**Supplementary methods**

Kinase assays

Compounds were profiled against various recombinant KIT single and double mutants (Reaction Biology Corp.). Compounds were tested in 10-dose IC50 mode, with 3-fold serial dilutions starting at 3 μM. All kinase reactions were performed using 10 μM ATP.

**Supplementary patient information**

Three patients with metastatic GIST, for whom all approved medical treatments had failed, were offered off-label treatment with ponatinib. Patient’s written informed consent to a nonconventional medical treatment, selected in the lack of alternative therapies known to be effective in the disease, was obtained, and all patients were aware that there was not any previously reported proof of antitumor activity. The clinical experience of these 3 patients, treated consecutively under the care of the same physician (treatment initiation between November 2013 and January 2014), is as follows.

Patient 1 was a previously healthy woman, diagnosed with gastric GIST in 2006 at the age of 49 and whose tumor was previously determined to harbor a primary KIT exon 11 (W557C, Del 558-560) mutation. In September 2007 she was diagnosed with metastatic disease to the liver, peritoneum and the adrenal glands and started imatinib (400 mg) treatment. Following disease progression in June 2010, her imatinib dose was increased (800 mg) prior to switching to sunitinib, due to disease progression in November 2010. The patient had further disease progression documented in May 2011 and was subsequently treated with regorafenib (160 mg), which resulted in disease stabilization until December 2012. She was then rechallenged with imatinib (800 mg total daily dose), which resulted in disease stabilization until September 2013, when she showed marked disease progression. Her treatment was then switched to pazopanib 800 mg once daily, but this was unsuccessful and progression was accompanied by loss of weight and decreased performance status due to increasing tumor burden. In November 2013, the patient started exploratory treatment with ponatinib (30 mg, qd). Within seven days of ponatinib treatment, the patient experienced improvement in fatigue and a follow-up CT scan after 4 weeks of treatment showed marked radiologic response of all visible metastases. A 4 month follow-up scan revealed increased contrast-enhancing nodules within the cyst-like lesions but no increase in size of any lesion. Against these findings ponatinib-dose was increased to 45 mg and a further follow-up showed ongoing disease-control 2 months later.

Patient 2 was a 62 year old male patient with type 1 diabetes and a history of DVT hypercholesterolemia, hypertension, who was a heavy smoker. The patient was diagnosed with GIST of the stomach in November 2010 and received neoadjuvant imatinib (12/2010). The patient’s tumor, shown to have a primary KIT exon 11 (Del/Ins P551-W557/L) mutation, was resected in June 2011 but extensive progression was noted in August 2012 and his imatinib escalated to 800 mg. Upon disease progression the patient was switched to sunitinib (11/2012) and then regorafenib (1/2013). Following symptomatic tumor progression the patient underwent debulking surgery on multiple metastases (4/2013). Patient was restarted on regorafenib but exhibited marked, symptomatic tumor progression as of December 2013. In January 2014, the patient began exploratory ponatinib (30 mg, qd) and was co-treated with acetylsalicylic acid (100 mg) given the high cardiovascular risk of the patient. After 3 weeks of treatment, the patient experienced a mild cerebellar ischemia and received LMW heparin. After 4 weeks of ponatinib treatment, shrinkage of multiple lesions was observed by CT scan and the patient reported a substantial alleviation of upper abdominal discomfort. Patient was therefore continued on ponatinib given the palliative benefit of the treatment. Patient was admitted to the local hospital in March 25th due to acute chest pain. A coronary angiography revealed a, previously unknown, severe coronary artery disease (3-vessel disease) but no myocardial infarction. Ponatinib treatment was stopped due to the high cardiovascular risk. Minimally invasive intervention or surgical treatment was deemed too risky for the patient. A CT-scan on April 2nd showed further shrinkage of tumor lesions. The patient died of myocardial infarction on April 22nd, 5 weeks after treatment discontinuation, which was deemed unrelated to ponatinib by the treating physician.

Patient 3 was a 43 year old male patient diagnosed with a localized 12 cm jejunal GIST in May 2008 showing a primary KIT exon 11 (Del 552-558) mutation. After resection of the primary the patient was treated with adjuvant imatinib for one year until May 2009. In December 2009 the patient relapsed with multiple hepatic and peritoneal lesions. Imatinib (400 mg) was restarted and patient underwent resection of multiple metastases after the patient achieved a partial remission (3/2010). Upon disease progression the patient received sunitinib (11/2010) and imatinib (800 mg) (3/2011) and further debulking surgery (4/2011 and 1/2012). Salvage treatment with sorafenib (12/2012), regorafenib (9/2013) and pazopanib following progression of disease (12/2013) did not provide clinical benefit. In January 2014 the patient received ponatinib (30 mg) but again global progression was observed after 4 weeks of ponatinib and palliative surgery was performed for symptom relief.

**Supplementary figure legends**

Figure S1. Chemical structures of imatinib, sunitinib, regorafenib and ponatinib

Figure S2. Expression and activation of KIT in engineered Ba/F3 cells

Immunoblot analysis of KIT expression and Y721 phosphorylation (pKIT) of Ba/F3 KIT cell lysates.

Figure S3. Ponatinib inhibits the phosphorylation of exon 11primary activating, and secondary resistant mutant forms of KIT

The cell lines were treated with increasing concentrations of drug (PO, ponatinib; IM, imatinib; SU, sunitinib; RE, regorafenib) for two hours, then cell lysates were immunoblotted with antibodies directed against the indicated proteins. The phosphospecific antibodies were to KIT Y721, AKT S473, and ERK (MAPK) T202/Y204. The actin stains are loading controls.

Figure S4. Secondary mutants reduce ponatinib potency in Ex9 ins and V560D cell lines

IC50 values (nM) of imatinib, sunitinib, regorafenib and ponatinib in Ba/F3 cell lines containing a V654A ATP pocket secondary mutation (blue bars) or a D816H A-loop mutation (red bars) in a background of two different primary mutations (green bars) (A) Ex9 and (B) V560D. The cell lines were treated with increasing concentrations of drug for three days, and cell viability determined using MTT.

Figure S5. Illustration of the optimal fit of ponatinib to KIT

Ponatinib (shown as space-filling spheres) displays an optimal fit to the binding cavity of KIT (indicated by a mesh pattern). The manners in which the hinge binding bicycle of ponatinib fills the ATP pocket and the CF3 of the ponatinib B-ring fills the DFG pocket are highlighted.

Figure S6. Impact of compound treatment on KIT signaling in GIST-derived cell lines

Cells were treated with drug (PO, ponatinib; IM, imatinib; SU, sunitinib; RE, regorafenib) for two hours, cell lysates were and immunoblotted with antibodies directed against the indicated proteins.