Advances in Small-Molecule C-KIT/PDGFRα Inhibitors for the Treatment of Gastrointestinal Stromal Tumors

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Abstract

Stem cell factor receptor (*C-KIT*) or platelet-derived growth factor receptor α (*PDGFRα*) gene mutations have been identified as oncogenic drivers for most gastrointestinal stromal tumors (GISTs). Thus, small-molecule inhibitors of C-KIT or PDGFRα have emerged as effective treatments for GISTs. Although the currently approved first- to fourth-line drugs are initially effective against GISTs, the inevitable development of drug resistance remains an unmet challenge. To address secondary mutations leading to drug resistance, several novel selective C-KIT/PDGFRα small-molecule inhibitors have been developed and clinically studied. This review summarizes the pathogenesis, treatment, and drug resistance mechanisms of GISTs and briefly describes current challenges and future efforts for GIST treatment using small-molecule kinase inhibitors.

Keywords
- PDGFRα
- C-KIT
- GISTs
- kinase inhibitors
- clinical resistance

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors in the gastrointestinal tract, accounting for 1 to 3% of gastrointestinal malignancies.[1] Most GISTs occur in the stomach (50–70%) and small intestine (20–30%), whereas these tumors are relatively uncommon in the rectum (10–20%) and esophagus (0–6%). Meanwhile, GISTs in the mesentery omentum and retroperitoneum are rare.[2] Mutations in the stem cell factor receptor (*C-KIT*) and platelet-derived growth factor receptor α (*PDGFRα*) genes have proven to be the main pathogenic drivers of GISTs, accounting for 85 to 95% of all cases.[3,4] *C-KIT* gene mutations are present in 60 to 85% of GIST tumors, whereas 5 to 10% of GISTs carry *PDGFRα* gene mutations. GISTs without *C-KIT* or *PDGFRα* gene mutations are defined as wild-type GISTs, and their pathogenesis has not been fully clarified. The possible pathogenic factors include the deletion of succinate dehydrogenase B, activation of insulin growth factor 1 receptor, mutation of the oncogene *BRAF*, and neurofibromatosis type I.[5] This article mainly focuses on GISTs driven by *C-KIT* and *PDGFRα* mutations, which have been effectively treated by small-molecule kinase inhibitors.

C-KIT and PDGFRα

C-KIT and PDGFRα, encoded by the *C-KIT* and *PDGFRα* genes, respectively, belong to the class III receptor tyrosine kinase (RTK) family. Both proteins consist of an extracellular region, a transmembrane region, and an intracellular region.[6] As shown in ►**Fig. 1**, the extracellular region encoded by *C-KIT* exon 9 or *PDGFR*α exons 3–10 consists of five immunoglobulin-like domains, which identify and bind to the corresponding ligand to cause receptor dimerization and tyrosine

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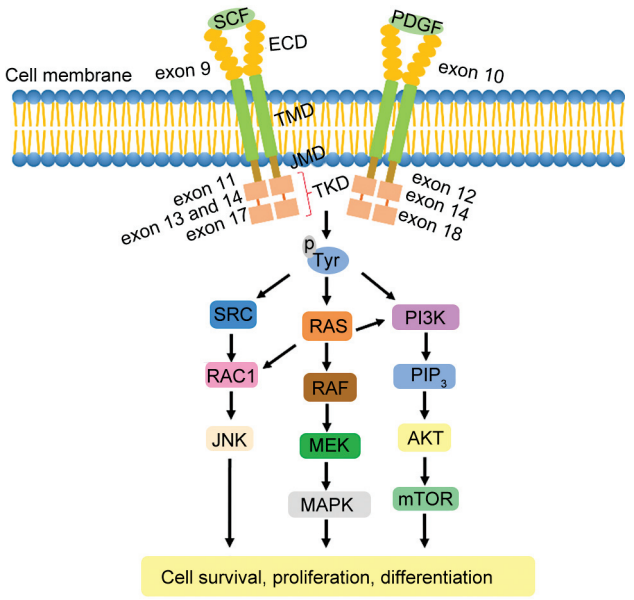


Fig. 1 C-KIT and PDGFRα signaling pathway.

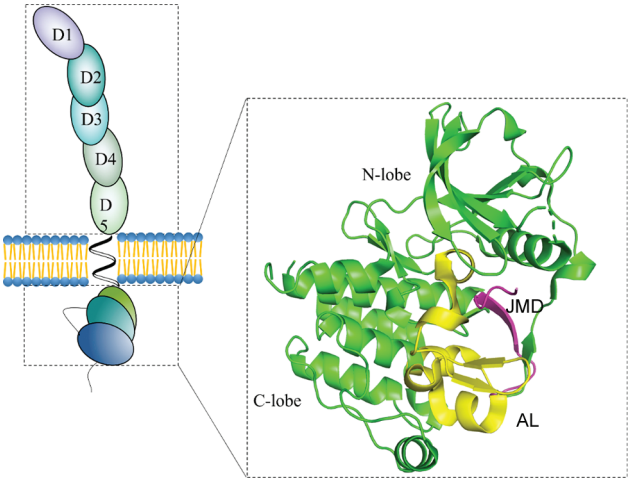


Fig. 2 General structure of PDGFRα (PDB: 5K5X). AL, activation loop; JMD, juxtamembrane domain.

kinase autophosphorylation. The intracellular region contains a juxtamembrane domain (JMD) region encoded by *C-KIT* exon 11 or *PDGFR*α exon 12 and a kinase domain. The kinase domain includes an adenosine triphosphate (ATP)-binding domain encoded by *C-KIT* exons 13–14 or *PDGFR*α exon 14 and an activation loop (AL) switch encoded by *C-KIT* exons 17–18 or *PDGFR*α exons 18–19.

C-KIT and PDGFRα both exist as inactive monomers in the absence of ligand binding. Once bound to ligands, the two monomer receptors form dimers on the membrane and activate protease activity and downstream signal transduction pathways, such as the SRC/RAC1, RAS/RAF, and PI3-K/AKT pathways, thereby regulating cell growth and proliferation.[7,8] Mutations allow C-KIT and PDGFRα to maintain dimerization and self-phosphorylation without ligand binding, perpetuating receptor-activated signaling and resulting in the activation of downstream effectors that ultimately mediate abnormal cell survival, proliferation, and differentiation (►**Fig. 1**).[6,9,10]

The configuration of these kinases is mainly controlled by JMD and AL moieties. In PDGFRα, JMD consists of residues 555–586, and it is associated with kinase folding. AL in PDGFRα is a long flexible peptide segment (►**Fig. 2**). JMD and AL regulate cellular kinase activity by controlling the kinase conformation through a double-switching mechanism.[11] When C-KIT or PDGFRα is in an autoinhibited inactivated state, JMD is embedded, and it has multiple contacts with the catalytic site between the N-lobe and C-lobe. Numerous conserved residues within this domain are indispensable for binding with hydrophobic interactions, preventing conformational changes in the active kinase state and substrate binding. Deletion or point mutation of key residues within JMD disrupts these mutual contacts and releases JMD from the kinase fold, resulting in nonautoinhibition activation. The conformation of AL, another key regulatory domain, controls the access to catalytic sites and kinase activity. AL of C-KIT/PDGFRα spans 27 residues from the conserved aspartate–phenylalanine–glycine (DFG) sequence to the alanine–proline–glutamate sequence. C-KIT D816 and PDGFRα D842 have multipolar interactions within the adjacent helix of AL, supporting the DFG-out conformation of AL. Therefore, missense mutations or insertions/deletions will maintain AL in a DFG-out conformation, resulting in constitutive kinase activity. For C-KIT/PDGFRα, a shift in AL from the DFG-out conformation to the DFG-in conformation is the primary mechanism of kinase activation (►**Fig. 3**).

Oncogenic kinase mutations disrupt one or multiple regulatory switching mechanisms, causing switch dysfunction and the loss of physiological conformational control. Mutations in GISTs mainly affect the exons encoding the functional domains of C-KIT/PDGFRα. Primary C-KIT mutations occur mostly in exons 9 and 11 and more rarely in exons 13 and 17 (►**Table 1**). Among them, primary C-KIT mutations in exon 11 are the most common (accounting for 60–70% of GISTs), and they are mainly induced by interstitial deletions of codons 550–579. Conversely, point mutations are limited to four codons (557, 559, 560, and 576).[1] Although the incidence is low, mutations of this switch will disrupt the inactive conformation of C-KIT, causing a conformational balance shift to the active form. The primary C-KIT mutation in exon 9 is the second most frequent mutation, being present in 10% of GISTs. Most of these lesions arise in the small intestine, and the mutations primarily involve repeats of residues 502–503.[2] The conformational changes caused by this mutation can simulate ligand binding, leading to dimerization and sustained activation. Contrarily, secondary mutations most commonly occur in exon 13 or 17 of C-KIT, and these mutations, primarily comprising point mutations, insertions, and repeat mutations, are relatively rare. PDGFRα is the second most commonly mutated oncogene in GISTs. PDGFRα exon 18 mutations are most common (6% of GISTs), whereas mutations in exons 12 and 14 are rare (<2% of GISTs).[12] Among them, single-base substitutions in exon 18, especially D842V, represent the most common PDGFRα mutation in GISTs (►**Table 1**).

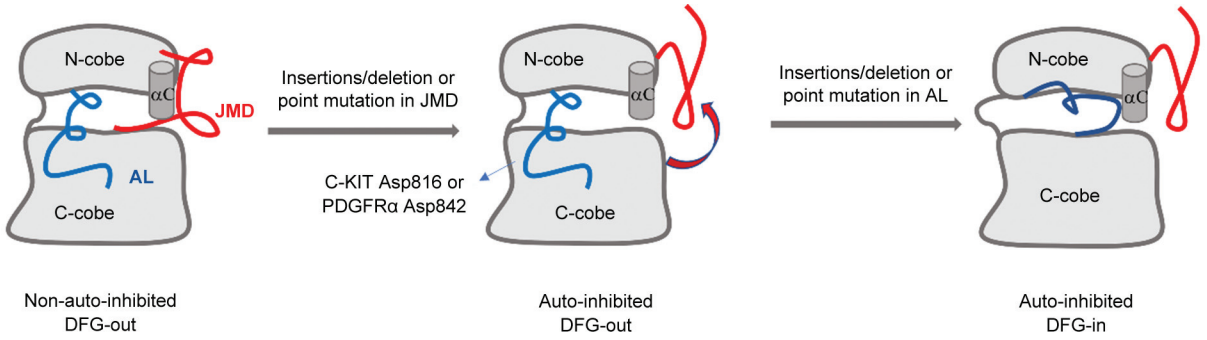


Fig. 3 Kinase domain conformation states for C-KIT and PDGFRα. AL, activation loop; JMD, juxtamembrane domain.

In general, a small number of primary mutations and almost all secondary mutations in C-KIT/PDGFRα are located either in the main AL switch or ATP switch pocket.[13] Secondary mutations that alter the tyrosine kinase domain of C-KIT or PDGFRα represent the primary mechanism of secondary resistance to targeted drugs in patients with GIST. However, some patients exhibit acquired drug resistance without secondary mutations, which might be associated with gene deletion, receptor amplification, signaling bypass, or the activation of other RTKs.

Small-Molecule Kinase Inhibitors

Surgical excision is the major treatment for early-stage GISTs. However, nearly 50% of patients with GISTs experience postoperative recurrence or metastasis after complete tumor resection.[14] The efficacy of C-KIT/PDGFRα inhibitors has been gradually affirmed for metastatic or unresectable GISTs. In this section, we provide a brief introduction to clinically applied therapeutic drugs for GISTs, including marketed drugs such as imatinib, sunitinib, regorafenib, ripretinib, and avapritinib, as well as crenolanib, bezuclastinib, NB003, THE-630, and IDRX-42, which are currently in clinical development.

Marketed Small-Molecule Kinase Inhibitors

The type II kinase inhibitors imatinib, sunitinib, and regorafenib, which are the approved first-, second-, and third-line treatments for GISTs, respectively, are effective targeted therapies for advanced GISTs. However, they only partially inhibit mutated C-KIT or PDGFRα, and they have limited activity against AL mutations. Ripretinib and avapritinib, two next-generation approved tyrosine kinase inhibitors (TKIs), exert stronger therapeutic effects against GISTs. The structure, type, date of approval, indication, and effects against various mutations in C-KIT and PDGFRα are summarized for the five aforementioned marketed drugs in ►**Table 2**.

Imatinib, Sunitinib, and Regorafenib

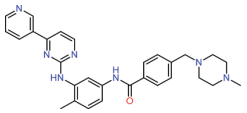
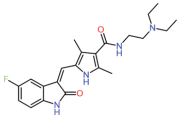
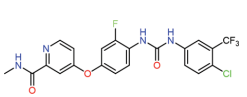
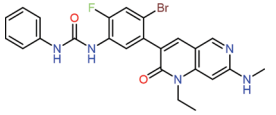
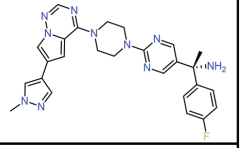
Imatinib is a multitargeted small-molecule TKI of C-KIT, PDGFRα, and ABL. This drug was approved for the first-line treatment of inoperable and metastatic GISTs in 2002. Imatinib is a type II kinase inhibitor that binds to the inactive conformation of C-KIT/PDGFRα. After treatment with imatinib, 80% of patients with advanced GISTs exhibit a response after 2 to 3 months. Median overall survival (OS) is approximately 57 to 60 months, and approximately 50% of patients

Table 1 Mutation types of C-KIT and PDGFRα and their frequency

Gene	Exon	Type of mutation	Domain affected	Frequency
C-KIT	9	Insertion	Ligand-binding	≈10%
	11	Deletion	JMD	60–70%
		Insertion		
		Single-base substitution		
	13	Single-base substitution	ATP-binding pocket	≈1%
	17	Single-base substitution	AL	≈1%
PDGFRα	12	Deletion	JMD	≈1%
		Insertion		
		Single-base substitution		
	14	Single-base substitution	ATP-binding pocket	<1%
	18	Single-base substitution	AL	≈6%
Wild type	/	/	/	10–15%

Abbreviations: AL, activation loop; ATP, adenosine triphosphate; JMD, juxtamembrane domain.

Table 2 Approved drugs for GISTs

Name		Imatinib	Sunitinib	Regorafenib	Ripretinib	Avapritinib
Structure						
Type		II	II	II	II	I
Approved time		2002	2006	2013	2021	2020
Indication for advanced GISTs		First-line	Second-line	Third-line	Fourth-line	PDGFRα exon 18
C-KIT mutation	Exon 9	☑	☑	☑	☑	Q
	Exon 11	☑	☑	☑	☑	☑
	Exon 13 (V654A)	Q	☑	Q	☑	Q
	Exon 14 (T670I)	Q	☑	☑	☑	Q
	Exon 17 (D816V/H)	Q	Q	Q	☑	☑
	Exon 17 (D816E)	Q	Q	☑	☑	☑
PDGFRα mutation	Exon 18 (D842V)	Q	Q	Q	Q	☑

Abbreviation: GISTs, gastrointestinal stromal tumors.
Note: ☑ sensitive; Q resistant.

survive longer than 5 years.[15] However, the majority of patients eventually experience disease progression. In total, 10 to 20% of patients with GISTs display primary resistance to imatinib, such as patients carrying the D842V mutation in PDGFRα exon 18 and patients with wild-type GISTs, and 50% of patients develop secondary resistance to imatinib. Most secondary C-KIT mutations after imatinib treatment occur in exons 13 (V654A), 14 (T670I), and 17 (e.g., D816H/V). Conversely, secondary PDGFRα mutations, especially D842V, mainly arise in exon 18 (►**Table 2**).[16,17] Resistance is caused by multiple secondary mutations within the ATP-binding domain and AL. The co-crystal of imatinib complexed with C-KIT suggests that imatinib makes contact with V654 in the side chain and forms a hydrogen bond with the gatekeeper (GK) residue T670. The mutations in the ATP-binding pocket, including V654A in exon 13 and T670I in exon 14, are predicted to decrease the binding affinity of imatinib for the kinase (►**Fig. 4**).[18,19] Meanwhile, AL mutations result in the conversion of C-KIT/PDGFRα from the DFG-out conformation to the DFG-in conformation, thereby interfering with the binding of imatinib and reducing its inhibitory activity.

Sunitinib, a multitargeted TKI with activity against PDGFRα/β, C-KIT, VEGFR1/2/3, and RET, was approved as a second-line treatment for patients with advanced GISTs after the development of resistance or intolerance to imatinib.[20] In addition to patients with wild-type C-KIT, those with exon 9 mutations, as well as those carrying secondary mutations in exon 13 or 14, including V654A and T670I, have better responses to sunitinib because the binding of sunitinib to C-KIT is not hindered by these mutations (►**Table 2**).[21,22] However, sunitinib binds to the DFG-out state of C-KIT/PDGFRα similarly to imatinib, resulting in poor inhibitory effects when these kinases carry AL mutations.[23]

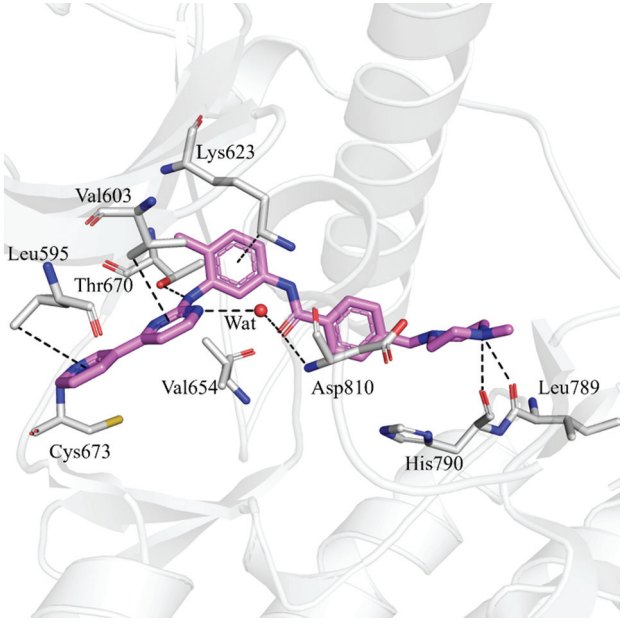


Fig. 4 The X-ray crystal structure of imatinib complexed with C-KIT (PDB ID: 1T46). C-KIT is shown by a white cartoon. Key residues and hydrogen bonds are represented by sticks and black dashed lines, respectively.

Regorafenib, a multitargeted kinase inhibitor targeting VEGFR, PDGFRα/β, C-KIT, RET, and RAF1, was approved as a third-line treatment for advanced GISTs after imatinib and sunitinib failure or intolerance by the Food and Drug Administration (FDA) in 2013.[24] Regorafenib also binds to the inactive conformation of C-KIT/PDGFRα. Concerning secondary C-KIT mutations, regorafenib exhibits a complementary activity profile to sunitinib. Regorafenib displays potent activity against several exon 17 mutations in C-KIT. but it is ineffective against the D816V/H mutation. The drug has poor efficacy against the common secondary imatinib resistance mutation V654A in C-KIT exon 13, but it is effective against the T670I mutation in exon 14. Furthermore, regorafenib also has a poor inhibitory effect against D842V-mutated PDGFRα (►**Table 2**). Additionally, the more severe side effects of sunitinib and regorafenib, such as diarrhea and fatigue, limit their clinic application.[25]

Whereas the aforementioned multitargeted TKIs can be used to treat advanced GISTs, clinical resistance attributable to AL mutations in C-KIT or PDGFRα remains a challenge. Imatinib, sunitinib, and regorafenib are type II inhibitors that only bind to the inactive forms of C-KIT and PDGFRα. Secondary mutations in AL cause a shift to the active conformation, reducing the ability of inhibitors to bind to the target. Ripretinib and avapritinib, as recently approved next-generation TKIs, are particularly designed to address these issues.

Ripretinib

C-KIT and PDGFRα are structurally similar dual-switch kinases consisting of an inhibitory switch and an AL switch, and their kinase activity is regulated by the kinase switch pocket. Ripretinib is a type II C-KIT/PDGFRα kinase inhibitor with a unique dual mechanism of action that permits it to act on both switches.[26] Ripretinib exhibits ideal inhibitory activity against the full spectrum of primary and secondary resistance mutations in C-KIT and PDGFRα, including AL mutations, which were previously believed to only be achieved by type I inhibitors. In a pivotal phase III clinical trial, ripretinib produced good outcomes in patients with GISTs, including progression-free survival (PFS) of 6.3 months and OS of 15.1 months.[27] Based on these promising clinical results, ripretinib was approved by the FDA in May 2020 for the fourth-line treatment of advanced GISTs in adults who previously received three or more kinase inhibitors.

Notably, ripretinib is a “switch-controlled” kinase inhibitor that binds to the ATP-binding pocket, blocking the kinase from adopting an active state and stabilizing the kinase in an inactive state. The co-crystal structure of DP-2976, a closely related analog of ripretinib, with C-KIT demonstrated the switch control design concept of the drug. Ripretinib binds to the DFG-out active conformation with the urea moiety, forming a hydrogen bond with switched E640, which plays an antagonist’s role in maintaining the AL switch in an inactive type II state. The terminal phenyl ring of ripretinib takes over the interior

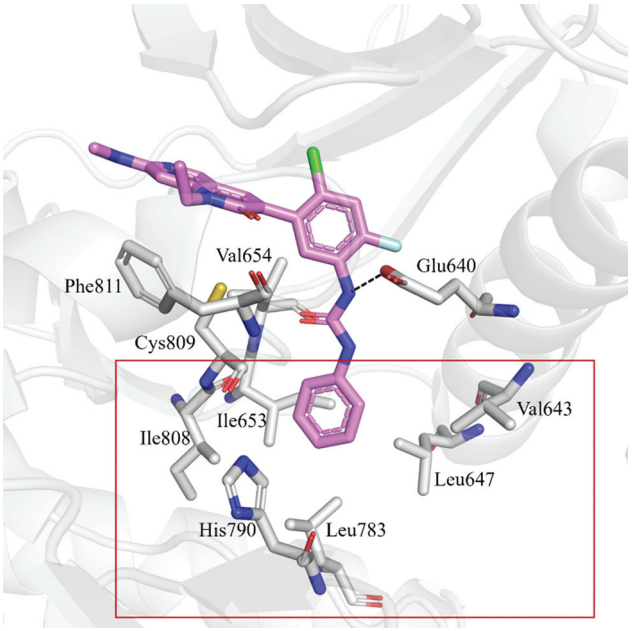


Fig. 5 The X-ray crystal structure of DP-2976 complexed with C-KIT (PDB ID: 6MOB). The red box presents the interior R-spine within the switch pocket formed by V643, L647, I653, V654, L783, H790, and I808.

R-spine within the switch pocket formed by surrounding residues, thereby stabilizing the AL switch phenyl side chain of F811 (DFG motif) in a type II inactive state. Furthermore, the fused pyridone ring of ripretinib forms extensive hydrophobic and van der Waals interactions with switched C809 and F811, further keeping the AL switch in a type II inactive state (►**Fig. 5**).[26]

Despite the ideal inhibitory activity of ripretinib against most C-KIT/PDGFRα mutations and the improvement in survival in patients with GISTs, disease progression occurs in some patients. The resistance mechanisms associated with disease progression have not been well defined, but it is possibly associated with resistance mutations in the ATP-binding pocket, as well as C-KIT/PDGFRα-independent mechanisms involving the PI3K and RAS/RAF pathways.[28] Therefore, it is necessary to develop the next generation of C-KIT/PDGFRα inhibitors with better therapeutic efficacy.

Avapritinib

Avapritinib is a potent and selective type I/II C-KIT/PDGFRα inhibitor targeting AL mutations such as D842V in PDGFRα ($IC_{50} = 0.24$ nmol/L) and D816V in C-KIT ($IC_{50} = 0.27$ nmol/L). For patients with GISTs harboring D842V in PDGFRα, the objective response rate following avapritinib therapy was 84% (complete response rate of 7%), and 61% of patients experienced remission for ≥ 6 months. In the dose expansion segment, 56 patients with AL mutations in PDGFRα were included, and 49 of these patients had a favorable overall efficacy evaluation, including complete responses in 5 patients (9%) and partial responses in 44 patients (79%) without dose-limiting toxicity. Based on these promising clinical results, avapritinib was approved by the FDA in January 2020 for the treatment of unresectable or metastatic GISTs in adults with PDGFRα exon 18 mutations, including D842V.

Although avapritinib is effective against GISTs carrying AL mutations in PDGFRα, recent clinical research has demonstrated that patients acquire resistance after treatment. Among them, the solvent front (SF) mutation G680R and the GK residue mutation T674I are the main causes of avapritinib resistance, accounting for more than 40% of cases. In particular, the D842V/G680R dual mutation in PDGFRα accounted for 30% of cases of drug resistance, including a high degree of acquired resistance. Avapritinib has strong activity against D842V-mutated PDFGRα with an IC_{50} of 0.24 nmol/L, whereas the D842V and G680R mutations are associated with IC_{50} values exceeding 1 μmol/L. Similarly, avapritinib had an IC_{50} of 40 nmol/L for D842V-mutated PDFGRα in a GIST cell model (GIST-T1), whereas its IC_{50} for G680R-mutated PDFGRα exceeded 10 μmol/L. In addition, the D842V/T674I dual mutation in PDGFRα has been linked to resistance in GISTs-T1 cells with an IC_{50} of 750 nmol/L, and median OS in patients with avapritinib-resistant GISTs is only 5.2 months. The X-ray crystal structure of avapritinib complexed with wild-type PDGFRα (►**Fig. 6A**) illustrates that the pyrrolotriazine scaffold N2 nitrogen of avapritinib interacts with C677 in the hinge region via a hydrogen bond. In addition, there is a water-mediated interaction between N4 of the pyrrolotriazine scaffold of avapritinib and T674. Although there is no direct interaction between avapritinib and G680, the structures are separated by approximately 4 Å, suggesting the mutation to arginine introduces a steric clash with the more sterically demanding guanidinium group. Conversely, the GK mutation T674I breaks the water-mediated interaction between avapritinib and T674, resulting in reduced binding affinity and ultimately resistance (►**Fig. 6B**).[29,30]

Similarly, avapritinib effectively inhibits C-KIT carrying the D816V mutation ($IC_{50} = 3.8$ nmol/L), but it has little activity against C-KIT carrying the GK mutation T670I ($IC_{50} = 270$ nmol/L).[31] The X-ray crystal structure of avapritinib complexed with wild-type C-KIT (►**Fig. 7A**) and T670I-mutated C-KIT (►**Fig. 7B**) revealed a comparable binding mode and resistance mechanism as observed for PDGFRα.[30]

In general, avapritinib can effectively overcome several forms of clinically acquired resistance to first- to third-line type II kinase drugs, especially resistance associated with AL mutations. However, secondary mutations that confer avapritinib resistance are found in the SF and GK regions. There is an urgent need for next-generation C-KIT/PDGFRα inhibitors to overcome avapritinib-resistant mutations.

Small-Molecule Kinase Inhibitors in Clinical Development

Although the approved C-KIT/PDGFRα inhibitors have provided therapeutic benefits in patients with GISTs, secondary mutations conferring drug resistance remain a challenge. In this section, we briefly summarize the progress and optimization strategies of C-KIT/PDGFRα small-molecule inhibitors that are in clinical development (►**Table 3**).

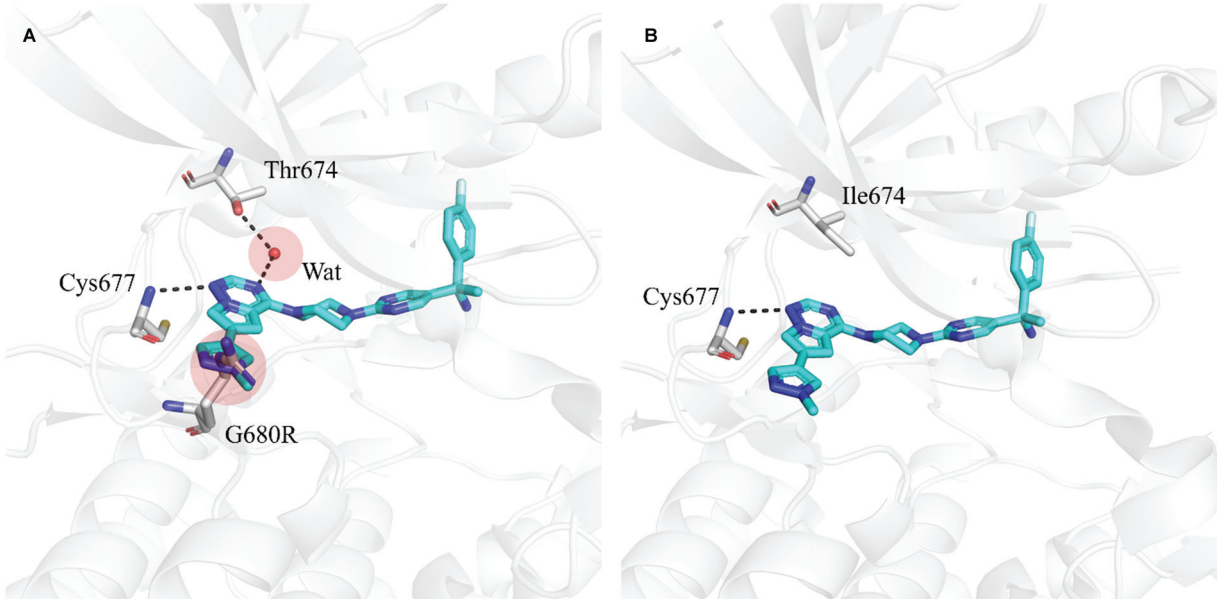


Fig. 6 The co-crystal structures of avapritinib bound to (**A**) wild-type PDGFRα (PDB ID: 8PQJ) and (**B**) PDGFRα T674I (PDB ID: 8PQH). Solvent front residue G680 is mutated to R680, which is displayed simply as G680R.

Crenolanib (CP-868596)

Crenolanib is a selective type I PDGFRα inhibitor with highly potent activity against the D842V mutation ($IC_{50} = 10$ nmol/L). Moreover, crenolanib has better inhibitory activity against the avapritinib resistance mutation D842V/T674I in PDGFRα with an IC_{50} of approximately 20 nmol/L in CHO cells.[32] However, crenolanib is relatively ineffective against the PDGFRα V561D and D842V/G680R mutants, with IC_{50} values of approximately 400 nmol/L in CHO cells and 10 μmol/L in GIST-T1 cells. The X-ray crystal structure of crenolanib complexed with PDGFRα T674I demonstrated that the imidazole nitrogen and amine of crenolanib form hydrogen bonds with C677 and R822, respectively, of the kinase. Moreover, crenolanib does not collide with the mutated GK residue T674I, which explains its ideal inhibitory activity against the GK mutants T674I and D842V/T674I (►**Fig. 8**). Crenolanib is currently being evaluated in a phase III clinical trial for the treatment of GISTs in patients carrying the D842V mutation (NCT02847429).

Bezuclastinib (CGT9486)

Bezuclastinib is a type I C-KIT inhibitor developed by Cogent Biosciences. The X-ray crystal structure of bezuclastinib complexed with C-KIT illustrated that the 1*H*-pyrrolo[2,3-*b*] pyridine scaffold forms two hydrogen bonds with C673 in the hinge region, and the NH of the amide forms a hydrogen

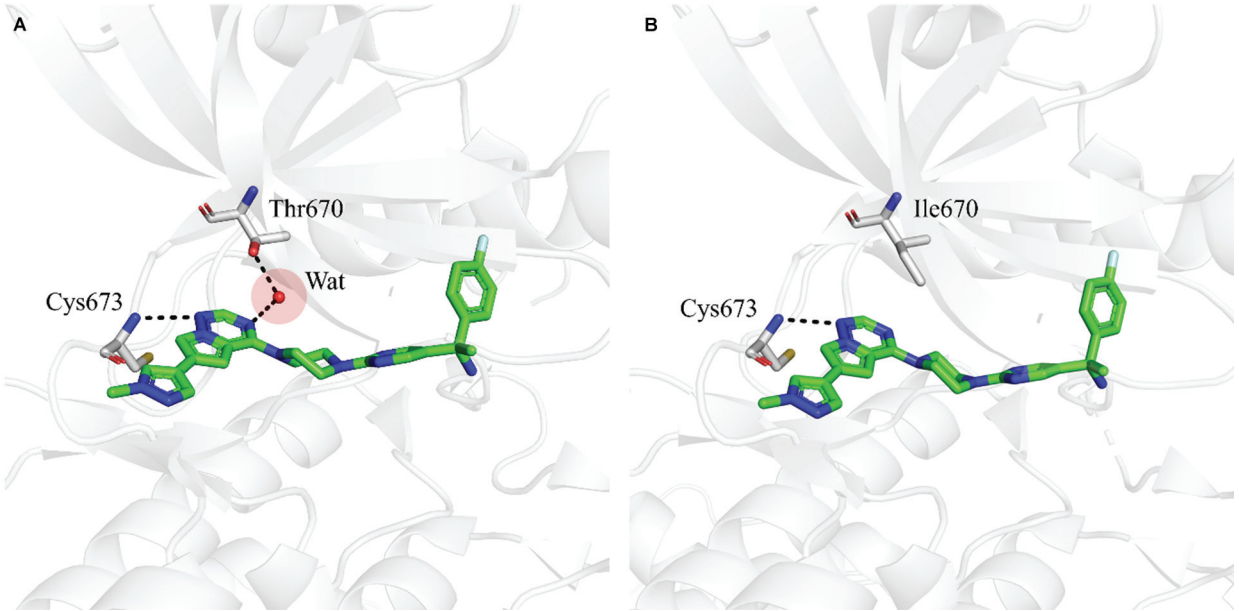
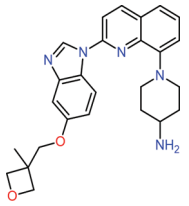
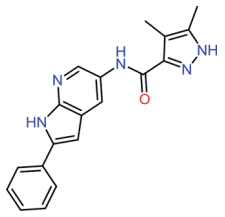
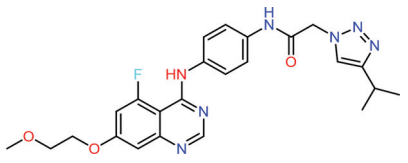
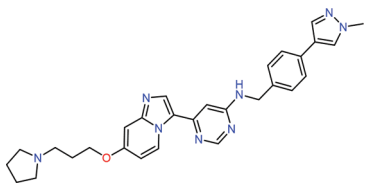


Fig. 7 The co-crystal structures of avapritinib bound to (**A**) wild-type C-KIT (PDB ID: 8PQ9) and (**B**) C-KIT T670I (PDB ID: 8PQG).

Table 3 Small-molecule C-KIT/PDGFRα inhibitors in clinical research for the treatment of GISTs

Name	Structure	Organization	Type	Target	Efficacy	Status (clinical trial number)
Crenolanib		Arog Pharmaceuticals	I	PDGFRα, FLT3	PDGFRα D842V	III (NCT02847429)
Bezuclastinib		Cogent Biosciences	I	C-KIT	C-KIT D816V, V560G/D816V	III (NCT05208047)
NB003		AstraZeneca	II	C-KIT, PDGFRα	pan C-KIT mutants	I (NCT04936178)
THE-630	/	Theseus Pharmaceuticals	/	C-KIT	pan C-KIT mutants	Discontinued
IDRX-42		IDRX	II	C-KIT	pan C-KIT mutants	I (NCT05489237)

Note: Data are updated up to February 24, 2024.

bond with the GK residue T670. In addition, the dimethylpyrazole forms hydrogen bonds with E640 and D810 (►**Fig. 9**). Bezuclastinib is effective against C-KIT carrying primary mutations (exons 9 and 11) and AL mutations (exons 17 and 18).[33] In imatinib-resistant BaF3 cells, bezuclastinib potently inhibited C-KIT carrying the D816V and V560G/D816V mutations with IC_{50} values of 6.6 and 7.1 nmol/L, respectively, representing approximately 300- to 600-fold greater potency than achieved by imatinib.[34] Because of its complementary C-KIT mutant inhibition profiles, bezuclastinib is a potent combination partner for sunitinib, which has activities against ATP-binding pocket mutations (exons 13 and 14). Preliminary data indicate that bezuclastinib and sunitinib can be safely combined to achieve improved clinical outcomes in patients with heavily pretreated GISTs, with median PFS reaching 12.1 months.[35] Currently, a phase III randomized trial of bezuclastinib plus sunitinib in subjects with GISTs (NCT05208047) is ongoing.

NB003 (AZD3229)

NB003, a selective type II C-KIT/PDGFRα inhibitor, displays potent inhibitory activity against multiple primary and imatinib-resistant secondary mutations in GISTs.[36] Kettle et al reported a detailed optimization process for NB003. Inspired by the potent C-KIT inhibitory activity of compound **1**[37] (AZD2932) and the high C-KIT/kinase insert domain receptor (KDR) selectivity of compound **2**,[38] compound **3** was designed by incorporating two potent fragments from both compounds (►**Fig. 10**). Indeed, compound **3** exhibited improved activity against three C-KIT cell lines, but its selectivity against vascular endothelial growth factor receptor 2 (VEGFR2; also known as KDR) was lost. Next, reversing the acetamide linker and removing the methoxy on the phenyl ring generated compound **4**, which displayed enhanced selectivity. Furthermore, replacement of the isopropyl imidazole of **4** with isopropyl triazole gave the more potent compound **5**, which had improved selectivity over

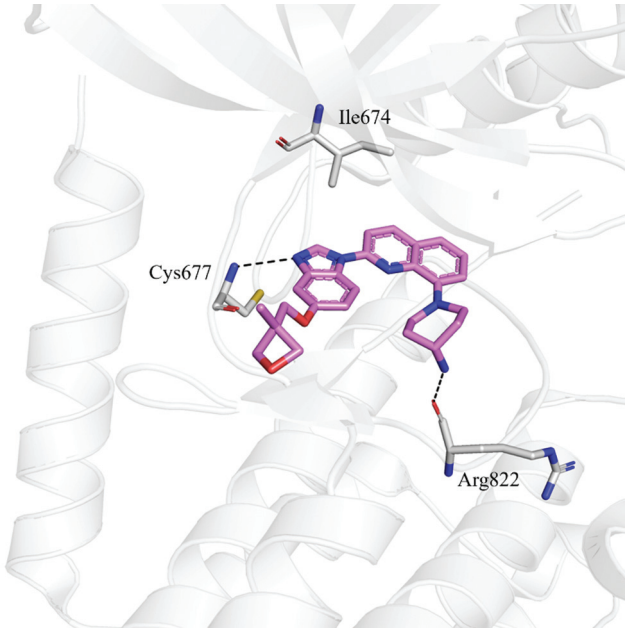


Fig. 8 The binding mode of crenolanib complexed with PDGFRα T674I (PDB ID: 6JOI).

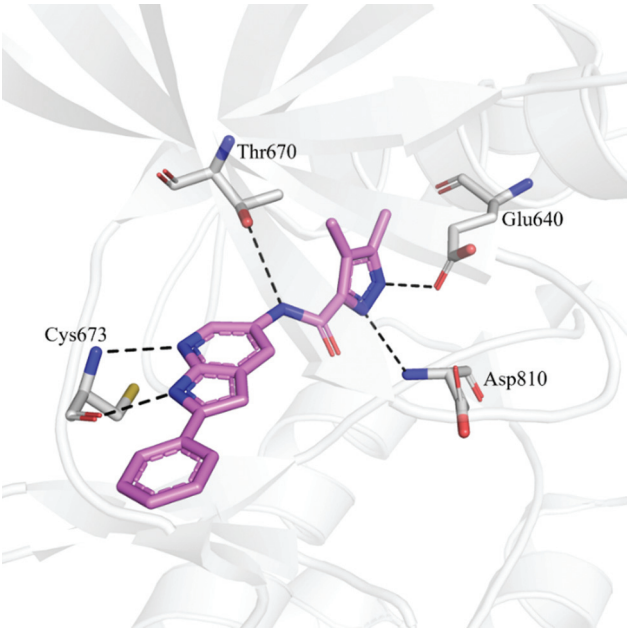


Fig. 9 The crystal structure of bezuclastinib complexed with C-KIT (PDB ID: 7KHK).

VEGFR2. Optimization of the two methoxyl groups in quinazoline with fluoro and methoxyethyl moieties led to NB003 (**6**), which exhibited at least 400-fold higher selectivity against KDR at least 400-fold. NB003 displayed excellent potency for C-KIT ($GI_{50} = 1$ nmol/L) and PDGFRα ($GI_{50} = 3$ nmol/L) with almost 1,000-fold selectivity over VEGFR2 ($GI_{50} = 1{,}378$ nmol/L) in Ba/F3 cells. *In vivo* studies also found that NB003 exerted durable inhibition in patient-derived xenograft models of

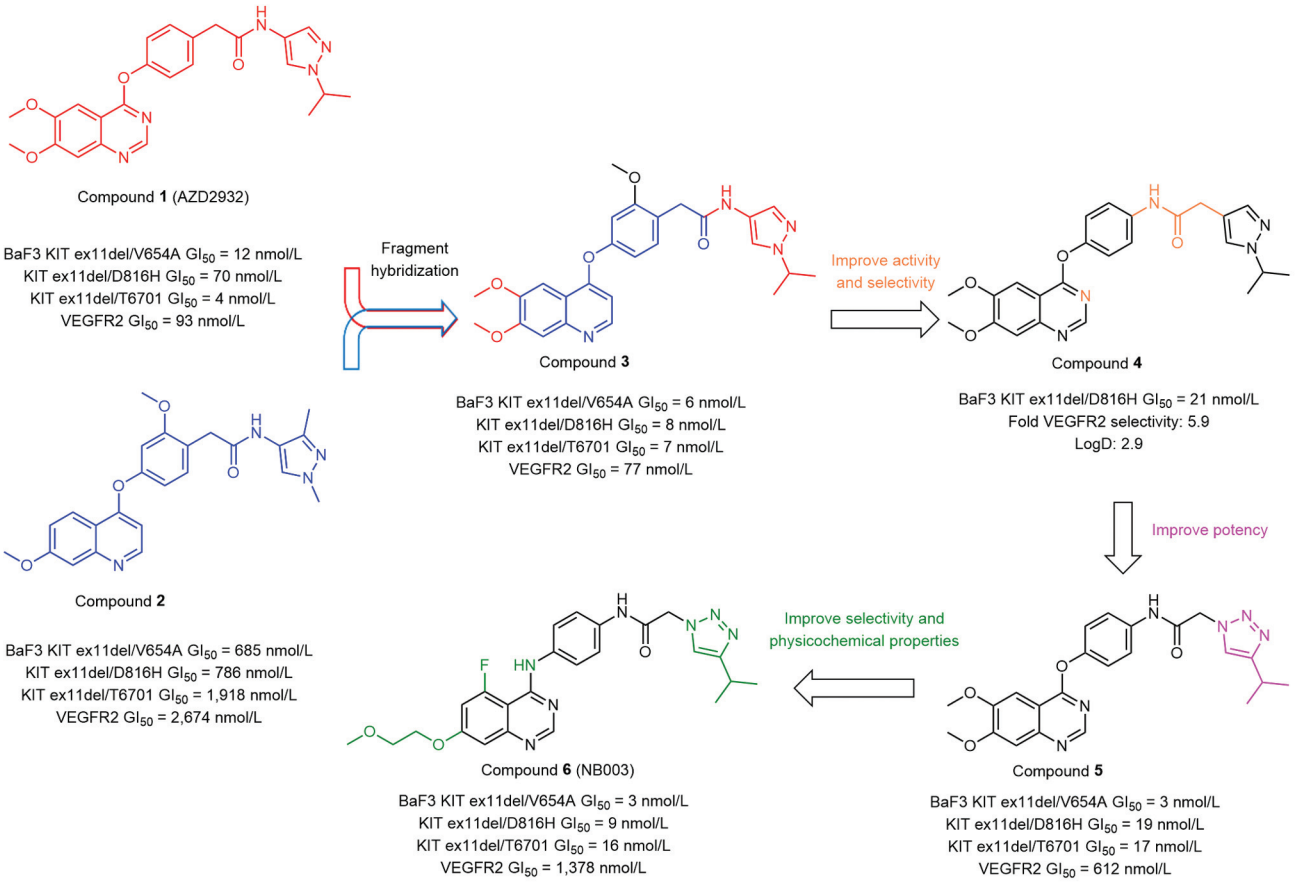


Fig. 10 Optimization process of NB003 based on compounds **1** and **2**.

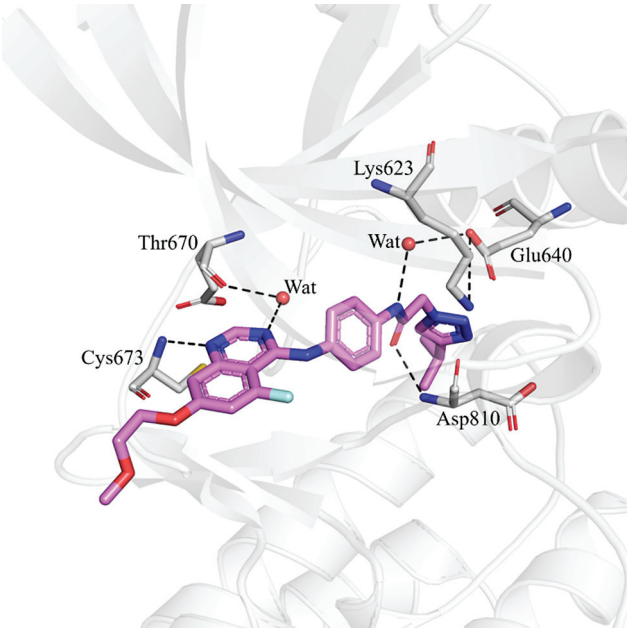


Fig. 11 The binding mode of NB003 complexed with C-KIT (PDB ID: 6GQM).

GISTs.[39] A co-crystal structure was solved between NB003 and C-KIT, illustrating that NB003 is a type II inhibitor that binds to the DFG-out inactive conformation of C-KIT. The quinazoline moiety interacts with C673 in the hinge region and forms a water-mediated interaction with the GK residue T670. The acetamide forms a water-mediated interaction with the conserved residues K623 and E640. The triazole fragment inserts into the DFG-out pocket and stabilizes the bound waters in C-KIT (►**Fig. 11**). Ultimately, NB003 has progressed to phase I clinical development based on its optimal C-KIT activity, selectivity over VEGFR2, and pharmacokinetic properties (NCT04936178).

THE-630

THE-630 has displayed extensive inhibition of primary and secondary C-KIT mutants in early clinical development. THE-630 exhibits high potency ($IC_{50} \leq 3$ nmol/L) in GIST-T1 and BaF3 cells featuring exon 11 deletion and an activating insertion in exon 9, respectively.[40] In addition, THE-630 has excellent inhibitory activities against ATP-binding pocket and secondary AL mutants (e.g., V654A, T670I, D816G/H) with $IC_{50} \leq 25$ nmol/L. THE-630 exhibited antitumor activity and tolerable safety in patients with advanced GISTs (NCT05160168) in a phase I/II study. However, the phase I/II clinical study of THE630 in patients with GISTs was terminated in 2023.

IDRX-42 (M-4205)

IDRX-42 is a selective type II C-KIT inhibitor with extensive activity against clinically relevant C-KIT mutants and outstanding biochemical and cellular properties. Blum et al reported the optimization process of IDRX-42 in detail.[41] Optimization of the hit compound **7** focused on improving its metabolic stability and cellular potency by incorporating pyrimidine and hydrophilic groups such as methylimidazole and ethoxymethoxy to obtain compound **8**, which featured improved cellular potency and metabolic stability. To explore its druggability, further optimization of **8** using various hydrophilic groups instead of ethoxymethoxy led to IDRX-42 (**9**), which displayed therapeutic efficacy and high metabolic stability (►**Fig. 12**). IDRX-42 exhibited an IC_{50} of 59 nmol/L in the imatinib-resistant cell line GIST430/654 (exon 11 and 13 mutations), but it was relatively ineffective in cells carrying the GK mutation T670I and AL mutation D816V in C-KIT ($IC_{50} = 367$ and 522 nmol/L, respectively). The co-crystal structure of IDRX-42 complexed with C-KIT illustrated that the imidazo [1,2-*a*] pyridine nitrogen forms a hydrogen bond with C673, and the NH moiety interacts with the GK residue T670, which explains the decreased activity of the drug against GK mutations. In addition, the terminal benzylic substituent forms a π-interaction with W557, and the pyrimidine nitrogen has water-mediated interactions with D810 and K623 (►**Fig. 13**). Currently, IDRX-42 is in phase I development for the treatment of metastatic and/or

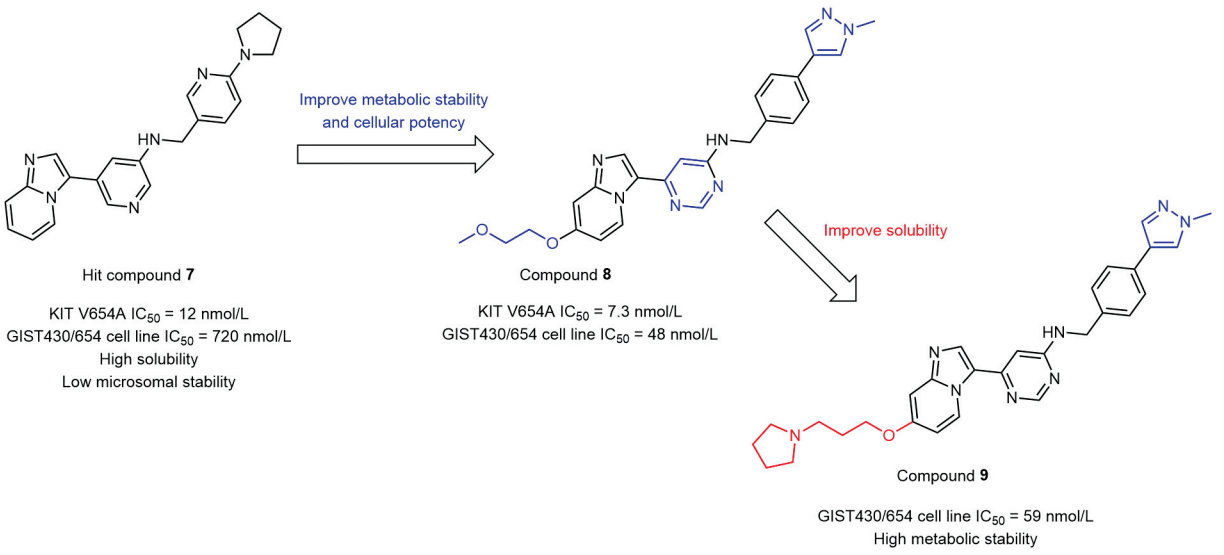


Fig. 12 Optimization process of IDRX-42 based on the hit compound **7**.

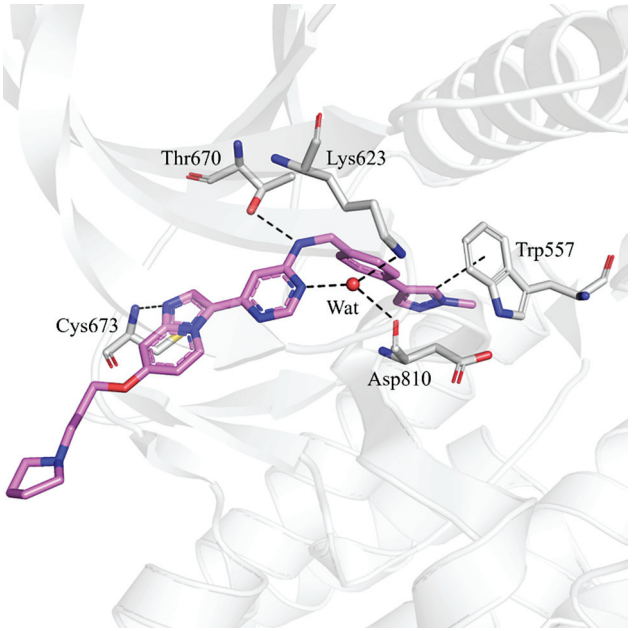


Fig. 13 The binding mode of IDRX-42 complexed with C-KIT (PDB ID: 7ZW8).

unresectable GISTs (NCT05489237), and it has received orphan drug designation from the FDA for the treatment of GISTs.

Conclusion and Perspectives

The approved first- to fourth-line drugs and avapritinib, as effective targeted therapies for GISTs, exert excellent inhibitory activity against the vast majority of C-KIT/PDGFRα mutations. Despite the significant clinical benefits of these treatments, their clinical application is limited by SF and GK mutation-mediated clinical resistance. The development of next-generation C-KIT/PDGFRα inhibitors that can overcome acquired resistance to avapritinib is a research hotspot and unmet clinical need. Currently, there are multiple TKIs in clinical development for GIST treatment, such as crenolanib, bezuclastinib, NB003, and IDRX-42. These drugs could represent additional treatment options for GISTs, especially for patients who harbor secondary drug resistance mutations.

The SF mutation G680R in PDGFRα causes steric hindrance with the methylpyrazole of avapritinib, leading to poor inhibitory potency. Similarly, an SF mutation also arises in RTK after treatment with the first-generation TKI larotrectinib. The second-generation macrocycle-based TKIs selitrectinib[42] and repotrectinib[43] can effectively inhibit SF mutations by avoiding clashes with the mutant residues. Therefore, reasonable macrocyclization could represent an effective strategy to overcome SF mutations in PDGFRα. In addition, GK mutations are the most common mutations in acquired drug resistance, and they inevitably emerge in C-KIT (T670I) and PDGFRα (T674I) with similar resistance mechanisms. GK mutations, namely T670I in C-KIT and T674I in PDGFRα, disrupt the water-mediated interaction of avapritinib with T670 in KIT and T674 in PDGFRα, resulting in reduced binding affinity. A GK mutation from lysine to leucine (T315I) also occurs in ABL kinase. Ponatinib avoids a steric clash with I315 via an alkyne linker and additionally forms favorable van der Waals interactions with I315, resulting in favorable inhibitory activity.[44] Thus, avoiding steric hindrance or increasing additional interactions, e.g., hydrogen bonds or hydrophobic interactions, to compensate for lost interactions attributable to GK mutations could represent a strategy to increase the binding affinity of compounds with GK mutant kinases.[45] In addition to on-target resistance, overactivation of bypass signaling or downstream pathways is also an important factor leading to resistance in GISTs. Nevertheless, the review provides a solid foundation for the discovery of next-generation C-KIT/PDGFRα inhibitors for overcoming clinical resistance in GIST treatment.

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Conflict of Interest

None declared.

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