SUPPLEMENTAL STUDY DESIGN

Our sample size ensured that, if the trial was not terminated early, a 95% confidence interval (95% CI) would have width at most 0.22 under the assumption of a 10% overall response rate. Our aim was to achieve response rate of at least 10%. To be certain that this target remained achievable while the trial was ongoing, accruing results were monitored after 10 patients were recruited with the intention to stop the trial if there was strong evidence that the target rate would not be achieved. Formally, accrual would be stopped if posterior probability of PROB (pi_R > 0.1 | data) < 0.1, where pi_R refer to overall response probability. The prior distribution on pi_R is beta (0.2, 1.8). In particular, the decision cut-offs were to stop if [number of responses]/[number of patients evaluated] was less than or equal to 0/15. The properties of this statistical design were studied by means of computer simulation of trials under various assumed response rates (10,000 each). Supplemental Table 1 summarizes the probability of stopping the trial early for the indicated true response rates.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Immunohistochemical staining

Paraffin-embedded tissue was sliced at 8µm thickness prior to mounting. Paraffin was removed by heating of the slides to 60°C for 30 minutes followed by placement in a bath of xylene followed by a series of increasingly diluted ethanol bath. For staining of Src and phosphorylated forms of Src, the slides were boiled in a pressure cooker for 5 minutes at 125°C in a bath of Borg decloaker soluation (Biocare Medical Inc.). For staining of FAK and phosphorylated forms of FAK, the slides were placed in a bath of EDTA buffer and bolied in a microwave oven for 5

minutes, followed by treatment with Dako target retrieval solution (Dako North America, Inc.) for one hour. Peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 12 minutes. The slides were rinsed with PBS for 3 minutes each for 3 times, followed by a protein block solution (Cyto Q immune-diluent buffer; Innovex) for 20 minutes at room temperature. Antibodies were diluted in the protein block solution at the specified dilution ratio in a volume of 50 to 100 μL and incubated at 4°C overnight. A negative control was incubated in protein block solution without the primary antibody added. Slides were then washed again in PBS (3 minutes x 3) followed by treatment with the secondary antibody (Mach 4 Universal HRP polymer, Biocare Medical Inc, or 4+Goat anti-rabbit biotinylated antibody, Biocare Medical Inc.). Seventy microliters of diaminobenzidine (DAB) was applied for 2-10 minutes followed by rinsing after sufficient staining was developed. Counterstaining was done with Gill's No. 3 hematoxylin (Sigma), followed by drying and mounting with Universal mount (Open Biosystems). Mounted slides were visualized using a bright field microscope.

Primary antibodies utilized were anti-Src antibody (1:100, Cell Signaling Technology), anti-phospho-Src family kinase Y416 (corresponding to human Y418, 1:100 to 1:500, Cell Signaling Technology), anti-FAK antibody (1:100, Cell Signaling Technology Inc), and anti-phospho-FAK Y816 (1:100, Biosource Invitrogen Co.), and anti-vinculin (Cell Signaling Technology).

Immunohistochemistry on Frozen Sections

For analysis of frozen sections by immunohistochemistry, tumor sample was placed in optimal cutting temperature compound as described above immediately after tumor harvesting. These were maintained at -80 degrees until cutting. Slides were fixed with cold acetone at room

temperature for 10 minutes followed by endogenous blocking of peroxidases with 3% hydrogen peroxide and phosphate buffered saline for 12 minutes. Protein blocking was completed with cyto Q and was incubated for 20 minutes at room temperature. 50 microliters of diluted antibody was added and was kept overnight at 4 degrees Celsius. The antibody utilized for frozen immunohistochemistry were total Src from cell signaling at 1-100 ratio and of phosphor-Src [Y418] from RND at 1-100 diluted in cyto Q. This was followed by a PBS rinse, three times for three minutes. A peroxidase labeled for immunohistochemistry a peroxidase labeled secondary antibody diluted in cyto Q blocking solution for 1 hour was added. The secondary antibody was Mach 4 HRP for 30 minutes followed by additional rinsing. The DAB chromogen was added for 3-10 minutes and intensity of the reaction checked on the Brightfield microscopy. Hematoxylin counter staining was added for 14 seconds followed by a wash of distilled water. The slides were mounted with PER mount. For immunofluorescence the same procedure was followed with the exception of a secondary antibody of anti-rabid IgG Dylight 649 conjugated at a dilution of 1:1000 in cyto Q. Nuclear staining was done with the Hoechst 3342 fluorochrome.

Serum and mononuclear cell collection

Serum and peripheral blood mononuclear cells were collected at baseline, day 8 of the second and fourth cycle (2 to 6 hours after taking the daily dose of dasatinib), and optionally at the time of study treatment discontinuation. In the expansion cohort, the cycle 2 day 8 blood draw was instead obtained at the time of liver biopsy (between days 8-14 of cycle 2 or 3), which was 2 to 6 hours after taking the daily dose of dasatinib. Two whole blood samples were collected using two cell preparation tubes with sodium citrate (CPT), with a total of 16cc drawn. The tubes were

gently inverted 8 times to mix the samples, followed by centrifugation immediately after collection for 30 minutes at 1700g at 18-25°C. The plasma layer was removed from each of the two CPT tubes and transferred in 1.5mL aliquots to separate labeled 1.8mL cryovials containing protease and phosphatase inhibitor prior to storage at - 80°C. The white blood cell band (buffy coat layer) was extracted with a pipette into a 15mL conical tube. Both samples were combined into a single centrifuge tube. PBS was added to a final volume of 14mL and centrifuged for 15 minutes at 300g at 4°C. The supernatant was removed and resuspended in 1.5mL PBS, followed by transferring to a labeled 1.5mL microfuge bullet tube. The sample was centrifuged for 5 minutes at 300g. The supernatant was removed and the sample was placed on dry ice followed by storage at -80°C. The sample was then lysed using standard techniques described above for immunoblotting.

Liver biopsy

Patients in the expansion cohort consented to paired liver biopsies to assess Src activity and modulation of Src activity by treatment with dasatinib. Biopsies were obtained prior to therapy (within 4 weeks of initiation of therapy), and between day 8 and day 14 of cycle 2 or 3. Samples were immediately processed with one core snap-frozen in liquid nitrogen, and the second sample rapidly frozen in Tissue-Tek OCT for storage at -80ºC. Using imaging guidance (CT-scan or ultrasound), an 18G Chiba needle was advanced towards the proximal edge of the lesion and a 20G Trucut core biopsy needle was placed coaxially so that the cutting portion of the needle would be within the lesion. A minimum of two core biopsies was obtained, with a maximum of 6.