Supplementary Materials and Methods:

Production of the Hu-Mikβ1 monoclonal antibody

Humanization of Mikβ1 was reported previously. The immunoglobulin sequence expressed in the hybridoma producing murine Mikβ1 was determined by Protein Design Labs, Inc., Palo Alto, CA[24]. Gene-coding design sequences of Hu-Mikβ1 antibody V_L and V_h regions were synthesized as many exon segments that were utilized in the expression vectors. In designing the hypervariable region of Hu-Mikβ1, the 3 light chain and 3 heavy chain complementary determining regions (CDRs) were transferred into the Lay Immunoglobulin framework. Using a molecular model previously described[25], a number of framework amino acids were identified that differed between Mikβ1 and the human Lay sequence and there were a number near the CDRs that made it likely that they could influence CDR confirmation and thus binding affinity. Amino acids from the murine Mikβ1 sequence were substituted at those positions in the humanized antibody to maintain the confirmation of the CDRs. This was done at residues 3, 4, 38, 44, 69 and 70 in the V_L domain and residues 28, 29, 30, 37, 47, 49, 67, 71, 72, 73, 78, 80 in the V_h domain. In addition, a number of amino acids in the Lay V_L and V_h sequences that were unusual at these positions relative to other human antibody sequences were identified and changed to the human consensus amino acids. This was done at residues 13 and 41 of V_L in residues 1, 84, 89 and 90 V_h. The host cell line used to express Hu-Mikβ1 was Sp2/0-A-g14 from ATCC (Manassas, VA). Sp2/0 was transfected with pBk- Hu-Mikβ1 and pVg1- Hu-Mikβ1 plasmid DNAs by electroporation using a Gene Pulser apparatus (BioRad, Hercules, CA) according to the manufacturer's instructions. The Hu-Mikβ1 for the clinical trial was produced by the Biopharmaceutical Development Program (BDP) SAIC-Frederick Incorporated. Hu-Mikβ1 was expressed and secreted in the 2D1 cell lines. Hu-Mikβ1 produced by the 2D1 cell

lines was clarified by Tangential Flow Filtration (TFF) through a 0.45 micron filter and purified by a series of chromatography steps including Protein A immobilized on Sepharose (SP-Sepharose High-Performance Chromatography) and Q-Sepharose Fast-Flow Chromatography. Specific robust steps for the activation and removal of virus were included in the purification process.

Spontaneous PBMC proliferation in patients with T-LGL

Spontaneous proliferation of PBMCs from 6 patients with T-LGL was studied. PBMCs obtained by Ficoll centrifugation were washed and cultured for 6 days in RPMI 1640 (BioSource, Rockville, MD), 10% FBS, 0.3 mg/mL glutamine and incubated at 37°C in 5% CO_2. PBMCs were incubated with medium alone or with the addition of 10 μg/mL UPC-10 antibody as a nonspecific IgG2 AIG control antibody (Sigma, St. Louis, MO) or Hu-Mikβ1 (anti-IL-2/IL-15Rβ, anti-CD122). Cells were pulsed for the last 4 hours of a 6-day culture with 1 μCi of [^{3}H] thymidine (1 Ci = 37 GBq), and uptake of radioactivity was determined.

Detection of infused Hu-Mikβ1 in patient sera

Levels of circulating Hu-Mikβ1 antibody in the serum of patients receiving this therapy were quantitated using a competitive inhibition ELISA developed in our laboratory. This assay specifically detects circulating Hu-Mikβ1 without regard to the presence of and without any interference from the normal human IgG that is present in the patient serum. The assay is performed in brief as follows.

1) 96 well ELISA plates were coated with an affinity purified goat anti- Hu-Mikβ1 antibody overnight at 4°C, washed, blocked with BSA/PBS for 1 hour at 37°C to prevent nonspecific binding, and then washed again.
2) A biotin conjugated Hu-Mikβ1 reagent was prepared as previously described.
3) A standard curve of known concentrations of Hu-Mikβ1 was prepared using points encompassing the dynamic range of 7.80 ng/mL to 2000 ng/mL. Each Hu-Mikβ1 at a known concentration point was added to a coated well followed by a standard amount of the biotin conjugated Hu-Mikβ1. The plates were incubated for 2 hours at 37°C and washed.
4) Both the unmodified standard Hu-Mikβ1 and the biotin conjugated Hu-Mikβ1 will bind to the goat anti- Hu-Mikβ1 coated wells. To determine the relative amount of biotin conjugated Hu-Mikβ1 was bound to the plate, alkaline phosphatase conjugated streptavidin was added to each well, and the plate was incubated for 2 hours at 37°C and washed. The plate was developed with the addition of p-nitrophenyl phosphatase substrate at 37°C for 1 hour. The resulting color development was detected at 405 nm using a microtiter plate reader.
5) The color developed was directly proportional to the amount of biotin conjugated Hu-Mikβ1 that was bound to the well. Because both the unmodified and biotin conjugated Hu-Mikβ1 reagents present compete for binding to the coated wells, the color development was inversely proportional to the amount of known standard Hu-Mikβ1 present in the well. This competition allows for the specific construction of a standard curve of Hu-Mikβ1 concentration versus color developed.

6) Unknown patient serum samples were quantitated for Hu-Mikβ1 concentration in a similar fashion. A serum sample of unknown Hu-Mikβ1 concentration was added to a coated well along with the same standard amount of biotin conjugated Hu-Mikβ1 that was used in the standard curve points and the wells were developed colorimetrically as above. The concentration of Hu-Mikβ1 in the unknown sample was quantitated by comparing the amount of color development in the unknown sample with that of the known Hu-Mikβ1 samples in the standard curve.

A Two-Arm capture ELISA for the detection of antibodies to Hu-Mikβ1

To assess the immunogenicity of Hu-Mikβ1 in patients infused with this monoclonal antibody, we developed a specific ELISA technique capable of quantitating the total of all classes of antibody produced *in vivo* against this therapeutic agent with a sensitivity to 156 ng/mL in serum. In this assay ELISA plate wells were coated with the Hu-Mikβ1 antibody as the antigenic target. Circulating serum multimeric antibodies directed toward this monoclonal bound to the antigenic target coated to the plate. The free arm of the bound antibody then bound to added biotin conjugated Hu-Mikβ1 and was detected and quantitated colorimetrically. The assay was performed in brief as follows.

1) 96 well ELISA plates were coated with Hu-Mikβ1 at 37^{o}C for 3 hours, washed, and then blocked with PBS/BSA at 37^{o}C for 1 hour and washed again.
2) A standard curve of antibody to Hu-Mikβ1 was constructed for each assay using serial dilutions of an affinity purified goat anti-Hu-Mikβ1 antibody prepared by Hoffmann-La Roche, Inc., for assay quantitation and quality control. The standard

curve points were assayed in triplicate and cover a dynamic range of 78 to 2000 ng/mL of purified antibody to Hu-Mikβ1. Normal human serum was included as a negative control, and serum from a cynomolgus macaque immunized with Hu-Mikβ1 was included as an independent positive control. Patient samples for anti- Hu-Mikβ1 quantitation were added to their wells in duplicate.

3) All standard curve, control, and unknown patient samples were added to the coated plates and incubated overnight at 4°C.
4) After the plates were washed, a standard amount of biotin conjugated Hu-Mikβ1 was added to each well for 2 hours at 37°C followed by washing.
5) For detection of plate-bound biotin conjugated Hu-Mikβ1, alkaline phosphatase conjugated streptavidin was added to each well for 2 hours at 37°C followed by washing.
6) The assay was developed with the addition of diethanolamine buffer with p-nitrophenyl phosphatase substrate at 37°C. The color developed was detected at 405 nm using a microtiter plate reader. The color produced was directly proportional to the amount of antibody to the Hu-Mikβ1 present. Unknown samples were quantitated for antibody to Hu-Mikβ1 concentration by interpolation from the standard curve constructed with the known affinity of goat anti- Hu-Mikβ1.

Supplementary Figure Legend: Hu-Mikβ1 at a dose of 0.5, 1.0 or 1.5 mg/kg administered to patients with LGL leukemia and neutropenia on a single occasion had only a modest effect on the total circulating absolute neutrophil count (ANC).

Supplemental Figure 1