Appendix S1

Flow Cytometry:

Flow cytometric staining of peripheral blood mononuclear cell (PBMC) was performed as described elsewhere[1]. Briefly, purified PBMCs were incubated with dog immunoglobulin G (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) to prevent non-specific binding of antibodies to Fc receptors. Cells were stained with the primary antibodies, CD4, CD5, CD8, CD11b, CD14, CD21, CD45 (Serotec, Raleigh, NC), and CD34 (BD Biosciences, San Jose, CA), conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), or labeled with Zenon Alexa-Fluor 647 probes (Invitrogen-Molecular Probes, Carlsbad, CA). Before running samples on flow cytometry, 0.5 µg/ml of *7*-amino-actinomycin D (7-AAD; eBioscience, San Diego, CA) was added to exclude dead cells from analysis. Flow cytometry was performed using LSRII cytometers (BD Immunocytometry Systems, San Jose, CA), and results were analyzed using FlowJo (Tree Star, Ashland, OR).

Appendix S2

Comparative Genomic Hybridization:

Oligonucleotide array comparative genomic hybridization (oaCGH) was performed using Agilent SurePrint G3 Canine Genome 180K microarrays which contain 171,534 coding and noncoding 60-mer oligonucleotide sequences spaced at ~13 kb intervals. Tumor DNA was isolated from cryopreserved peripheral blood cells obtained from the dog at day 1 and day 7 (Table 1) using a Qiagen Blood & Cell Culture DNA Mini kit. A sex-matched equimolar pool of genomic DNA from 16 healthy dogs (eight breeds, two of each breed) was used as the reference to prevent detection of natural constitutional copy number variations. Tumor and reference DNA

(500 ng) were labeled with cyanine 3-dCTP and cyanine 5-dCTP, respectively, using the Agilent enzymatic labeling kit and purified using a Qiagen Nucleotide Removal kit. Labeled test and reference DNA were co-precipitated in the presence of 10 μg Herring Sperm DNA to serve as a carrier, and resuspended in 44 μl HPLC water, 11 μl 10x blocking agent, and 55 μl Agilent HI-RPM hybridization buffer. The hybridization mix was denatured at 95ºC for 5 min, incubated at 37ºC for 30 min and applied to the array using an Agilent microarray gasket and hybridization chamber. The chamber was placed in a 65 ºC rotating oven at 20 rpm for 40 hours. Washes were completed using commercially available wash buffers (Agilent Technologies) according to the manufacturer's guidelines. Arrays were protected with an ozone barrier and scanned at 3 μm resolution using an Agilent DNA G2565CA Microarray Scanner with SureScan High resolution technology.

Appendix S3

oaCGH Analysis:

Scanned data were extracted using Feature Extraction Software v10.10 (Agilent Technologies), and assessed for data quality using Agilent QC metrics. Extracted data were analyzed using Genomic Workbench 6.6 (Agilent Technologies). The Aberration Detection Method 2 (ADM-2) algorithm was used to identify regions of aberrant copy number change. A threshold value of 6 was used to reduce sample noise without losing true aberrations. The mean aberration log2 ratio threshold was set to equivalent or exceeding 0.2. An estimate of noise was determined by calculating the spread of log ratio difference between consecutive probes (DLRspread). Data was also centralized, and the Fuzzy Zero algorithm was applied to correct for global error.

Appendix S4

Fluorescence *in situ* hybridization:

Fluorescence *in situ* hybridization was completed as previously described [2,3]. Briefly, bacterial artificial chromosomes (BAC) were selected from the CHORI-82 dog BAC library (http://bacpac.chori.org/library.php?id=253) from within a subset of the regions of the CanFam v2.0 canine genome assembly (http://genome.ucsc.edu) that were aberrant in the oaCGH profiles, as well as from known regions of interest in canine leukemia (CFA 9 (*BCR, BRCA1*) and 26 (*ABL, PTEN*) ; CFA 22 (*RB1*)) [4] and regions showing aberrant copy number ratios in aCGH.

Each BAC DNA was isolated from a 10 ml bacterial culture using a Qiagen REAL Prep 96 kit, and 500ng were labeled via a nick translation reaction with one of five fluorochromes: Spectrum Red/Orange/Green dUTP, diethylaminomethylcoumarin-5-dUTP, or Cyanine5-dUTP. Groups of five differently labeled probes (30ng each) were pooled and precipitated with 15 μg of sonicated dog DNA used as a competitor.

Cellular preparations were generated from heparinized peripheral blood specimens drawn from the dog on day 1, just prior to receiving the first dose of vincristine, and then seven days later. Tumor cells were harvested directly using conventional techniques of hypotonic treatment and methanol-glacial acetic acid fixation. Hybridization, post-hybridization washes, and DAPI staining were performed according to routine protocols [5]. Images were acquired using a semi-automated multicolor FISH workstation as described previously [2]. Mulitiplane images of 50 cells for reactions verifying aCGH aberrant copy number ratios and 150 cells for reactions evaluating known regions of interest were analyzed for copy number or structural changes in both pre- and

post-treatment samples. The same number of cells from a healthy control dog was analyzed for each reaction. Differences in the frequency of aberrations in pre-treatment and post-treatment cells were analyzed using Fisher's Exact test.

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