FGFR3 Fusion *In Vivo* Xenograft Platform

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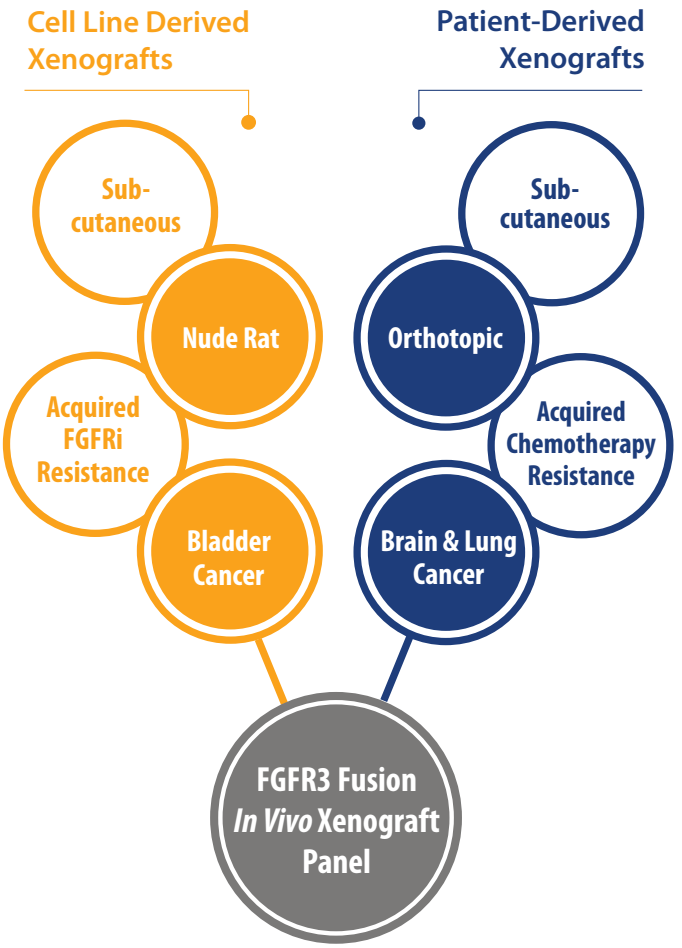
Advance preclinical FGFR inhibitor development with a comprehensive FGFR3 fusion *in vivo* xenograft panel

FGFR3 fusions are known to have oncogenic potential, and could provide highly 'druggable' targets for specific cancer patients, including those with acquired chemotherapeutic resistance.

FGFR inhibitors (FGFRi) to target these fusions require development and evaluation *in vivo*, in models recapitulating the FGFR3 fusion genotypes and resistance phenotypes observed in the clinic.

CrownBio provides a comprehensive FGFR3 Fusion *In Vivo* Xenograft Panel, with a variety of models available for all stages of preclinical development:

- Wide ranging panel features:
  - Cell line derived xenograft models available for initial lead compound development.
  - Patient-derived xenograft (PDX) models providing highly predictive efficacy data.
- FGFR3-TACC3 and FGFR3-JAKMIP1 fusions across bladder, lung, and brain cancer corresponding to clinically relevant fusion events.
- Acquired resistance models, available for validation with novel FGFRi:
  - Including cell line derived xenografts resistant to current FGFRi.
  - Chemoresistant PDX models with FGFRi sensitivity to mimic the clinical setting.



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FGFR3 Fusion *In Vivo* Xenograft Platform Key Facts

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CrownBio provides a fully comprehensive FGFR3 Fusion *In Vivo* Xenograft Panel to efficiently move novel FGFRi through preclinical studies, ready for clinical trials:

- Panel comprises of ValidatedXeno™ cell line derived xenografts utilized for a range of lead compound development studies:
  - RT4 and RT112/84 subcutaneous bladder cancer models with FGFR3-TACC3 fusion.
  - Models responsive and resistant to standard of care (SoC) agents including docetaxel, paclitaxel, and cisplatin, and sensitive to multitargeted tyrosine kinase inhibitors (TKIs) e.g. sunitinib, lenvatinib.
  - Experimental treatment data available including models with FGFRi sensitivity, and the development of FGFRi resistant models available for validation.
  - Nude rat RT4 and RT112 models for PK studies.
- Complemented by our HuPrime® PDX models providing highly predictive efficacy data for key translational decision making as you move towards clinical trials:
  - Lung (NSCLC, SCC) and brain cancer models featuring FGFR3-TACC3 and FGFR3-JAKMIP1 fusions, fully profiled through NGS.
  - Subcutaneous and orthotopic models.
  - Models sensitive and resistant to a range of SoC including cisplatin, with an acquired combination paclitaxel + carboplatin resistance model available for validation.
  - FGFRi sensitivity in both chemosensitive and acquired chemoresistant PDX model settings for clinically relevant resistance modeling studies.

Targeting FGFR3 Fusions for Oncology Drug Development

The FGFR family of tyrosine kinase receptors are involved in a wide range of cellular activities including proliferation, differentiation, and survival. Mutations, single nucleotide polymorphisms (SNPs), amplifications, and translocations of family members FGFR1 through FGFR4 have been implicated as causative factors in a variety of cancer types[1].

Included within these translocations are FGFR3 fusions caused by chromosomal rearrangements in solid tumors, usually observed in bladder and lung cancer, glioblastoma, and cholangiocarcinoma[2]. Some of the identified fusions have oncogenic potential, and understanding their downstream signaling and how to block this could provide novel therapeutic opportunities[2].

For example, FGFR3-TACC3 fusion proteins have been investigated across a range of cancer types, proving to:

- Be sensitive to FGFR TKIs in glioma, bladder, and lung cancer cells[3,4]
- Be responsive to a multi-kinase TKI targeting FGFR in clinical trials, with a cervical cancer patient showing stable disease, suggesting FGFR inhibition[5]
- Promote resistance to EGFR-targeted therapy in HNSCC cancer cell lines driven by EGFR signaling[4].

These treatment responses and emerging data suggest that FGFR3 fusions could provide 'druggable' targets for a selection of cancer patients, and could be the key to treating treatment acquired resistance in certain cancer types.

Appropriate models capturing the fusion genotypes observed in patients are now needed to evaluate novel agents targeting FGFR3 fusions, and for further research into the functional capabilities of these fusion events.

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CrownBio Provides a FGFR3 Fusion *In Vivo* Xenograft Platform for FGFRi Development

Our integrated platform provides two complementary strands for *in vivo* evaluation of FGFR3 fusion targeted drugs:

- ValidatedXeno cell line derived xenografts for initial lead compound development. Our range of FGFR3 fusion models allow clients to advance their preclinical development programs in a timely and cost-effective manner.
- For clients looking to move towards clinical study, we offer HuPrime PDX models. Derived directly from primary tumor tissue (never having contact with plastic), PDX are known to be the most predictive preclinical models available for drug evaluation[6], fully recapitulating the histopathological and genetic profiles of original patient tumors. Our large collection of 2,500 PDX models provide robust preclinical data for key next step decision making.

ValidatedXeno FGFR3 Fusion Xenograft Models for FGFRi Lead Compound Development

For initial *in vivo* studies we provide the RT4 and RT112/84 bladder cancer models, which harbor FGFR3-TACC3 fusions[7,8]. These xenograft models can be used to trial novel drugs targeting the FGFR3 fusion, as well as SoC agents for bladder cancer. The model types, information, and background are shown in **Table 1**.

Table 1: Summary of ValidatedXeno FGFR3 Fusion Models

Model	Cancer Type	Model Type	Model Information
RT4	Urinary bladder	Subcutaneous	A human Caucasian male epithelial transitional cell papilloma, which is tumorigenic. Aneuploid cell line[9]
RT112/84	Urinary tract; bladder	Subcutaneous	A human female epithelial bladder carcinoma, which is tumorigenic in nude mice. A clonal derivative of the RT112 cell line[10,11]

All of our xenograft models are well-characterized and validated, with background information, mutation status, and treatment data fully searchable through our collated online cell line/cell line derived xenograft database XenoBase®. XenoBase can be accessed from the CrownBio website at **www.crownbio.com** or directly from **xenobase.crownbio.com**.

FGFR3 Fusion Xenograft Models Respond to FGFRi, with Resistance Models Under Development

Our RT4 and RT112/84 models have been validated with a range of SoC agents, and experimental treatments targeting the FGFR3-TACC3 fusion in both in-house and client studies (in-house data summarized in **Table 2**).

Table 2: RT4 and RT112/84 SoC and Experimental Treatment Data

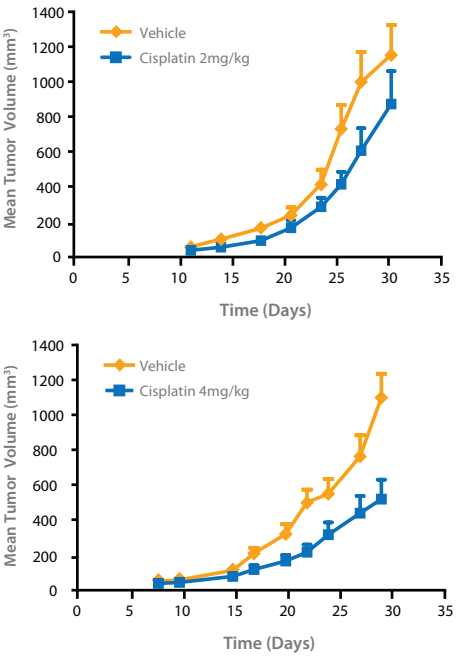
RT4: data acquired using BALB/c mice. RT112/84: data acquired using MF-1 female nude mice and BALB/c female nude mice.

Model	Targeted Therapy		Chemotherapy	
	Resistant	Sensitive	Resistant	Sensitive
RT4	-	Lenvatinib, lucitanib, sunitinib	-	-
RT112/84	-	Lenvatinib, lucitanib, sunitinib. Regression/ stabilization with AZD4547 (FGFR inhibitor)	Docetaxel, paclitaxel	Partial response with cisplatin
RT112/84R	AZD4547 (FGFR inhibitor) resistant model being generated	-	-	-

SoC treatments for bladder cancer include cisplatin for tumors which cannot be treated with surgery or radiotherapy, and taxanes for patients who cannot receive cisplatin. The RT112/84 model shows a partial response to cisplatin (**Figure 1**). Resistance to both docetaxel and paclitaxel is also observed for the RT112/84 model, with all data and tumor growth curves available within XenoBase.

Figure 1: Partial RT112/84 Tumor Response to Cisplatin

Tumors established in MF-1 female nude mice (n=9/10 mice per group) from cells in PBS.



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We have also targeted the FGFR-TACC3 fusion with AZD4547, an experimental FGFR1, 2, and 3 inhibitor which is currently in clinical trials for a range of FGFR expressing cancer types[12]. The RT112/84 model was shown to be highly sensitive to this agent (**Figure 2**).

As acquired resistance is an inevitable feature of TKI use, we are also developing a RT112/84 model resistant to AZD4547. This will allow clients to trial both first and second generation FGFR inhibitors in related *in vivo* models. A RT112/84 model resistant to AZD4547 *in vitro* has been established (shown in **Figure 3**) which is available for validation *in vivo* studies, where the mechanism of action of resistance can be characterized.

Figure 2: RT112/84 Subcutaneous Model Highly Sensitive to FGFR Inhibitor Treatment

AZD4547 treatment initiated at tumor volume of 200mm³ in MF-1 female nude mice.

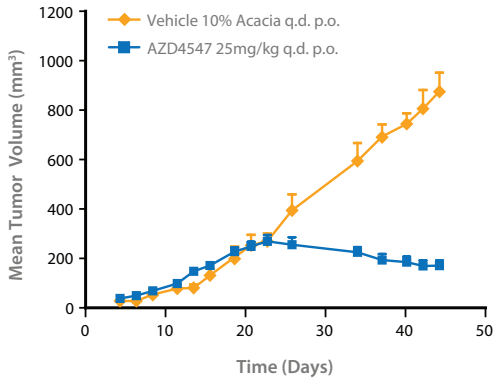
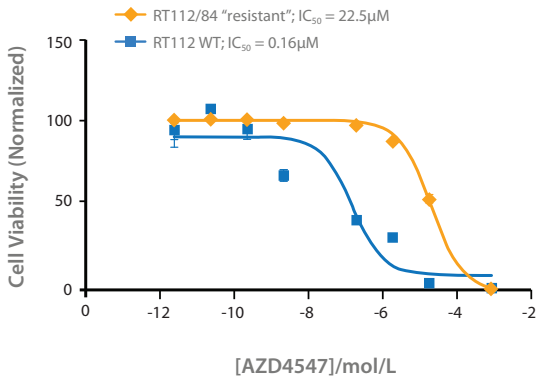


Figure 3: RT112/84 *In Vitro* Resistance to AZD4547 Developed, *In Vivo* Model Available for Validation



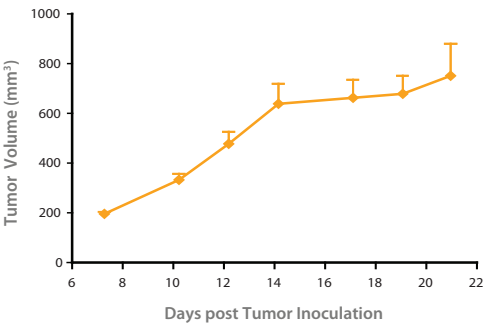
Multitargeted TKIs which act against FGFR have also been trialed with these models. Both RT4 and RT112/84 models were sensitive to sunitinib, lucitanib, and lenvatinib, with all data and tumor growth curves available within XenoBase.

Need to Evaluate Your Agent in a Rodent Platform More Similar to Humans?

Rats are more physiologically similar to humans than mice, providing an attractive option for a range of studies, with a compounds PK properties typically closer to those observed in humans.

We have therefore established nude rat models for RT4 and RT112 (the parental cell line from which RT112/84 is derived). Example growth data for the RT112 model are shown in **Figure 4**, with further RT4 data available in XenoBase.

Figure 4: RT112 Subcutaneous Rat Model Growth Rate



HuPrime FGFR3 Fusion PDX Models: A More Predictive Alternative for Preclinical Drug Evaluation

Within the HuPrime collection, we have identified three PDX models harboring FGFR3 fusion events with either TACC3 or JAKMIP1 (summarized in **Table 3**), derived from US, European, and Asian populations.

All our PDX models are well characterized and validated, with PDX background information, characterization and QC, mutation status, and treatment data searchable through our collated online PDX database HuBase™. HuBase can be accessed from our website at **www.crownbio.com** or directly from **hubase.crownbio.com**.

PDX and cell line derived xenografts can be searched simultaneously via Onco*Express*™, our integrated online oncology search engine also available from our website or directly at **oncoexpress.crownbio.com**.

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Table 3: Summary of HuPrime FGFR3 Fusion PDX Models

hMSC: human mesenchymal stem cells. #RNAseq gene expression, Log_2(FPKM) values.

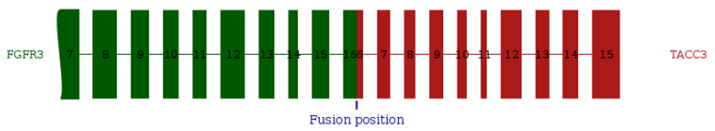
HuPrime Model	FGFR3 Fusion Partner	Patient Background	Tumor Pathology Diagnosis	PDX Tumor Pathology QC	Background & Model Type	Genomic Profiling	FGFR3 Gene Expression#
BN2289	TACC3	Asian female, aged 75 years. Treatment naïve.	Anaplastic oligodendrogliomas (WHO Grade 3). IHC results: GFAP(+), S-100(+), Syn(-), Ki-67(10% +), MMP-9(-), MGMT(-), P53(-), PCNA(+), P170(+)	Pa, P2: Oligodendrogliomas, Grade 3	BALB/c nude mice. Subcutaneous and orthotopic	P5: RNAseq P2: Affy U219 P1: Affy SNP 6.0	5.5694
LU5147	JAKMIP1	Caucasian male, aged 65 years. Unknown prior treatment.	NSCLC, SCC	SCC	NOD SCID. Subcutaneous	P3: RNAseq	7.2623
LU6426	TACC3	Caucasian male, aged 80 years, smoker. Treatment naïve.	NSCLC, SCC	P11: Poorly differentiated SCC	MF-1 nude mice, hMSC supplement; BALB/c nude mice. Subcutaneous	P10: RNAseq	8.904

The FGFR3 fusions have been confirmed in all models by RNAseq, and validated in the BN2289 model by PCR. **Figure 5** exemplifies fusion data at exon 16 of the FGFR3 gene and exon 6 of the TACC3 gene on chromosome 4 for model BN2289. Further fusion data for all models are included within HuBase, and sequencing validation data are available on request.

FGFR3 gene expression data for the models is included within **Table 3**. Amplified DNA copy number for FGFR3 was observed for the LU6426 model (DNA copy number of 6).

Figure 5: HuPrime FGFR3-TACC3 Fusion PDX Model Genomic Profiling: BN2289

The chromosome 4 position of the FGFR3-TACC3 fusion.



PDX model	Upstream Fusion Gene	Up-stream Chromo-some	Up-stream Strand	Upstream Genome Junction Position	Down-stream Fusion Gene	Down-stream Chromo-some	Down-stream Strand	Down-stream Genome Junction Position
BN2289	FGFR3	4	+	1808661	TACC3	4	+	1737000

PDX Models Respond to FGFRi in Chemosensitive and Chemoresistant Settings

Our PDX models are used to provide clients with robust, highly predictive efficacy data for key translational decision making. We have validated our models with a range of SoC and experimental treatments (summarized in **Table 4**, data available with HuBase), as well as developing clinically relevant SoC resistant PDX models for FGFRi evaluation.

Table 4: FGFR3 Fusion PDX Models SoC and Experimental Treatment Data

BN2289: data acquired in BALB/c nude mice; LU5147: data acquired in NOD SCID mice; LU6426: data acquired in MF-1 nude mice .

Model	Chemotherapy		Targeted Therapy	
	Resistant	Sensitive	Resistant	Sensitive
BN2289	-	Temozolomide	-	BGJ398 (FGFR inhibitor)
LU5147	-	Cisplatin	-	-
LU6426	Paclitaxel + carboplatin (acquired resistance)	Paclitaxel + carboplatin	-	AZD4547 (FGFR inhibitor)

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Standard of care chemotherapy agents have been trialed with our FGFR3 fusion models, appropriate for each specific disease type. The BN2289 model was sensitive to temozolomide, LU5147 was sensitive to cisplatin, and the LU6426 model was sensitive to paclitaxel and carboplatin combination therapy.

As FGFR3 fusions could be potential drivers and 'druggable' targets in tumors with acquired resistance, we are developing drug resistant PDX models for FGFRi evaluation. A proof-of-concept LU6426 model of acquired resistance to paclitaxel and carboplatin is available for validation, derived via cycled dosing of the agents *in vivo* until a resistant phenotype emerged (**Figure 6**). Both the chemosensitive and chemoresistant models respond to treatment with AZD4547 (**Figure 7**).

Figure 6: LU6246 PDX Model Response and Acquired Resistance to Paclitaxel + Carboplatin

*p≤0.001

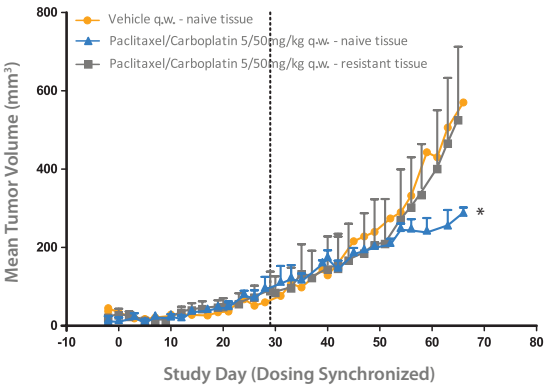
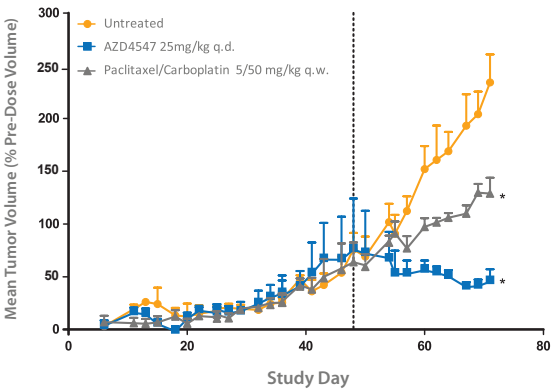


Figure 7: LU6426 PDX Model Responds to FGFRi in Chemosensitive and Chemoresistant Settings

A: Response to AZD4547 in LU6426 model sensitive to paclitaxel and carboplatin treatment. B: Response to AZD4547 in LU6426 model with acquired resistance to paclitaxel and carboplatin treatment. *p≤0.001.

A.



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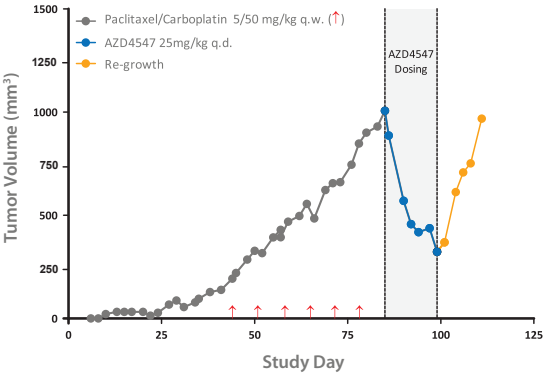
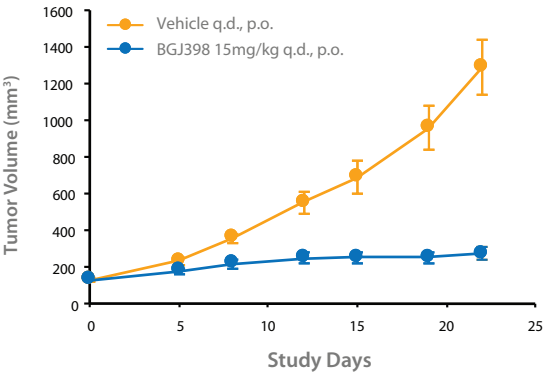


Further multi targeted TKIs have also been tested with our PDX models, with the BN2289 model showing a robust response to the pan FGFR inhibitor BGJ398 (**Figure 8**).

Figure 8: BN2889 PDX Model Responds to FGFR Inhibitor BGJ398



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Summary

FGFR3 gene fusions produce constitutively active oncogenic kinase protein products, which are druggable targets for oncology research. Relevant fusions could also potentially be used to stratify patients in the clinic for FGFR inhibitor treatment. To further the preclinical evaluation of FGFRi to target these fusion events, CrownBio provides a FGFR3 Fusion *In Vivo* Xenograft Panel comprising of both cell line and patient-derived xenograft models.

Our ValidatedXeno cell line derived xenografts are used for a range of lead compound development studies. Our FGFR3 fusion resources include the RT4 and RT112/84 models. Our xenograft models provide a range of responses to SoC chemotherapy, and include models highly sensitive to a range of multitargeted TKIs and also an experimental FGFR1, 2, and 3 inhibitor.

We are currently generating an RT112/84 model resistant to FGFRi (AZD4547) treatment, *in vitro* resistance has been established which can be transitioned to *in vivo* research for the development of agents to overcome this resistance.

Our cell line derived xenografts are complemented by HuPrime PDX models, fully recapitulating the histopathological and genetic profiles of original patient tumors, and delivering efficacy data for key translational next step decision making.

Our FGFR3 fusion PDX resources cover models of glioma and NSCLC SCC. The clinically relevant FGFR3-TACC3 and FGFR3- JAKMIP1 fusions in our models are confirmed by RNAseq with full sequencing data available.

The PDX models are sensitive to SoC agents specific to their disease type, as well as experimental FGFR inhibitors. The LU6426 NSCLC model is available for validation with acquired resistance to the SoC paclitaxel and carboplatin treatment regimen, with FGFR inhibitor sensitivity still maintained, for investigating FGFRi to overcome FGFR fusion driven acquired resistance.

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