

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome–positive ALL. *N Engl J Med* 2006;354:2542-51.

## Supplement A

A two-sample (daily and twice daily doses) modified continuous reassessment method (MCRM) (18) was used for dose-level selection and maximum tolerated dose (MTD) determination. The MCRM uses the estimated probability of dose limiting toxicity at the posterior means from a Bayesian model for decision-making. The model supports a provisional dose for dose escalation if its estimated probability of dose-limiting toxicity is  $\leq 0.33$ . The MCRM estimate of MTD was the dose at which the estimated probability was closest to 0.33 without exceeding it.

The starting dose was 50 mg daily in a 28-day cycle and was based upon 1/10th of the highest dose in a 4-week toxicity study in rats. Cohorts of patients received escalating doses until MTD was reached. The MCRM specified no maximum cohort size but at least 3 patients were to be treated in each cohort, and at least 6 patients were to be treated at the MTD. The dose level recommended for the next cohort by the MCRM was one which closely approximated the target toxicity rate of  $\leq 33\%$ .

The MTD was defined as the highest dose of nilotinib given for at least 1 cycle where the likelihood of patients experiencing dose-limiting toxicity is  $\leq 33\%$ .

A dose-limiting toxicity was a nilotinib-related adverse event occurring during cycle 1 and meeting standard criteria for toxicity in acute leukemia. The final recommended dose was based on the MCRM MTD estimate and an assessment of safety and tolerability data from subsequent cycles at all dose levels.

## **Supplement B**

### **Pharmacokinetics**

Serum samples for pharmacokinetic analysis were collected on days 1, 8, 15, and 28. Serum concentrations of nilotinib were determined using a validated method of liquid chromatography-tandem mass spectrometry assay. Serum concentration-time data were analyzed by standard non-compartmental methods to derive pharmacokinetic parameters.

### **Assessment of Biomarker Inhibition**

Blood samples were taken pre-dose and post-dose at steady state (day 15). Mononuclear cells were isolated and analyzed for expression and phosphorylation of CRKL, STAT1, STAT5, and AKT using quantitative flow cytometry. Monoclonal antibodies were obtained from Cell Signaling Technology (Beverly, MA). P-values were computed using the Wilcoxon signed rank test. The Bonferroni-Holm procedure was used to control for multiplicity.

## Comparison of Biomarker Phosphorylation at Baseline and Day 15 in

### Patients treated with twice daily Nilotinib therapy

	n	Baseline		Day 15		p-value
		(% Positive Cells)		(% Positive Cells)		
		Mean +/-SD	Median	Mean +/-SD	Median	
P-AKT	20	49.6 +/- 30.0	41.7	30.8 +/- 25.8	18.6	0.036
P-CRKL	19	50.6 +/- 30.1	46.3	29.9 +/- 23.8	24.4	0.008
P_STAT1	19	68.1 +/- 27.6	79.5	47.7 +/- 33.3	61.6	0.003
P-STAT5	18	38.8 +/- 26.0	33.0	21.1 +/- 22.0	15.0	0.024

### Assessment of BCR-ABL Mutational Status

Baseline blood samples were obtained and mutation analysis was performed on Abl codons 50-550 using direct sequencing technology at Transgenomics Inc, Omaha Nebraska. When a duplicate baseline blood sample was available, the mutation analysis was repeated at one of two academic

laboratories. The sequencing template was created by semi-nested PCR using primers located in the Bcr and Abl regions of the *BCR-ABL* gene.

### Response to Nilotinib by Mutation Status in 86\* Patients with CML

<b>CML Phase</b>	<b>Presence of Abl mutation</b>	<b>No. Patients</b>	<b>Response rate to nilotinib (%) HR/ CG</b>
<b>Chronic</b>	<b>P-loop</b>	<b>2</b>	<b>2 (100) / 0 (0)</b>
	<b>non P-loop</b>	<b>4</b>	<b>2 (50) / 3 (75)</b>
	<b>None</b>	<b>9</b>	<b>6 (67) / 5(56)</b>
<b>Accelerated</b>	<b>P-loop</b>	<b>16</b>	<b>10(63) / 6 (38)</b>
	<b>non P-loop</b>	<b>10</b>	<b>8 (80) / 8(80)</b>
	<b>None</b>	<b>22</b>	<b>15 (68) / 13 (59)</b>
<b>Blastic</b>	<b>P-loop</b>	<b>2</b>	<b>0 (0) / 0 (0)</b>
	<b>non P-loop</b>	<b>3</b>	<b>1 (33) / 1 (33)</b>
	<b>None</b>	<b>18</b>	<b>8 (44) / 8 (44)</b>
<b>Any</b>	<b>P-loop</b>	<b>20</b>	<b>12(60) / 6 (30)</b>
	<b>non P-loop</b>	<b>17</b>	<b>11 (65) / 12 (71)</b>
	<b>None</b>	<b>49</b>	<b>29 (59) / 26 (53)</b>

\*Mutations were: G250E 9 patients (10%); E255K 4 (5%); E355G, F317L, H396R, M351T 3 (3%) each; E255V, E459K, F359V, M244V, Q252H, T315I 2 (2%) each; and E334G, F311I, F311S, H396P, H490L, L248L, M290T, Y253F 1 (1%) each. Three mutations were observed in 1 patients and 2 mutations were observed in 8 patients each. Patients with Ph+ ALL not reported in this table

HR = hematologic; CG = cytogenetic, includes minimal and minor cytogenetic responses

## **Assessment of Gilbert's Genotype**

Baseline blood samples were obtained and DNA was extracted by Quintiles (Atlanta, GA) using PUREGENE DNA isolation kits (Gentra Systems, Minneapolis, MN, USA). Numerical variations in dinucleotide repeats in the bilirubin UDP-glucuronosyl transferase 1 promoter region (A[TA]<sub>n</sub>TA motif) were genotyped by Epidauros (Bernried, Germany), sequencing on an ABI PRISM 3700 or ABI PRISM 3100 (Applied Biosystems, Foster City, CA). Sequences were analyzed using commercially available software suite (University of Washington: Phred, Phrap, PolyPhred and Consed). Genotyping calls were compiled using proprietary software suite (SNP suite). A logistic regression of hyperbilirubimbia (based on laboratory data) versus genotype was used for data analysis.