugiyama, et al., 2010). NFκB signaling can be negatively regulated through the interaction of FGFR4 and IKKβ (Drafahl, et al., 2010). Created with [BioRender.com](https://www.biorender.com)

Figure 3. FGFR4 and FGF19 overexpression in cancer.

(A) FGFR4 expression level in primary cancer compared to adjacent normal tissues based on pan-cancer TCGA analysis (Cancer Genome Atlas Research, et al., 2013). n: number of tumor samples included in study; FC: FGFR4 mRNA fold change in primary tumors compared to adjacent normal tissues. (B) FGF19 expression level in primary cancer compared to adjacent normal tissues based on pan-cancer TCGA analysis. Cancers with FGFR4 or FGF19 expression fold change more than 1.5 and reported in literature or with fold change more than 2 are presented in A and B. Data accessed via Firebrowse (<http://firebrowse.org>, TCGA data version 2016_01_28). Abbreviations: BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COADREAD, colorectal adenocarcinoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell

carcinoma; KICH, kidney chromophobe; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; SARC, sarcoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma.

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Figure 4. FGFR4 nonsynonymous mutations.

(A) Different types of FGFR4 nonsynonymous mutations with frequency ≥ 3 in the Cosmic database (Harsha, et al., 2018). * represents nonsense mutation. (B) Mutation rate of FGFR4 across different types of cancer in the MSK-IMPACT database for breast (Razavi, et al., 2018) and other cancers (Zehir, et al., 2017). Only cancers with at least two samples harboring FGFR4 mutations and with frequency $\geq 1\%$ in either primary tumor or metastasis are included. Only in breast cancer, the mutation rate of FGFR4 is significantly higher in metastases versus primary tumors (Fisher exact test, $p=0.0006$). (C) FGFR4 hotspot mutations (N535 and V550) are enriched in breast metastases compared to non-breast

metastases, both in MSK-IMPACT (Fisher exact test $p=5.8e-6$) and Foundation Medicine (Fishes exact test $p<2.2e-16$) database. **(D)** FGFR4 hotspot mutations are enriched in metastatic invasive lobular carcinoma versus invasive ductal carcinoma (MSK-IMPACT Fisher exact test $p=0.02$; Foundation Medicine: Fisher exact test $p<0.0007$). Figures C and D are modified from Levine et al. (Levine, et al., 2019), using a CC-BY 4.0 License (<https://creativecommons.org/licenses/by/4.0/>).

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Table 1.

FGF activation potential for FGFR4

FGF subfamily	FGF	FGFR4 activity
FGF1 subfamily	FGF1	High
	FGF2	High
FGF4 subfamily	FGF4	High
	FGF5	Weak
	FGF6	High
FGF7 subfamily	FGF3	Weak
	FGF7	Weak
	FGF10	Weak
	FGF22	Weak
FGF8 subfamily	FGF8	High
	FGF17	High
	FGF18	Medium
FGF9 subfamily	FGF9	Weak
	FGF16	Weak
	FGF20	Weak
FGF19 subfamily	FGF19	Weak
	FGF21	Weak
	FGF23	Weak

Note: **1.**The table above is based on two studies from Ornitz and Zhang (Ornitz, et al., 1996; X. Zhang, et al., 2006); **2.**Activity was measured in BaF3 cells expressing exogenous FGFRs. Mitogenic activity after FGF stimulation was used for evaluating the response. **3.**The biological response of FGF19 family was measured without the co-expression of beta-Klotho, but with increased concentration of FGFs and heparin. NB: Without beta-Klotho expression, it is difficult to interpret the FGF19 subfamily binding affinity for FGFR4. **4.**FGFR4 tested in this study is transcript variant 2 in NCBI database (NM_022963.3), which contains two Ig-like loops in the extracellular domain.

Table 2

Current clinical trials with a selective FGFR1–3 inhibitor, pan-FGFR inhibitor or FGFR4-specific inhibitor.

Drug name (company)	IC/EC 50 (nM)	ClinicalTrials.gov Identifiers	Conditions	Drugs combined	Phase	Reference
FGFR1–3 selective inhibitors						
AZD4547 (AstraZeneca)	FGFR1: 0.2	NCT02546661	Urothelial Cancer	Durvalumab	I	(Gavine, et al., 2012)
	FGFR2: 2.5	NCT02664935	Lung Cancer	No	II	
	FGFR3: 1.8	NCT02965378	Lung Cancer	No	II / III	
	FGFR4: 165	NCT02117167	Lung Cancer	No	II	
Debio1347 (Debiopharm)	FGFR1: 9.3	NCT01948297	Solid Tumors	No	I	(Brichory, et al., 2017; Nakanishi, et al., 2014)
	FGFR2: 7.6	NCT03344536	Metastatic Breast Cancer	Fulvestrant	I/II	
	FGFR3: 22	NCT03834220	Solid Tumors	No	II	
	FGFR4: 290					
E7090 (Eisai)	FGFR1: 0.7	NCT02275910	Solid Tumors	No	I	(Watanabe Miyano, et al., 2016)
	FGFR2: 0.5	NCT04238715	Cholangiocarcinoma	No	II	
Pemigatinib/INCB054828 (Incyte)	Cells with FGFR1–3 alterations: 30–50 nM	NCT04088188	Cholangiocarcinoma	Gencitabine +Cisplatin	I	(P. C. Liu, et al., 2015)
		NCT03235570	Solid Tumors	No	I	
		NCT03822117	Solid Tumors	No	I	
		NCT02393248	Solid Tumors	Chemo or Targeted Agent	I/II	
	Cells without FGFR1–3 alterations: >1500 nM	NCT03011372	Myeloproliferative Neoplasms	No	II	
		NCT02872714	Urothelial Cancer	No	II	
		NCT02924376	Cholangiocarcinoma	No	II	
		NCT04003623	Solid Tumors	No	II	
		NCT04096417	Colorectal Cancer	No	II	
		NCT03914794	Urothelial Cancer	No	II	
		NCT04003610	Urothelial Cancer	Pembrolizumab	II	
		NCT03656536	Cholangiocarcinoma	No	III	
		NCT04258527	Solid Tumor	No	I	

Drug name (company)	IC/EC 50 (nM)	ClinicalTrials.gov Identifiers	Conditions	Drugs combined	Phase	Reference
		NCT04256980 NCT04294277	Cholangiocarcinoma Urothelial Cancer	No No	II II	
Pan-FGFR inhibitors						
BGJ398/Infigratinib (Novartis)	FGFR1: 0.9	NCT04228042	Urothelial Cancer	No	I/II	(Guagnano, et al., 2011)
	FGFR2: 1.4	NCT02150967	Cholangiocarcinoma	No	II	
	FGFR3: 1	NCT04197986	Urothelial Cancer	No	III	
	FGFR4: 60	NCT03773302	Cholangiocarcinoma	No	III	
		NCT04233567	Solid tumors	No	II	
Erdafitinib/JNJ-42756493 (Janssen)	FGFR1: 1.2	NCT03238196	Metastatic Breast Cancer	Fulvestrant +Palbociclib	I	(Perera, et al., 2017)
	FGFR2: 2.5	NCT03473743	Urothelial Cancer	JNJ-63723283	I / II	
	FGFR3: 3.0	NCT03210714	Pediatric Tumors	No	II	
	FGFR4: 5.7	NCT02699606	Solid Tumors	No	II	
		NCT02365597	Urothelial Cancer	No	II	
		NCT02952573	Multiple Myeloma	Dexamethasone	II	
		NCT04083976	Solid Tumors	No	II	
		NCT04172675	Urothelial Cancer	No	II	
		NCT03827850	Lung Cancer	No	II	
		NCT03390504	Urothelial Cancer	No	III	
PRN1371 (Principia)	FGFR1:0.6 FGFR2: 1.3 FGFR3: 4.1 FGFR4: 19.3	NCT02608125	Solid Tumors	No	I	(Brameld, et al., 2017)
Rogaratinib/BAY 1163877 (Bayer)	FGFR1: 12–15	NCT03517956	Solid Tumors	Copanlisib	I	(Collin, et al., 2018)
	FGFR2: <1	NCT01976741	Solid Tumors	No	I	
	FGFR3: 19	NCT03788603	Solid Tumors	No	I	
	FGFR4: 33	NCT03473756	Urothelial Cancer	Atezolizumab	I/II	
		NCT04125693	Solid Tumors	No	II	
		NCT03762122	Lung Cancer	No	II	
		NCT04077255	Gastric Cancer	Paclitaxel	II	
		NCT03410693	Urothelial Cancer	No	II / III	

Drug name (company)	IC/EC 50 (nM)	ClinicalTrials.gov Identifiers	Conditions	Drugs combined	Phase	Reference
TAS120/Eutibatinib (Taiho)	FGFR1: 3.9 FGFR2: 1.3 FGFR3: 1.6 FGFR4: 8.3	NCT03088059 NCT02052778 NCT04024436 NCT04189445 NCT04093362 NCT03784014	HNSCC Solid Tumors Metastatic Breast Cancer Solid/Myeloid/Lymphoid Cholangiocarcinoma Soft Tissue Sarcoma	No No Fulvestrant No No No	II I / II II II III III	(Kalyukina, et al., 2019)
FGFR4 specific inhibitors						
Blu-554 / Fisolatimib (Blueprint Medicines)	FGFR4: 5	NCT02508467	HCC	No	I	(R. Kim, et al., 2017)
H3B-6527 (H3 Biomedicine)	FGFR4:<1.2	NCT02834780	HCC/Cholangiocarcinoma	No	I	(Joshi, et al., 2017)
INCB062079 (Incyte)	FGFR4: FGF19+ cells <200 nM FGF19-cells >5000 nM	NCT03144661	HCC/Advanced Solid Tumors	No	I	(P. C. C. Liu, et al., 2017; Ruggeri, et al., 2017)