認された研究計画書、「個人情報保護法」、「臨床研究に関する倫理指針（平成２０年厚生労働省告示第４１５号）」に準じて実施した。対象患者に対し、大阪大学医学部附属病院の共同研究者である医師が説明資料に従い研究について説明し、十分の理解を得た上で、文書に同意を得た。

個人情報保護

大阪大学医学部附属病院においては消化器外科　高橋剛助教を個人情報管理者とし連結可能匿名化することで個人情報の管理を行った。医薬基盤研究所には大阪大学医学部附属病院において連結可能匿名化された情報が試料とともに提供され、提供される情報は年齢、性別、病名、生化学データとした。

C. 研究結果

結果はD項にまとめて記載した。

D. 結果・考察

①

GIST 細胞株（GIST-T1）に対してイマチニブを添加することで、IC50 値が 100 倍を示す耐性株（GIST-T1R2, R8, R9）を作成した。エキソーム解析を行い親株と比較し、これら耐性株が共通に獲得した遺伝子変異を 19 か所検出した。

②

大阪大学医学部附属病院において、切除されたイマチニブ耐性 GIST 腫瘍サンプル 25 検体（初発、耐性がそろったものを 3 組含む）から genomic DNA を抽出した。現在、これらから耐性に関わる遺伝子変異の同定中である。その結果を踏まえ、①と対比し今後の方向性を決定する。

E. 結論

細胞株を用いた耐性誘導実験の中で、耐性株には、親株に認めなかった遺伝子変異を確認することができた。現在、臨床検体を用いてさらなる検討中である。

F. 健康危険情報

該当無し。

G. 研究発表

G-1.　論文発表

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G-2. 学会発表

1. 第73回日本癌学会（パシフィコ横浜）
2014年9月25日(木) SOCS-1 induces apoptosis of ovarian cancer cell lines via JAK/STAT3 dependent and independent pathways. Nakagawa S, Serada S, Morimoto A, Takada T, Kimura T, Ueda Y, Yoshino K, Fujita M, Kimura T, **Naka T**.
2. 第73回日本癌学会（パシフィコ横浜） 2014年9月25日(木) The effect of cell cycle by gene therapy for gastric cancer using SOCS-1 in vitro. Nakatsuka R, Takahashi T, Serada S, Fujimoto M, Miyazaki Y, Kurokawa Y, Yamasaki M, Miyata H, Nakajima K, Takiguchi S, Mori M, Doki Y, **Naka T**.
3. The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA" April 5-9 Quantitative proteomic analysis of cell-surface membrane proteins: biomarker discovery in esophageal squamous cancer. Harada E, Serada S, Takahashi T, Fujimoto M, **Naka T**.
4. The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA" April 5-9 CpG oligodeoxynucleotide enhances the efficacy of anticancer monoclonal antibody in an in vivo xenograft model

using human endometrial cancer cell. Hiramatsu K, Serada S, Kobiyama K, Morimoto A, Fujimoto M, Ishii K, **Naka T**.

5. The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA" April 5-9 Gene therapy for peritoneal dissemination model of gastric cancer using SOCS-1 by Adenoviral Vector. Nakatsuka R, Takahashi T, Serada S, Fujimoto F, Souma Y, Miyazaki Y, Kurokawa Y, Yamasaki M, Miyata H, Nakajima K, Takiguchi S, Mori M, Doki Y, Doki Y, **Naka T**.
6. The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA" April 5-9 Suppressor of cytokine signaling (SOCS)-1 suppresses a proliferation of malignant melanoma cells via the suppression of JAK/STAT and the activation of p53 signaling pathways. Tagami N, Serada S, Fujimoto F, Tanemura A, Katayama I, **Naka T**.

H. 知的財産権の出願・登録状況

名称　　：食道がんのマーカーおよびその利用
発明者　　：**仲哲治**、世良田聡、藤本穣、豊浦雅義、庄屋雄二
出願人　　：独立行政法人医薬基盤研究所
出願日　　：2013年12月27日
出願番号　　：特願2013-272085
国際出願番号：PCT/JP2014/006455
出願日　　：2014年12月25日

名称　　：悪性腫瘍の治療薬
発明者　　：仲哲治、世良田聡、藤本穣、豊浦雅義、庄屋雄二
出願人　　：独立行政法人医薬基盤研究所
出願日　　：2013年12月27日
出願番号　　：特願2013-272084
国際出願番号：PCT/JP2014/006456
出願日　　：2014年12月25日

I. 研究協力者

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世良田　聡　医薬基盤研究所 免疫シグナルプロジェクト
髙橋　剛　大阪大学医学部附属病院 消化器外科

別紙３　　厚生労働科学研究委託費（革新的がん医療実用化研究事業）

委託業務成果報告（業務項目）

網羅的ドライバー遺伝子変異検索に基づく耐性GISTの治療薬開発に関する研究
KIT・PDGFRA遺伝子解析体制の構築

研究分担者　　廣田　誠一　兵庫医科大学付属病院　病理学

研究要旨

研究参加病院からの手術または生検の各種検体を用い、HE 染色および免疫染色（KIT, CD34, DOG1, SDHB, pfetin, Ki-67 等）を行い、同時に *c-kit* 遺伝子のエクソン 9, 11, 13, 17 と *PDGFRA* 遺伝子のエクソン 12, 14, 18 の変異解析を行った。

各施設から提供されたパラフィンブロックを用い、中央判定として上記病理遺伝子診断を行った。研究グループの GIST 診断は十分なレベルで診断されており、中央での *KIT・PDGFRA* 遺伝子変異の検索も可能であることが確認できた。

A. 研究目的

別に行われている GIST 登録事業では、一般病院で GIST と判定された症例の約 5%が中央判定では非 GIST と最終診断されている。同じく GIST 登録事業では、一般病院で再発高リスク GIST と判定された症例の約 15%が中央判定では再発非高リスク GIST と診断されている。これらの事実は、高価な分子標的薬が使用される GIST の領域において、無駄な分子標的薬治療が行われ、医療費が無駄に使用されている可能性を示唆している。GIST の確定診断・リスク評価の適切な遂行が GIST 診療領域では重要であり、それが適切に行われているかを検証し、また、本研究の精度の向上に寄与することが本研究の目的である。

B. 研究方法

手術または生検の各種検体において、HE 染色および免疫染色（KIT, CD34, DOG1, SDHB, pfetin, Ki-67 等）を行い、GIST の病理診断が各施設で適切に行われているかどうかについて検討した。また、上記各種検体について、パラフィンブロックから DNA を抽出し、c-kit 遺伝子のエクソン 9, 11, 13, 17 と PDGFRA 遺伝子のエクソン 12, 14, 18 の変異解析を行い、GIST の確定診断の確認をするとともに、GIST の発生に関与する他の遺伝子の検索体制の整備や、GIST と鑑別すべき非 GIST 症例における遺伝子変異検索体制の整備を行った。

＜倫理面への配慮＞

GIST およびそれに関連した疾患における遺伝子変異の解析については、当施設での倫理委員会で承認されており、また、検体は匿名化されており、個人情報の漏洩の心配はない。

C. 結果

C. 研究結果

これまで検索した手術または生検の各種検体においては、概ね GIST の病理診断が各施設で適切に行われているものと考えられた。また、遺伝子変異検索の結果も概ね、これまでの報告に矛盾しない頻度で認められた。

D. 考察

研究グループの研究者は GIST に関する知識や経験が豊富であり、それらの施設での GIST の病理診断は十分なレベルで行われているものと評価される。遺伝子変異の検索からは各施設での GIST 症例に大きな偏りはないものと思われた。

E. 結論

研究グループの研究者の施設での GIST の病理診断はほぼ十分なレベルで行われている。遺伝子変異の検索に各施設での偏りはない。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

Ito T, Yamamura M, Hirai T, Ishikawa T, Kanda

T, Nakai T, Ohkouchi M, Hashikura Y, Isozaki K, Hirota S. Gastrointestinal stromal tumors with exon 8 c-*kit* gene mutation might occur at extragastric sites and have metastasis-prone nature. **Int J Clin Exp Pathol.** 7:8024-8031, 2014.

2. 学会発表

劉寧寧, 井出良浩, 梶本仙子, 木村尚美, 松田育雄, 廣田誠一：KIT-Dup-Ser501Ala502 変異を持つ GIST の臨床病理学的特徴と変異分子の生物学的特徴の検討. 第 103 回病理学会総会（示説）, 2014 年 4 月 24 日

H. 知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

Ⅲ. 学会等発表実績

様式第19

学 会 等 発 表 実 績

委託業務題目「網羅的ドライバー遺伝子変異検索に基づく耐性GISTの治療薬開発に関する研究」
機関名 独立行政法人 国立がん研究センター東病院

1．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
ﾚｯｸﾘﾝｸﾞﾊｳｾﾞﾝ病に伴う消化管間質腫瘍(GIST)に対する臨床試験の提案	**西田俊朗、内藤陽一、佐々木政興、野村尚吾、佐藤暁洋、土井俊彦**	第6回日本ﾚｯｸﾘﾝｸﾞﾊｳｾﾞﾝ病学会 東京	2014/11/15～16	国内
稀少がんの臨床研究－消化管間質腫瘍(GIST)	**西田俊朗、佐藤暁洋 土井俊彦**	第114回日本外科学会定期学術集会 京都	2014/4/3～5	国内
高リスクGISTに対する完全切除後の治療に関するレジストリ研究－病理診断の重要性	**西田俊朗**、長晴彦 尾阪将人 **小松嘉人** 黒川幸典 **土井俊彦** 平井敏弘 北川雄光 馬場秀夫 **山田康秀** 杉山敏郎 掛地吉弘 羽藤慎二 平島詳典 **廣田誠一**	第52回日本癌治療学会学術集会 横浜	2014/8/28～30	国内
KIT-Dup-Ser501Ala502変異を持つGISTの臨床病理学的特徴と変異分子の生物学的特徴の検討．第103回病理学会総会（ポスター）	劉寧寧，井出良浩，梶本仙子，木村尚美，松田育雄，**廣田誠一**	第103回病理学会総会・広島	2014年4月24日	国内
SOCS-1 induces apoptosis of ovarian cancer cell lines via JAK/STAT3 dependent and independent pathways. (Poster)	Nakagawa S, Serada S, Morimoto A, Takada T, Kimura T, Ueda Y, Yoshino K, Fujita M, Kimura T, **Naka T.**	第73回日本癌学会 パシフィコ横浜	2014年 9月25日(木)	国内
The effect of cell cycle by gene therapy for gastric cancer using SOCS-1 in vitro. (Poster)	Nakatsuka R, Takahashi T, Serada S, Fujimoto M, Miyazaki Y, Kurokawa Y, Yamasaki M, Miyata H, Nakajima K, Takiguchi S, Mori M, Doki Y, **Naka T.**	第73回日本癌学会 パシフィコ横浜	2014年 9月25日(木)	国内
Quantitative proteomic analysis of cell-surface membrane proteins: biomarker discovery in esophageal squamous cancer. (Poster)	Harada E, Serada S, Takahashi T, Fujimoto M, **Naka T.**	The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA"	2014 April 5-9	国外
CpG oligodeoxynucleotide enhances the efficacy of anticancer monoclonal antibody in an in vivo xenograft model using human endometrial cancer cell. (Poster)	Hiramatsu K, Serada S, Kobiyama K, Morimoto A, Fujimoto M, Ishii K, **Naka T.**	The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA"	2014 April 5-9	国外

Gene therapy for peritoneal dissemination model of gastric cancer using SOCS-1 by Adenoviral Vector. (Poster)	Nakatsuka R, Takahashi T, Serada S, Fujimoto F, Souma Y, Miyazaki Y, Kurokawa Y, Yamasaki M, Miyata H, Nakajima K, Takiguchi S, Mori M, Doki Y, Doki Y, Naka T.	The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA"	2014 April 5-9	国外
Suppressor of cytokine signaling (SOCS)-1 suppresses a proliferation of malignant melanoma cells via the suppression of JAK/STAT and the activation of p53 signaling pathways. (Poster)	Tagami N, Serada S, Fujimoto F, Tanemura A, Katayama I, **Naka T.**	The 105th Annual Meeting of the American Association for Cancer Research (AACR Annual Meeting 2014) San Diego, USA"	2014 April 5-9	国外
cDNA and miRNA microarray analysis comparing gastric and metastatic liver gastrointestinal stromal tumors、ポスター発表	菊池寛利、飯野一郎太、宮崎真一郎、尾崎裕介、平松良浩、太田学、神谷欣志、坂口孝宣、馬場聡、瀬藤光利、椙村春彦、今野弘之	American Association for Cancer Research Annual Meeting 2014	平成26年4月	国外
消化管間質腫瘍における肝転移機構の探索、口頭発表	菊池寛利、飯野一郎太、松本知拓、尾崎裕介、宮崎真一郎、高橋善明、藤田剛、平松良浩、太田学、神谷欣志、坂口孝宣、今野弘之	第114 回日本外科学会定期学術集会	平成26年4月	国内
マイクロアレイ解析を用いた胃・小腸GISTの生物学的相違の検討、ポスター発表	原竜平、菊地寛利、松本知拓、尾崎裕、宮崎真一郎、高橋善明、飯野一郎太、藤田剛、川端俊貴、平松良浩、太田学、神谷欣志、坂口孝宣、今野弘之	第114 回日本外科学会定期学術集会	平成26年4月	国内
進行GISTに対する術前イマチニブ療法の検討、口頭発表	菊池寛利、宮崎真一郎、松本知拓、尾崎裕介、川端俊貴、平松良浩、太田学、神谷欣志、坂口孝宣、**今野弘之**	第52回日本癌治療学会学術集会	平成26年8月	国内
消化管間質腫瘍の肝転移におけるマイクロRNA発現変化、ポスター発表	菊池寛利、飯野一郎太、宮崎真一郎、尾崎裕介、平松良浩、太田学、神谷欣志、馬場聡、瀬藤光利、坂口孝宣、**今野弘之**	第73回日本癌学会学術総会	平成26年9月	国内
肝転移GISTに対する治療戦略、ポスター発表	菊池寛利、松本知拓、尾崎裕、宮崎真一郎、川端俊貴、平松良浩、太田学、神谷欣志、坂口孝宣、**今野弘之**	第12回日本消化器外科学会大会	平成26年10月	国内
マイクロアレイ解析を用いた胃・小腸GISTの生物学的相違の検討、口頭発表	原竜平、菊地寛利、宮崎真一郎、川端俊貴、平松良浩、太田学、神谷欣志、坂口孝宣、**今野弘之**	第25回日本消化器癌発生学会総会	平成26年11月	国内

2. 学会誌・雑誌等における論文掲載

掲載した論文(発表題目)	発表者氏名	発表した場所 (学会誌・雑誌等名)	発表した時期	国内・外の別
Tyrosine kinase inhibitors in treatment of unresectable or metastatic gastrointestinal stromal tumours	**Nishida T**, Doi T, Naito Y	Expert Opin Pharma	2014年2月	国外
Impact of rechallenge with imatinib in patients with advanced gastrointestinal stromal tumor after failure of imatinib and sunitinib.	Sawaki A, Kanda T, **Komatsu Y**, **Nishida T**.	Gastroenterol Res Pract.	2014年6月	国外
Subgroups of Patients with Very Large Gastrointestinal Stromal Tumors with Distinct Prognoses: A Multicenter Study.	Wada N, Kurokawa Y, **Nishida T**, Yanagimoto, Y, Takahashi T, Nakajima K, Shuji Takiguchi S, **Hirota S**, Tsujinaka T, Mori M, Doki Y	J Surg Oncol	2014年10月	国外
Gastrointestinal stromal tumors with exon 8 c-kit gene mutation might occur at extragastric sites and have metastasis-prone nature.	Ito T, Yamamura M, Hirai T, Ishikawa T, Kanda T, Nakai T, Ohkouchi M, Hashikura Y, Isozaki K, **Hirota S**	Int J Clin Exp Pathol.	2014年10月	国外
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Ⅲ. 学会等発表実績　刊行物・別刷

EXPERT OPINION

Tyrosine kinase inhibitors in the treatment of unresectable or metastatic gastrointestinal stromal tumors

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Introduction: Gastrointestinal stromal tumor (GIST) is the most common sarcoma of the gastrointestinal tract. Proliferation of GIST is driven by activating mutations in the KIT or PDGFRA genes that found in most sporadic GISTs. Surgery is the main remedial measure for primary GIST, and imatinib is the principal therapeutic of choice for unresectable or metastatic GIST. Imatinib revolutionized treatment for unresectable or metastatic GISTs; however, resistance to imatinib has inevitably developed for most GIST patients.
Areas covered: PubMed was searched to find biological studies of GIST and clinical trials of molecularly targeted agents on unresectable or metastatic GISTs, and the key papers found have been reviewed. In this paper, the standard therapy which includes imatinib, sunitinib and regorafenib for unresectable or metastatic GIST has been reviewed and molecular mechanisms of resistance for tyrosine kinase inhibitors (TKIs) have been postulated and discussed. Treatment measures for resistant GIST and therapeutic choices after the standard therapy have also been described.
Expert opinion: The standard therapy for unresectable or metastatic GISTs is first-line imatinib, second-line sunitinib and third-line regorafenib. After standard therapy, best supportive care or clinical trials is recommended in the guidelines. However, patients may benefit from continuation of TKIs beyond disease progression and from rechallenge of TKIs used previously.

Keywords: imatinib, molecularly targeted therapy, regorafenib, resistance, sunitinib, tyrosine kinase inhibitor

Expert Opin. Pharmacother. (2014) 15(14):1979-1989

1. Introduction

1.1 Epidemiology of gastrointestinal stromal tumor

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the gastrointestinal tract. GIST tumor cells (70 – 75%) consist mainly of spindle cells; however; 20% of the morphology is made up of epithelioid cells, the other 5 – 10% show mixed morphology in pathological examinations. Almost all GISTs are positive for KIT (95%), also known as CD117, and for DOG1 (95%), a calcium-dependent, receptor-activated chloride channel protein, in immunohistochemistry [1]. Activating mutations in the *KIT* gene are found in approximately 70 – 80% of primary GISTs, and mutations in the *PDGFRA* gene are found in approximately 10% of primary GISTs (**Figure 1**) [1]. Familial GISTs, which are a rare entity, are caused by germ-line mutations in either *KIT* or *PDGFRA*; they have multiple GISTs and specific features. Patients with familial GISTs are younger and are typically associated with hyperplasia of interstitial cells of Cajal (ICC) in the myenteric plexus [2]. Thus, GISTs may be considered to be tumors with neoplastic transformation of immature mesenchymal cells, in the lineage of which KIT-positive

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Article highlights.

- The standard therapy for unresectable or metastatic gastrointestinal stromal tumors (GISTs) is imatinib in the first-line. After imatinib, sunitinib is indicated as second-line therapy for imatinib-resistant GIST, and, as third-line, regorafenib is indicated for sunitinib-resistant GIST.
- Most GISTs have activating mutations either in the *KIT* gene or in the *PDGFRA* gene, and genotype including primary and secondary mutations is correlated with activities of imatinib, sunitinib and regorafenib.
- Imatinib inhibits wild-type and also most mutated KIT and PDGFRA tyrosine kinases. Sunitinib and regorafenib inhibit several kinases including VEGFRs in addition to KIT and PDGFRA kinases, which may explain some activities of the drugs on wild-type GISTs. Profiles of KIT-inhibition including binding sites of the drugs are distinctive; hence, the three drugs have different activities on mutated KIT and PDGFRA tyrosine kinases.
- It may require dose modification according to genotype and patient conditions to optimize GIST therapy. However, standard doses of imatinib, sunitinib and regorafenib should be kept without any interruption when the drugs are tolerable.
- Patients with pediatric GISTs and/or *SDH*-mutated GISTs, which are thought to be insensitive to imatinib, may demonstrate some benefits from sunitinib and regorafenib.
- Imatinib resistance is divided into two: primary and secondary. Primary resistance is defined as disease progression within 6 months, and secondary (or acquired) resistance is practically defined as progressive disease (PD) after 6 months.
- Mutations newly appeared in the *KIT* and *PDGFRA* genes after treatment may be major causes of resistance to imatinib, sunitinib and regorafenib.
- Therapeutic options for imatinib-resistant GIST may include change to sunitinib, dose increase and surgical interventions to limited progression. The latter two may have limited evidence.
- After the standard therapy of tyrosine kinase inhibitors, continuation of the drug beyond PD or rechallenge of previously used drugs may be feasible and may have benefits for some GIST patients. In this situation, the drug that showed effectiveness and tolerability in previous treatment should be considered.
- It may require individualized approaches based on patterns of disease progression and causes of resistance to optimize the prognosis of unresectable or metastatic GIST patients. Genotyping of GIST is important in the selection of targeted agents, and mutation testing is recommended prior to medical therapy.

This box summarizes key points contained in the article.

ICC in normal myenteric plexus may be included [3]. Taken together, GIST may be diagnosed when mesenchymal tumors in the gastrointestinal tract have spindle and/or epithelioid features and are positive for KIT- and/or DOG1- immunostaining or for a mutation test of *KIT* and *PDGFRA*.

Incidence of GIST is clinically reported to be 10 – 20 cases per million per year. Clinical GIST is mainly found in the stomach (60 – 70%) and small intestine (20 – 30%), and < 5% of GISTs are found in the colon, esophagus or peritoneum including the mesentery, retroperitoneum and omentum [4,5]. The median age of patients with clinical GIST is around 60 years at diagnosis, although GIST has been reported in all age groups. By pathological and endoscopic examinations, preclinical small GISTs are shown to be more prevalent in middle age than expected [1,5]. Furthermore, microscopic GISTs, which may harbor *KIT* mutations, were frequently found in the proximal stomach (10 – 30%) of middle-aged or more elderly individuals, and their incidences were varied at each gastrointestinal site [1,5-8]. The natural history of these small tumors, including growth and malignant potential, are largely unknown. Most microscopic GISTs are supposed to be biologically indolent and remain stable for a long time or may even show involution [1,9]. Only a tiny fraction of microscopic GIST and a small fraction of small GISTs may be considered to progress and become clinically malignant GISTs [5].

1.2 Therapeutic outcomes of surgery for primary disease

The treatment for primary and resectable GIST is complete resection (R0) by surgery. The treatment with tyrosine kinase inhibitors (TKI) including imatinib, sunitinib, and regorafenib, is indicated for unresectable, metastatic or recurrent GIST. In addition, imatinib is indicated for adjuvant therapy of GIST patients with significant risk of recurrence. Most patients (nearly 60%) with primary localized GIST are considered to be cured only by R0 surgery [10]. GIST metastasizes to the liver and peritoneal cavity but rarely to the lymph nodes. As recurrences of GISTs after surgery are usually observed in the abdominal cavity, postoperative monitoring could be covered by abdominal CT with contrast media [4,11]. The important prognostic factors for recurrence after complete surgery include mitotic rate high power field, tumor size (cm), tumor location (gastric or non-gastric) and the presence or absence of tumor rupture [4,10,11]. Recently, genotype has not been indicated to be an independent prognostic factor [12]. Using these four prognostic factors, several risk stratification methods have been proposed [10,11,13,14].

2. Focus of the review

This review focuses on standard therapy with imatinib, sunitinib and regorafenib for unresectable or metastatic GIST and also discusses treatment of TKI-resistant GIST as well as postulated resistant mechanisms. Guidelines indicate that initial therapy for unresectable or metastatic GIST is imatinib (**Figure 2A**). When GIST is refractory or resistant or patients are intolerable, sunitinib is recommended [11]. After sunitinib, regorafenib may be used. To optimize medical therapy, we should be acquainted with tumor nature, drug action, clinical features of drug resistance and its basic mechanisms.

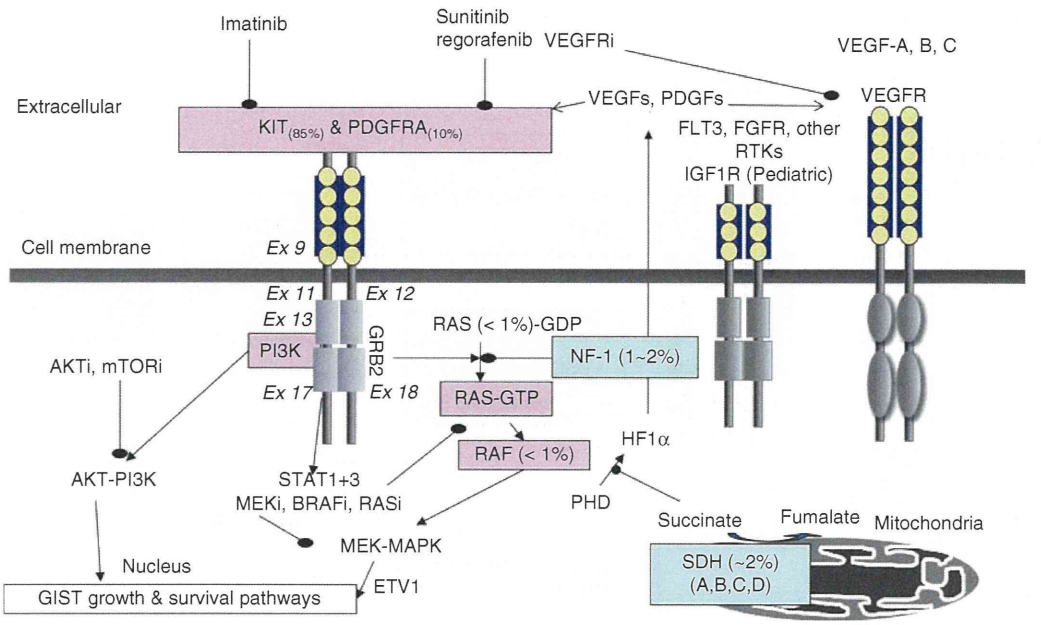

Figure 1. Driver mutations in GIST. Possible driver mutations in GISTs are illustrated with their frequency.
AKTi: Inhibitors of AKT; BRAFi: Inhibitors of BRAFi; GISTs: Gastrointestinal stromal tumors; MEKi: Inhibitors of MEK; mTORi: Inhibitors of mTOR; PDGF: Platelet-derived growth factor; RASi: Inhibitors of RAS; VEGFRi: Inhibitors of VEGFR.

3. Heterogeneous causes of GIST

As mentioned above, most GISTs have mutations either in the *KIT* or in the *PDGFRA* gene (**Figure 1**). Affecting *KIT* mutations are typically found in the juxtamembrane domain encoded by exon 11 (70%), followed by the extracellular domains encoded by exons 9 (about 6%) and exon 8 (very infrequent), and by mutations in the kinase I and II domains (mainly encoded by exons 13 and 17, respectively; each 1%) [1,4]. In the *PDGFRA* gene (prevalence in the primary disease is about 10%), mutations are found in the juxtamembrane domain (nearly 1%), the kinase domain I (< 1%) and the kinase domain II (9%). Prevalence rate of mutations in each exon may be different between primary and advanced diseases by some degree, where *KIT* exon 9 mutations account for higher proportion (nearly 10%), and PDGFRA mutations appears to be lower (nearly 5%) in unresectable or metastatic GISTs [1,4]. Most primary mutations found in the *KIT* and *PDGFRA* genes are stabilized in autoinhibited form except the two rare mutations of D816H/V in the *KIT* gene and D842V in the *PDGFRA* gene, which show conformational equilibrium stabilizing in activated form [15,16]. In general, mutations stabilized in autoinhibited form may be considered sensitive to imatinib, sunitinib and regorafenib, and those in activated form are resistant to these drugs. The *KIT* exon 9 insertion mutation, however, is usually less sensitive to the standard dose of imatinib (400 mg/day) than *KIT* exon 11 mutations and requires a higher dose of imatinib (800 mg/day) [17].

Nearly 10% of GISTs have mutation neither in *KIT* nor in *PDGFRA*. These GISTs are called 'wild-type GIST' forming a heterogeneous group with various mutations in the *BRAF*, RAS-family (*HRAS*, *NRAS*, *KRAS*), succinate dehydrogenase (*SDH*) or *NF1 gene* (**Figure 1**) [1,18]. Wild-type GIST usually express the wild-type KIT protein and thus, is KIT-positive in immunohistochemistry. Mutations in *SDHs* and *NF1* are loss-of-function mutations, and those in *BRAF* and *RAS* are gain-of-function mutations. Later, we will discuss wild-type GIST in a separate section. Mutations in GISTs including *KIT* and *PDGFRA* are mutually exclusive.

4. TKIs available for GIST

4.1 Imatinib

Imatinib, a derivative of 2-phenylaminopyrimidine, inhibits the BCR-ABL protein, ABL, KIT and PDGFRs. Imatinib is water soluble and is efficiently absorbed from the gastrointestinal tract (**Table 1**). The drug mostly binds to albumin and, in a part, to α1-acid glycoprotein in the blood and is metabolized in the liver, mainly by CYP3A4. Its half-life in the blood is 16 – 18 h. The response rates (RR) are around 50 – 70% and median progression-free survival (PFS) is nearly 2 years [19-22]. Biomarkers of imatinib activity may include blood level of the drug, genotype of GIST, initial tumor volume, performance status (PS) of patients and initial white blood cell count and serum albumin [20,22-25].

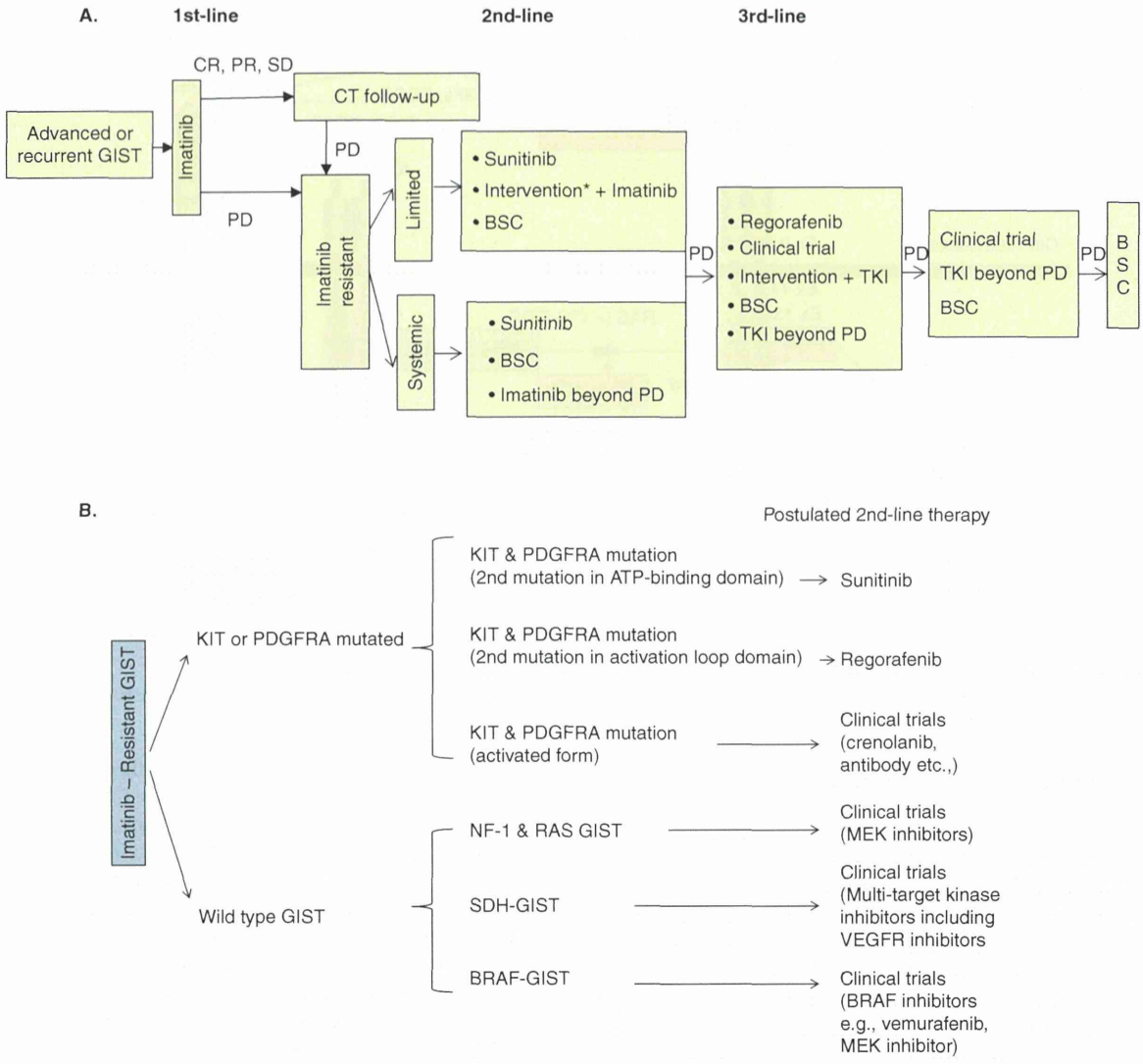

Figure 2. Therapeutic flow of tyrosine kinase inhibitors in unresectable or metastatic GIST. A. Standard therapy for unresectable or metastatic GIST is shown. **B.** Postulated genotype-guided treatment option for imatinib-resistant GIST in future is indicated.

*Intervention includes surgery, transcatheter arterial embolization (TAE), or radiofrequency ablation (RFA) for limited progression.

BSC: Best supportive care; CR: Complete response; GIST: Gastrointestinal stromal tumor; PD: Progressive disease; PR: Partial response; SD: Stable disease; TKI: Tyrosine kinase inhibitor; VEGFRI: VEGFR inhibitors.

4.2 Sunitinib

Sunitinib, a derivative of oxindole, is a multi-targeted receptor TKI, which specifically inhibits KIT, VEGFR-1,-2,-3, PDGFRA, PDGFRB, KIT, RET and FLT-3. Sunitinib has less bioavailability than imatinib, and its metabolic pathways are similar to imatinib. Half-life of sunitinib is 40 – 50 h (**Table 1**). Second-line sunitinib for imatinib-resistant GISTs showed 7% RR, and median PFS was 8 months [26,27]. The median overall survival (OS) from the starting date of sunitinib and imatinib are reported to be nearly 1.5 and 5 years, respectively [22,28]. Clinical activities of sunitinib are thought to be related with blood level of sunitinib, genotype and PS [29].

4.3 Regorafenib

Regorafenib is a diphenyl urea-based multi-targeted kinase inhibitor and inhibits KIT, VEGFRs, PDGFRs, TIE2, FGFR, RET, RAF-1 and BRAF (both wild type and the V600E mutant). Absorption from the gastrointestinal tract

Table 1. Pharmacological profile of imatinib, sunitinib and regorafenib.

	Imatinib	Sunitinib	Regorafenib
Targets	BCR-ABL, KIT, PDGFRs, ABL	VEGFRs, KIT, PDGFRs, CSF-1R, FLT3, RET	VEGFRs, KIT, PDGFRs, RET, RAF-1, BRAF, FGFR
Molecular weight	C29H31N7O · CH4O3S: 589.7	C22H27FN4O2 · C4H6O5: 532.6	C21H15ClF4N4O3 · H2O: 500.8
$t_{1/2}$ in blood	16 – 18 h	40 ~ 50 h	30 h
Absorption form the gastrointestinal tract	> 98%	55 – 80%	70 – 85%
Binding in blood	> 95% binding to albumin and α1- acid glycoprotein	95 – 90% binding to albumin and others	> 99.5% binding to albumin and others
Metabolism	Liver (CYP3A4)	Liver (CYP3A4)	Liver (CYP3A4, UGT1A9)
Doses	400 mg/day continuous	50 mg/day 4 weeks-on and 2 weeks-off	160 mg/day 3 weeks-on and 1 week-off
Biomarkers	Blood level, genotype, initial tumor volume, performance status, initial white blood cell count and serum albumin	Blood level (or doses), genotype, performance status	Genotype

and protein binding in the blood are almost similar to sunitinib. The drug is mainly metabolized in the liver by CYP3A4 and UGT1A9, and its half-life is nearly 30 h (**Table 1**). Third-line use of regorafenib for sunitinib-resistant GISTs was 4.5% in RR, and median PFS is 4.8 months [30,31]. Biomarkers of regorafenib may include genotype; however, a number of biomarkers are still under investigation [30].

The first metabolites of imatinib, sunitinib and regorafenib have similar inhibitory activities to their original drugs. Although these three TKIs directly inhibit the activities of KIT and PDGFRA tyrosine kinases, which may result in apoptosis of GIST cells and in tumor stabilization or shrinkage, their inhibition profiles to the tyrosine kinases, such as binding sites, are distinctive. Importantly, activities of these TKIs on GIST are largely dependent on mutations.

5. Resistance to TKI in GIST

Resistance to TKI therapy such as imatinib can be divided into two types: primary and secondary [1,4]. Primary resistance is disease progression without any responses and is practically defined as PD (progressive disease) within 6 months. Secondary (or acquired) resistance is disease progression after showing some sign of stable disease, partial response or complete response and is practically defined as PD after 6 months.

5.1 Primary resistance

Primary resistance was seen in nearly 10% of GISTs treated with imatinib. Primary resistance appeared as increased tumor size or appearance of new lesions in radiographic examinations with enhanced CT scan. The major cause of primary resistance is considered to be mutations [1,23-25]. Mutations, of which kinase domains are stabilized in activated form, such as *PDGFRA* exon 18 D842V mutations, are considered to be resistant to the all available three TKIs [1,32]. GIST showing KIT-independent proliferation including wild-type GIST and is also considered to be insensitive to imatinib [1,4]. Some GISTs with *KIT* exon 9 mutations are clinically insensitive to the standard dose of imatinib (400 mg/day) [33].

5.2 Secondary resistance

Secondary resistance is thought to eventually occur in the majority of GISTs after initial benefits from the TKI therapy are seen. Secondary resistance to imatinib is diagnosed by the significant enlargement of tumors, new nodules in pre-existing lesions or new lesions by enhanced CT, and, in certain cases, by re-uptake of 2-deoxy-2-(^{18}F)fluoro-D-glucose in positron emission tomography-CT [34]. Resistant GISTs may have secondary mutations in the *KIT* or *PDGFRA* gene (70 – 80%) as described below, KIT over-expression or copy-number increase of mutated *KIT* (< 10%), *de novo* activation of alternative pathways (> 10%) or decreased cellular level of imatinib (not confirmed in GIST) [1,4,23-25,35]. Genetic analysis of lesions resistant to imatinib revealed that most resistant tumors have secondary mutations either in the ATP-biding domain (exon 13 and 14 of the *KIT* gene) or in the activation loop domain (exons 16, 17 and 18) [23-25]. Secondary mutations in the ATP-binding domain were sensitive to sunitinib and those in the activation-loop were resistant to sunitinib [29], whereas *in vivo* and *in vitro* experiments have indicated that regorafenib may be potentially active for some secondary mutations in the activation-loop domain and for T670I mutations in *KIT* exon 14 [30,36]. These results indicate that patients with imatinib-resistant GISTs due to secondary mutations in the ATP-biding domain may benefit from sunitinib and that those with activation loop mutations may benefit from regorafenib (**Figure 2B**), if the genotypes of resistant lesions are available. Recent progress in sequencing technology of plasma DNA (liquid biopsy) indicates that this will hopefully be available in practice in the near future [37-39].

Secondary resistant tumor cells may exist before imatinib or may appear after treatment. Studies from CML and lung cancer

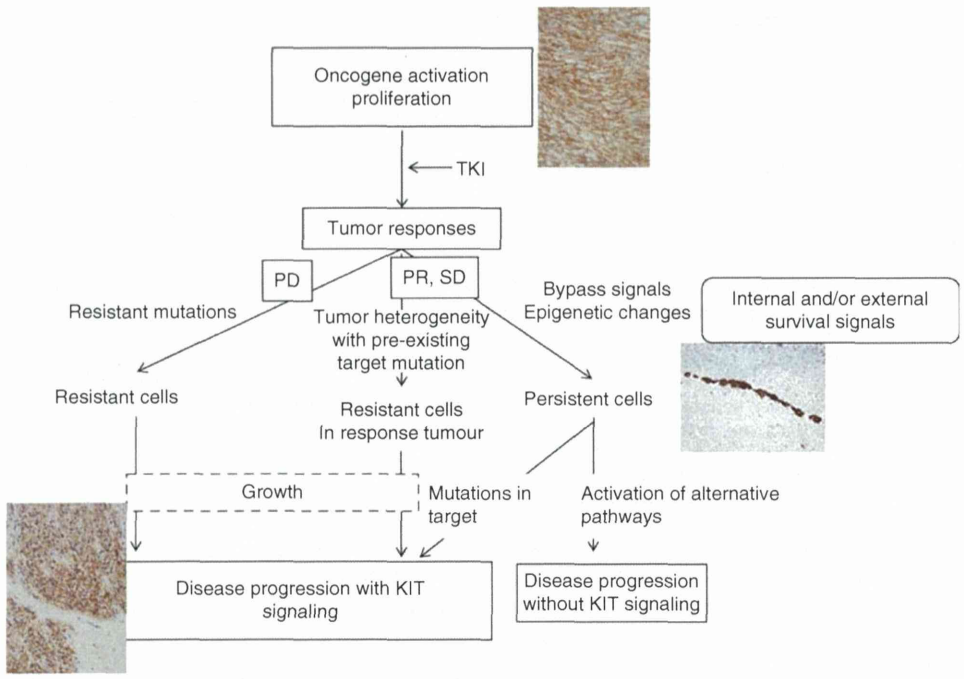

Figure 3. Schematic illustration of tumor responses to tyrosine kinase inhibitors. Postulated tumor responses to TKI, such as imatinib, and gain of resistance are illustrated with photographs of KIT-immunostaining.
PD: Progressive disease; PR: Partial response; SD: Stable disease; TKI: Tyrosine kinase inhibitor.

have indicated that some secondary resistant tumor cells, to be precise, tumor cells with secondary mutations, which are clinically emerging after imatinib, may already exist before the therapy (**Figure 3**) [40-42]. Other secondary mutations could be considered to arise after imatinib. The proliferation of KIT-dependent tumors depends on activated *KIT* mutations and, thus, tumor cells may go for apoptotic death by imatinib. There are, however, various amounts of surviving tumor cells even in sensitive tumors that show responses. These cells may be speculated to be stem-like cells or persistent cells without secondary mutations [43]. After shutdown of KIT- and PDGFRA-signaling, the persistent cells are envisaged to survive with signals from alternative kinases and/or from extracellular stimuli [43,44], such as FAK in GIST and MET-hepatocyte growth factor pathways as indicated in lung cancer [41-45]. Some of these cells may acquire secondary mutations in *KIT* subsequently, although these are all hypotheses. Thus, it has not been settled when resistant secondary mutations occur.

6. Therapy after disease progression

6.1 Potential therapy for resistant GIST

PFS of TKI therapy appears to be shortened with treatment-lines in GIST, and RR also decreases in the late therapeutic course (third line compared to first line). This may be partly due to an increase in genetic heterogeneity of target diseases and due to increased malignant potential of the tumors [46,47]. In fact, after imatinib therapy, resistant lesions may have various secondary mutations in the kinase domains, although primary mutations are the same in a patient. For instance, some metastatic lesions having ATP-binding domain mutations may still respond to sunitinib, and others having activation loop mutations may show resistance to the drug [29]. PFS of the placebo arm in the third-line trial (0.9 months) was much shorter than that in the second-line (1.6 months), suggesting an increase in tumor aggressiveness [26,31].

When GIST shows resistance to imatinib, a substantial number of patients, especially patients with small intestinal GISTs, which may have *KIT* exon 9 mutations, may have benefits from cross-over to high-dose imatinib (800 mg/day) [25,33,48]. At present, it is still unknown whether OS may be prolonged by dose-increase, although therapeutic options are increased. Other therapeutic options for imatinib-resistant GISTs may include surgical interventions to limit progression, where only one or two lesions among many metastatic lesions show resistance to imatinib [11]. In these approaches, imatinib-resistant lesions are treated by surgical resection, transcatheter arterial embolization or radiofrequency ablation, and the other sensitive lesions are controlled by imatinib. Several retrospective studies have indicated that surgery for limited progression may have benefits, where median PFS was extended for another 8 – 10 months with imatinib therapy

after intervention [49-52]. This therapeutic option also lacks evidences in OS. Surgical interventions to limit progression during sunitinib therapy do not always appear to be beneficial [53].

6.2 Treatment beyond disease progression and rechallenge

It has been reported that imatinib rechallenge for patients with sunitinib-resistant GISTs have extended PFS (1.8 months) as compared with placebo (0.9 months) [54]. Regorafenib, however, demonstrated median PFS of 4.8 months in the third-line therapy [31]. Thus, regorafenib is considered to be indicated for patients with sunitinib-resistant GISTs if available. When regorafenib is intolerable or is not available, rechallenge of imatinib may be an option [55]. Although PFS is usually shorter and objective responses weaker at rechallenge, toxicity is not cumulative, and rechallenged TKI may be tolerable even in the late stages. Another optional treatment after regorafenib may include regorafenib continuation beyond PD when tolerable. In fact, preliminary data indicated that patients with treatment continuation even after disease progression showed a median PFS of 4.5 months [56]. There are several reasons to advise rechallenge of TKI or treatment beyond PD. First, target mutations are varied in every lesion and responses to TKI differ for each lesion. Although some lesions may show resistance to the drug, others may still be sensitive to the drug. Second, the presence of TKI may slow down proliferation activities of tumor cells and clinical progression may appear to be slowed under TKI even if TKI can not completely inhibit target kinase activities as well as disease progression. Third, when tumors have negative feedback on other survival signals downstream of target kinases, resistance after TKI therapy may be caused by feedback activation of alternative kinases as seen in *BRAF*-mutated (V600E) melanomas [57-59]. Under these conditions, drug holidays may restore activities of alternative kinases and some tumors may regain sensitivity to TKIs. Together, even after the standard therapy, TKI beyond PD or rechallenge may be feasible and may have some benefits for some patients. In the selection of TKIs, the drugs should have been effective and tolerable in previous therapy.

7. Wild-type GIST

Wild-type GIST is caused by various causal mutations. Proliferation of some wild-type GIST is driven by oncogenic mutations in downstream kinases of the KIT and PDGFRA receptor tyrosine kinases, including *BRAF*, RAS-family (*HRAS*, *NRAS*, *KRAS*) and *NF1*. As wild-type GISTs show KIT-independent growth, KIT inhibition by imatinib is considered to be ineffective [1].

The product of the *NF1* gene, neurofibromin, is a negative regulator of RAS and its loss of activity may constitutively activate RAS signaling [60]. Because BRAF, neurofibromin and the RAS proteins are constituents of the MAPK-signaling cascade, their alterations may result in KIT-independent growth and inactivation of the MEK-MAPK pathway, suggesting a possibility of MEK inhibitors for GIST with these alterations. In fact, experimental studies *in vitro* have implicated that patients with *NF1*-mutated or *RAS*-mutated tumors may have potential benefits from MEK inhibitors (**Figure 2B**) [61-63].

Others may include loss of SDH (complex II of the mitochondrial respiratory chain) activity due to mutations in subunits (*SDHA*, *SDHB*, *SDHC* and *SDHD*) or post-translational defects [64,65]. *SDH*-mutated GIST may be diagnosed as KIT-positive and SDHB-negative tumors in immunohistochemistry [66]. Functional loss in the SDH complex is postulated to increase the cellular level of succinate, which in turn negatively regulates prolyl hydroxylase [1]. The decreased activity of the enzyme induces HIF1α, which in turn activates transcriptional activities of IGF2, VEGF and platelet-derived growth factor (PDGF). Hence, expression of VEGF and PDGF is considered to be increased in *SDH*-mutated GISTs. This may indicate that although GISTs with *SDH* mutations may be insensitive to imatinib, they may be sensitive to multi-targeted kinase inhibitors such as sunitinib. Recent reports suggested that sunitinib had substantial activities against pediatric GIST that may have frequent *SDH* alterations [67], and that pazopanib, a VEGFR inhibitor, stabilized unresectable or metastatic GIST with *SDH* mutations [68]. These possibilities described above, however, should be confirmed by clinical studies.

8. Conclusion

Although unresectable or metastatic GISTs had ominous prognosis, TKIs including imatinib, sunitinib and regorafenib revolutionized treatment of unresectable or metastatic GISTs, and the prognosis of GIST patients has been improved tremendously. However, drug resistance subsequently evolves to each TKI, and cure is rarely obtained by TKI therapy alone. The standard TKI therapy in GIST includes first-line imatinib, second-line sunitinib and third-line regorafenib. Major causes of drug resistance are additional mutations in the kinase domains of the *KIT* or *PDGFRA* genes. Some lesions may progress with secondary mutations, whereas the others still show responses to the drug. Thus, responses to the drug are heterogeneous and second mutations differ for each resistant tumor in the same patients. Treatment continuation with TKI or rechallenge may have benefits to some patients even after the standard treatment. To optimize TKI therapy, individualized approaches based on patterns of disease progression and causes of resistance are required for patients with unresectable or metastatic GISTs.

9. Expert opinion

9.1 Strategy of TKI therapy in unresectable or metastatic GIST

The proliferation of GIST tumor cells are driven by mutated kinases and subsequently activated downstream kinases. Thus,

activities of TKIs may be well correlated with clinical outcomes as seen in first-line imatinib therapy. Inhibitory activities of imatinib are considered to be affected by binding abilities to the KIT protein and intracellular concentration of the drug. The former may be influenced by genotype and the latter may be affected by blood level of imatinib and dose intensity, although blood levels of the drug vary greatly individually. Antitumor activities of imatinib and sunitinib are shown to be correlated with dose intensity as well as blood levels [69,70]. Drug interruption inevitably induced disease progression in advanced disease [71,72]. Although most tumors progressing after interruption responded to imatinib rechallenge, quality of responses after reintroduction did not reach the prior tumor status observed at interruption [73]. Thus, we should keep standard doses of imatinib, sunitinib and regorafenib without any interruption when tolerable.

Imatinib binding to the KIT tyrosine kinase is another important determinant of drug activities. Imatinib binding may be influenced by genotype of the *KIT* gene as mentioned above. Antitumor activities of imatinib are correlated with primary mutations in the *KIT* and *PDGFRA* genes [1,29-31]. Most secondary-resistant tumors have secondary mutations in the kinase domains, which were also indicated to be correlated with activities of sunitinib and regorafenib [35,36]. Wild-type GISTs may be insensitive to imatinib [1]. Wild-type GISTs are heterogeneous. Wild-type GISTs with *SDH* inactivation may have some sensitivity to multi-targeted inhibitors, such as sunitinib, as described above. In all events, genotype of GISTs may be important in the selection of targeted agents and mutation testing is recommended prior to the medical therapy [74]. In the future, genotype-guided treatment may optimize drug activities and patient outcomes (**Figure 2B**).

9.2 After standard therapy

After standard therapy, best supportive care or clinical trials are recommended in the guidelines. However, patients with good PS could have further treatment, and clinical trials are not always available. In such a situation, regorafenib beyond PD as well as rechallenge of imatinib or sunitinib may works for some patients. In drug selection, it is important for drugs to have had good clinical activities and acceptable tolerability in prior treatment [55].

Declaration of interest

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