Additional Details by Experimental Protocol Subsection

Cell Culture and Microarray Hybridization.
Cells were harvested at nine specific time points during treatment: 0 hr (no IM), 2 hr, 4 hr, 6 hr, 9 hr, 12 hr, 18 hr, 24 hr, and 48 hr. Total RNA was shipped from the Fox Chase Cancer Center to Johns Hopkins for microarray analysis. Total RNA was quantified with NanoDrop ND-1000 followed by quality assessment with 2100 Bioanalyzer (Agilent Technologies) according to manufacturer's protocol. Samples met the following quality parameters: OD280/260 and OD260/230 equal or higher than 1.5, 28S/18S ribosomal RNA ratio equal or higher than 1.2, and RNA Integrity Number(RIN) equal or higher than 8.0.

Agilent human 4xG4112a microarrays were utilized for obtaining transcript level estimates with cohybridization against Stratagene Human Reference mRNA using the microarray core at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. Sample amplification and labeling procedures were carried out using Low RNA Input Fluorescent Linear Amplification Kit (Agilent Technologies). 0.4 microgram of total RNA was reverse transcribed into first strand and second strand cDNA by MMLV-RT using an oligo dT primer (System Biosciences) that incorporated a T7 promoter sequence. The cDNA was then used as a template for in vitro transcription in the presence of T7 RNA polymerase and Cyanine labeled CTPs (Perkin-Elmer). The labeled cRNA was purified using RNeasy mini kit (Qiagen) and dye labeled. RNA spike-in controls (Agilent Technologies) were added to RNA samples before amplification and labeling according to the manufacturer's protocol.

0.828 microgram of each sample labeled with Cy3 or Cy5 were mixed with control targets (Agilent Technologies). Fragmentation was carried out by incubating at 60C for 30 minutes and stopped by adding equal volume of 2x GE Hi-RPM hybridization buffer (Agilent Technologies). Fragmented targets were placed on the 4xG4112a slides, assembled into a hybridization chamber (Agilent Technologies) and hybridized at 65ºC for 17 hours in a hybridization oven with rotation. Hybridized microarrays were washed and dried according to the Agilent microarray processing protocol.

Agilent Microarray Scanning and Data Preprocessing.
Microarrays were scanned using Agilent Scan Control 7.0 software on the G2505B Scanner. Data were extracted with Agilent Feature Extraction 9.5.3.1 software. LOESS normalization was performed with width 0.7 and no background correction. MA plots were reviewed together with Agilent summary files to confirm the quality of all arrays.

Estimation of Signaling Activity.
As noted in the text, Bayesian Decomposition as a Markov chain Monte Carlo method has poorly defined convergence criteria. As such, in addition to performing three separate runs that obtained almost identical χ^2 fits to the data, we also took readings of χ^2 from the posterior every 100 samples to confirm that all runs showed no ongoing improvement in χ^2 during sampling.

The statistic presented in equation 2 represents the the *Z*-score for a transcription factor and it is essentially the average *Z*-score for all genes $g_i \in G_t$ that are regulated by the transcription factor *t*. It is compared to

$$Z_{rand,p} = \frac{1}{R}\sum_{r\in\mathbf{A}} Z_{rp}$$

where R genes are chosen at random, which varies by number of genes regulated by a transcription factor (R) and the strength of the assignment of genes to the pattern (Z_{rp}). Sample values are plotted in figure 2, and these provides a distribution based on permutations of assignment of genes to the transcription factor for determination of statistical significance.

COMET Assays, qRT-PCR, and Western Blots

After treatments, cells were trypsinized and resuspended in PBS with a final concentration of 1 x 10^5 cells/ml cold PBS. 50 µl of cell suspension was mixed with 0.5 ml of melted agarose solution (1% Low Temperature Melting Agarose Solution containing 0.5 g LTMA agarose in 49.5 ml PBS). 50 µl of the cell-agarose mixture was then transferred to a CometSlide (Trevigen) and spread with a pipet tip to cover the entire sampling area. After cooling at 4 °C, the slides were immersed in ice-cold Lysis buffer (2.5 M NaCl; 0.1 M EDTA; 10 mM Tris; 0.1% sarkosyl; 0.1% Triton X-100, pH 10) for 45 min in dark, followed by immersion in ice-cold Alkali buffer (0.3 M NaOH; 1 mM EDTA) for additional 45 min in dark. Slides were then subjected to electrophoresis in Alkali buffer at 22 V for 20 min on ice. After electrophoresis, slides were fixed with 70% EOTH for 5 min, neutralized with 0.4 M Tris pH 7.8 for 5 min at room temperature. The DNA was stained with SYBR fluorescent dye and visualized with an immunofluorescent microscope.

For qRT-PCR, RNA was isolated from GIST-T1 cell lines untreated or treated with 10µM IM for 2, 6, 12, 24 or 48 hours. RNA was reverse transcribed to cDNA by SuperScript II reverse transcriptase (Invitrogen). Expression of RNA for JAK3, SOCS3 and CDC25A and the endogenous control gene, HPRT, was measured in each sample by real-time PCR (with TaqMan Gene Expression Assay products on an ABI PRISM 7900 HT Sequence Detection System, Applied Biosystems, Foster, CA) following protocols recommended by the manufacturer and as previously described (1). The primer/probe (FAM) sets for JAK3, SOCS3 and CDC25A, and HPRT were obtained from Applied Biosystems.

For immunoblotting, all antibodies used in immunoblotting were obtained from Cell Signaling Technology (Beverly, MA).

Tumor Samples

All GIST samples were screened for CD117 (KIT) positivity by standard IHC to validate appropriateness for participation in the clinical trial. Patients were required to have adequate hematologic, renal, and hepatic function as well as tumor mass greater than 3 cm in size. All patients signed informed consent following IRB approval for this study. All patients received IM at 600 mg daily by mouth which was continued until the day of surgery, with dose modifications for protocol defined toxicities.

1. Chen X, Arciero CA, Wang C, Broccoli D, Godwin AK. BRCC36 is essential for ionizing radiation-induced BRCA1 phosphorylation and nuclear foci formation. Cancer research 2006; 66: 5039-46.