Supplemental Figure 1. Constitutive and ligand-induced pStats in Kasumi-1 cells. Representative examples, all taken from the same experiment, are shown. A) Dot plots illustrate pY-Stat3 and pY-Stat5 signals in unstimulated cells (left), cells stimulated with 100 ng/ml G-CSF (center), and cells stimulated with 50 + 100 ng/ml IL-6 + sIL-6R (right). The position of the quadrant was established with the isotype control condition. B) Histogram overlays demonstrate differences in each pStat parameter under 4 conditions: isotype control (shaded), unstimulated (solid black line), G-CSF stimulated (dotted red line), and IL-6 stimulated (dashed blue line). For G-CSF stimulation, the mean ± SD of the log₂(ΔMFI) was 3.4 ± 0.6 for pY-Stat3 (n=49); 1.4 ± 0.4 for pS-Stat3 (n=49); and 2.9 ± 0.7 for pY-Stat5 (n=42). For IL-6 stimulation, the mean ± SD of the log₂(ΔMFI) was 3.5 ± 0.6 for pY-Stat3 (n=51); 1.0 ± 0.3 for pS-Stat3 (n=51); and 0.4 ± 0.6 for pY-Stat5 (n=44).
Supplemental Figure 2. Constitutive pY418-Src and total Stat3 levels in AML and NBM samples. Waterfall plots illustrate the distributions of values for additional baseline Stat pathway parameters, including A) pY418-Src and B) Total Stat3. The red bars with asterisks indicate the values for 9 normal bone marrow samples that were analyzed similarly. Values represent the percent of events in the positive region.
Supplemental Figure 3. Expression of the negative Stat pathway regulators SOCS3 and SHP1. Waterfall plot demonstrates the range of expression of A) SOCS3 and B) SHP1. The red bars with asterisks indicate the values for 10 normal bone marrow samples that were analyzed similarly. The green bar with the arrow indicates the Kasumi-1 value, defined as 1. Bar values represent the densitometry value, normalized first to the loading control, then to the Kasumi-1 value from the same blot.
Supplemental Figure 4. Bivariate correlations reveal relationships between Stat pathway parameters. A) Constitutive pY-Stat5 and pY418-Src were significantly associated. B) Constitutive pY-Stat5 and G-CSF-induced pY-Stat5 were inversely related. The level of CD33 expression was significantly associated with C) Constitutive pY-Stat5, D) constitutive pY418-Src, and E) gp130 expression. F) Samples with high CD33 expression had smaller responses to IL-6 stimulation. R=Spearman correlation coefficient
Supplemental Figure 5. Constitutive pY-Stat3 and total Stat3 levels did not significantly correlate with survival. The threshold for low v. high levels was defined as the mean + 2SD of the corresponding parameter for the NBM samples. Additional cutpoint analyses using all AML sample values between the 10th and 90th percentiles confirmed that there is no threshold value that identifies patients with a significantly different EFS or OS. A) The 5 year OS for patients whose constitutive pY-Stat3 levels were < 63.2% (n=121) was not different from those with higher pY-Stat3 levels (n= 18). B) The 5 year OS for patients whose total Stat3 levels were < 86.3% (n=69) was not different from those with higher total Stat3 levels (n= 70).
Supplemental Figure 6. Survival rates associated with three G-CSF response categories. Kaplan-Meier survival curves show that patients whose blasts demonstrated a response (defined as $\log_2(\Delta MFI) \geq 1$) to G-CSF at Dose 1 or 2 (1 or 10 ng/ml dose) had a trend toward superior EFS (left) and OS (right) compared to patients whose blasts responded only at Dose 3 (100 ng/ml) or did not respond at any tested dose. There was no difference in EFS or OS between the latter two response groups.