

GIST PDX Models

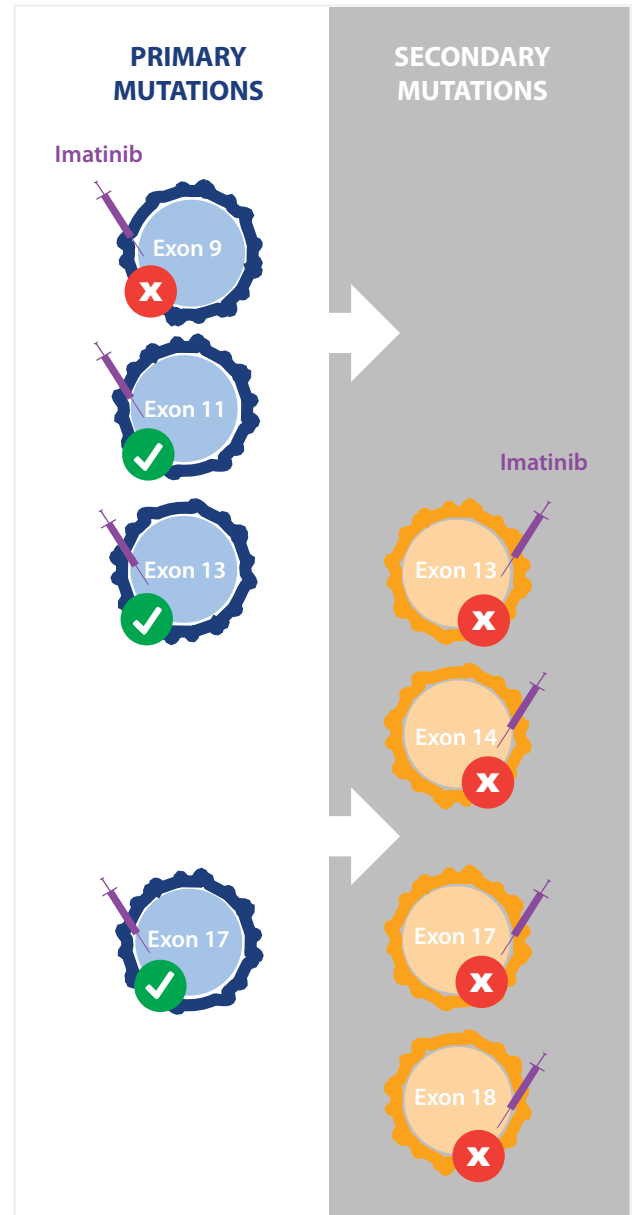
Discover the world's most comprehensive GIST PDX collection, fully characterized for KIT mutations

Accelerate your GIST targeted agent drug discovery programs with CrownBio's panel of well validated, clinically relevant PDX models.

While the genetic reasons behind imatinib acquired resistance in gastrointestinal stromal tumors (GIST) are well known, the development of new agents to overcome this resistance has been limited. This is due to a lack of preclinical models which fully recapitulate the complex interplay of KIT primary and secondary mutations seen in the clinical population.

CrownBio provides the world's most comprehensive panel of GIST patient-derived xenograft (PDX) models, ideal for the preclinical evaluation of novel therapies to circumvent imatinib resistance:

- Full model characterization to link drug response/resistance to mutational status.
- Models with KIT primary exon 11 mutations only, as well as common double mutations across multiple KIT exons, to truly reflect the complex genetic backgrounds and resistance genotypes observed in the clinic.
- Double mutant models preclinically validated in PDX pharmacology studies as patient-selection criteria for imatinib resistance.
- Therefore providing clinically relevant, highly predictive research models for evaluating the next generation of drugs to overcome imatinib resistance.





GIST PDX Panel Key Facts

CrownBio provides the world's most comprehensive GIST PDX panel representing a unique platform for the evaluation of new drug candidates to better delay and overcome the imatinib resistance found in the clinic:

- To progress the preclinical evaluation of novel GIST agents to overcome imatinib resistance driven by KIT acquired secondary mutations.
- Panel comprises 13 PDX models from a US population.
- Models closely reflect patient tumors for histopathological and genetic profiles, and are highly predictive of patient response.
- Full model background, QC, and RNAseq genetic profiling data searchable through our online PDX database, [HuBase™](#).
- Panel of models includes:
 - › those derived from treatment naïve patients with primary exon 11 mutations only
 - › double mutant models across exons 9, 10, 11, 13, and 17, representing acquired resistance through a wide range of mutations including the common V654A and N822K SNPs.
- Observed imatinib resistance correlating with exon 11 (delW557K558)/exon 17 (Y823D) and exon 13 (K642E)/exon 17 (N822K) double mutations.
- *In vivo* pharmacology data overcoming imatinib resistance for agents such as regorafenib, ponatinib, and dasatinib.

Improved Preclinical GIST Models are Needed to Overcome Imatinib Resistance

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumor within the GI tract⁽¹⁾, affecting around 4,000-5,000 people in the US each year^(2,3). Despite best efforts in the treatment of GIST, patients continue to face a poor prognosis, with 5-year relative survival rates of only 48% for patients diagnosed with metastatic disease⁽⁴⁾.

GIST treatment was revolutionized following characterization of the genomic and molecular events driving tumor development. Around 80% of GISTs harbor a KIT mutation⁽⁵⁾, and the introduction of the KIT inhibitor imatinib mesylate (Gleevec™) vastly improved the outcome for many KIT positive patients. The most common primary KIT mutations in GIST occur in exon 11, and confer increased sensitivity to imatinib⁽⁶⁾.

However, as with many kinase inhibitors, acquired resistance occurs rapidly - observed in approximately half of patients within 20 months of imatinib therapy⁽⁷⁾. This resistance is linked to secondary single nucleotide KIT mutations in exons 13, 14, 17, and 18^(7,8). Second and third generation KIT inhibitors have been shown to only provide limited clinical benefit e.g. for imatinib resistant clinical trial patients treated with sunitinib, only 12-19% showed a significant response. Second and third line treatments also aren't potent against all acquired mutations.

This highlights the unmet need for new therapies to target GIST, specifically imatinib resistant cases. However, there is a lack of available preclinical models which fully replicate all of the resistance genotypes seen within the clinical population.

Preclinical models are therefore needed which mimic the complex mutational interplay of patient resistance genotypes. These models can be utilized to provide robust efficacy data on which agents to move forward in GIST research, and for overcoming imatinib resistance.

CrownBio Provides the World's Most Comprehensive GIST PDX Collection

Xenografts derived directly from primary tumor tissue (which have never been in contact with plastic) are known to be the most predictive preclinical models available for drug evaluation⁽⁹⁾, fully recapitulating the histopathological and genetic profiles of original patient tumors.

CrownBio provides the world's largest commercial collection of PDX models for translational research programs, [HuPrime®](#). Within this collection is the most comprehensive global panel of 13 GIST PDX models, fully characterized for a range of clinically recognized KIT mutations, and available for preclinical drug evaluation programs.

Full Patient Background and Genomic Profiling Available for Our GIST PDX Panel

The CrownBio GIST PDX collection comprises of models derived from a US population, from both treatment naïve and pretreated patients. **Table 1** summarizes a selection of our available model data, including patient background and treatment history, tumor biopsy site within the GI tract or at a metastatic location, and tumor histology diagnosis.

GIST PDX Panel Factsheet

Table 1: HuPrime GIST PDX Panel of Well-Characterized Models

HuPrime ID	Patient Background	Biopsy Site	Histology	RNAseq Available?
Treatment Naïve Patients				
GS11328	Caucasian male, aged 54	Stomach	GIST	Yes (P0)
GS11329	Caucasian female, aged 75	Colon tumor	Stromal high grade tumor	Yes (P0)
GS11342	Caucasian male, aged 82	Stomach	GIST	Yes (P0)
GS11353	Caucasian male, aged 67	Abdomen	Malignant GIST	Yes (P0)
GS11354	Caucasian female, aged 81	Small bowel serosa	Malignant GIST	Ongoing
Pretreated Patients				
GS5107	Caucasian male, aged 60	Abdominal nodule	--	Yes (P1)
GS5108	Caucasian female, aged 43	Right lobe liver	Multiple metastatic GI stromal sarcoma	Yes (P3)
GS11331	Caucasian male, aged 42	Abdomen	--	Ongoing
GS11351	Caucasian female, aged 54	Small intestine	--	Ongoing
Patients with Unknown Treatment History				
GS5106	Caucasian male, aged 58	Small bowel implant	--	Yes (P1)
GS11327	Caucasian female, aged 66	Abdominal mass	--	Yes (P0)
GS11339	Caucasian female, aged 37	Omentum	GI tumor	Yes (P0)
GS11360	Caucasian female, aged 49	Omentum	--	Yes (P0)

All CrownBio models undergo in house pathology QC to confirm disease type and subtype; original histology QC information, and pathology images can be found in [HuBase](#) our easily searchable PDX database and [OncoExpress™](#) our comprehensive oncology search engine. These resources are accessed from our website at www.crownbio.com or directly at hubase.crownbio.com and oncoexpress.crownbio.com.

Full genetic characterization data for our GIST panel can also be found within [HuBase](#). **Table 1** confirms the RNAseq data available for our models, allowing detailed searching, comparison, and selection of appropriate PDX based on their genetic background.

Full KIT Mutational Analysis Available, Plus Further GIST Related Genes

Using genomic profiling data we have fully detailed the KIT mutations found within our PDX panel. This allows clients to select specific models harboring genetic features of interest for their agent under investigation.

Table 2 summarizes the CrownBio GIST PDX panel by KIT mutation status, by models which:

- are from treatment naïve patients and harbor recognized primary exon 11 mutations only.
- feature double mutations across a range of KIT exons. This includes common published mutations such as V654A and N822K which are known to confer imatinib resistance to GISTs.

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Further mutated genes of interest in GIST include PDGFR α , and published PDGFR α mutations found in our models which also result in imatinib resistance⁽¹⁰⁾ are shown in **Table 2**. All supporting information is found within [HuBase](#), where any other genes of interest can be searched for further mutational status data.

Table 2: A Range of KIT Mutated Models Available, Clinically Relevant for Imatinib Resistance Studies

All models are KIT exon 12 and 14 wild type. Mutations determined by KIT and PDGFR α targeted sequencing, RNAseq data availability in [HuBase](#) for these models is detailed in **Table 1**. Further RNAseq data is available for GS11339 and GS11360 however, all KIT exons are not covered by sufficient reads to discover mutations.

HuPrime ID	KIT Exons					PDGFR α Exon 18
	9	10	11	13	17	

KIT Exon 11 Mutations Only, Developed from Treatment Naïve Patients

GS11328	WT	WT	c.TGGAAG1669del p.WK557del	WT	WT	WT
GS11329	WT	WT	c.GAT1735del p.D579del	WT	WT	WT
GS11342	WT	WT	c.GGAAGG1670del p.WKV557fs	WT	WT	WT

Other KIT Mutations + PDGFR α Mutations

GS5106	WT	WT	WT	c.a1924g p.K642E homo	c.t2466a p.N822K	WT
GS5107	WT	WT	WT	c.a1924g p.K642E homo	c.t2466a p.N822K	WT
GS5108	WT	WT	c.TGGAAG1669del p.WK557del	WT	c.t2467g p.Y823D homo	WT
GS11327	WT	WT	c.AAACCCATGTATGAAGTACAGTGGAA 1648del p.KPMYEVQWK550fs	WT	WT	c.c2472t p.V824V homo
GS11331	WT	WT	c.TGGAAG1669del p.WK557del	c.t1961c p.V654A	WT	WT
GS11351	c.ins1510GCCTAT p.ins504AY	c.a1638g K546K	WT	WT	WT	c.c2472t p.V824V
GS11353	WT	WT	c.ATGTATGAAGTA1654del p.MYEV552del	WT	WT	c.c2472t p.V824V

Evaluate Novel Agents to Overcome Imatinib Resistance using Clinically Relevant Models

CrownBio PDX models are used to evaluate the preclinical efficacy of newly developing GIST agents, and to provide robust next step decision making data. Our full KIT mutation characterization provides models especially useful for assessing agents to overcome imatinib resistance, and for the further validation of resistance/sensitivity mutations.

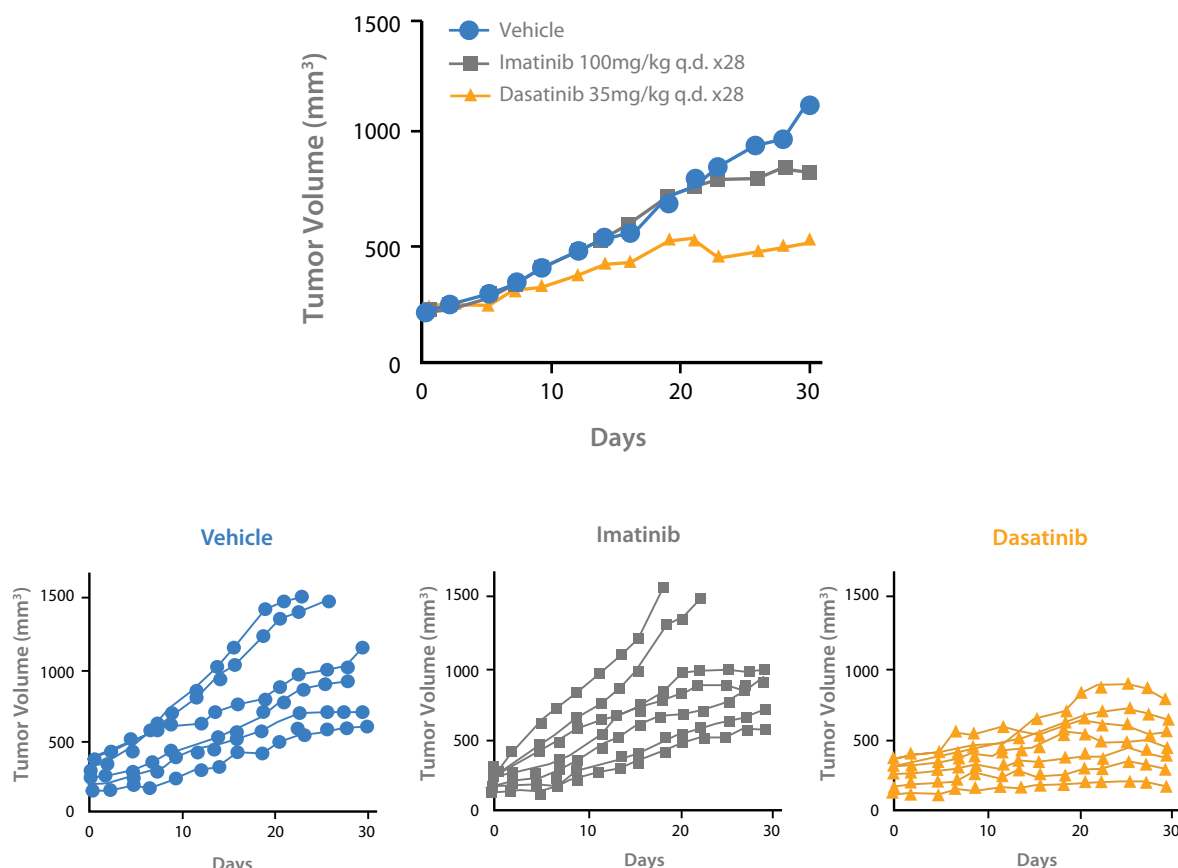
Through in house PDX pharmacology studies using the GS5108 model treated with imatinib and dasatinib (a multi TKI which targets KIT, as well as ABL, SRC, PDGFR, and other tyrosine kinases), the exon 11 (delW557K558)/exon 17 (Y823D) double mutation^(1,11) has been preclinically validated as patient selection criteria for imatinib resistance.

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Figure 1 shows model resistance to imatinib which is overcome by dasatinib treatment. Dasatinib showed significant efficacy in inhibiting tumor growth when compared with both vehicle ($p=0.0019$) as well as imatinib ($p=0.0221$).

Figure 1: Exon 11 (delW557K558)/Exon 17 (Y823D) Double Mutant Resistance to Imatinib and Sensitivity to Dasatinib⁽¹⁾

Median tumor volume and individual spider plots. Day 0: stratification; Day 1: start of dosing.



Client case studies have further utilized these models to confirm resistance genotypes, overcome imatinib resistance, and examine the genetics behind disease relapse⁽¹²⁾.

In collaboration with Array BioPharma, the GS5108 model detailed above, as well as the GS5107 exon 13 (K642E)/exon 17 (N822K) double mutant PDX, were used to evaluate new agents for imatinib-resistant GIST and investigate if acquired resistance models retain KIT-dependence⁽¹²⁾.

The models were treated with a range of approved KIT inhibitors such as:

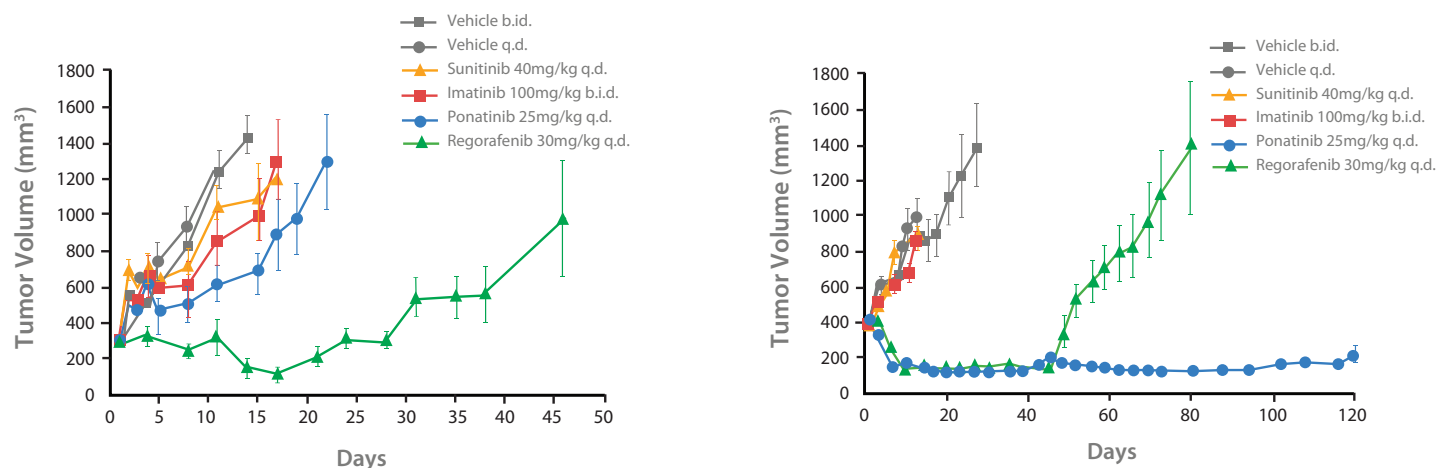
- imatinib (approved GIST first line treatment)
- sunitinib (approved GIST second line treatment)
- regorafenib (approved GIST third line treatment)
- as well as ponatinib which is in clinical trials for this indication (**Figure 2**)⁽¹²⁾.

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Figure 2: Overcoming Double Mutant Imatinib Resistance with Regorafenib or Ponatinib⁽¹²⁾

Data generated in collaboration with Array BioPharma. Drug administration from Day 0 to 12.

A: GS5107 exon 13 (K642E)/exon 17 (N822K); B: GS5108 exon 11 (delW557K558)/exon 17 (Y823D).



For both models (as expected for their double mutant genotypes) resistance to imatinib was observed. Resistance was also seen following treatment with sunitinib, which is known to lack potency against KIT mutations in exon 17, which are present in both models^(12,13).

However, durable complete regression was observed for both PDX:

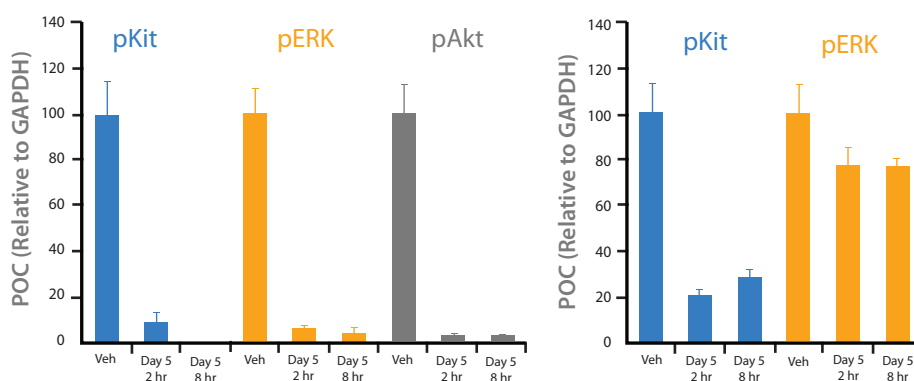
- for model GS5107 using regorafenib (with limited efficacy for ponatinib)
- for model GS5108 using both regorafenib and ponatinib⁽¹²⁾

Ponatinib efficacy was shown to correlate with inhibition of pKIT for both models, as well as pERK for GS5108 (**Figure 3**), suggesting that resistance is linked to a strong persistence of oncogene addiction⁽¹²⁾.

These data suggest that patients suffering from relapsed GIST still retain KIT-dependence and would therefore benefit from treatment with inhibitors that engaged both exon 11 and activation loop mutations, or a treatment combination that covers both targets⁽¹²⁾.

Figure 3: Ponatinib Efficacy Correlates with Inhibition of pKIT⁽¹²⁾

A: GS5108 no detectable signal for STAT1/3/5; B: GS5107 no detectable signal for pAKT STAT3/5, no quantifiable signal for STAT1.



This case study validates our PDX panel of GIST models as a valuable preclinical tool in understanding imatinib resistance mechanisms and evaluating/developing the next generation of inhibitors to overcome this resistance.

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Summary

Imatinib acquired resistance is a major obstacle to improving survival rates and outcomes in GIST. While the genetic basis of patient resistance is well understood, preclinical models recapitulating clinical genotypes are lacking.

CrownBio has developed the world's most comprehensive collection of 13 GIST PDX models, available for preclinical efficacy studies. The models are fully characterized for KIT mutations, and represent a wide diversity of published mutation events. Our PDX can be selected for drug evaluation studies based on client mutations of interest and drug response, providing the most highly predictive efficacy data for next step decision making.

Through in house and client case studies, CrownBio has modeled imatinib-resistant disease, preclinically validating double mutant genotypes as patient selection criteria for drug resistance. The associated *in vivo* pharmacology data show that resistance can be circumvented through the use of next generation drugs such as regorafenib, ponatinib, and dasatinib.

Overall, the CrownBio GIST PDX panel represents a novel platform for the evaluation of new drug candidates to better delay and overcome the acquired imatinib resistance now found in the clinic.

Acknowledgments

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