

FGFR inhibitors: Effects on cancer cells, tumor microenvironment and whole-body homeostasis (Review)

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Abstract. Fibroblast growth factor (FGF)2, FGF4, FGF7 and FGF20 are representative paracrine FGFs binding to heparan-sulfate proteoglycan and fibroblast growth factor receptors (FGFRs), whereas FGF19, FGF21 and FGF23 are endocrine FGFs binding to Klotho and FGFRs. *FGFR1* is relatively frequently amplified and overexpressed in breast and lung cancer, and *FGFR2* in gastric cancer. BCR-FGFR1, CNTRL-FGFR1, CUX1-FGFR1, FGFR1OP-FGFR1, MYO18A-FGFR1 and ZMYM2-FGFR1 fusions in myeloproliferative neoplasms are non-receptor-type FGFR kinases, whereas FGFR1-TACC1, FGFR2-AFF3, FGFR2-BICC1, FGFR2-PPHLN1, FGFR3-BAIAP2L1 and FGFR3-TACC3 fusions in solid tumors are transmembrane-type FGFRs with C-terminal alterations. AZD4547, BGJ398 (infigratinib), Debio-1347 and dovitinib are FGFR1/2/3 inhibitors; BLU9931 is a selective FGFR4 inhibitor; FIIN-2, JNJ-42756493, LY2874455 and ponatinib are pan-FGFR inhibitors. AZD4547, dovitinib and ponatinib are multi-kinase inhibitors targeting FGFRs, colony stimulating factor 1 receptor (CSF1R), vascular endothelial growth factor (VEGF)R2, and others. The tumor microenvironment consists of cancer cells and stromal/immune cells, such as cancer-associated fibroblasts (CAFs), endothelial cells, M2-type tumor-associated macrophages (M2-TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells. FGFR inhibitors elicit antitumor effects directly on cancer cells, as well as indirectly through the blockade of paracrine signaling. The dual inhibition of FGF and CSF1 or VEGF signaling is expected to enhance the antitumor effects through the targeting of immune evasion and angiogenesis in the tumor microenvironment. Combination therapy using tyrosine kinase inhibitors (FGFR or CSF1R inhibitors) and immune checkpoint blockers (anti-PD-1 or anti-CTLA-4 monoclonal antibodies) may be a promising choice for

cancer patients. The inhibition of FGF19-FGFR4 signaling is associated with a risk of liver toxicity, whereas the activation of FGF23-FGFR4 signaling is associated with a risk of heart toxicity. Endocrine FGF signaling affects the pathophysiology of cancer patients who are prescribed FGFR inhibitors. Whole-genome sequencing is necessary for the detection of promoter/enhancer alterations of *FGFR* genes and rare alterations of other genes causing FGFR overexpression. To sustain the health care system in an aging society, a benefit-cost analysis should be performed with a focus on disease-free survival and the total medical cost before implementing genome-based precision medicine for cancer patients.

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1. Introduction

Receptor tyrosine kinases (RTKs) are transmembrane-type receptors with cytoplasmic tyrosine kinase domains (1), which transduce extracellular signals to a variety of intracellular signaling cascades, such as RAS-ERK, PI3K-AKT, IP₃-Ca²⁺ and DAG-PKC (Fig. 1A). Phylogenetic analyses of 518 protein kinases revealed that RTKs are clustered with non-receptor-type tyrosine kinases (2), and analyses of 54 human RTKs using the Clustal Omega program revealed that RTKs are classified into the epidermal growth factor receptor (EGFR) group (EGFR, ERBB2, MET, RYK, etc.), the fibroblast growth factor receptor (FGFR) group [FGFRs, colony stimulating factor 1 receptor (CSF1R), vascular endothelial growth factor (VEGF)R2, etc.], the insulin receptor (INSR) group (INSR, IGF1R, ALK, ROS1, etc.), the RAR-related orphan receptor (ROR) group (ROR1, ROR2, DDR2, NTRK1, etc.) and the EPH receptor (EPH) group (EPHA1, EPHB1, PTK7, etc.) (Fig. 2).

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Since the aberrant activation of RTKs is a driving force of human carcinogenesis, small-molecule inhibitors targeting RTKs have been developed for cancer therapy (3-12). For example, erlotinib and gefitinib target EGFR; afatinib and lapatinib target EGFR and ERBB2; and ponatinib (AP24534) targets multiple RTKs, such as CSF1R, FGFRs, PDGFRs, RET and VEGFRs (Fig. 1B).

FGFR1, FGFR2, FGFR3 and FGFR4 constitute the FGFR family of RTKs with three immunoglobulin-like domains in the extracellular region (13-16). FGF1 (acidic FGF), FGF2 (basic FGF), FGF3-FGF10, FGF16, FGF17, FGF18, FGF20 and FGF22 bind to heparin-sulfate proteoglycan for paracrine signaling through FGFRs, whereas FGF19, FGF21 and FGF23 bind to Klotho proteins for endocrine signaling through FGFRs. FGFRs are involved in the regulation of cell survival, proliferation, differentiation and motility during embryogenesis, adult-tissue homeostasis and carcinogenesis (17-20).

Gene amplification, gain-of-function coding mutation and gene fusion are three major classes of *FGFR* alterations in human cancer (Fig. 3) (14,21-24). Clinical trials of several tyrosine kinase inhibitors (TKIs) targeting FGFRs are ongoing (25-28), while TKI resistance and tumor-stromal interaction related to FGFRs are hot issues (29-32). Knowledge of FGFRs has been exponentially growing as a result of the advancement of massively parallel sequencing technology combined with the global trend toward translational medicine. In this review, recent progress in the field of FGFR medicine is reviewed with emphases on *FGFR* alterations in human cancer, the classification of small-molecule FGFR inhibitors and the effects of FGFR inhibitors on the tumor microenvironment and whole-body homeostasis.

2. *FGFR* genetic alterations in human cancer

FGFR alterations in major cancers. Lung cancer is the most common malignancy worldwide (33), which is histologically classified into adenocarcinoma, large cell carcinoma, small cell carcinoma and squamous cell carcinoma. *FGFR1* amplification preferentially occurs in squamous cell lung cancer (34); 9.3% of stage I cases, 22% of stage II cases (35) and 19% of stage IV cases with brain metastasis (36). *FGFR2-CCAR2*, *FGFR2-CIT* and *FGFR3-TACC3* fusions and gain-of-function mutations of *FGFR2* (A266_S267insSTVVGDD and 290_291WI>C) are also observed in lung cancer (37-39). Aberrant FGFR signaling may promote the proliferation and survival of tumor cells in the early stage, and invasion and metastasis in the later stage. As smoking is a risk factor of squamous cell lung cancer, the smoking-induced DNA damage of bronchial epithelial cells may cause lung carcinogenesis in part through *FGFR1* gene amplification.

Breast cancer is the second most common malignancy worldwide (33). Breast cancers have been classified based on immunohistochemical analyses of estrogen receptor (ER), progesterone receptor (PR) and ERBB2 (HER2) receptor. The prognosis for luminal breast cancer (ER⁺) is better than that for non-luminal HER2⁺ breast cancer (ER/PR/HER2⁺) and triple-negative breast cancer (ER/PR/HER2⁻) (40). Endocrine therapy using tamoxifen or aromatase inhibitors is the standard therapy for patients with luminal breast cancer without lymph node metastasis; however, recurrence as a result of resistance

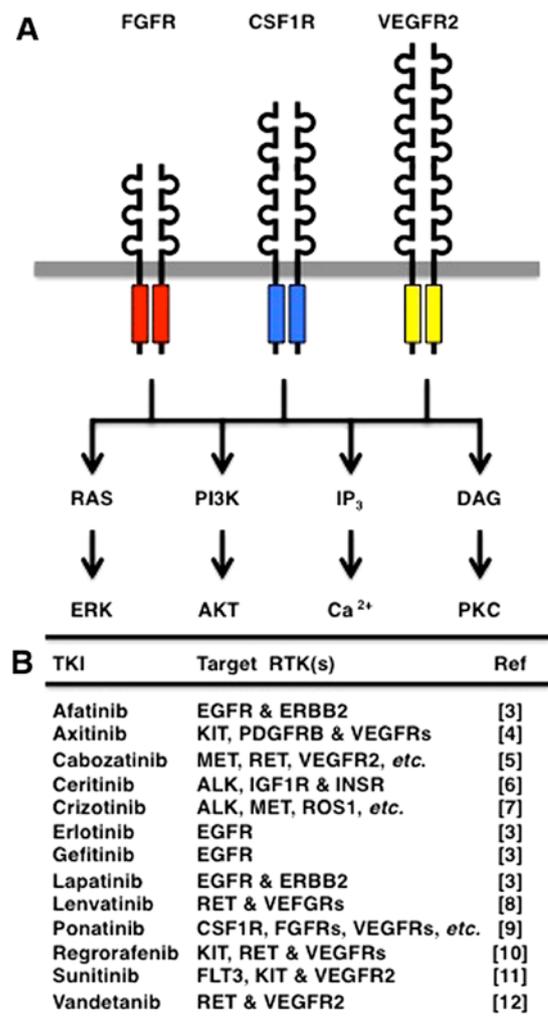


Figure 1. Receptor tyrosine kinase (RTK) signaling cascades and RTK inhibitors. (A) RTK signaling cascades. Multiple RTKs, such as fibroblast growth factor receptors (FGFRs), CSF1R and VEGFR2, redundantly activate the RAS-ERK, PI3K-AKT, IP₃-Ca²⁺ and DAG-PKC signaling cascades. (B) Representative tyrosine kinase inhibitors (TKIs) and their targets.

to endocrine therapy is a serious issue in clinical practice (41). *FGFR1* gene amplifications and *FGFR2* gain-of-function missense mutations are detected in circulating tumor cells of two cases and one case of breast cancer patients, respectively (42,43). The *FGFR1* gene is more frequently amplified in metastatic breast cancer than the *FGFR2* and *FGFR3* genes (44), which leads to FGFR1 overexpression and resistance to endocrine therapy (45). The preclinical studies mentioned above indicate that FGFR1-targeted therapy is applicable for a subset of breast cancer patients.

Gastric cancer is the fifth most common malignancy worldwide, although its incidence and mortality have been decreasing (33,46). The amplifications of genes encoding RTKs, such as *EGFR*, *ERBB2*, *FGFR2* and *MET*, occur in gastric cancer (47,48). Gastric cancer with *FGFR2* amplification is significantly associated with lymphatic invasion and a poor prognosis (49,50); however, the molecular mechanisms through which *FGFR2* amplification promotes lymph node metastasis remain unclear. Preclinical studies using small-molecule FGFR2 inhibitor and patient-derived cancer

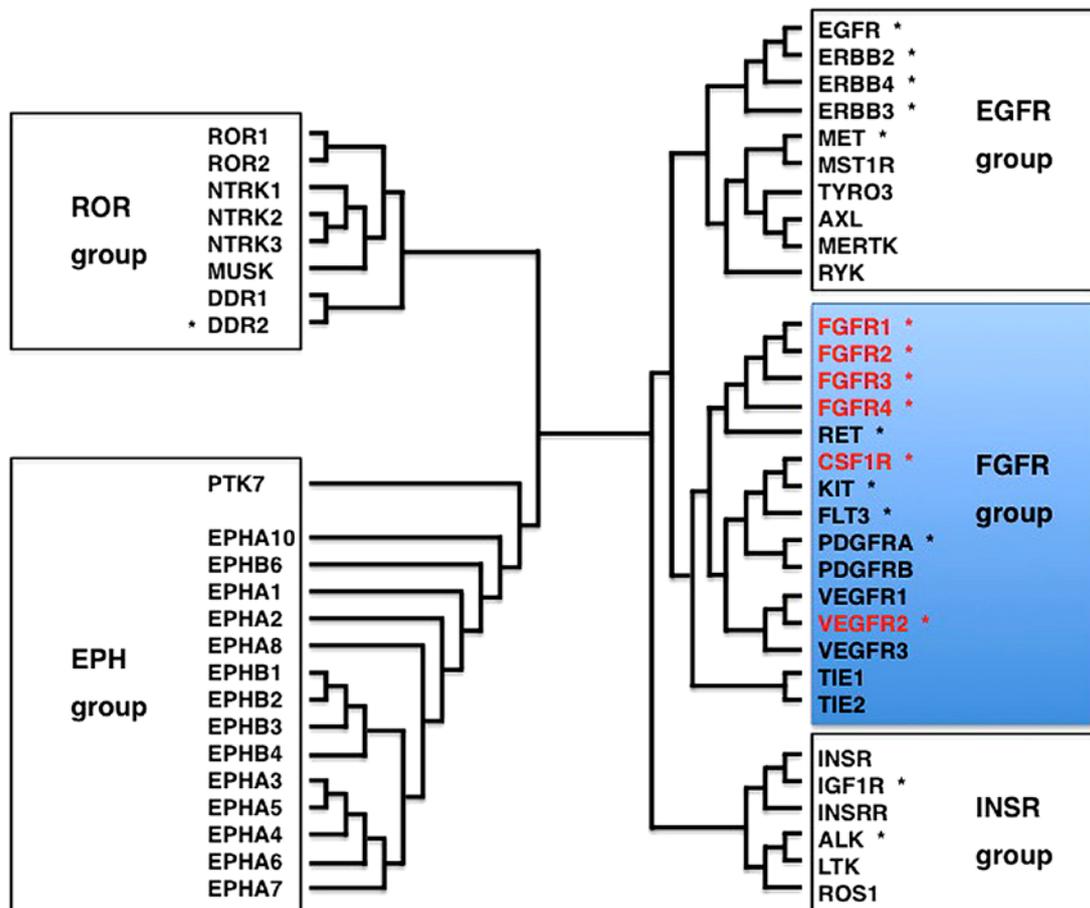


Figure 2. Receptor tyrosine kinase (RTK) superfamily. Phylogenetic tree of 54 human RTKs are shown. *, 18 RTKs in the OncoPrint Comprehensive Panel (137). RTKs are classified into the EGFR, FGFR, INSR ROR and EPH groups. FGFR1, FGFR2, FGFR3, FGFR4, CSF1R and VEGFR2, shown in red, belong to the FGFR group.

xenografts revealed that *FGFR2* amplification in human gastric cancer is a promising therapeutic target (51,52).

Gain-of-function and loss-of-function mutations. Somatic *FGFR1* mutation (N546K) in Ewing sarcoma (53), *FGFR2* mutations (R203C, N549K and K659N) in breast cancer (43,54), *FGFR2* mutations (S252W and N549K) in endometrial cancer (55), *FGFR2* coding in/del mutations in lung cancer (39) and *FGFR3* mutations (R248C and S249C) in bladder cancer (56) are gain-of-function mutations (Fig. 3), whereas somatic *FGFR2* mutations (D530N, I642V and A648T) in melanoma are loss-of-function mutations (57).

Missense mutations of *FGFRs* involved in congenital disorders rather than cancer have been well characterized. Gremlin *FGFR1* mutations (P252R and Y372C) and *FGFR2* mutations (S252W, P253R, K526E, N549K, K641R, etc.) in patients with craniosynostosis and *FGFR3* mutations (R248C, S249C, G380R, N540K, K650E, etc.) in patients with skeletal dysplasia are gain-of-function mutations (17-20); however, germline *FGFR1* mutations (R254W and V429E) in patients with congenital hypogonadotropic hypogonadism are loss-of-function mutations (58,59).

These facts clearly indicate that there are gain-of-function, as well as loss-of-function mutations in *FGFRs*. Therefore, validation of gain-of-function based on kinase or cell-based

assay is mandatory before prescribing *FGFR* inhibitors to cancer patients with *FGFR* coding mutations.

Two types of *FGFR* fusions. *FGFR* fusions in human cancers are classified into type 1 fusions caused by chromosomal translocations in hematological malignancies, and type 2 fusions caused by chromosomal rearrangements in solid tumors (Fig. 3). Chromosomal translocations involving the *FGFR1* gene occur in patients with myeloproliferative neoplasms initially presenting with eosinophilia and lymphadenopathy and then developing acute myeloid or mixed-lineage leukemia (60,61); chromosomal translocations involving the *FGFR3* gene have been shown to occur in patients with peripheral T cell lymphoma (62). Type 1 *FGFR* fusion proteins, including BCR-*FGFR1*, CNTRL-*FGFR1*, CUX1-*FGFR1*, *FGFR1OP*-*FGFR1*, *FGFR1OP2*-*FGFR1*, LRRFIP1-*FGFR1*, MYO18A-*FGFR1*, RANBP2-*FGFR1*, TPR-*FGFR1*, TRIM24-*FGFR1*, ZMYM2-*FGFR1* and ETV6-*FGFR3* are non-receptor-type *FGFR* kinases (Fig. 3). CNTRL-*FGFR1* fusion kinase promotes the expansion of the stem cell population and causes myeloid and lymphoid malignancies (63). By contrast, type 2 *FGFR* fusion proteins in solid tumors, including *FGFR1*-TACC1, *FGFR2*-AFF3, *FGFR2*-BICC1, *FGFR2*-CASP7, *FGFR2*-CCAR2, *FGFR2*-CCDC6, *FGFR2*-CIT, *FGFR2*-OFD1, *FGFR2*-PPHLN1, *FGFR3*-BAIAP2L1, *FGFR3*-JAKMIP1

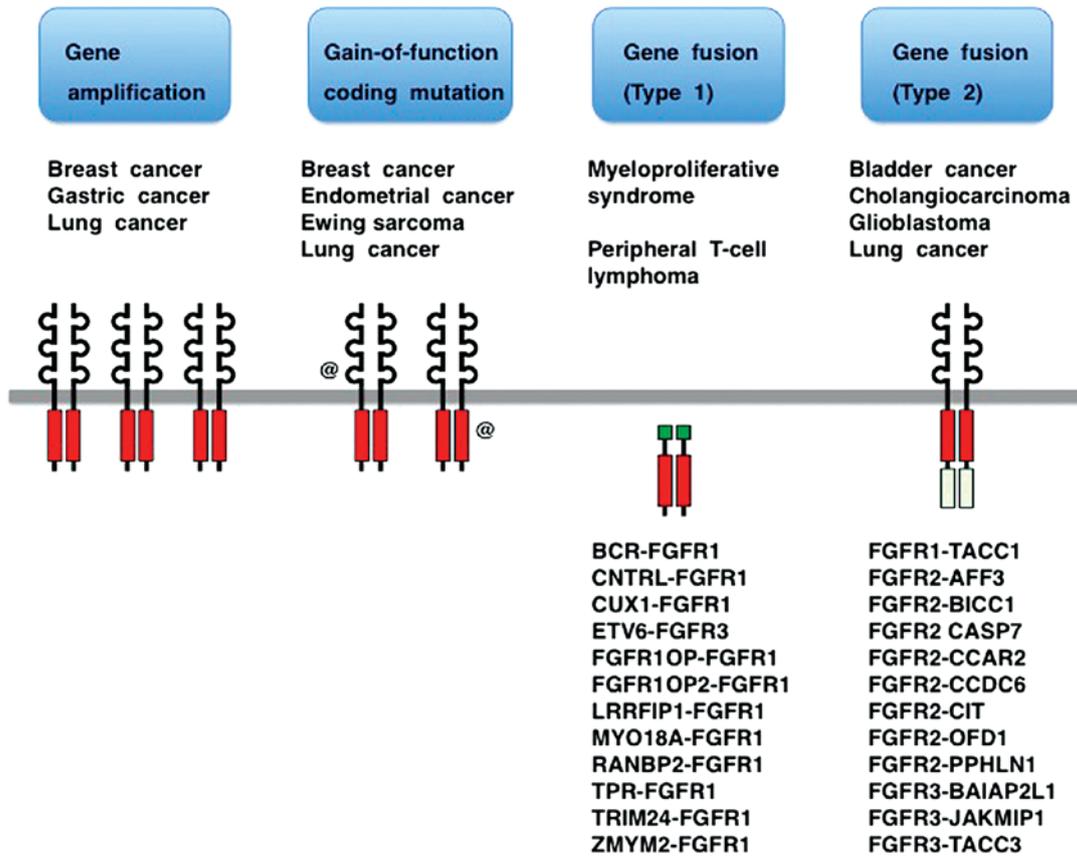


Figure 3. Fibroblast growth factor receptor (*FGFR*) alterations in human cancer. *FGFR* genes are activated in human cancer as a result of gene amplification, coding mutation and gene fusion. *FGFR* gene fusions are further classified into two groups. Type 1 *FGFR* fusions in hematological malignancies encode non-transmembrane-type *FGFR* kinases. Type 2 *FGFR* fusions in solid tumors encode transmembrane-type *FGFR*s with C-terminal substitution to the region of fusion partners.

and *FGFR3-TACC3*, are transmembrane-type *FGFR*s with C-terminal substitution to the region of fusion partners (37,38,56,64,65) (Fig. 3).

Both types of *FGFR* fusion proteins are endowed with oncogenic potential through the acquisition of protein-protein-interaction modules from fusion partners for ligand-independent dimerization and/or recruitment of aberrant substrates. Type 1 *FGFR* fusion proteins acquire oncogenic potential through altered subcellular localization as a result of the loss of the extracellular and transmembrane domains of wild-type *FGFR*s. Type 2 *FGFR* fusion proteins lose the PLC- γ -binding tyrosine (Tyr or Y) residue (Y766 in *FGFR1*, Y769 in *FGFR2* or Y760 in *FGFR3*) owing to the C-terminal alterations. To understand the mechanisms of carcinogenesis caused by the *FGFR* fusions, substrates and downstream signaling cascades of *FGFR* fusion proteins need be elucidated.

3. *FGFR* inhibitors targeting cancer cells

Classification of *FGFR* inhibitors. AZD4547 (66,67), BGJ398 (infigratinib) (68), BLU9931 (69), Debio-1347 (70), dovitinib (TKI258) (27,71), FIIN-2 (72), JNJ-42756493 (73), LY2874455 (74) and ponatinib (9) are small molecule compounds that inhibit *FGFR*s at low nM levels (IC_{50} value <20 nM) (Fig. 4A). AZD4547, BGJ398, Debio-1347, dovitinib, JNJ-42756493 and LY2874455 are reversible *FGFR* inhibitors that occupy the ATP-binding pocket in the

kinase domain, whereas BLU9931 and FIIN-2 are irreversible *FGFR* inhibitors that covalently bind to each specific cysteine (Cys or C) residue in the kinase domain. These *FGFR* inhibitors reduce phosphorylation of *FGFR*s themselves and their direct targets, FRS2 and PLC- γ , and inactivate downstream signaling, such as the RAS-ERK, PI3K-AKT, IP_3 - Ca^{2+} and DAG-PKC signaling cascades.

AZD4547, BGJ398, Debio-1347 and dovitinib are *FGFR1/2/3* inhibitors that are less effective on *FGFR4*; BLU9931 is a selective *FGFR4* inhibitor; FIIN-2, JNJ-42756493, LY2874455 and ponatinib are pan-*FGFR* inhibitors (Fig. 5A). Phylogenetic analysis on 54 RTKs revealed diversification of *FGFR4* from other *FGFR*s (Fig. 2), and amino-acid alignment of the tyrosine kinase domains in the *FGFR* family members revealed relatively frequent amino-acid substitutions specifically in *FGFR4* (Fig. 4B). Phospho-tyrosine residues involved in catalytic activation (Y653 and Y654 in *FGFR1*), STAT recruitment (Y677 in *FGFR1*) and PLC- γ recruitment (Y766 in *FGFR1*) are conserved in all members of the *FGFR* family. By contrast, one tyrosine residue in the hinge region (Y563 in *FGFR1*, Y566 in *FGFR2* and Y557 in *FGFR3*) is changed to C552 in *FGFR4*, and phospho-tyrosine residue in the kinase insert region (Y583 in *FGFR1*, Y586 in *FGFR2* and Y577 in *FGFR3*) is changed to L572 in *FGFR4* (Fig. 4B). Y563 in *FGFR1* is necessary for the interaction with Debio-1347 (70), whereas C552 in *FGFR4* is necessary for the covalent binding with BLU9931 (69). As the diversification of *FGFR4* signifi-

A

	AZD4547	BGJ398	BLU9931	Debio-1347	Dovitinib (TKI258)	FIIN-2	JNJ-42756493	LY2874455	Ponatinib* (AP24534)
FGFR1	0.2	0.9	591	9.3	8.0	3.1	< 1	2.8	2.2
FGFR2	1.8	1.4	493	7.6	40	4.3	< 1	2.6	1.6
FGFR3	2.5	1.0	150	22	9.0	27	1.05	6.4	18.2
FGFR4	165	60	3.0	290		45	< 1	6.0	7.7
RET									0.2
VEGFR1				1000	10				3.7
VEGFR2	24			2100	13				1.5
VEGFR3					8.0				2.3
KIT	24			5500	2.0				12.5
CSF1R	9.7				36				8.6
FLT3	85			6400	1.0				12.6
PDGFRA					200				1.1
PDGFRB				560	27				7.7
Reference	[66, 67]	[68]	[69]	[70]	[27, 71]	[72]	[73]	[74]	[9]

B

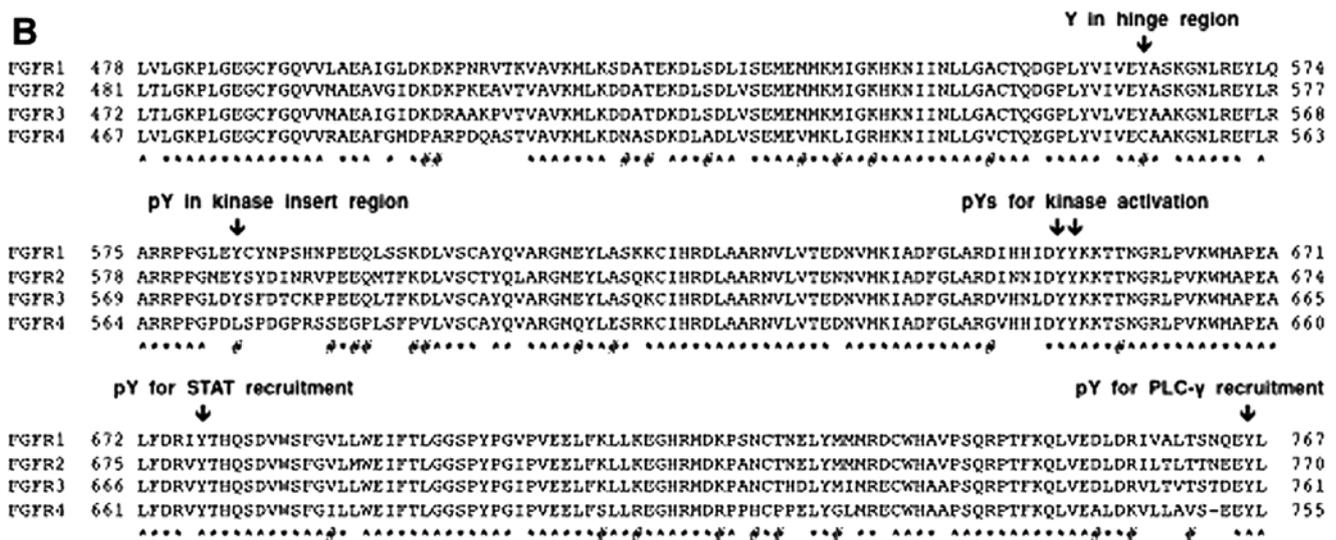


Figure 4. Small-molecule fibroblast growth factor receptor (FGFR) inhibitors and FGFRs. (A) Small-molecule FGFR inhibitors. Enzymatic IC₅₀ values for FGFRs and other substrates are listed. (B) Alignment of the tyrosine kinase domain of FGFR1, FGFR2, FGFR3 and FGFR4. Amino-acid position is shown on both sides of the alignment. Amino-acid residues conserved in all members of FGFRs are shown by asterisk, whereas amino-acid residues conserved in FGFR1, FGFR2 and FGFR3 but not in FGFR4 are shown by sharp. FGFR4 is relatively divergent from FGFR1, FGFR2 and FGFR3. Tyrosine residue in the hinge region (Y563 in FGFR1, Y566 in FGFR2 and Y557 in FGFR3) is substituted to C552 in FGFR4, which divergence is involved in the selectivity of FGFR inhibitors for FGFR1/2/3 and FGFR4.

cantly affects the binding affinities of TKIs, it is reasonable to functionally classify FGFR-targeting TKIs into FGFR1/2/3 inhibitors, FGFR4 inhibitor and pan-FGFR inhibitors.

Clinical trials and adverse effects. AZD4547, BGJ398, Debio-1347, dovitinib, JNJ-42756493 and ponatinib are currently being investigated in clinical trials (<https://clinicaltrials.gov>): phase II studies of AZD4547 in patients with breast, gastric and squamous-cell lung cancer (*FGFR1* or *FGFR2* amplification) and metastatic breast or non-small-cell lung cancer (*FGFR* genetic alterations, umbrella trial); phase II studies of BGJ398 in patients with solid tumors or hematological malignancies (*FGFR* genetic alterations); phase II study of dovitinib in patients with gastric cancer (*FGFR2* amplification); phase II study of JNJ-42756493 in patients with urothelial cancer (*FGFR* genetic alterations); phase II study of ponatinib in patients with advanced biliary cancer (*FGFR2*

fusion) or refractory metastatic solid tumors (genetic alterations in *FGFRs* and other targets).

TKIs have been approved for cancer therapy by regulatory authorities in expectation of an improved risk/benefit ratio; however, adverse effects on vital organs, such as the cardiovascular system and liver, are serious issues that may occur in the clinic (75). Hypertension, bleeding and thrombosis are adverse effects of anti-angiogenic therapy targeting the VEGF signaling pathway (76), while cardiovascular events are serious adverse effects of ponatinib for the treatment of chronic myeloid leukemia (77). AZD4547, dovitinib and ponatinib are representative multi-kinase inhibitors targeting FGFRs and other tyrosine kinases (Fig. 5A). Selective FGFR targeting is expected to reduce adverse effects, whereas the dual targeting of FGFR and VEGFR/CSF1R is expected to enhance the anti-tumor effects indirectly through the normalization of tumor microenvironment.

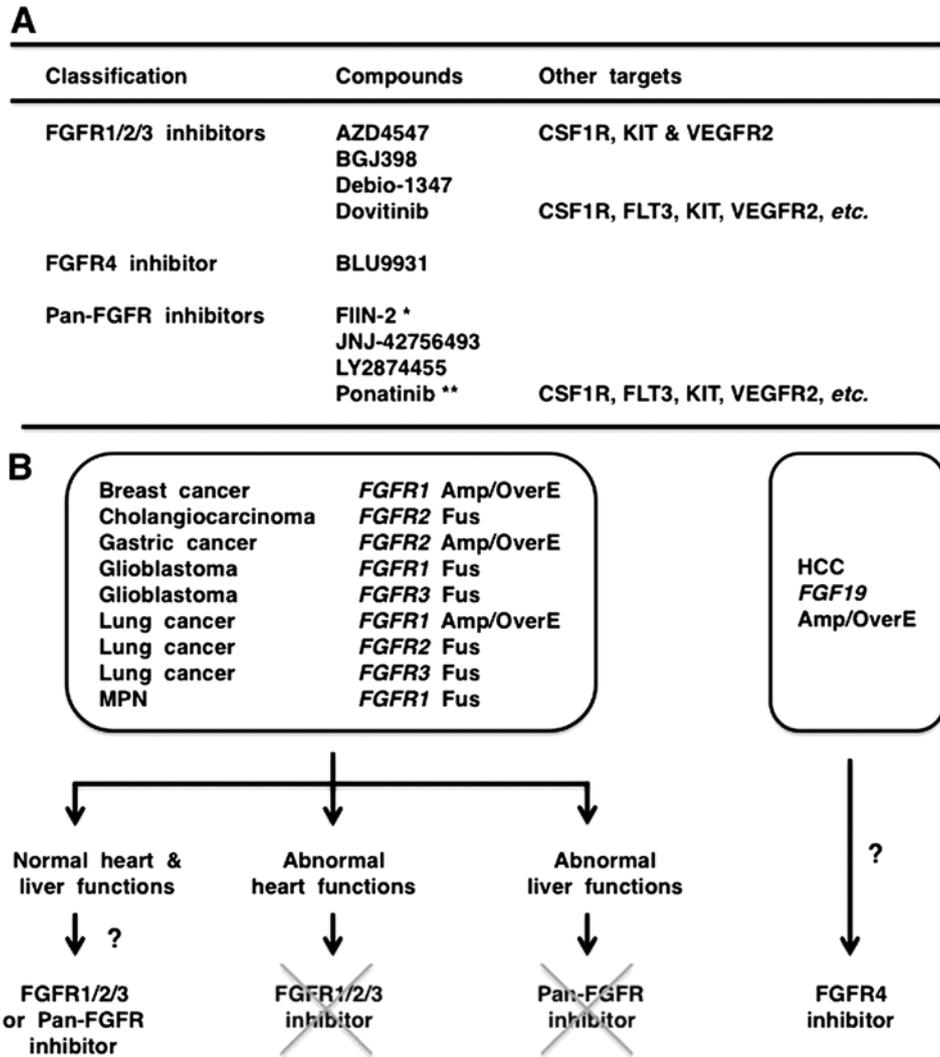


Figure 5. Selection of fibroblast growth factor receptor (FGFR) inhibitor for precision medicine. (A) Classification of FGFR inhibitors based on substrate specificities. *, FIIN-2 is for experimental use only. **, Severe adverse effects of ponatinib have been reported in patients with chronic myeloid leukemia. (B) Flow-chart for the choice of FGFR inhibitor in clinic. MPN, myeloproliferative neoplasm; Amp, gene amplification; OverE, overexpression; Fus, fusion. FGFR1/2/3 inhibitors and pan-FGFR inhibitors are applicable for human cancer with genetic alteration in *FGFR1*, *FGFR2* or *FGFR3*. FGFR1/2/3 inhibitors are not the choice for patients with heart diseases, whereas pan-FGFR inhibitors are not the choice for patients with liver dysfunction. FGFR4 inhibitor is applicable for HCC with genetic alteration in *FGF19*.

4. FGFR inhibitors targeting the tumor microenvironment

Tumor microenvironment and paracrine signaling. The tumor microenvironment consists of cancer cells and stromal/immune cells, such as fibroblasts, endothelial cells, lymphocytes, macrophages, monocytes and neutrophils (Fig. 6). The interactions of tumor cells and stromal cells are involved in almost all stages of tumor development, including neoplastic transformation, proliferation, invasion and metastasis, through the regulation of various cellular processes in a context-dependent manner (78–80). FGFs derived from cancer cells, as well as stromal cells play a key role in the tumor microenvironment.

Cancer-associated fibroblasts (CAFs) are activated stromal fibroblasts that support tumorigenesis (Fig. 6). FGF2 activates human dermal fibroblasts through transcriptional downregulation of the *TP53* gene, whereas BGJ398 or ponatinib treatment induces their senescence through the upregulation and activation of TP53 (81). By contrast, FGF2 signaling through FGFR1

causes resistance to EGFR inhibitor in lung cancer cells, and combination therapy using EGFR inhibitor and AZD4547 is effective to overcome drug resistance (82). Multiple myeloma cells induce FGF23 secretion from osteocytes, and then FGF23 signaling through FGFR3 to multiple myeloma cells promotes proliferation and induces heparanase upregulation, which explains the pathogenesis of osteolytic ‘punched-out lesion’ in patients with multiple myeloma. BGJ398 treatment inhibits FGF23-dependent growth and heparanase expression of multiple myeloma cells (83). These results indicate the rational for the application of FGFR inhibitors to target paracrine FGF signaling in the tumor microenvironment.

Angiogenesis. Tumor angiogenesis is largely classified into sprouting angiogenesis and vasculogenesis (84). Sprouting angiogenesis is the formation of new blood vessels as a result of endothelial sprouting from preexisting blood vessels, whereas vasculogenesis is the *de novo* formation of blood vessels

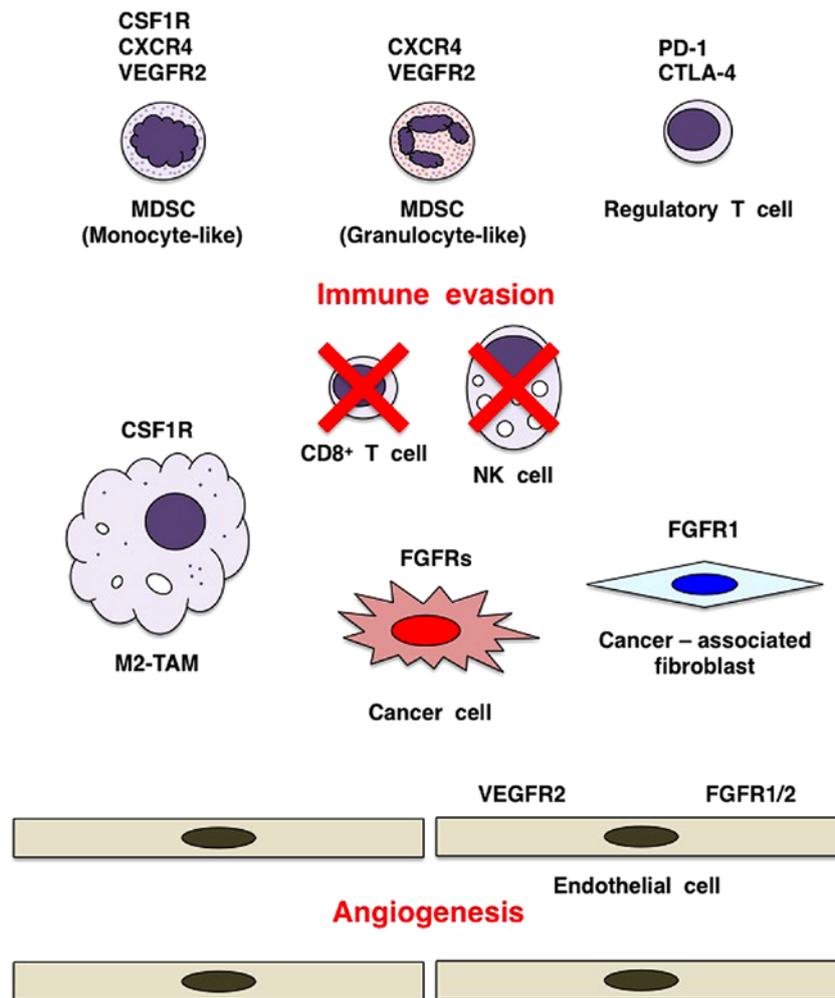


Figure 6. Cancer therapy targeting tumor microenvironment. Cancer cells, cancer-associated fibroblasts (CAFs), endothelial cells, myeloid-derived suppressor cells (MDSCs), tumor-associating macrophages of M2 type (M2-TAMs) and regulatory T cells are representative components of tumor microenvironment. Interactions between cancer cells and stromal/immune cells are involved in almost all steps of carcinogenesis. CSF1 signaling through CSF1R induces proliferation and differentiation of MDSCs and M2-TAMs. Dual inhibition of fibroblast growth factor receptor (FGFR) and CSF1R/VEGFR2 is expected to increase antitumor effects through targeting immune evasion and angiogenesis in the tumor microenvironment. Combination of FGFR/CSF1R inhibitor targeting cancer cells and stromal/immune cells and anti-PD-1/CTLA-4 monoclonal antibody targeting regulatory T cells and de-repressing CD8⁺ T cells may be a promising choice for cancer patients.

owing to endothelial differentiation of progenitor cells and endothelial-like differentiation of cancer cells. Tumor angiogenesis is involved in the supply of oxygen and nutrient (mature blood vessels), as well as the formation of hypoxic environment (immature blood vessels).

VEGF, FGF, angiopoietin (ANGPT) and Notch signaling cascades are major players of tumor angiogenesis (85,86). VEGF signaling through VEGFR2 promotes endothelial cell proliferation via the DAG-PKC signaling cascade, endothelial cell survival via the PI3K-AKT signaling cascade, endothelial cell migration via the FAK-Paxillin signaling cascade as well as vascular permeability and vasodilatation via the IP₃-eNOS (NOS3) signaling cascade (76,87,88). Pro-angiogenic FGF2 also promotes the proliferation and migration of endothelial cells directly through FGFR1 (or FGFR2) signaling activation (89,90) and indirectly through the induction/secretion of VEGF and ANGPT2 from endothelial cells (91,92). ANGPT1 is secreted from pericytes and maintains endothelial quiescence or stabilization through TIE2 signaling activation. ANGPT2 is secreted from endothelial cells and

promotes the endothelial activation or sprouting through TIE2 signaling inhibition (92,93). VEGF signaling in endothelial tip cells induces DLL4 expression, which subsequently activates Notch signaling in endothelial stalk cells for vascular quiescence through VEGFR downregulation (94-96). VEGF, FGF2 and ANGPT2 are involved in endothelial activation, whereas ANGPT1 and Notch are involved in endothelial quiescence. VEGFR2 and FGFR1/2 on endothelial cells are representative RTKs that promote tumor angiogenesis (Fig. 6).

VEGF signaling is targeted using anti-VEGF monoclonal antibody (mAb) or small-molecule VEGFR inhibitors in cancer patients; however, some tumors do not respond to the VEGF blockade therapy and other tumors recur after transient response [(Gacche and Meshram (76); Jain (78)]. As FGF signaling activation in endothelial cells is one of the mechanisms responsible for intrinsic and acquired resistance to the VEGF blockade therapy (97), FGFR inhibitors may be applicable to overcome the resistance to the VEGF blockade therapy. There are two options for the dual blockade of FGF and VEGF signaling cascades. Combination therapy using

FGFR inhibitor and anti-VEGF mAb is a preferable choice to reduce adverse effects, whereas monotherapy using small-molecule FGFR/VEGFR2 dual inhibitors, such as AZD4547 and dovitinob, may be a preferable choice to reduce medical cost. FGF/VEGF dual blockade therapy should be optimized in consideration of safety issues and medical costs.

Immune evasion. Cancer immunity and immune tolerance in the tumor microenvironment are regulated by the interaction between cancer cells and immune cells (98). CD8⁺ T cells, NK cells and NKT cells are immune effector cells involved in tumor elimination (98), whereas myeloid-derived suppressor cells (MDSCs) (99), tumor-associating macrophages of M2 type (M2-TAMs) (100) and regulatory T (Treg) cells (101) are immune modifier cells involved in immune evasion and tumor growth (Fig. 6).

MDSCs are heterogeneous populations of immature myeloid cells, including monocyte-like MDSCs (CD14⁺, CXCR4⁺, CSF1R⁺ and VEGFR2⁺), granulocyte-like MDSCs (CD15⁺, CXCR4⁺, KIT⁺ and VEGFR2⁺) and endothelial progenitor cells (CD31⁺, CXCR4⁺, KIT⁺ and VEGFR2⁺) (99). CSF1 (M-CSF), CSF2 (GM-CSF) and CSF3 (G-CSF) are secreted from the tumor microenvironment and stimulate the growth and survival of MDSCs and other myeloid-lineage cells, while CXCL12 (SFD-1 α) and VEGF promote the recruitment of MDSCs to the tumor microenvironment (102-106). MDSCs activate M2-TAMs and Treg cells, but inhibit CD8⁺ T cells and NK cells, leading to immune evasion in the tumor microenvironment. In addition, endothelial progenitor cell-like MDSCs are involved in tumor angiogenesis (104). MDSC infiltration and tumor angiogenesis during mammary tumorigenesis in MMTV-Wnt1/iFGFR1 bi-genic mice are significantly enhanced in comparison with MMTV-Wnt1 transgenic mice, and BGJ398 treatment results in tumor regression and disappearance of MDSCs from the residual mammary gland (107). By contrast, AZD4547 treatment inhibits the proliferation and lung metastasis of 4T1 mouse mammary tumor cells, and reduces MDSCs in the tumor microenvironment and systemic circulation (108). FGFR inhibitors induce the reduction or disappearance of MDSCs from the tumor microenvironment, partly by targeting cytokine-producing CAFs.

CSF1 signaling through CSF1R on monocyte/macrophage-lineage cells are involved in their proliferation, survival and differentiation (109-111). CSF1R inhibitors (GW2580 and PLX3397) and anti-CSF1R mAb (RG7155) have been developed as therapeutics for CSF1 signaling blockade in monocyte-like MDSCs and M2-TAM (112-115). Combination therapy of CSF1R inhibitor PLX3397 and paclitaxel inhibits tumor-infiltration of MDSCs and M2-TAM and suppresses mammary tumorigenesis (113,114). IC₅₀ value of PLX3397 to CSF1R is 20 nM (113), whereas IC₅₀ values of AZD4547 (67), ponatinib (9) and dovitinib (71) to CSF1R are 9.7, 8.7 and 36 nM, respectively. By contrast, PI3K is one of common signaling effectors CSF1R and FGFRs (Fig. 1A), and PI3K activation enhances immune suppressor and pro-angiogenic potentials of M2-TAMs (116). Therefore, as CSF1 and FGF signals are both involved in the accumulation of tumor-infiltrating/promoting MDSCs and M2-TAMs, the dual inhibition of CSF1R and FGFRs may be more effective for cancer therapy than selective CSF1R inhibition.

5. FGFR inhibitors affecting whole-body homeostasis

Endocrine FGF signaling. FGF19, FGF21 and FGF23 are endocrine FGFs that transduce signals to target organs through FGFRs and the Klotho family of co-receptors, α -Klotho (KL) and β -Klotho (KLB) (117). FGF19 is upregulated by bile acid in the intestine to transduce endocrine signaling through FGFR4 and β -Klotho in the liver. FGF21 is upregulated by fasting in the liver and adipose tissue to transduce paracrine signaling through FGFR1 and β -Klotho locally and endocrine signaling to the pancreas and brain. FGF23 is upregulated by serum phosphate, vitamin D and parathyroid hormone in bone to transduce endocrine signaling through FGFR1 and α -Klotho in the kidneys and negative feedback signaling through FGFR3 and α -Klotho in the parathyroid gland (118). As endocrine FGFs are involved in the maintenance of whole-body homeostasis, FGFR inhibitors elicit endocrine or metabolic abnormalities. This section will be focused on adverse effects of FGFR inhibitors on endocrine FGF signaling in cancer patients.

FGF19-FGFR4 signaling in liver homeostasis. FGF19 signaling through FGFR4 in the liver stimulates hepatocyte proliferation and glycogen synthesis but reduces bile acid synthesis and triglyceride synthesis (69,119). FGF19-FGFR4 signaling blockade in cynomolgus monkeys using anti-FGF19 monoclonal antibody causes hepatotoxicity, increased bile acid secretion and severe diarrhea (120). *Fgfr4* knockout in mice also causes increased bile acid secretion in the liver, which leads to induction of *Fgf15* (mouse ortholog of human FGF19) in the intestine and subsequent improvement of insulin resistance and glucose metabolism (121). The selective FGFR4 inhibitor, BLU9931, may be applied for the treatment of patients with hepatocellular carcinoma depending on FGF19-FGFR4 signaling. By contrast, as FGFR4 blockade is associated with a risk of liver toxicity, FGFR1/2/3 inhibitors rather than pan-FGFR inhibitors are preferable for the treatment of cancer patients with genetic alterations in *FGFR1*, *FGFR2* or *FGFR3*, especially those with liver dysfunction (Fig. 5B).

FGF23-FGFR4 signaling in heart homeostasis. Serum FGF23 elevation is a biomarker indicating on-target effects of FGFR1/2/3 and pan-FGFR inhibitors in cancer patients, whereas serum FGF23 levels are also elevated in patients with non-cancerous diseases, such as hypophosphatemic rickets and chronic kidney diseases (118). Physiological FGF23 signaling through FGFR1 and α -Klotho in the kidneys decreases the serum phosphate level through the downregulation of phosphate reabsorption. FGFR inhibitors, hindering FGF23 signaling in the kidneys, promote hyperphosphatemia and subsequent FGF23 secretion from bone and soft-tissue mineralization. Pathological FGF23 upregulation is associated with endothelial dysfunction and arterial stiffness (122). Pathological FGF23 signaling through FGFR4 in cardiac myocytes then induces phosphorylation of PLC- γ and activation of the IP₃-Ca²⁺ signaling cascade, which results in cardiac remodeling, such as cardiac hypertrophy and cardiac fibrosis (123). As FGF23-FGFR4 signaling activation is associated with a risk of cardiac toxicity, pan-FGFR inhibitors rather than FGFR1/2/3 inhibitors may be selected for the treatment of cancer patients with *FGFR* genetic alterations, particularly those with heart dysfunction (Fig. 5B).

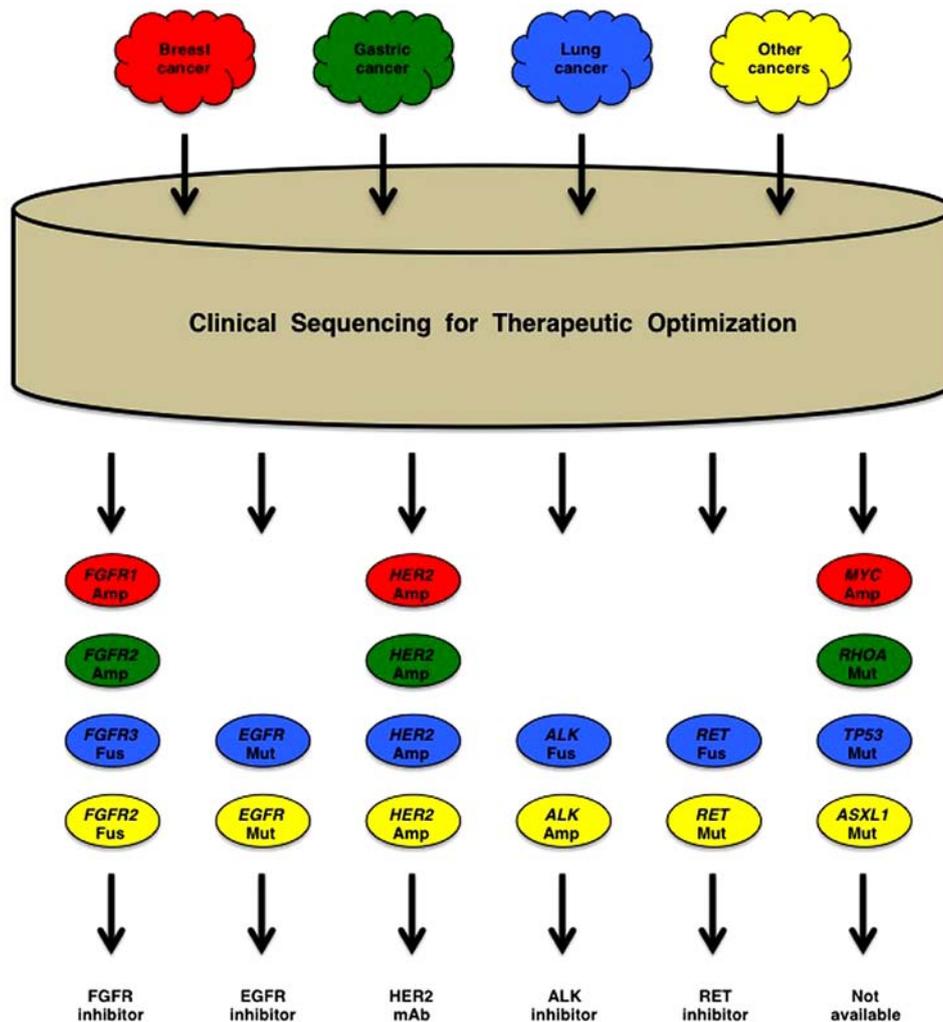


Figure 7. Genome-based precision medicine for cancer patients. Amp, amplification; Fus, fusion; Mut, mutation. Patients with breast cancer (red), gastric cancer (green), lung cancer (blue) and other cancers (yellow) are reorganized into the groups of cancer patients with specific genetic alterations. Patients with fibroblast growth factor receptor (FGFR), EGFR, HER2, ALK and RET alterations are prescribed FGFR inhibitor, EGFR inhibitor, HER2 monoclonal antibody (mAb), ALK inhibitor and RET inhibitor, respectively. However, at present, there is no targeted therapy for cancer patients with *MYC* Amp, *RHOA* Mut, *TP53* Mut, *ASXL1* Mut, etc.

6. Conclusion

FGFR genetic alterations in human cancer are classified into gene amplification, gain-of-function coding mutation and two types of fusions. AZD4547, BGJ398, Debio-1347 and dovitinib are FGFR1/2/3 inhibitors; BLU9931 is a selective FGFR4 inhibitor; FIIN-2, JNJ-42756493, LY2874455 and ponatinib are pan-FGFR inhibitors. FGFR inhibitors elicit antitumor effects directly on cancer cells as well as indirectly through normalization of tumor microenvironment, especially paracrine signaling, angiogenesis and immune evasion. FGF19-FGFR4 signaling inhibition is associated with a risk of liver toxicity, whereas FGF23-FGFR4 signaling activation is associated with a risk of heart toxicity. Endocrine FGF signaling affects pathophysiology of cancer patients with FGFR-targeting therapy.

7. Perspectives

Massively parallel sequencing technology for the whole-exome or whole-genome sequencing has been used to clarify genomic landscapes in various types of human cancer (124). The over-

expression of FGFR occurs in human cancers through gene amplification, as well as other types of aberrations. Rearrangement in the distal enhancer region and point mutation in the proximal promoter region are both able to induce FGFR overexpression. Repression of FGFR-targeting microRNA (miRNA) precursor gene or upregulation of long-non-coding RNA (lncRNA) sequestering FGFR-targeting miRNA leads to FGFR overexpression. SWI/SNF mutation dysregulating chromatin remodeling, as well as cancer-associated fusion transcription factor also cause FGFR overexpression. For example, lung cancer cells with FGFR1 upregulation rather than *FGFR1* copy number gain are sensitive to ponatinib (125). Rhabdoid tumor cells with FGFR overexpression as a result of *SMARCB1* (*SNF5*) deletion are sensitive to BGJ398 (126). Myxoid liposarcoma cells with FGFR2 upregulation owing to FUS-DDIT3 or EWS1R-DDIT3 fusion are sensitive to BGJ398 and dovitinib (127). Taken together, these facts clearly indicate that FGFR inhibitors are applicable for the treatment of cancers with FGFR overexpression in the absence of gene amplification, particularly rare cancers with their specific alterations inducing FGFR overexpression. However, development of biomarkers for FGF dependence is necessary before clinical

application of FGFR inhibitors for the treatment of cancers with FGFR overexpression.

Genomic heterogeneity is the major mechanism of tumor evolution for recurrence after cancer therapy (128). *FGFR2-BICC1* and *FGFR2-PHLLN1* fusions in mostly distinct, but some overlapping cases of cholangiocarcinomas (65) suggest convergent evolution and intra-tumor heterogeneity, respectively. Resistance to EGFR-targeted therapy occurs based on paracrine FGF signaling from tumor-stromal cells (82) or activating *FGFR* alterations, such as *FGFR1* amplification and *FGFR3* mutation, in cancer cells (129,130). On the other hand, resistance to FGFR-targeted therapy occurs based on paracrine signaling through EGFR/HER2/MET (29,107) or secondary *FGFR* alterations (131). FGFR1 V561M (34), FGFR2 V564M (72), FGFR3 V555M (131) and FGFR4 V550M (132) are gatekeeper mutations that cause resistance to ATP-competitive FGFR inhibitors, such as AZD4547 and BGJ398, whereas FGFR2 V564M is sensitive to a covalent pan-FGFR inhibitor, FIIN-2 (72). Because antitumor effects of FIIN-2 are limited to cell-based assays, orally bioavailable derivatives of FIIN-2 should be developed for the treatment of cancer resistant to ATP-competitive FGFR inhibitors.

Immune-checkpoint blockade therapy is a frontier in the field of clinical oncology. PD-1 ligand (PD-L1) is expressed on cancer cells and stromal/immune cells, whereas PD-1 and CTLA-4 are expressed on CD8⁺ T cells and Treg cells (133-136). As PD-1 signaling and CTLA-4 signaling are both involved in functional suppression of cytotoxic T cells directly or indirectly through Treg cells, anti-PD-1 mAb, anti-PD-L1 mAb and anti-CTLA-4 mAb are clinically applied for cancer immunotherapy, which leads to sustainable remission in a fraction of patients. By contrast, FGFR and CSF1R inhibitors are shown to target immune cells, such as MDSCs and M2-TAMs, in the tumor microenvironment (Fig. 6) and are expected to indirectly repress PD-L1 expression on tumor cells and stromal/immune cells through normalization of tumor microenvironment. Therefore, combination therapy using TKI (FGFR or CSF1R inhibitor) and immune checkpoint blocker (anti-PD-1 or anti-CTLA-4 mAb) may be a promising choice for cancer patients.

Cancer patients are prescribed appropriate drug based on their genetic alterations to reduce the costs of diagnosis and to increase the amounts of knowledge (Fig. 7). Partial-exome sequencing of a panel of cancer-associated genes are utilized for therapeutic optimization of cancer patients in the field of clinical oncology, which is relatively inexpensive but unable to detect cis-acting enhancer/promoter alterations and trans-acting rare coding alterations. By contrast, integrative genomic analyses based on whole-genome sequencing are utilized for precise characterization of human cancers in the field of basic oncology, which is expensive but comprehensive and informative. As a benefit-cost ratio is a critical issue to sustain health care system of aging society, it is necessary to discuss the benefit-cost issue with a focus on disease-free survival and total medical cost before implementation of genome-based precision medicine for cancer patients.

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