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A place for precision medicine in bladder cancer: targeting the FGF receptors

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Running head: Targeting FGF receptors in bladder cancer

ABSTRACT

Bladder tumours show diverse molecular features and clinical outcome. Muscle-invasive bladder cancer has poor prognosis and novel approaches to systemic therapy are urgently required. Non-muscle-invasive bladder cancer has good prognosis, but high recurrence rate and the requirement for life-long disease monitoring places a major burden on patients and healthcare providers. Studies of tumour tissues from both disease groups have identified frequent alterations of FGF receptors, including mutations of *FGFR3* and dysregulated expression of FGFR1 and FGFR3 that suggest that these may be valid therapeutic targets. We summarize current understanding of the molecular alterations affecting these receptors in bladder tumours, preclinical studies validating them as therapeutic targets, available FGFR-targeted agents and results from early clinical trials in bladder cancer patients.

Keywords: Bladder, urothelial, cancer, FGF receptors, FGFR1, FGFR3, targeted therapy

Bladder cancer: diverse diseases with major unmet clinical needs

Urothelial carcinoma of the bladder is the fifth most common cancer in men in Western countries with approximately 380,000 new cases and 150,000 deaths per year worldwide [1]. This is a heterogeneous disease with diverse clinical course and major challenges in management. No new agent for the treatment of advanced bladder cancer has been approved during the past three decades and despite recent advances in molecular understanding, application of targeted therapies remains in its infancy.

The majority of patients (~70%) at diagnosis have non-muscle invasive bladder cancer (NMIBC). This includes tumours that have not penetrated the epithelial basement membrane (stage Ta) and those that invade through the basement membrane into the submucosa, but not into muscle (stage T1). Following transurethral resection, a single instillation of a chemotherapy agent (commonly Mitomycin C) is recommended [2], and patients are then monitored by regular cystoscopy. For those with high risk NMIBC (e.g. multifocal disease, high grade or stage T1) [3] a course of BCG instillations is given. Recurrence of NMIBC is common, often on multiple occasions over many years, but only a relatively small proportion (15-20%) progress to muscle-invasive disease and these are predominantly patients with stage T1 tumours [4]. Because the disease does not progress in most cases, prevalence of NMIBC is high and the need for repeated monitoring and surgery makes this the most expensive of all cancers to treat [5, 6]. New approaches to localized therapy for this very large group of patients are needed.

Muscle-invasive bladder cancer (MIBC) presents a much less favorable prognosis. Of the 25-30% of patients with muscle invasion at diagnosis, some (~5%) have distant metastases and a significant proportion (25%) have undetected metastatic disease in the regional lymph nodes. Five-year survival rate for patients with locally advanced or metastatic disease is approximately 15% [7]. When no metastatic disease is detected, MIBC is treated by cystectomy or radiotherapy with curative intent. The only approved systemic therapies for locally advanced and metastatic diseases are cisplatin-based chemotherapy combinations [8]. These are also used in the neoadjuvant setting where they provide a small improvement in patient survival [9, 10]. Although approximately 50% of patients with systemic disease initially respond to chemotherapy, duration of response is usually short, and as there are currently no effective second line treatments, prognosis is poor [11].

Molecular studies of bladder cancers have revealed major molecular differences between NMIBC and MIBC [12] and have identified several oncogenic targets that hold promise for therapy. Amongst these, most complete preclinical information is available for the FGF receptors. Both FGFR1 and FGFR3 are implicated as oncogenes with a role in urothelial cancer and early trials of FGFR-targeted agents are currently underway. Other molecular targets identified in MIBC have recently been described in detail [13] and will not be discussed here, though it is likely that combination therapies targeting some of these and FGFRs will be required in the future. Here, we focus on how FGF receptors and their ligands are altered in bladder cancers, the preclinical evidence that they are rational therapeutic targets, the therapies available, considerations for patient selection for therapy and potential mechanisms of resistance.

FGF receptor structure

FGF receptor signaling plays major roles in embryonic development and in regulation of cell proliferation, differentiation, migration, angiogenesis and tumorigenesis in the adult organism. The receptor family includes four highly conserved receptor tyrosine kinases (FGFRs 1-4) that share a common structure comprising an extracellular portion with three immunoglobulin (Ig)-like domains, a hydrophobic transmembrane domain and an intracellular split kinase domain (Figure 1A). In conjunction with heparan sulphate proteoglycans (HSPG) the receptors bind FGF ligands, leading to receptor dimerization and autophosphorylation and activation of downstream signaling pathways [14]. Eighteen biologically active FGFs have been described (FGF1-FGF10, FGF16-FGF23). These bind to Ig-like domains II and III [15], with specificity conferred not only by the receptor family member and ligand but also by alternative splicing of the receptors [16]. For example, the C-terminal half of FGFR3 Ig domain III may be encoded by either of two exons, resulting in two isoforms, FGFR3-IIIb and FGFR3-IIIc [17, 18]. A fifth receptor (FGFRL1) lacks the intracellular kinase domain and is predicted to influence FGFR ing as a decoy receptor [19]. The receptors and their isoforms are expressed in a cell- and tissue-related manner, leading to specific roles in different tissues and at different stages in development.

Multiple mechanisms increase FGFR3 signaling in bladder tumours

FGFR3 point mutation

FGFR3 is aberrantly activated by several mechanisms in bladder cancer. Activating missense mutations were the first mechanism identified. To date, 13 different missense mutations have been reported [20-28], with three alterations, (R248C, S249C, and Y375C) accounting for more than 85% of mutations (Figure 1A). These three mutations generate novel cysteine residues and have been proposed to activate the receptor via ligand-independent dimerization resulting from the formation of disulphide bonds [29]. Ectopic expression of these mutant forms in normal urothelial cells has shown that S249C- and Y375C-FGFR3 proteins are constitutively phosphorylated. Phosphorylation of S249C-FGFR3 is not enhanced following ligand stimulation but Y375C-FGFR3 shows some ligand dependence [30]. Recent findings using Förster Resonance Energy Transfer (FRET) to examine dimerization of R248C, S249C and Y373C (equivalent to Y375C in the IIIb isoform), found only modest stabilisation of the dimer, suggesting that structural perturbation rather than constitutive dimerization may play an important role [31]. These features could have critical importance in selection of approaches to target these proteins. Infrequently (~1%), mutations are identified in the kinase domain (K652E/M/Q/T). Ectopically expressed K652E is constitutively phosphorylated in urothelial cells, but is not dimerized [30], and structural analyses indicate that this mutation introduces hydrogen bonds that act to stabilise the active state conformation [32].

Activating point mutations are most common in bladder tumours of low grade and stage [22, 25, 33]. 86% of papillary urothelial neoplasms of low malignant potential (PUN-LMP) and low-grade papillary urothelial carcinomas (LG-PUC) according to the 2004 WHO classification system [34] were found to contain a mutation [35]. Mutations are also found in urothelial papilloma [35], a predicted precursor for low-grade urothelial carcinoma, suggesting an early role in the development of NMIBC. Fewer MIBC (12-16%) contain mutations [22, 33] and mutations have not been identified in carcinoma *in situ* (CIS), which is a precursor for MIBC [36]. *FGFR3* mutation is also common in upper tract urothelial tumours [37, 38], where it is more common in tumours of ureter than renal pelvis [37].

Stage T1 tumours, which penetrate the urothelial basement membrane, have an intermediate mutation frequency [33, 39]. Whilst it is clear that stage Ta and MIBC are distinct at the molecular level, the status of T1 tumours remains unclear. Molecular analysis typically reveals features of both the major groups. For example, analysis of *TP53* mutation (a feature of MIBC) and *FGFR3,* revealed mutation frequencies of 58% and 17% respectively. Overall, 49% had *TP53* mutation alone, 7.6% had *FGFR3* mutation alone and 9% had both mutations [39].

Mutant forms of FGFR3 are highly oncogenic in rodent fibroblasts [30, 40]. Although these are not sufficient to induce cell transformation in human telomerase-immortalised normal urothelial cells (TERT-NHUC), expression of mutant FGFR3 stimulates continued proliferation at higher density, with relative effect proportional to the frequency of specific mutations in bladder tumours (S249C>Y375C>K652E=wildtype). Cell-cell and cell-matrix adhesion is also altered in TERT-NHUC expressing point mutant forms of FGFR3 [41]. Such alterations may be critical early in the development of papillary NMIBC.

FGFR3 fusion proteins

A second FGFR3-activating mutational event involves the generation of fusion proteins. These were initially identified in bladder cancer cell lines and bladder tumour tissues that expressed aberrant high molecular weight forms of FGFR3 [42]. Several subsequent studies have reported similar fusions [43-45]. The fusions create FGFR3-TACC3 and FGFR3-BAIAP2L1 chimeric proteins that contain the entire sequence of FGFR3 apart from the final exon (amino acids 1-760) fused in-frame to different C-terminal regions of the fusion partners (Figure 1B). To date nine FGFR3-TACC3 fusions and five FGFR3-BAIAP2L1 fusions have been reported in bladder tumour cell lines and bladder tumours. None contained activating point mutations. As the majority of samples analyzed have been MIBC, any association with tumour grade or stage is not yet apparent, though the very high frequency of point mutations in low-grade tumours suggests that fusions may be more common in tumours of stage T1 and higher. This is compatible with the finding of FGFR3-TACC3 fusions in 5 of 59 high-grade but in no low-grade upper tract tumours (0 of 23) [38].

FGFR3 fusions with TACC3 and BAIAP2L1 are highly oncogenic in immortal rodent fibroblasts, inducing morphological transformation, anchorage independence and tumorigenicity [42, 45]. The contribution made

by the fusion partners is not fully elucidated, but the presence of a coiled-coil domain in TACC3, which is retained almost intact in all fusion proteins indicates that constitutive dimerization is likely to be important for their function. Indeed, FGFR3-TACC3 fusions show ligand-independent activation and some constitutive dimerization [42]. Similarly BAIAP2L1 contains a BAR (Bin-Amphiphysin-Rvs) domain which includes a coiled-coil region, and oncogenic functions of FGFR3-BAIAP2L1 depend on dimerization via the BAR domain [45]. As the BAR domain is predicted to have specific interaction with membranes [46], it is possible that FGFR3-BAIAP2L1 fusions may show activity in altered cellular locations. TACC3 in complex with clathrin and ch-TOG (colonic hepatic tumour overexpressed gene) localises to centrosomes and to the mitotic spindle [47] and thus it has been suggested that re-localised FGFR3-TACC3 may contribute to the development of aneuploidy [48]. However, as the majority of FGFR3-TACC3 fusion proteins lack TACC3 Ser 558, whose phosphorylation by Aurora kinase is required for TACC3-clathrin-ch-TOG complex formation [47], this remains unclear.

Isoform switching and upregulated expression

Exquisitely fine-tuning of FGFR activity is required for normal physiological processes, and multiple layers of regulation of expression, activity and downstream signaling have evolved to achieve this. Dysregulation of some of these mechanisms are implicated in bladder cancer.

In normal urothelial cells, FGFR3 is expressed at a low level, but more abundantly than other members of the FGF receptor family and this is exclusively as the IIIb isoform, which mainly binds FGF1 [16]. A splice variant (Δ8-10) encoding a secreted form of FGFR3 lacking the transmembrane domain is also expressed. This shows regulated expression in relation to proliferative state and appears to perform a negative regulatory function, possibly by binding and sequestering FGFs or by binding to and inhibiting signaling by full-length receptors [49]. In bladder cancer cell lines that have detectable FGFR3 expression, some show a relative reduction in expression of full-length IIIb and Δ8-10 isoforms and a splicing switch to the full-length IIIc isoform, normally expressed by mesenchymal cells [49]. This can bind a wider range of FGF ligands [50], which may allow aberrant paracrine or autocrine signaling.

In normal urothelium, FGFR3 protein is barely detectable by immunohistochemistry [51]. However, upregulated expression is detected in a proportion of bladder tumours of all grades and stages. There is significant association with the presence of a mutation [51-53] and consequently a higher proportion of NMIBC show upregulated expression [51-55]. Although mutations are less frequent in stage T1 and MIBC, more than 40% of these tumours also show upregulated expression [51, 53]. Figure 2 shows the distribution of *FGFR3* point mutations and protein expression according to tumour grade and stage in a one-year cohort of newly diagnosed bladder cancers from a single institution [51]. It is possible that some tumours lacking point mutations but with high levels of FGFR3 expression contain FGFR3 fusion proteins, but this has not yet been examined systematically.

The exact mechanisms of FGFR3 overexpression in bladder cancer are not fully clarified. Upregulated expression of oncogenic proteins is often caused by gene amplification. However, high-level amplification

of *FGFR3* has not been reported, and low-level gain of the 4p16.3 region is found only at low frequency. In a study of 84 stage T1 tumours, three or more copies of *FGFR3* were detected in only 6 cases, though upregulated protein expression was detected in 63% [53]. Similarly in MIBC and metastatic lesions, low frequencies of copy number gain were observed [56], which are insufficient to account for the relatively high frequency of upregulated expression of wildtype FGFR3 in these tumours.

MicroRNAs that target FGFR3 including miR-99a and -100 are downregulated in bladder tumours, particularly NMIBC, and there is inverse correlation of their expression and FGFR3 [57]. These microRNAs also appear to play a role in some MIBC, where a subset of invasive tumours with mutation or upregulated expression of FGFR3 is characterised by loss of expression of miRs-99a/100 [43]. FGFR3 fusion transcripts all lack the 5' UTR of FGFR3, which contains recognition sites for these regulatory microRNAs, leading to upregulated expression [58].

Transcription factors implicated in FGFR3 regulation include HIF-1α, which induces FGFR3 upregulation under hypoxic conditions [59] and the p53 family transcription factors p73 and p63 [60]. p63 is expressed in basal and intermediate cell layers in the normal urothelium and is widely expressed in low-grade/stage tumours [61], where it is associated with upregulated expression of FGFR3 [62]. High levels of p63 are also expressed in some MIBC [61-63], though it is not clear whether this is also the subgroup that contains *FGFR3* mutation or upregulation.

FGFR3 status and prognosis

In NMIBC, where the frequency of *FGFR3* mutation is highest, several studies have assessed the relationship to recurrence and disease progression. In stage Ta tumours overall, several studies indicate that those with mutation appear at lower risk of recurrence and progression [25, 64]. Similarly, in stage T1 tumours, favourable outcome is associated with the presence of mutation [65]. In upper tract invasive tumours, the presence of *FGFR3* mutation also indicates better survival [37]. It is suggested that the inclusion of *FGFR3* mutation analysis can add valuable information to the currently used grading and staging systems [66, 67].

In MIBC, the presence of *FGFR3* mutation was found to be associated with a higher frequency of *CDKN2A* deletion than in other MIBC [68] and this was confirmed in the recent TCGA study of MIBC [43]. Interestingly, in *FGFR3*-mutated NMIBC but not in wildtype tumours, hemizygous or homozygous deletion of *CDKN2A* was a predictor of disease progression that was independent of tumour grade and stage [68]. Data from this study is shown in Figure 3. These findings strongly suggest that MIBC with *FGFR3* mutation and *CDKN2A* deletion represent tumours that have progressed from NMIBC. It is not yet clear whether such patients have distinct disease outcome.

FGFR3 signalling and its consequences

FGFR dimerization, kinase activation and trans-autophosphorylation lead to context-dependent activation of downstream signalling pathways [69] (Figure 4). Binding and phosphorylation of FGFR substrate 2 (FRS2) [70] leads to recruitment of the adapter proteins growth factor receptor-bound 2 (GRB2) and son of sevenless (SOS) which activates the RAS/MAPK pathway [71]. Binding of GRB2-associated binding protein 1 (GAB1) recruits PI3K, which leads to activation of AKT1. Independently of FRS2, PLCγ binds to FGFR3 leading to generation of diacylglycerol and activation of protein kinase C (PKC)[72]. STAT [73, 74] and RSK2 [75] are also activated in some cell types.

In cultured normal human urothelial cells, expression of point mutant FGFR3 leads to constitutive activation of the RAS/MAPK pathway and PLCγ signalling but not activation of the PI3K pathway or SRC, both of which are strongly activated when these proteins are expressed in mouse fibroblasts [30]. Activation of the RAS/MAPK pathway by FGFR3 provides a rationale for the finding that mutations in *FGFR3* and the RAS genes are mutually exclusive in bladder cancer [76]. Both TACC3 and BAIAP2L1 fusions also promote RAS/MAPK signalling [42, 45, 77]. BAIAP2L1 fusions but not TACC3 fusions have also been reported to promote STAT1 phosphorylation [77].

The effects of FGFR activation are highly context dependent. Thus it cannot be assumed that the same pathways are activated in all urothelial tumours as in normal urothelium. Indeed, a range of responses is possible depending on FGFR3 isoform, the availability of docking and effector proteins and the presence of other mutations.

Transcriptional profiling of a bladder tumour cell line that contains an FGFR3-TACC3 fusion before and after FGFR3 knockdown, identified an expression signature linking FGFR3 to *de novo* sterol and lipid biosynthesis and metabolism [78], and this phenotype was confirmed in a second cell line containing the most common point mutant form of FGFR3 (S249C). Interestingly, these effects were inhibited by PI3K and mTORC1 inhibitors but not a MEK inhibitor, indicating that downstream signalling in some tumour cells is distinct from that in normal urothelial cells. Compatible with this, some tumours with *FGFR3* mutation in the absence of PI3K pathway mutations were associated with immunohistochemical detection of phospho-AKT [79].

Downstream signalling by FGFR3 fusions is almost certainly altered but this has not been examined in detail. The last exon of FGFR3, that is lost in all fusions identified in bladder tumours, includes Y762 that is implicated in PLCγ activation [72] and p85 binding [80], and part of a region (amino acids 589-806) involved in interaction with and phosphorylation of TGFβ-activated kinase 1 (TAK1). Interaction with TAK1 and its phosphorylation has been shown to lead to activation of NF□B [81]. Thus, it is predicted that downstream signalling by these fusions will differ from that of intact FGFR3.

Signalling by FGF receptors is subject to a range of feedback regulatory mechanisms, loss of which may affect signalling. These include the Sprouty proteins (SPRY 1-4) that are upregulated in response to FGFR

signalling through MAPK and provide feedback inhibition by binding to adapter proteins GRB2 and SOS1 [82]. Regulation of Sprouty turnover by CBL may also play a role [83], as may changes in expression of key regulatory proteins such as FRS2 or phosphatases that act on FGFR3 [84, 85]. Other potential negative regulatory proteins include SEF and DUSP proteins [86]. Detailed discussion of these aspects is beyond the scope of this review but consideration of these complex regulatory mechanisms may ultimately be important in the interpretation of clinical responses to inhibition of FGFRs.

Taken together, mutation and expression data indicate that more than 80% of NMIBC and more than 40% of MIBC have activated FGFR3 signalling [51] and these frequencies may be even higher if elevated ligand expression (see below), altered isoform expression and alterations to regulatory mechanisms are included.

FGFR1 alterations in bladder cancer

FGFR1 has been less intensively studied than FGFR3. Activating mutations have not been reported and DNA amplification appears relatively infrequent [87, 88]. A single fusion (FGFR1-NTM) has been reported following targeted next generation sequencing in a case of relapsed MIBC, though its structure was not described [88]. However increased expression at mRNA and protein level is found in a large proportion of tumours [89, 90]. A similar splicing mechanism to that found in FGFR3 gives rise to IIIb and IIIc isoforms that differ in the composition of the second half of Ig loop III [91]. Two other major splice variants of FGFR1 exist; FGFR1β, which lacks the first Ig loop and FGFR1α which retains the entire extracellular region [92].

Bladder tumours were found to express exclusively the FGFR1 IIIc isoform, but diversity in expression of α and β isoforms was identified with a significant increase in the FGFR1β: FGFR1 α mRNA ratio with increasing tumour grade and stage [90]. Ectopic expression of either isoform in TERT-NHUC led to activation of MAPK and several other signaling pathways. Multiple FGF ligands led to ERK activation in these cells and by titration of FGF, it was found that FGFR1β was more sensitive to low concentrations of FGF1, indicating the potential importance of the change in ratio of these isoforms in MIBC [90].

Activation of ectopically expressed FGFR1α by FGF2 in TERT-NHUC led to increased proliferation and decreased apoptosis that was dependent on both direct MAPK activation, and indirect activation via PLCγ [89]. However, the effects of FGFR1 activation in bladder cancer cell lines are more striking. In several cell lines, activation of ectopically expressed FGFR1 induced an epithelial-mesenchymal transition (EMT) characterized by change to a more elongated mesenchymal cell shape, increased cell motility and invasion. As in normal urothelial cells, multiple signaling pathways were activated and the combined activation of MAPK and PLCγ was required for a full EMT. Induction of COX2 was a major consequence of FGFR1 activation in these cells, leading to increased synthesis of prostaglandin E2 [93]. Compatible with these findings, FGFR1 expression is high in bladder cell lines with mesenchymal phenotype, suggesting a role in

invasion and metastasis. Significantly lower levels are detected in cell lines with FGFR3 upregulation, which show epithelial phenotype [94].

Altered expression of other FGFR receptors and FGFR ligands

Expression of FGFR4 is very low in normal urothelium [49] and has not been reported in bladder tumours. FGFR2 appears to play context dependent oncogenic or tumour suppressive roles. Activating mutations and/or gene amplification are found in endometrial, lung, breast and other cancers [95]. Although bladder cancer was reported in a case of Apert Syndrome (FGFR2-P253R) [96], this mutation has not been found in sporadic bladder cancers [97], and recent genome sequencing studies have not identified mutations elsewhere in the gene. In contrast, decreased FGFR2 expression is found in some bladder tumours. This is associated with decreased survival [98] and with loss of E-cadherin expression, indicating a relationship of loss of expression to a less epithelial phenotype [99]. Indeed, subsequent functional studies of FGFR2-IIIb in bladder tumour cells suggested a tumour suppressor role [100]. However, increased expression of the FGFR2 IIIc isoform in a model of bladder cancer metastasis was related to an epitheloid phenotype and enhanced capability to generate metastases following intracardiac inoculation, indicating a role for FGFR2 in the mesenchymal–epithelial transition (MET) that is required during the later stages of metastatic tumour establishment. Thus FGFR2 may play distinct roles at different stages in bladder tumour progression.

As activation of wildtype FGFR3 and FGFR1 require ligand binding, it is predicted that autocrine or paracrine signalling must operate in tumours with upregulated expression of non-mutant forms of these receptors. Several studies have reported high levels of ligands in patient urine [101-104]. Elevated levels of FGF1 and FGF2 mRNA and/or protein have been detected in tumour tissues, associated with high tumour stage and grade [104-107]. To date there has been no comprehensive analysis of expression of other FGFs. Expression of these FGFs is reported in both tumour cells and stroma, with no detectable expression in normal urothelium [64, 105, 106]. Thus autocrine or paracrine stimulation of upregulated FGFR3 and FGFR1 may drive the transformed phenotype in many cases.

Preclinical evaluation of FGFRs as therapeutic targets in bladder cancer

Therapeutic target validation requires evidence for alteration of the target and for a role as an oncogenic driver upon which tumour cells depend for survival or proliferation. This has been confirmed for both FGFR3 and FGFR1 using gene knockdown approaches and treatment with relevant inhibitors.

Based on frequencies of alterations, it is possible that the vast majority of NMIBC and a significant proportion of MIBC could benefit from FGFR3-targeted therapies. FGFR1 may also be a valid target in many tumours. Table 1 summarises preclinical analyses conducted in a range of tumour cell lines. Many groups have used RT112 and RT4 cell lines that initially were thought to have wildtype FGFR3, but have subsequently been shown to contain FGFR3-TACC3 fusions. All reports indicate sensitivity of these lines,

including IC50 values in the low nanomolar range for some of the FGFR3 selective small molecules (e.g. [108]) and good responses to FGFR3-specific antibodies [109-111]. Dimerization of both wildtype FGFR3 and of mutant forms containing novel cysteine residues is inhibited by an antibody targeting Ig domains II-III [111]. Cell lines with point mutations (e.g. 97-7, UMUC14) also show response, but in general these are less sensitive than those with fusions. Effects of Dovitinib, a multi-targeted kinase inhibitor, on proliferation and colony forming ability were found to be associated with epithelial rather than mesenchymal phenotype [112]. These studies provide encouraging data to support the exploration of FGFR3 as a therapeutic target in clinical studies. However it is noted that in general the anti-proliferative effects of inhibitors are cytostatic (cell cycle arrest in G1 or G0) rather than cytotoxic.

Many bladder tumours and tumour cell lines express elevated levels of FGFR1 [89, 90, 94]. All cell lines expressing high levels were established from MIBC and these have been shown to exhibit a more "mesenchymal" or EMT phenotype with low expression of E-cadherin and high expression of ZEB1. This contrasts with cell lines with "epithelial" phenotype which have high E-cadherin and express FGFR3 [94]. Dependence on FGFR1 has been examined using stable shRNA knockdown and small molecule FGFR inhibitors (PD173074 and NVP-BGJ398) [89, 94] (Table 1) and this has revealed distinct dependencies. Whilst the cell line JMSU1 showed a major dependence on FGFR1 for proliferation, this was not affected in UMUC3. However knockdown of FGFR1 in UMUC3 caused loss of anchorage independence and reduced tumorigenicity *in vivo* [89]. Lack of dependence for proliferation in UMUC3 was confirmed in a later study and a second FGFR1-expressing line (UMUC13) was identified that also lacked FGFR1-dependence for proliferation. However, both of these lines showed FGFR1-dependence for cell invasion and anchorage independent growth. In a highly metastatic derivative of UMUC3, no significant effect of BGJ398 on growth of established orthotopic tumours was measured, but formation of metastases was significantly reduced and was associated with a reduction in the number of circulating tumour cells [94]. Taken together these data indicate that MIBC cells may be FGFR1-dependent for distinct aspects of their phenotype, and suggest that effects on EMT and the metastatic phenotype rather than on cell proliferation may dominate.

Inhibition of FGFR signaling

FGFR inhibitors

The finding of aberrant FGFR signaling in a wide range of cancer types, including breast, lung and prostate [113], has led to major interest in exploitation for therapy and during the past decade, a range of inhibitors has been developed and many early phase clinical trials conducted [114, 115]. Table 2 lists agents in clinical development with activity against FGFR1 and/or FGFR3. These include non-selective and selective small molecule tyrosine kinase inhibitors (TKIs), a monoclonal antibody and an FGF ligand trap.

TKIs inhibit the kinase activity of the receptors by preventing binding of ATP. Initial development was of non-selective TKIs that have most potent activity against PDGF and VEGF receptors but also inhibit related

receptors including FGFRs. These include Dovitinib, Ponatinib, Pazopanib, Nintedanib, Lucitanib, Brivanib and Lenvatinib, which tend to have higher activity against FGFR1 than FGFR3. These have progressed from phase I to phase II or III trials in tumour types other than bladder. Where bladder cancer patients were included in phase I studies, few encouraging results have been noted. Although Dovitinib (TKI258) had shown promise in preclinical studies (Table 1), a recent phase II trial of this agent as second line therapy for advanced bladder cancer with known *FGFR3* mutation status reported disappointing results [116]. All patients discontinued treatment, mainly due to disease progression, no *FGFR3* status-related responses were recorded and the trial was terminated. Brivanib, a VEGFR2 and FGFR1 inhibitor similarly showed disappointing results in patients with advanced bladder cancer [117]. Reports of phase I trials of Nintedanib (BIBF 1120), Lenvatinib (E7080) and Lucitanib did not describe any bladder cancer patients, but these drugs have progressed for other indications.

A phase II trial of Pazopanib as single agent in highly pre-treated patients with advanced urothelial cancer reported partial responses in 7 and stable disease 14 of 41 patients [118]. Tumours from three patients from this trial, including 2 responders and one refractory case have subsequently been analysed for copy number alterations and mutations in a panel of cancer-relevant genes with the aim of identifying molecular signatures of sensitivity. Point mutated *FGFR3* (S249C) was found in the refractory patient and no FGFR-related alterations in the two responders, suggesting that response was related to other targets of this agent, such as VEGFRs or PDGFRs, which are known to be altered in some urothelial cancers. However, another study has reported a durable (> 6 months) response to Pazopanib in a patient whose tumour contained amplified FGF19 and a point mutation in *FGFR3* (S249C) [119]. These studies illustrate the difficulty of identifying predictive biomarkers for multi-targeted agents, particularly in tumour types where more than one of the targets may show alterations compatible with oncogenic driver status.

To limit the toxicities of multitargeted agents and improve potency against the FGFRs, selective FGFR inhibitors have now been developed. Encouraging results have been reported in bladder cancer patients in phase I studies of such drugs. BGJ398 [120] has shown good activity in advanced bladder cancer. In an ongoing phase I study (NCT01004224) for patients with FGFR genetic alterations, 4 of 5 advanced bladder cancer patients with FGFR3 mutation or fusions showed durable responses (> 16 weeks) [121]. Results for a phase I study of JNJ-42756493 in patients with advanced solid tumours are also encouraging [122]. Of 23 patients with FGFR1-4, FGF3 or FGF4 alterations, three bladder cancer patients showed durable responses, one of whose tumours contained an FGFR3-TACC3 fusion and one an FGFR2 truncation.

AZD4547, a selective small molecule inhibitor of FGFRs 1-3 also has reported activity in advanced bladder cancer. In a phase I trial using patient selection based on amplification of FGFRs 1 or 2, two of three bladder cancer patients showed prolonged response. Of these, both had high-level expression of FGFR1 and FGFR3 and one had a ligand binding domain mutation in *FGFR3* [123]. A subsequent publication has reported prolonged progression-free survival of the latter patient on drug (32 months). The mutation detected in this tumour (S236N) is not a known activating mutation and was found to be present in the

germline. Interestingly however, the tumour showed high-level co-amplification of *FRS2* and *MDM2* (12q14-q15), and very high levels of FRS2 mRNA, suggesting that such upregulation may be sufficient to drive FGFR oncogenic addiction in the absence of alteration to the receptors themselves [124]. AZD4547 is currently under evaluation in a phase Ib trial in combination with gemcitabine and cisplatin, with initial dose escalation in any solid tumour type normally treated with Gem/Cis as first line therapy, followed by an expansion phase in advanced bladder cancer (FIESTA; CRUKD/12/009). Other selective inhibitors currently in phase I evaluation include LY287445 [125], which shows good activity against RT112 xenografts, Debio 1347 [126], which demonstrates preclinical activity in SW780, a tumour cell line containing a FGFR3-BAIAP2L1 fusion (Table 1) [45] and TAS-120 [127].

The FGFR3-specific antibody MFGR1877s (R3Mab), which shows excellent activity in preclinical bladder models [111] has undergone phase I studies in advanced solid tumours and multiple myeloma. Long-term stable disease was reported in 5 of 10 bladder cancer patients [128]. Finally, an FGF ligand trap (FP-1039) has been developed, which sequesters FGFs, preventing FGFR activation. This agent uses the extracellular region of FGFR1-IIIc to trap FGFs [129] and is currently undergoing phase Ib study in patients with FGFR1-amplified metastatic non-small cell lung cancer and mesothelioma (NCT01868022).

Multitargeted TKIs induce a wide range of toxicities that mainly reflect their effects on VEGFR, including hypertension, proteinuria and hypothyroidism [130]. FGFR-selective agents have a different toxicity profile, including hyperphosphataemia and tissue calcification due to inhibition of FGF23 signalling, nail toxicity, hair modifications, mucositis, retinal detachment and muscle and joint pains [122]. These effects are reported to be clinically manageable and reversible, but can lead to discontinuation of therapy or dose reduction. Targeting specific FGFRs might alleviate some of these effects. However, apart from the development of an FGFR3-specific antibody, this has not yet been achieved. As clinical studies are at an early stage, strategies to manage these effects are not yet proven and long-term effects of these inhibitors remain unknown.

HSP90 Inhibitors

FGFR3 is an HSP90 client protein. Wildtype, kinase dead and point-mutant FGFR3 all show strong interaction with HSP90 and the co-chaperone Cdc37. As this interaction stabilises FGFR3, inhibition of HSP90 function leads to reduced FGFR3 signalling, its ubiquitination and degradation via the proteasome. Other FGFRs interact more weakly with these chaperones [131]. This suggests that HSP90 inhibitors may be therapeutically beneficial in FGFR3-driven bladder cancer. The HSP90 inhibitor Ganetespib has shown potent activity in bladder tumour cell lines with FGFR3 fusions and point mutations [132]. Importantly, Ganetespib induced apoptosis in RT112, a line that shows only G1 cell cycle arrest in response to FGFR inhibitors. Good activity in the cell line 97-7 (FGFR3-S249C) is also in contrast to the relatively poor response of this cell line to small molecule FGFR inhibitors [108]. Synergy of the HSP90 inhibitor 17-AAG with cisplatin, gemcitabine and docetaxel in bladder tumour cell lines including RT4 (FGFR3-TACC3) has also been reported [133]. Although HSP90 inhibitors have been widely tested in the clinic and have entered

phase II and phase III trials in some cancer types, there has been virtually no representation of bladder cancer patients in phase I studies.

Other approaches to therapy

It is likely that other approaches to therapy for FGFR-driven bladder tumours will emerge as non-canonical interactions and details of downstream signaling are clarified. For example, FGFRs can signal via interactions with other cell surface proteins that do not involve canonical FGF binding [134]. It is also hypothesized that interactions of the FGFR3 fusion proteins with adapters will be different from those of wildtype receptor and may be targetable.

Other possibilities include approaches designed to target the signaling pathways downstream of FGFRs, for example RAS/MAPK and PI3K, that are also relevant for tumours driven by other receptor tyrosine kinases, mutant RAS genes, mutations in components of the PI3K pathway and others. The effect of FGFR1 signaling on COX2-driven EMT also suggests that COX2 inhibition and inhibition of effects downstream of COX2 could have a role in advanced bladder cancer.

Patient selection for FGFR-targeted therapy

Relevant assays to identify FGFR alterations in tissue samples have been developed; *FGFR3* mutations can be detected rapidly in FFPE tissues using primer extension assays [135], mass spectrometry-based approaches [136] or targeted sequencing [137], antibodies to detect upregulated expression of FGFR3 and FGFR1 have been identified, FGFR3-TACC3 fusions can be detected using RT-PCR [42] and FGFR3-BAIAP2L1 fusions detected using RT-PCR or break apart FISH [45]. Thus, selection of patients with some relevant alterations for trials of FGFR-targeted therapies is not a major barrier.

However, other issues may prevent ideal patient selection. For example there may be other unrecognized alterations in the FGFR signalling pathway such as the FRS2 amplification described above, which may represent *bona fide* oncogenic drivers. Importantly, the presence of specific molecular alterations does not guarantee tumor dependence. Initial drivers of the transformed phenotype may become redundant later during tumour progression. Similarly, molecular alterations required for progression may involve alterations that are absent in the primary tumour.

Induction of EMT by FGFR1 signaling in preclinical models [93], suggests that upregulated expression could exist in distant metastases but not in the primary tumor and it will be important to examine this. However, availability of appropriate tissue for this purpose is a problem as samples are most commonly from localized disease taken at transurethral resection or cystectomy that may not be representative of metastatic tissues. Although access to distant metastatic tissues is limited, paired primary tumour and matched regional lymph node metastases can be assessed. This has been done for FGFR3 expression and reveals that in the majority of cases, matched primary tumour and lymph node metastases share the same FGFR3 expression profile [138]. FGFR3 mutation and FGFR1 status have not yet been compared in such samples.

A group of MIBC that may be suitable for FGFR-targeted therapy are those that are predicted to have progressed from NMIBC [68] and contain *FGFR3* mutation or a fusion protein and deletion of *CDKN2A* (encoding p16). These tumours show upregulated expression of FGFR3 and p63 and down-regulation of MiRs-99/100 [43]. As clinical trials of FGFR inhibitors in bladder cancer patients expand, the hypothesis that this subgroup will contain the likely responders can be tested.

A key requirement for the future is better preclinical models. A few relevant cell lines are available (Table 1) but it is unlikely that these represent the full spectrum of tumours that may be suitable for treatment. Patient-derived xenografts (PDXs) or tumour organoids may be superior models. A recent study described several PDXs derived from MIBC, one of which contained an *FGFR3* mutation and showed good response to the FGFR3-specific antibody R3Mab [139]. To date no organoid models of bladder cancer have been reported.

FGFR-targeted therapies for non-muscle-invasive bladder cancer?

The frequency of FGFR3 alteration in NMIBC far exceeds that in MIBC. However, all of the systemic therapies developed to date show toxicity profiles that would not be acceptable in the majority of NMIBC patients, who despite recurrent localized disease usually have long life expectancy. However, in the highest risk group of NMIBC patients, including those who have failed BCG therapy, the possibility of systemic therapy may be considered as an alternative to cystectomy. Of particular interest may be FGFR-specific agents such as FGFR3 targeted antibodies, which may avoid the major toxicities that are associated with inhibition of all FGFRs.

For low-risk disease, one possibility is the development of topical treatments to target disease within the bladder. Such an approach might be used post-resection to eliminate floating tumor cells and tumour cells remaining within the urothelium. This would require re-formulation of existing agents and to date this has not been undertaken. However, the lack of induction of apoptosis by FGFR-targeted agents may indicate that it is unlikely to be efficacious as a sole agent and relevant drug combinations might be required. The use of nanomedicine approaches to target cytotoxic drugs to FGFR-expressing cells is another possibility, yet to be explored. As FGFR3 mutation and expression is so common in these tumours, this is an attractive possibility.

The problem of resistance

In addition to innate resistance to targeted therapy, a major consideration for all treatments involving inhibition of a critical oncogenic driver is the almost inevitable development of acquired resistance. This can arise via acquisition of new mutations in the target or other genes, through epigenetic mechanisms or via plasticity in signaling [140]. Understanding of potential mechanisms of resistance may suggest potential

approaches to avoid its development, for example combination therapies that target likely escape mechanisms.

Two approaches have been used to elucidate potential mechanisms of resistance to FGFR-targeted therapies. Large-scale screens have been conducted to identify mechanisms of escape from FGFR inhibition. Two studies assessed the ability of secreted factors to rescue tumour cell lines from the effects of a range of inhibitors, and included the FGFR3-dependent bladder cancer cell lines RT112 and RT4. RT112 was rescued from the effects of PD173074 by EGFR ligands, NRG1/2 and HGF [141, 142], and RT4 could be rescued by EGF and NRG1 [141]. Subsequently, an RNAi screen to identify mechanisms of escape from FGFR inhibition showed that RT112 was indeed capable of activating an escape mechanism via EGFR. Differential dependencies of bladder cell lines on EGFR and FGFR3 signaling were revealed and excellent responses to dual receptor inhibition in preclinical assays were demonstrated [143].

In a second approach, cell lines with increased resistance to drug have been derived following chronic exposure. Resistant variants of the multiple myeloma line KMS-11, which contains Y373C (equivalent to Y375C in FGFR3 IIIb), were found to contain a mutation in the so-called "gatekeeper" residue (V555M) in the ATP-binding pocket of FGFR3 [144]. This mutant form shows slightly enhanced kinase activity in the absence of ligand, though this is much lower than the activity of the disease-associated kinase domain mutant K650E (IIIc isoform) and is not sufficient for transformation of rodent fibroblasts. Interestingly, it was found that whilst the equivalent mutation in both FGFR1 and FGFR3 confers resistance to the FGFR-selective inhibitors PD173074 and AZD4547, it does not affect sensitivity to the multi-targeted kinase inhibitor TKI258 (Dovitinib). High-resolution crystal structure data indicate that TKI258 occupies a smaller region within the ATP-binding pocket of FGFRs than FGFR1-3 selective inhibitors and the multi-kinase inhibitor Ponatinib [145]. This highlights the potential of TKI258 or related compounds as second line treatments to target gatekeeper mutation-related resistance and the possibility that based on structural data of TKI258 interaction with the gatekeeper mutation, selective inhibitors that retain activity against such mutants can be developed.

Acquired resistance in RT112 cells cultured in BGJ398 was associated with a change to an EMT-like phenotype and increased activation of ERBB2/ERBB3 signaling. The derived resistant cells were sensitive to ERBB family inhibitors (AZD8931 or Lapatinib) and combined treatment of parental RT112 induced greater effects than FGFR inhibition alone. Assessment of secreted factors in the resistant cells identified NRG1 as a major effector of ERBB dependence in the FGFR inhibitor resistant state. Importantly, the induced resistance and EMT phenotype was reversible, indicating significant plasticity in these cells [146]. As similar effects have been recorded after short-term treatment with FGFR inhibitors [143, 146], these data provide a rationale for the use of combinations of FGFR and ERBB family inhibitors in the clinic. It is hoped that current clinical trials with FGFR inhibitors alone will provide further information regarding resistance mechanisms.

Future perspective

To realise the maximum potential of FGFR-related precision medicine in bladder cancer, four key areas require further research during the coming years; identification of optimal criteria for selection of patients for therapy, improved understanding of resistance, identification of mechanisms that can drive cytotoxic rather than cytostatic responses and development of improved preclinical models.

The mutation and expression status of FGFRs in bladder cancer is well described and sufficient information can be gained from formalin-fixed paraffin-embedded tissue samples to allow rational selection of patients for clinical trials involving FGFR-targeted agents. As early data from clinical trials indicates that tumours with *FGFR3* mutation or FGFR3 fusions are responsive to these agents, it is likely that further trials involving patient pre-selection based on these molecular features will now be developed. Trials that include evaluation of FGFR1 are also desirable. However the field does not yet understand the determinants of innate resistance to such agents, or the full spectrum of mechanisms that will inevitably allow escape or the development of resistance in treated patients. Thus, re-evaluation of selection criteria may be required in the future and it will be essential for all trials to include the collection of good clinical samples that will allow relevant retrospective analyses.

Current preclinical data shows that escape from FGFR3 dependence via activation of ERBB pathway signalling may be a major mechanism of both innate and acquired resistance to FGFR inhibitors, and this requires confirmation in patient samples. If confirmed, then combinations with ERBB inhibitors may be indicated. As many of the tumours that contain *FGFR3* mutations also contain mutations that activate the PI3 kinase pathway, it is possible that inhibitors of this pathway may also be relevant. Similarly, the recent identification of a higher frequency inactivating mutations in chromatin modifier genes than are found in other cancer types may suggest combination with epigenetic therapies.

The cytostatic rather than cytotoxic effects of FGFR inhibitors will require combination with agents that pre-empt the development of resistance and facilitate rapid cell killing. Although it is envisaged that some cancers may be converted to chronic and non-life-threatening diseases by long-term treatment with targeted cytostatic agents, existing FGFR inhibitors appear too toxic for long-term treatment. In particular, they are not suitable for systemic treatment of the very large population of NMIBC patients with low and moderate risk disease. Thus a major challenge will be to develop novel agents or re-formulate existing agents to allow intravesical instillation. Localised treatment has the significant advantage that highly toxic combinations may be tolerated, potentially overcoming cytostatic effects.

Perhaps the biggest barrier to evaluation of novel agents and combination therapies in bladder cancer is the lack of well-characterised preclinical models. In the coming years it will be crucial to develop fully-characterised isogenic models for preclinical drug screening and models that allow rapid evaluation of drug sensitivity of patient samples. NMIBC are more genomically stable and contain fewer molecular alterations than MIBC. Thus the feasibility of engineered preclinical model development is higher. Overall, although advanced disease is the indication where novel therapies are most likely to be tested via systemic

administration, there is immense scope for improvement of outcomes and quality of life for patients with NMIBC and this should not be overlooked.

Executive summary

- Bladder cancer comprises two major disease entities, NMIBC and MIBC that differ in their molecular features and have unmet clinical needs.
- Dysregulation of FGFRs has been identified in bladder cancers of all grades and stages.
- In NMIBC, activating point mutations of FGFR3 are common (70%).
- In MIBC, point mutations are less common (12-16%) but many tumours (>40%) show upregulated expression of non-point mutated FGFR3 protein.
- Translocations generating oncogenic FGFR3 fusion proteins containing FGFR3 amino acids 1-760 including the kinase domain fused in frame to various portions of TACC3 or BAIAP2L1 have been identified in some cases (2-5%).
- Isoform switching to a form (FGFR3-IIIc) that binds a wider range of FGF ligands has been found in bladder tumour cell lines but remains to be examined in detail in tumour samples.
- FGFR1 shows upregulated expression in bladder tumours of all grades and stages, with increased ratio of the two major isoforms (β:α) with increasing tumour grade and stage.
- In tumour cell lines FGFR1 activation can induce an EMT suggesting an important role in tumour progression and metastasis.
- Both FGFR3 and FGFR1 can activate PLCγ and MAPK pathway signalling in normal urothelial cells. As FGFR3 fusion proteins lack amino acids that are critical for certain phosphorylation events and interactions with other proteins, it is predicted that their downstream effects are altered.
- Preclinical studies have confirmed FGFR3 as a relevant therapeutic target in bladder cancer. Most cell lines show a cytostatic rather than cytotoxic response.
- A range of inhibitors including selective or multitargeted small molecule inhibitors, antibodies and a ligand trap have been developed and assessed in phase I clinical trials.
- As FGFR3 is an HSP90 client protein, HSP90 inhibitors may also have a role in the clinic.
- Early results indicate durable responses to selective FGFR inhibitors in some bladder cancer patients, particularly those with tumours containing *FGFR3* mutations or fusions.
- Although FGFR3 alterations are most common in NMIBC, existing inhibitors are not suitable for treatment of this patient group due to their toxicity. Thus localised approaches to FGFR inhibition are needed.
- Studies of the mechanisms of resistance to FGFR inhibitors in bladder cancer are in their infancy. Early *in-vitro* data indicate that ERBB receptor signalling may allow escape both during initial treatment and as a mechanism of acquired resistance.

- Mutations in the "gatekeeper" residue of FGFR3 induce resistance to some but not all FGFR inhibitors, suggesting that the development of second-line FGFR inhibitors may be possible.

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Table 1. Preclinical evaluation of FGFR1 and 3 as therapeutic targets.

Approach/treatment	Urothelial cell lines/FGFR status	Results	Reference
FGFR3-specific human single-chain Fv antibodies recognising extracellular domain	RT112; FGFR3-TACC3*	Decreased proliferation	[109]
Immunotoxin fused to FGFR3-specific human single-chain Fv antibodies	RT112; FGFR3-TACC3*	Apoptosis in vitro Delayed and reduced xenograft growth	[110]
Stable knockdown	97-7; S249C	Cell flattening, decreased proliferation, reduced clonogenicity on plastic and in soft agar	[147]
siRNA knockdown Small molecule ATP binding site competitor SU4502 (FGFRs 1 and 3)	MGHU3; Y375C	Decreased proliferation and anchorage independent growth	[40]
Inducible knockdown; Antibody targeting extracellular domain (R3Mab)	RT4 and RT112; FGFR3-TACC3* UMUC14; S249C	Reduced proliferation in vitro Suppression of xenograft growth	[111]
PD173074; small molecule ATP binding site competitor	UMUC14; S249C MGHU3; Y375C	50% inhibition of proliferation at 10 nM (cell cycle arrest) Reduced FGFR3 phosphorylation Inhibition of xenograft growth	[148]
Small molecule ATP binding site competitors: PD173074 (FGFRs 1 and 3), TKI258 (Dovitinib) multi-kinase inhibitor, SU5402 (FGFRs 1 and 3)	Tumour cell line panel with differing FGFR3 and RAS gene status and normal human urothelial cells.	Range of sensitivities. Inhibition of FGFR3 phosphorylation and proliferation. Cells with FGFR3 fusions* more sensitive than those with point mutations. JMSU1 cell line with upregulated FGFR1 expression shown to be sensitive. RAS-mutant cells resistant. Normal cells resistant. Cell cycle arrest in vitro. Inhibition of xenograft growth.	[108]
Ponatinib; Multi-targeted kinase inhibitor inhibiting FGFR1-4	MGHU3; Y375C UMUC14; S249C T24; wildtype FGFR3	Reduced FGF2 phosphorylation Decreased proliferation Suppression of xenograft growth (UMUC14) No effect on T24	[149]
NVP-BGJ398; small molecule ATP binding site competitor FGFRs 1-3	Large panel of cell lines from range of tumour types, including 18 urothelial.	Inhibition of proliferation in four bladder lines; RT112, RT4, SW780*, and JMSU1.	[120]
NVP-BGJ-398	Panel of cell lines with different FGFR3 status.	Inhibition of proliferation of cells expressing high levels of FGFR3 independent of FGFR3 mutation status. Cell cycle arrest	[94]
Dovitinib	Panel of cell lines with measured EMT status	Correlation of effect on proliferation and colony forming ability with epithelial phenotype.	[112]
LY287445	RT112; FGFR3-TACC3	Suppression of xenograft growth	[125]

Debio 1347	UMUC14; S249C RT112; FGFR3-TACC3 SW780; FGFR3-BAIAP2L1 fusion Rat 2 cells expressing FGFR3-BAIAP2L1 fusion	Inhibition of proliferation	[45, 126]
R3Mab	Panel of urothelial tumour cell lines with different FGFR3 status.	Modest in vitro response in RT4 and RT112 (FGFR3-TACC3)*, and UMUC14 (S249C). Highest response in UMUC1 (high expression of wildtype FGFR3). Orthotopic xenografts of RT112, UMUC3 and UMUC1 showed response.	[150]
Stable knockdown PD173074	UMUC3 and JMSU1; high FGFR1 expression.	JMSU1 showed marked dependence on FGFR1 for proliferation and anchorage independent growth. In UMUC3, no effect on proliferation but marked effect on anchorage independent growth and tumorigenicty.	[89]
Stable knockdown NVP-BGJ398	UMUC3 and UMUC13; high FGFR1 expression.	No effect on proliferation but cell invasion and anchorage independence inhibited in both lines.	[94]

* FGFR3 fusions in these cell lines was identified later [42].

Table 2. Targeted fibroblast growth factor receptor agents in clinical development

Drug	Company	Target(s)	IC50 FGFR1 (nM)	IC50 FGFR3 (nM)	Stage of development[1]	Ref.
Multitargeted tyrosine kinase inhibitors						
Dovitinib (TKI258)	Novartis Pharmaceuticals	FGFR1-3, VEGFR, PDGFR, CSF-1R, CKIT, RET, TRKA, FLT3	8	9	Phase III	[116]
Pazopanib	Novartis	VEGFR, PDGFR, FGFR1-3, KIT, LTK, LCK	>100	>100	Phase III	[151]
Ponatinib	ARIAD Pharmaceuticals	BCR-ABL, FGFR1-2, VEGFR2, PDGFRα, KIT, LYN	2.2	18	Phase III	[152]
Lucitanib (E-3810)	Clovis Oncology	VEGFR, FGFR1 and 2	17.5	> 200	Phase II	[153]
Brivanib	Bristol-Myers Squibb	VEGFR, FGFR1	148	-	Phase III	[117]
Nindetanib (BIBF 1120)	Boehringer Ingelheim	VEGFR, FGFR1-3, PDGFR, SRC, LYK, LYN	69	108	Phase III[2]	[154, 155]
Lenvatinib (E7080)	Eisai	VEGFR, PDGFRα, KIT, RET, FGFR1-4	46	52	Phase III	[156, 157]
FGFR-selective tyrosine kinase inhibitors						
BGJ298	Novartis Pharmaceuticals	FGFRs 1-4	0.9	1	Phase II	[120] [121]
AZD4547	Astrazeneca	FGFRs 1-3	0.2	< 5	Phase II	[158] [124]
LY2874455	Eli Lilly and Company	FGFRs 1-4	2.8	6.4	Phase I	[125]
JNJ-42756493	Janssen	FGFRs 1-4	<10	< 10	Phase II[3]	[122] [159]
Debio 1347	Debiopharm	FGFRs 1-3	9.3	22	Phase I	[126]
TAS-120	Taiho Oncology	FGFRs 1-4	<1	<1	Phase I	[160]

FGFR antibodies

MFGR1877S (R3Mab)	Genentech	FGFR3	-	0.3 (binding of FGF1 to FGFR3-IIb)	Phase I	[111] [128]

FGF ligand trap

FP-1039 (GSK3052230)	Five Prime Therapeutics	FGFR1c ligand trap; blocks multiple FGFs	-	-	Phase I	[161]

[1] Trials in any cancer type. Some agents have been approved for specific indications.
[2] In progress, A Phase II Trial of BIBF1120 in Patients With Advanced FGFR3 Mutated, FGFR3 Overexpressed, or FGFR3 Wild Type Urothelial Carcinoma of Urinary Bladder, Urethra, Ureter, and Renal Pelvis and Who Have Failed Platinum-based Chemotherapy (NCT02278978).
[3] In progress, A Phase 2, Two-arm Multicenter, Open-Label Study to Determine the Efficacy and the Safety of Two Different Dose Regimens of a Pan-FGFR Tyrosine Kinase Inhibitor JNJ-42756493 in Subjects With Metastatic or Surgically Unresectable Urothelial Cancer With FGFR Genomic Alterations (NCT02365597).