FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547

Liang Xie*1, Xinying Su*1, Lin Zhang1, Xiaolu Yin1, Lili Tang1, Xiuhua Zhang1, Yanping Xu1, Zeren Gao1, Kunji Liu1, Minhua Zhou1, Beirong Gao1, Danping Shen2, Lianhai Zhang3, Jiafu Ji3, Paul R. Gavine1, Jingchuan Zhang1, Elaine Kilgour4, Xiaolin Zhang1 and Qunsheng Ji1

*Liang Xie and Xinying Su contributed equally to this study

Author affiliations: 1) Innovation Center China, AstraZeneca R&D, China; 2) Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; 3) Department of Surgery, Peking University Cancer Hospital, Beijing Institute for Cancer Research, Key laboratory of Carcinogenesis and Translational Research (Ministry of Education), Beijing, China; 4) AstraZeneca Oncology, Macclesfield, Cheshire, UK

Corresponding author: Qunsheng Ji, AstraZeneca Innovation Center China, Building 7, 898 Halei Road, Zhangjiang Hi-Tech Park, Shanghai, 201203, P.R.China E-mail: Qunsheng.Ji@astrazeneca.com

Keywords: AZD4547, tyrosine kinase inhibitor, FGFR2 amplification, gastric cancer

Running title: AZD4547 sensitivity in FGFR2-amplified gastric cancer

Word count: 4600 (maximum = 5000)
Acknowledgments

We wish to thank Yao Wang, Min Shi, Katherine Ye, Zengquan Wang for data generation; Jie Zang for drafting the manuscript. This study was sponsored by AstraZeneca. Editorial assistance was provided by Claire Routley from Mudskipper Bioscience, funded by AstraZeneca.
Abstract

**Purpose:** *FGFR* gene aberrations are associated with tumor growth and survival. We explored the role of *FGFR2* amplification in gastric cancer (GC) and the therapeutic potential of AZD4547, a potent and selective ATP competitive receptor tyrosine kinase inhibitor of FGFR1–3, in patients with *FGFR2*-amplified GC.

**Experimental design:** Array comparative genomic hybridization and FISH were used to identify *FGFR2* amplification in GC patient tumor samples. The effects of FGFR2 modulation were investigated in GC cells with *FGFR2* amplification and in patient-derived GC xenograft models using two approaches; inhibition with AZD4547 and shRNA knockdown of *FGFR2*.

**Results:** Amplification of the *FGFR2* gene was identified in a subset of Chinese and Caucasian patients with GC. GC cell lines SNU-16 and KATOIII, carrying the amplified *FGFR2* gene, were extremely sensitive to AZD4547 *in vitro* with GI50 values of 3nM and 5nM, respectively. AZD4547 effectively inhibited phosphorylation of FGFR2 and its downstream signaling molecules and induced apoptosis in SNU-16 cells. Furthermore, inhibition of FGFR2 signaling by AZD4547 resulted in significant dose-dependent tumor growth inhibition in *FGFR2*-amplified xenograft (SNU-16) and PDGCX models (SGC083), but not in non-amplified models. ShRNA knockdown of *FGFR2* similarly inhibited tumor growth *in vitro* and *in vivo*. Finally, compared to monotherapy, we demonstrated enhancement of *in vivo* antitumor efficacy using AZD4547 in combination with chemotherapeutic agents.

**Conclusion:** FGFR2 pathway activation is required for driving growth and survival of GC carrying *FGFR2* gene amplification both *in vitro* and *in vivo*. Our data support therapeutic intervention with FGFR inhibitors, such as AZD4547, in patients with GC carrying *FGFR2* gene amplification.
Translational relevance: Amplification of the FGFR2 gene may be involved in oncogenesis and progression of gastric cancer (GC). This report describes the incidence of FGFR2 amplification in GC and a preclinical assessment of the therapeutic potential of AZD4547, a selective FGFR kinase inhibitor. Following identification of FGFR2 gene amplification in a subgroup of Chinese and Caucasian GC patients, the dependency of FGFR2 signaling (activated by FGFR2 amplification) for gastric tumor growth and survival, both in vitro and in vivo, was systematically demonstrated.

AZD4547 treatment resulted in tumor regression in GC xenografts derived from patients carrying FGFR2 gene amplification, but not in non-amplified models. Regression was accompanied by inhibition of phospho-FGFR2 and downstream pathway inactivation. The comprehensive translational studies presented here support a tumorigenic role for FGFR2 gene amplification in patients with GC and warrant clinical investigation of FGFR inhibitors, including AZD4547, in this setting.

Word count: 144 words (maximum 150)
Introduction

Gastric cancer is the second leading cause of death from cancer worldwide and accounts for nearly one in ten of all cancer deaths (1). Most new cases and deaths occur in East Asian countries, including China, Japan and Korea (2). Such high incidence rates in this region may be due in part to the preference for a high-salt diet, a high proportion of smokers and a high prevalence of *Helicobacter pylori* infection (2, 3). Despite advances in diagnostic methods and improvement of both surgical skills and systematic chemotherapy regimens, the overall prognosis for gastric cancer remains disappointing, especially for those with late-stage disease; the 5-year survival rate for patients with stage III/IV metastatic gastric cancer is only around 10% (4, 5). Better management of this disease, particularly through the use of targeted therapeutic agents, is required.

Genetic abnormality and intra-tumor heterogeneity are two typical features of cancer cells which play crucial roles in carcinogenesis and tumor progression (6). Although detailed mechanisms of tumor development driven by gene amplification, mutation or translocation have not been fully characterized (7), aberrant genes have been demonstrated clinically as valid targets for cancer treatment (8-10). A key challenge that remains, however, is how to distinguish these so-called ‘driver’ oncogenes from other co-existing ‘passenger’ genes (11). In order to facilitate the identification and validation of ‘driver’ genes, patient-derived specimen-based molecular analysis techniques and *in vivo* disease models have been widely employed (12-14). Consequently, a number of candidate oncogenes, including several within the receptor tyrosine kinase (RTK)/RAS pathway, have been discovered in gastric cancers (15,16).

Fibroblast growth factor receptor family members (FGFR1–4) belong to the receptor tyrosine kinase superfamily. Through interaction with FGF ligands, the receptors are involved in diverse cellular functions including regulation of development processes, mediation of cell proliferation and differentiation, as well as angiogenesis and tissue regeneration (17-19). Binding of the FGF ligand to the receptor induces dimerization of the FGF:FGFR complex, leading to kinase activation and autophosphorylation of multiple tyrosine residues in the cytoplasmic domain of the receptor. This results in
activation of downstream signaling of the PI3K-AKT and MAPK-ERK pathways (20). Genetic modifications or over-expression of FGFRs have been associated with tumorigenesis and progression in breast, prostate, stomach and hematological malignancies (21, 22). In particular, abnormal activation of FGFR2 signaling has been linked with several types of human cancers, and somatic FGFR2 mutations have been reported in lung, gastric and ovarian cancers (23-25). FGFR2 amplification has been associated with tumor cell proliferation and survival of gastric cancer cell lines (26). Furthermore, FGFR2 amplification may correlate with a poor prognosis for gastric cancer patients (27). Thus, FGFR2 has attracted significant attention as a potential therapeutic candidate for the development of targeted therapeutic anticancer agents (28).

AZD4547 is an orally bioavailable, highly selective and potent ATP-competitive small-molecule tyrosine kinase inhibitor of FGFR1–3. We have previously described the chemical structure and selectivity profile of this compound and shown that it can significantly inhibit FGFR phosphorylation and repress proliferation of cancer cell lines via inhibition of FGFR signaling (29). AZD4547 treatment has also exhibited antitumor activity in a representative FGFR-driven human tumor xenograft model. Currently, the compound is under investigation in several Phase I and II clinical trials (ClinicalTrials.gov identifiers: NCT01202591; NCT01457846; NCT00979134; NCT01213160).

In the current study, amplification of the FGFR2 gene was identified in a subset of Chinese and Caucasian gastric cancer patients. Subsequently, we aimed to investigate the preclinical activity of AZD4547 in FGFR2-amplified gastric cancer models and ultimately demonstrated potent antitumor activity with tumor regression selectively occurring in FGFR2-amplified gastric cancer xenografts.
Materials and methods

Materials

The following chemical reagents were all obtained from Sigma-Aldrich: cisplatin (Cat# P4394), fluorouracil (Cat# F6627), docetaxel (Cat# 01885) and irinotecan (Cat# I1406).

The following antibodies were purchased from Cell Signaling Technology (CST): phospho-FGFR2 (tyr653/654), phospho-FRS2α (tyr436), phospho-PLCγ 1 (Tyr783), phospho-p44/p42 MAP kinase (Erk1/2), total-Erk, phospho-S6 (ser240/244), total-S6, cleaved caspase-3, poly-(ADP-ribose) polymerase (PARP) and horseradish peroxidase (HRP)-linked anti-rabbit IgG. Other antibodies included total-FRS2 (R&D systems), total-FGFR2 (Abcam), phospho-PLCγ (tyr783; Epitomics) and Ki67 (DAKO). An antibody against β-actin (Sigma) was used as a control.

A panel of 29 gastric cancer cell lines was obtained from the American Type Culture Collection (ATCC) or from internal collections. SNU-16 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 2mM L-glutamine (Invitrogen). All cell lines were genetically tested and authenticated using the StemElite ID™ System Kit (Promega, #G9530) and were not cultured for more than 3 months prior to performing the work described here.

Female Balb/c nude mice aged 6–8 weeks old and 8–10 week old female (nu/nu) SCID nude mice were purchased from Vital River, Beijing, China. Animals were housed in a specific pathogen-free animal facility in accordance with the Guide for Care and Use of Laboratory Animals (Eighth edition) and the regulations of the Institutional Animal Care and Use Committee (IACUC). All animal studies were approved by the IACUC committee.

All studies using human tissues were performed with the patients’ consent and the approval of the Local Research Ethics committee.
Array comparative genomic hybridization (aCGH) analysis of gastric cancer patient samples

FGFR2 gene copy number was analyzed in frozen patient tissue samples using the Agilent 244K array CGH platform. The quality of the raw data was checked with Agilent CGH Analytics software, using the derivative of the log ratio spread (DLRSpread) as a surrogate for assay quality. Any sample with a DLRSpread >0.3 was excluded from further analysis. Data for those samples that qualified for further assessment were subsequently analyzed using Nexus software (version 4) for recovery of FGFR2 segmental structure (segmentation) and the discrete copy number value at the single-sample level (calling). Segments with an array CGH logRatio (copy number of sample versus control) >0.8, were classified as amplified.

Fluorescence in situ hybridization (FISH) analysis

The FGFR2 FISH probe was generated from BAC (RP11-62L18) DNA directly labeled with Spectrum Red (ENZO, #02N34-50). The Spectrum Green-labeled centromere of chromosome 10 (CEP10) probe (Vysis #32-132010) was used as an internal control. FISH analysis was performed according to routine methodology (30). In brief, 4 μm sections from formalin fixed and paraffin embedded (FFPE) gastric cancer patient tissue samples were deparaffinized. After pretreatment with the SpotLight Tissue pretreatment kit (Invitrogen, #00-8401), tissue sections and the FGFR2/CEP10 probes were denatured at 80°C for 5 minutes, and then incubated together at 37°C for 48 hours. Following this hybridization step, excess and unbound probes were removed with post-hybridization wash buffer (0.3% NP40/1xSSC at 75.5°C for 5 minutes followed by two washes of 2xSSC at room temperature for 2 minutes), and nuclei were counterstained with DAPI (4’, 6 diamidino-2-phenylindole). Visualization of the fluorescent signals was performed using a fluorescence microscope (Olympus) equipped with appropriate filters and image analysis was performed with CytoVision (Leica). The enumeration of the FGFR2 gene and chromosome 10 was conducted in 50 tumor nuclei for each tissue section. Amplification of FGFR2 was defined as a FGFR2:chromosome 10 ratio of ≥2, or tight FGFR2 gene clusters in ≥10% of the nuclei analyzed per tissue section.
**Immunohistochemistry**

Immunohistochemical (IHC) analysis was performed using a Lab Vision autostainer. In brief, 3 μm formalin fixed paraffin embedded (FFPE) sections were dewaxed and rehydrated on the Leica XL autostainer and underwent antigen retrieval for 5 minutes in pH 6 retrieval buffer (S1699; DAKO) followed by washing in running tap water for 5 minutes. Sections were rinsed in Tween–Tris buffered saline (TBST) and incubated with endogenous peroxidase block for 10 minutes. Slides were washed in TBST and then incubated with primary antibodies: phospho-Erk (CST #4376, 1:50) or phospho-S6 (CST #2215, 1:100) for 60 minutes at room temperature and finally washed in TBST twice. Slides were reacted with EnVision+ System-HRP Labeled Polymer Anti-Rabbit for 30 minutes and washed in TBST twice, developed in diaminobenzidine substrate for 5 minutes, and rinsed in tap water. Sections were counter stained, dehydrated and cleared in Leica XL autostainer, and finally sealed in the ClearVue automated cover slipper. Normal rabbit IgG (DAKO X9003) at 1:18000 and 1:20000 dilution was used as negative control for phospho-ERK and phospho-S6, respectively. For IHC detection of the proliferation marker Ki67, FFPE sections were pre-treated with an Animal Research Kit (DAKO, K3954) according to manufacturer's instructions. Sections were then incubated with anti-Ki67 primary antibody (DAKO, M7240; 1:100) for 15 minutes at room temperature, and washed twice in TBST, followed by incubation with streptavidin-peroxidase for 15 minutes, washed twice in TBST, counter-stained with DAPI and visualized by chemiluminescence, as described earlier. In order to evaluate apoptotic cell death within the xenografts, TUNEL assay was performed on xenograft sections using the *In Situ* Cell Death Detection kit (Roche, 12156792910) according to the manufacturer's instructions.

**In vitro anti-proliferative cell panel screening**

All 29 gastric cancer cell lines were seeded at optimized density in 96-well culture plates. Adherent cells were seeded the night before the start of treatment; suspension cells were seeded concurrently with treatment. Cells were treated with AZD4547 for 72 hours at concentrations ranging from 0–30 μmol/L. Control cells were treated with DMSO only. Cell proliferation was measured using the
CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit (Promega), according to the manufacturer’s instructions. Evaluation of cell proliferation of SNU5 and SNU16 cells was conducted using AlamarBlue Proliferation Assay Kit (Invitrogen), an assay with a similar detection mechanism and a better signal window.

**In vitro pharmacodynamics (PD) study**

SNU-16 or KATO-III cells were seeded at a density of 2x10^5 cells/ml in RPMI-1640 medium containing 10% FBS and cultured overnight. The cells were then pre-incubated with AZD4547 0.001, 0.003, 0.01, 0.03 or 0.1 µmol/L for 1 hour before being lysed in cell lysis buffer (CST) containing phosphatase and protease inhibitors (Sigma) and then 20 µg of protein was loaded onto 4–12% (w/v) NuPAGE gels; following electrophoresis, the lysates were transferred to PVDF membranes and probed with the following antibodies: phospho-FGFR (tyr653/654; CST #3471; 1:1000), phospho-FRS2 (tyr436; CST #3861; 1:1000), phospho-PLCγ (tyr783; Epitomics, #2325-1; 1:1000), phosphor-p44/p42 MAPK kinase (Erk1/2) (SCT #4370) or phospho-S6 (ser240/244; CST #2215; 1:1000). The appropriate secondary antibody was applied and visualization was performed using SuperSignal West Dura chemiluminescence substrate (Pierce) according to the manufacturer’s instructions. Membranes were re-probed to assess total-Erk1/2 (SCT #4960) and total-S6 levels (CST #2317; 1:1000).

**In vitro cell-cycle analysis study**

SNU-16 cells were exposed to AZD4547 0 (DMSO control), 3, 10 or 30 nmol/L for 48 hours. For fluorescence-activated cell sorting (FACS), cells were washed with PBS and then incubated with staining solution containing 0.2 mg/mL RNase A (Fermentas), 0.05 mg/mL propidium iodide (Invitrogen) and 0.1% Triton (Sigma) in PBS for 20 minutes at room temperature. Then cell-cycle distributions were assessed with a FACSCanto instrument (BD).

**Establishment of standard gastric cancer xenograft and patient-derived gastric cancer xenograft (PDGCX) mouse models**

SNU-16 cancer cells (5x10^6) were suspended in 200 µl of 50% Matrigel and inoculated subcutaneously (s.c) on either sides of the flank of balb/c nude mice.
Once the volume of the tumor xenograft reached approximately 300–500 mm$^3$, it was excised and cut into approximately 2 mm$^3$ segments, which were further implanted subcutaneously via Trocar needle into nude mice.

PDGCX mouse models were generated using tumor tissue from Chinese gastric cancer patients. In brief, surgically excised tumor tissue (F0) was cut into approximately 2 mm$^3$ segments and implanted s.c. into immune-compromised nude mice or SCID mice (F1). Transplantation occurred within 2 hours following excision. Subsequent passages were made into additional nude mice once the grafted tumors had reached a size of 400–600 mm$^3$. PDGCX models within ten passages (between F3 to F10) were used to evaluate antitumor efficacy.

For all \textit{in vivo} studies, tumors were measured in two dimensions with calipers and the tumor volume was calculated using the following formula: \text{tumor volume} = \text{(length} \times \text{width}^2) \times 0.5.

\textbf{\textit{In vivo} antitumor efficacy studies}

SNU-16 xenograft model and PDGCX mouse models were used to study the efficacy of AZD4547. For single agent studies, mice were randomized into groups of eight when tumor xenograft volumes reached 150–250 mm$^3$, and treated with either control vehicle (DMSO) or AZD4547 at doses ranging from 1.56–25 mg/kg, administered orally, once daily for several weeks. For combination studies with chemotherapy, animals were treated with low doses of AZD4547 3.125 mg/kg/qd, either alone, or in combination with FC (5-FU 10 mg/kg/qd plus cisplatin 4 mg/kg/qw), docetaxel 3 mg/kg/qw, irinotecan 17.5 mg/kg/qw or control vehicle (1% Tween 80, 5-FU 10 mg/kg/qd, 5 days on, 2 days off treatment). The doses of cytotoxic agents were based on the clinically recommended dosage for human use. Tumor volume and body weight of the mice were measured twice weekly.

\textbf{\textit{In vivo} single dose PD study and pharmacokinetics/pharmacodynamics (PK/PD) modeling}

SNU-16 tumor-bearing mice were administered a single oral dose of AZD4547 12.5 mg/kg once the tumor volume reached 400–500 mm$^3$. Plasma and xenograft
tissue samples were collected from each animal at the following time-points post dose: 0, 1, 2, 4, 8 and 24 hours. The total plasma concentration of AZD4547 was determined by liquid chromatography/mass spectrometry (LC/MS) methods. Each xenograft tissue sample was divided into two: one half underwent formalin fixation for IHC analysis and the other half was for snap frozen in liquid nitrogen. Phospho-FGFR and phospho-PLCγ expression levels for the PK/PD correlation study were determined by Western blot analysis on snap frozen samples and phospho-Erk expression was determined by IHC analysis on formalin-fixed samples (Phospho-FGFR, CST #3471; 1:1000; Phospho-Erk, CST #4370; 1:1000; phospho-PLCγ, CST #2821; 1:1000).

The PDGCX-based PD study was performed on xenograft tissue samples derived from animals treated as part of the AZD4547 efficacy study. Mice that had previously been administered AZD4547 1.56 mg/kg/qd during the efficacy study were given a single dose of AZD4547 12.5 mg/kg. Two hours post dose, xenograft tissue samples were collected and formalin-fixed sections were probed for phospho-Erk and phospho-S6 levels using IHC methods described earlier.

**Inducible shRNA knockdown of FGFR2 in vitro and in vivo**

A construct containing FGFR2-specific (exon 17) shRNA sequence under the control of the tetracycline promoter was used for this study. The shRNA sequences were 2B: GAATGAAGAACACGACCAA and 3C: GACTTGGATCGAATTCTCA (Invitrogen). SNU-16 cells were transfected with the FGFR2 construct and stable clones were selected using the antibiotic Puromycin (Sigma-Aldrich, P9620). A construct containing enhanced green fluorescent protein (EGFP)-specific shRNA sequence was used as a control, in which expression of shRNA can be induced by doxycycline (Sigma-Aldrich, D9891). SNU-16 parental cells and four stable clones (SNU-16_sh-EGFP control, SNU-16_sh-FGFR2 2B and SNU-16_sh-FGFR2 3C6 and 3C8) were established and used for the FGFR2 knockdown studies.

For the in vitro study, SNU-16 cells were treated with doxycycline 1 µg/ml for 1, 2 or 4 days, and the levels of FGFR2, phospho-FGFR and cleaved caspase-3 in the cell lysates were determined by Western blot analysis.
For the *in vivo* study, xenografts from stable clones SNU-16_sh-EGFP control, SNU-16_sh-FGFR2 2B and SNU-16_sh-FGFR2 3C8 were established following the methods described earlier. For each established xenograft, two small fragments (approximately 15 mm$^3$) were implanted s.c. with 50% Matrigel into the right flank of Balb/c nude mice. Mice bearing tumors with volumes approximating 150–250 mm$^3$ were randomized into two groups, with eight mice per group. For mice receiving treatment, doxycycline was dissolved in 5% sucrose solution to a working concentration of 2 mg/ml and administered via the drinking water which was protected from light and replaced every 2–3 days. Control groups received drinking water with 5% sucrose solution only. Treatment duration was 2 weeks for SNU-16_sh-FGFR2 2B-implanted mice and 3 weeks for SNU-16 parental, SNU-16_sh-FGFR2 3C8 and SNU-16_sh-EGFP mice.
Results

**FGFR2 is genetically amplified in tumor samples from patients with gastric cancer**

Tumor samples for array CGH analysis were provided by the Beijing Cancer Hospital. Eligible samples (tumor content of ≥70%) were collected from 131 Chinese patients with gastric cancer; the median age of the patients was 62 years (range 34–81) and the majority were male (n=94; 71%). Twenty age-matched tumor-free samples were used as control tissue. Amplification of the **FGFR2** gene was identified in three (2%) of the 131 samples analyzed (Fig 1A).

For FISH analysis, 197 Chinese GC patient samples were provided by the Shanghai Renji Hospital (median age 62 years [range 17–87]; male n=133 [67.5%]). In addition, 97 Caucasian GC patient samples were also analyzed (78 tissue microarray samples [TriStar technology] and 19 samples from the AstraZeneca Biobank; median age 67 years [range 38–93]; male n=66 [68%]). **FGFR2** amplification was identified in nine (5%) of the 197 samples from Chinese patients and seven (7%) of the 97 samples from Caucasian patients (Supplementary Table 1). Representative **FGFR2** amplified and control FISH images are shown in Figure 1B.

**FGFR2-amplified gastric cancer cell lines are hypersensitive to AZD4547 treatment**

Of 29 human GC cell lines screened, two **FGFR2**-amplified human gastric cancer cell lines, KATO-III and SNU-16, exhibited extreme sensitivity to AZD4547 treatment, with GI50 values of 3 and 5 nM respectively. The remainder of the cell lines screened (all non **FGFR2**–amplified), showed insensitivity to AZD4547 with GI50 values ranging from 1.6 to 30 μM (Fig 2A).

**AZD4547 modulation of FGFR2 signaling and shRNA knockdown of FGFR2 expression independently result in apoptotic induction in GC cells in vitro**

To validate a role for **FGFR2** amplification and expression in driving tumorigenesis within gastric cancer cell lines, short hairpin RNA (shRNA) technology was used to modulate **FGFR2** expression. Stably expressing inducible shRNA SNU16 cell line
clones were generated and tested in an in vitro proliferation assay. FGFR2 knockdown potently inhibited the growth of SNU-16 gastric cancer cells in vitro (Fig 2B). Following transfection with the construct incorporating FGFR2 clone 3C8, total-FGFR2 levels were reduced in a time-dependent manner following doxycycline treatment, with phospho-FGFR2 being undetectable after 1 day. Importantly, cleaved caspase-3 levels were substantially increased under the same conditions (Fig 2C). In contrast, both total- and phospho-FGFR2 were increased in parental or EGFP (vector control) transfected SNU-16 cells after doxycycline treatment (Fig 2C). Similar results were obtained with the control clone 3C6 (data not shown).

Following incubation with 30 nM AZD4547, levels of phosphorylated FGFR2 and its downstream signaling molecules; PLCγ, FRS2, pErk1/2 and S6 were all reduced in SNU-16 cells and similar modulation of these phospho-markers was observed in the KATOIII cell line (Fig 2D). Furthermore, dose-dependent increases in sub-G1 population of SNU-16 cells were also detected at 48 hours post-AZD4547 treatment (Fig 2E).

**FGFR2 inhibition leads to tumor regression in SNU-16 xenografts in vivo**

To extend the findings above, stable SNU-16 cell tet-controlled FGFR2 expression clones were used in in vivo studies. Doxycycline treatment of mice harboring established SNU-16 xenografts led to tumor regression only in those tumors stably expressing tet-controlled FGFR2 shRNA (clones 3C8 or 2B; Fig 3A). Tumor regrowth was observed following cessation of doxycycline treatment (Fig 3A).

Similarly, treatment of mice harboring FGFR2-amplified SNU-16 cells with AZD4547 over a 25-day period resulted in dose-dependent tumor growth inhibition, with significant tumor regression achieved using a daily dose of 12.5 mg/kg AZD4547 (Fig 3B).

To assess PD effects, mice bearing SNU-16 xenografts were treated with a single oral dose of 12.5 mg/kg AZD4547. Two hours post dose, greater than 95% inhibition of tumor FGFR2 phosphorylation was achieved, with partial recovery observed by Western blot at 24 hours (Fig 3C). Using both Western blot and IHC approaches, levels of the downstream markers phospho-ERK and phospho-PLCγ were
suppressed by ≥90% at 10 hours, with partial recovery apparent by 24 hours post dose (Fig 3D). The overall total plasma concentration of AZD4547 that was required to maintain 24-hour coverage of phospho-FGFR modulation correlated well with the AZD4547 concentration required for 90% inhibition (IC\textsubscript{90}, 50 nM) of SNU-16 cell growth in an \textit{in vitro} anti-proliferation assay. In addition, 50% inhibition of phospho-Erk and phospho-PLC\textsubscript{γ} was also observed at the AZD4547 IC\textsubscript{90} (50 nM) concentration in the SNU-16 PK/PD tumor xenograft model (Fig 3D).

Importantly, AZD4547 treatment led to a significant reduction in the proportion of SNU-16 tumor xenograft cells staining positive for Ki-67 and an increase in those positive for apoptotic markers (Fig 3E).

**AZD4547 induces tumor regressions in a PDGCX model carrying \textit{FGFR2} amplification**

To extend the translational relevance of our findings, we next sought to identify and test AZD4547 in a relevant PDGCX model. FISH analysis identified the patient-derived gastric cancer xenograft model, SGC083, as being \textit{FGFR2}-amplified. Antitumor efficacy studies confirmed this model to be highly sensitive and dose responsive to AZD4547 treatment, with significant tumor regression achieved using a daily 6.25 mg/kg dose of AZD4547 and complete tumor regression (undetectable tumors) was observed at doses of 12.5 mg/kg and 25 mg/kg AZD4547 (Fig 4A). Moreover, using quantified IHC staining, significant modulation (\( p<0.05 \)) of phospho-Erk and phospho-S6 was demonstrated using a dose of 12.5 mg/kg AZD4547 (Fig 4C), thus confirming pathway modulation \textit{in vivo}.

In contrast to the marked tumor regressions observed upon AZD4547 treatment of the \textit{FGFR2}-amplified SNU-16 and SGC083 xenograft models, only minimal to partial tumor growth inhibition was observed in the AZ521, MGC803, G001 and G009 \textit{FGFR2} non-amplified gastric cancer xenograft models (Table 1). Furthermore, treatment of the \textit{FGFR2} low polysomy model, G001, with 12.5 mg/kg AZD4547 did not result in significant modulation of pFGFR, pFRS2 or pErk (data not shown). Indeed, baseline expression of pFGFR2 and corresponding pathway activation were
low or undetectable across the FGFR2 non-amplified models we tested (data not shown).

To investigate the durability of the AZD4547 antitumor effect, mice bearing established SGC083 tumors were treated with 12.5 mg/kg AZD4547 for 20 days, at which point full tumor regression was achieved. Of seven mice, four were still tumor free at Day 116 when measurements were stopped. Three mice began to show tumor regrowth around Day 35, thus providing the opportunity for drug rechallenge. One mouse (SGC083F5-007) was rechallenged with 12.5 mg/kg AZD4547 at day 48, and complete regression was re-established after a further 20 days of treatment (Fig 4D).

**Combined treatment with AZD4547 enhances the antitumor efficacy of cytotoxic agents in the FGFR2-amplified SNU-16 xenograft model**

In order to assess the effects of combining AZD4547 treatment with existing standard of care chemotherapies, the SNU-16 xenograft model was tested preclinically with 5-Fluorouracil(10 mg/kg/qd)/Cisplatin(4 mg/kg/qw) (FC) and a low dose of AZD4547(3.1 mg/kg/qd). Single agent FC or AZD4547 gave tumor growth inhibition (TGI) values of 25% and 34% respectively, whilst 68% TGI was achieved when the same doses of AZD4547 and FC were combined (Fig 5A). Similar results were observed when AZD4547 was combined with either docetaxel or irinotecan. Single-agent docetaxel treatment achieved 48% TGI, whilst the combination with AZD4547 resulted in approximately 82% TGI. Monotherapy usage of irinotecan or AZD4547 generated TGI values of 60% and 14% respectively, whilst the combination resulted in significant tumor regression (100% TGI) (Fig 5B). The combination regimens were well tolerated as determined by minimal body weight loss (data not shown).
Discussion

Genetic aberrations of FGFR family members have been identified in different types of cancers. For example, FGFR1 amplification has been observed in breast cancer, ovarian cancer and lung cancer (31-33) and FGFR3 mutation has been identified in bladder cancer (34). FGFR2 genetic mutation or amplification leads to abnormal activation of the FGFR2 signaling pathway and contributes to carcinogenesis and tumor development in melanoma and gastric cancers, respectively (35-38). In this study, we report FGFR2 gene amplification incidence rates of 4.5% and 7% in cohorts of Chinese and Caucasian gastric cancer patients, respectively, consistent with previously published reports (36, 37). Interestingly, FGFR2 amplification was found to be mutually exclusive with HER2 and c-MET amplifications in these patient samples (data not shown). Thus, our data confirms the presence of a distinct segment of gastric cancer patients harboring tumors molecularly characterized by FGFR2 amplification, not only in the East Asian population, but also in Caucasian patients. The percentage of gastric cancer patients who have FGFR2 amplification is low at 5–7%, and hence identification of this cohort will present a significant challenge to the clinical development and treatment strategy. However, recent examples such as crizotinib in lung cancer (39) and trastuzumab in both breast and gastric cancer (40), demonstrate that in common cancers niche patients can be identified and successfully treated by highly targeted drugs.

Two approaches were used to functionally inhibit FGFR2 in gastric cancer cell lines and patient-derived xenograft models; pharmacological modulation with the small molecule inhibitor AZD4547, and specific shRNA knockdown of the FGFR2 gene. The results of these studies demonstrate that FGFR2 gene amplification is an oncogenic driver in gastric cancer. Once-daily oral administration of AZD4547 or shRNA knockdown of FGFR2 resulted in a rapid tumor regression in FGFR2-amplified models, and this was accompanied by inhibition of tumor phospho-FGFR2 levels and downstream signaling through phospho-PLCγ, phospho-Erk and phospho-S6. At a dose sufficient to induce tumor regression in a FGFR2-amplified model, AZD4547 displayed complete inhibition of tumor phospho-FGFR2, phospho-
Erk and phospho-PLC$\gamma$ for almost 24 hours. Indeed, the total AZD4547 in vivo drug exposure was maintained above the level required for complete in vitro inhibition of phospho-PLC$\gamma$ and phospho-Erk and the anti-proliferation IC$_{90}$ value in the SNU-16 cell line. Furthermore, antitumor efficacy was accompanied by inhibition of tumor Ki67 staining and induction of apoptosis. AZD4547 treatment did not, however, result in PD modulation of FGFR or downstream markers in a FGFR2 non-amplified xenograft model (G001). These data together suggest that AZD4547 exerts potent antitumor efficacy through direct inhibition of pFGFR2, with resulting PD pathway modulation only in those models with amplified FGFR2.

Chemotherapy regimens are commonly used in gastric cancer, including cisplatin/5FU doublet therapy in first line and taxane or irinotecan monotherapy for second-line disease. Here we have shown that AZD4547 can be combined with these chemotherapies in the SNU16 FGFR2 amplified model, resulting in antitumor efficacy greater than that achieved with either AZD4547 monotherapy or chemotherapy alone. Although these studies provide preliminary evidence for the feasibility of combining AZD4547 with chemotherapies, further studies are required to investigate the mechanistic interactions between AZD4547 and these standards of care.

Compared with standard cell-line derived tumor xenografts, patient-derived gastric cancer xenograft models offer the benefits of more informative disease models through maintenance of tumor heterogeneity and preservation of tumor architecture. Importantly, the FGFR2 amplification status was maintained between the patient-derived gastric cancer xenograft (PDGCX) models used and the original patient tumor samples from which they were derived (data not shown). Interestingly, we were able to perform some limited investigation of the rates and durability of complete tumor regression using the PDGCX model, SGC083. Following once-daily dosing of 12.5 mg/kg AZD4547, all seven mice showed complete regression of tumors within 3 weeks, and importantly, four of the mice were still tumor free at the end of the study on Day 116. Of the three tumors which regrew, one was rechallenged with AZD4547 and regression re-established, indicating that the tumor
remained sensitive to FGFR2 inhibition and that FGFR2 was likely still the ‘driver’ oncogene within this tumor. To our knowledge, this is the first observation of a FGFR2-amplified PDGCX tumor model showing such robust and durable antitumor response to a targeted pharmacological agent. Taken together, the studies above demonstrate that FGFR2 amplification leads to constitutive activation of the FGFR2 signaling pathway in gastric cancer, and furthermore that inhibition of this pathway using a well-tolerated, potent and selective inhibitor can lead to rapid and durable tumor regressions in FGFR2-amplified gastric cancer xenograft models.

In summary, our data demonstrates the existence of a cohort of Chinese and Caucasian patients with gastric cancer harboring FGFR2 gene amplification. Moreover, we have demonstrated the dependency of activated FGFR2 and downstream signaling on gastric cancer growth and survival in vitro and in vivo, using selective FGFR2 shRNA and the FGFR inhibitor, AZD4547. Thus, the preclinical data reported here support further development of FGFR inhibitors, such as AZD4547, as potential therapeutic agents for the treatment of patients with FGFR2 amplified gastric cancer.
References


Figure legends

Figure 1. FGFR2 gene amplification in Chinese and Caucasian gastric cancer patient samples

A. Representative example of FGFR2 gene amplification in a gastric tumor from a Chinese patient detected by array CGH (n=131).
B. Representative example of focal FGFR2 gene amplification from a cohort of Chinese GC specimens (n=197) detected by FISH analysis. Left panel is a non-amplified sample, right panel is an amplified sample. Red signals represent FGFR2 genes whilst green signals are CEP10.

Figure 2. Validation of FGFR2 gene amplification as an oncogenic driver in SNU-16 cells using pharmacological and shRNA approaches

A. AZD4547 sensitivity correlates with FGFR2 gene amplification status. In vitro MTS proliferation assay across a panel of 29 gastric cancer cell lines (upper panel) demonstrated that KATOIII and SNU16 cells were extremely sensitive to AZD4547 with GI₅₀ values of 3 and 5 nM, respectively. FGFR2 gene copy number in these two cell lines was confirmed by FISH (lower panel); both sensitive cell lines, KATOIII and SNU-16, showed strong FGFR2 gene amplification, while AZD4547 insensitive lines (eg N-87), which were not FGFR2-amplified, had normal FGFR2 gene status.
B. In vitro growth curve of SNU-16 cells stably expressing two FGFR2 specific shRNAs (one clone for 2B; two clones for 3C, 3C6 and 3C8), vector control (EGFP), or untreated (SNU-16) with/without doxycycline (Dox) treatment.
C. Inducible knockdown of FGFR2 by shRNA3C8 in SNU-16 cells resulted in a decrease of FGFR2 expression and FGFR2 phosphorylation compared with parental (SNU-16) or EGFP (vector control) transfected cells after doxycycline treatment. Increased caspase-3 cleavage was observed following shRNA treatment.
D. Inhibition of FGFR2 pathway activation in the AZD4547 sensitive cell lines SNU-16 and KATOIII. Cells were incubated with AZD4547 at the indicated doses. Cell lysates were immunoblotted for phospho-FGFR, phospho-FRS2, phospho-PLCγ, phospho- and total Erk, and phospho- and total-S6.
E. The mechanism of in vitro growth inhibition by AZD4547 in FGFR2-amplified SNU-16 cells. SNU-16 cells were incubated with increasing concentrations of AZD4547 for 48 hours. Cell-cycle distribution was analyzed using propidium iodide and a FACSCanto flow cytometric system.

**Figure 3.** AZD4547 treatment leads to tumor regression in FGFR2-amplified SNU-16 gastric cancer xenografts

A. Effect of FGFR2 shRNA treatment on growth of SNU-16 xenografts in vivo. SNU-16 xenografts developed from cells carrying either stably expressing tet-controlled FGFR2 shRNA (SNU16-ShRNA3C8 or 2B), EGFP control (SNU16-ShEGFP) or parental control (SNU16) were randomized into two groups and treated with/without doxycycline 2 mg/ml in 5% sucrose solution.

B. Effect of AZD4547 treatment on tumor growth in the SNU-16 xenograft model. AZD4547 was administered by oral gavage once daily to nude mice bearing established subcutaneous SNU-16 xenografts at the doses indicated. Statistical analysis of tumor growth inhibition was performed using a Student’s t-test.

C. Analysis of SNU-16 xenograft tumor phospho-FGFR levels following AZD4547 treatment. Phospho-FGFR was significantly inhibited in SNU-16 tumor tissues 1 hour after a single oral dose of AZD4547 12.5 mg/kg and was partially restored 24 hours following treatment.

D. PK and PD modeling in a SNU-16 xenograft model. AZD4547 12.5 mg/kg was administrated by oral gavage to tumor bearing nude mice; plasma and tumor xenografts were collected at various time points indicated after dosing. Total concentration of AZD4547 in plasma was plotted on the left axis, and phospho-Erk and phospho-PLCγ levels in xenografts were plotted on the right axis. IC₅₀ and IC₉₀ values of AZD4547 in an in vitro SNU-16 anti-proliferation assay are also indicated.

E. Ki67 immunohistochemical staining and TUNEL assay images of xenograft sections following treatment with a single dose of 12.5 mg/kg AZD4547.

**Figure 4.** AZD4547 displays potent antitumor efficacy in a FGFR2-amplified patient-derived gastric cancer xenograft (PDGCX) model
A. Growth curve of model SGC083 treated with AZD4547. Tumor-bearing nude mice were treated daily for 7–25 days with AZD4547 (or vehicle) at concentrations indicated. Tumor volume was measured at the time indicated. Statistical analysis of tumor growth inhibition was performed using a Student’s t-test.

B. FISH analysis of FGFR2 gene copy number in model SGC083. Red signals represent FGFR2 genes whilst green signals represent CEP10.

C. PD study of AZD4547 in model SGC083. IHC study of phospho-Erk and phospho-S6 (S240/244) after AZD4547 treatment. PD data are from 2 hours following a single AZD4547 12.5 mg/kg dose after 25 days of dosing with AZD4547 1.56 mg/kg/qd.

D. Re-challenge of FGFR2-amplified SGC083 PDTX models. AZD4547 was dosed daily for 20 days after which it was withdrawn and complete regression maintained in 4/7 tumors to day 116. One of three tumors that progressed at day 35 was re-challenged with AZD4547 (12.5 mg/kg) at day 48 for 20 days; this xenograft remained sensitive to AZD4547.

Figure 5. AZD4547 enhances the efficacy of cytotoxic agents in the SNU-16 FGFR2-amplified xenograft model

A. SNU-16 xenograft bearing nude mice were treated with either vehicle, AZD4547 3.1 mg/kg or FC (5-Fu 10 mg/kg and cisplatin 4 mg/kg) alone, or a combination of AZD4547 and FC as indicated. AZD4547 and 5-Fu were administered in a 5 day on, 2 day off treatment schedule. Cisplatin was administered once weekly. Statistical analysis of tumor growth inhibition was performed using a Student’s t-test.

B. AZD4547 enhanced the efficacy of docetaxel and irinotecan chemotherapy in the SNU-16 xenograft model. Statistical analysis of tumor growth inhibition was performed using a Student’s t-test.
**Table 1.** Correlation between *FGFR2* gene amplification and sensitivity to AZD4547 in a range of standard and PDGCX xenograft models

<table>
<thead>
<tr>
<th>Model</th>
<th>FGFR2 status (by FISH)</th>
<th>AZD4547 12.5 mg/kg</th>
<th>TGI (%)</th>
<th>Tumor regression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNU-16</td>
<td>Amplified  &gt;100</td>
<td>&gt;100</td>
<td>64**</td>
<td></td>
</tr>
<tr>
<td>AZ521</td>
<td>Disomy  63</td>
<td></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>MGC803</td>
<td>Disomy  37</td>
<td></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SGC083*</td>
<td>Amplified  &gt;100</td>
<td>&gt;100</td>
<td>90***</td>
<td></td>
</tr>
<tr>
<td>G001*</td>
<td>Polysomy   34</td>
<td></td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>G009*</td>
<td>Trisomy    23</td>
<td></td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

TGI, tumor growth inhibition
*Patient-derived gastric cancer xenograft (PDGCX) model
**Tumor regression was assessed at the end of the study
***AZD4547 was administrated for 7 days and tumor regression was assessed on day 25 post first dose
Figure 1

A

FGFR2 gene cluster

B

FGFR2
CEP10

Non-amplified

10 μm

Amplified
Figure 2

A

B

C

D

Days after doxycycline (1 µg/ml) treatment

0 1 2 4 0 1 2 4 0 1 2 4

Snu-16 Snu-16-EGFP Snu-16-FGFR2-3C8

Fluorescence intensity

0 1 2 3 4

Doxycycline (1 µg/ml)

Snu-16 treated with Dox Snu-16 control

Snu-16-EGFP treated with Dox Snu-16-EGFP control

Snu-16-FGFR2-2B treated with Dox Snu-16-FGFR2-2B control

Snu-16-FGFR2-3C6 treated with Dox Snu-16-FGFR2-3C6 control

Snu-16-FGFR2-3C8 treated with Dox Snu-16-FGFR2-3C8 control

Days after treatment with doxycycline (1 µg/ml)

0 1 2 3 4

AZD4547 (nM)

0 1 3 10 30 100

SNU-16 KATO III

AZD4547 (nM)

0 1 3 10 30 100

pFGFR2 (Tyr653/654)
pFGFR (Tyr436)
pPLCr (Tyr783)
pFRS2 (Tyr436)
pErk (Tyr202/204)
pS6 (Ser240/244)
Total Erk
Total S6

Snu-16 Snu-16-EGFP Snu-16-FGFR2-3C8

 FGFR2

 pFGFR

 Cleaved caspase-3

 GAPDH

0 1 3 10 30 100

pFGFR (Tyr653/654)
pFRS2 (Tyr436)
pPLCr (Tyr783)
pErk (Tyr202/204)
pS6 (Ser240/244)
Total Erk
Total S6

Snu-16 Snu-16-EGFP Snu-16-FGFR2-3C8

Snu-16 treated with Dox Snu-16 control

Snu-16-EGFP treated with Dox Snu-16-EGFP control

Snu-16-FGFR2-2B treated with Dox Snu-16-FGFR2-2B control

Snu-16-FGFR2-3C6 treated with Dox Snu-16-FGFR2-3C6 control

Snu-16-FGFR2-3C8 treated with Dox Snu-16-FGFR2-3C8 control

Days after treatment with doxycycline (1 µg/ml)

0 1 2 3 4

AZD4547 (nM)

0 1 3 10 30 100

SNU-16 KATO III

AZD4547 (nM)

0 1 3 10 30 100

pFGFR2 (Tyr653/654)
pFGFR (Tyr436)
pPLCr (Tyr783)
pFRS2 (Tyr436)
pErk (Tyr202/204)
pS6 (Ser240/244)
Total Erk
Total S6

Snu-16 Snu-16-EGFP Snu-16-FGFR2-3C8

FGFR2

pFGFR

Cleaved caspase-3

GAPDH

0 1 3 10 30 100

pFGFR (Tyr653/654)
pFRS2 (Tyr436)
pPLCr (Tyr783)
pErk (Tyr202/204)
pS6 (Ser240/244)
Total Erk
Total S6

Snu-16 Snu-16-EGFP Snu-16-FGFR2-3C8

Snu-16 treated with Dox Snu-16 control

Snu-16-EGFP treated with Dox Snu-16-EGFP control

Snu-16-FGFR2-2B treated with Dox Snu-16-FGFR2-2B control

Snu-16-FGFR2-3C6 treated with Dox Snu-16-FGFR2-3C6 control

Snu-16-FGFR2-3C8 treated with Dox Snu-16-FGFR2-3C8 control

Days after treatment with doxycycline (1 µg/ml)

0 1 2 3 4

AZD4547 (nM)

0 1 3 10 30 100

SNU-16 KATO III

AZD4547 (nM)

0 1 3 10 30 100

pFGFR2 (Tyr653/654)
pFGFR (Tyr436)
pPLCr (Tyr783)
pFRS2 (Tyr436)
pErk (Tyr202/204)
pS6 (Ser240/244)
Total Erk
Total S6

Snu-16 Snu-16-EGFP Snu-16-FGFR2-3C8

FGFR2

pFGFR

Cleaved caspase-3

GAPDH

0 1 3 10 30 100

pFGFR (Tyr653/654)
pFRS2 (Tyr436)
pPLCr (Tyr783)
pErk (Tyr202/204)
pS6 (Ser240/244)
Total Erk
Total S6

Snu-16 Snu-16-EGFP Snu-16-FGFR2-3C8

Snu-16 treated with Dox Snu-16 control

Snu-16-EGFP treated with Dox Snu-16-EGFP control

Snu-16-FGFR2-2B treated with Dox Snu-16-FGFR2-2B control

Snu-16-FGFR2-3C6 treated with Dox Snu-16-FGFR2-3C6 control

Snu-16-FGFR2-3C8 treated with Dox Snu-16-FGFR2-3C8 control
Figure 3

A. Tumor volume (mm³) over time for different treatment conditions:
- Snu-16 control
- Snu-16 treated with Dox
- Snu-16-FGFR2-3C8 control
- Snu-16-FGFR2-3C8 treated with Dox
- Snu-16-FGFR2-2B control
- Snu-16-FGFR2-2B treated with Dox
- Snu-16-EGFP control
- Snu-16-EGFP treated with Dox

B. Tumor volume (mm³) over time for different AZD4547 doses:
- Vehicle control
- AZD4547 1.56 mg/kg/qd
- AZD4547 3.125 mg/kg/qd
- AZD4547 6.25 mg/kg/qd
- AZD4547 12.5 mg/kg/qd

C. Western blot analysis showing:
- AZD4547 12.5 mg/kg
- 0 h, 1 h, 2 h, 24 h
- 80 µg protein load
- p-FGFR 120 Kd
- GAPDH 37 Kd

D. Total AZD4547 concentration (nM) over time:
- AZD4547 in plasma
- pERK
- pPLCγ

E. Immunohistochemical staining:
- Control
- AZD4547 12.5 mg/kg
- Ki67
- TUNEL
Figure 4

A

- Control
- AZD4547 1.56 mg/kg/qd, 25 days
- AZD4547 3.125 mg/kg/qd, 25 days
- AZD4547 6.25 mg/kg/qd, 25 days
- AZD4547 12.5 mg/kg/qd, 7 days
- AZD4547 25.0 mg/kg/qd, 7 days

P = 0.0072
P < 0.0001
P < 0.0001
P < 0.0001

B

FGFR2 CEP10

10 μm

C

pERK IHC (40X)

- Control
- AZD4547 12.5 mg/kg

P < 0.05

D

AZD4547 12.5 mg/kg:
Day 20 TGI > 100% and tumor regression 100%,
P = 0.0009

- Vehicle control
- SGC083F5-007
- SGC083F5-003
- SGC083F5-006
- SGC083F5-011
- SGC083F5-024
- SGC083F5-031
- SGC083F5-032

Tumor volume (mm³)

Study period (days)
Figure 5

A

Tumor volume (mm³)

Control
AZD4547 3.125 mg/kg/qd, 5 days on & 2 days off
SoC: 5-FU 10.0 mg/kg/qd, 5 days/week +
cisplatin 4 mg/kg/qw, 1st day/week
AZD4547 3.125 mg/kg/qd, 5 days on &
2 days off + SoC

P = 0.2185
P = 0.0549
P = 0.0007

Time (days)

B

Tumor volume (mm³)

Control
AZD4547 3.125 mg/kg/qd
Docetaxel 3 mg/kg/qw, day 1/week
Irinotecan 17.5 mg/kg/qw, day 1/week
AZD4547 3.125 mg/kg/qd +
docetaxel 3 mg/kg/qw, day 1/week
AZD4547 3.125 mg/kg/qd +
irinotecan 17.5 mg/kg/qw, day 1/week

P = 0.2125
P = 0.0124
P < 0.0001
P < 0.0001
P < 0.0001
FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547

Liang Xie, Xinying Su, Lin Zhang, et al.

Clin Cancer Res Published OnlineFirst March 14, 2013.