SUPPLEMENTAL MATERIAL

Supplemental Methods

Preparation of the liposomal C61 formulation. Liposomal nanoparticle (LNP) formulation, 25A, was prepared using the standard thin film evaporation method, in a round bottom flask using a fixed 26.2:13.8 ratio of DPPC to CHL. The standard thin film evaporation method was employed and the chloroform used in the LNP formulation was removed using a rotary evaporator at 40°C. Entrapment of C61 was obtained using the pH gradient procedure with 0.3 M Lactobionic acid (LBA) as a low pH buffer inside the LNP. Dipalmitoyl phosphatidylcholine (DPPC), Cholesterol (CHL), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) were obtained from Avanti Polar Lipids (Alabaster, AL). LBA and sucrose were obtained from Sigma (St Louis, MO). PD-10 desalting columns packed with Sephadex G-25 medium were obtained from GE Healthcare (Piscataway, NJ).

Physicochemical Characterization of Nanoparticles. Size measurement by the dynamic light scattering (DLS) technique was performed on a DynaPro Titan Instrument (Wyatt Technology Corp., Santa Barbara, CA) at the USC Nano BioPhysics Laboratory. For the measurement of C61 concentrations, NP samples were analyzed on an Agilent 1100 HPLC system equipped with a Hypersil ODS reverse phase analytical column (Hewlett Packard Hypersil 5 µm ODS, 125 x 4.6 mm) and a diode array detector with detection and reference wavelengths set at 230 nm and 420 nm, respectively. The Zeta potential measurements were carried out on a Brookhaven Instrument ZetaPlus (Holtsville, NY).

Transmission Electron Microscopy (TEM). The morphology of the C61 nanoparticles was examined using TEM. In brief, samples of liposomal nanoparticle formulations were diluted 1:1 with 2% uranyl acetate and placed on Formvar-coated copper grids (300 Mesh) for 5 minutes. The samples were then gently blotted against clean filter paper and air dried for 10 minutes. Grids were examined on a JEOL JEM-2100 LaB6 (Peabody, MA) transmission electron microscope and photographed with the Orius SC1000B Gatan (Pleasanton, CA) digital camera.

Apoptosis Assays. Cells were treated with increasing concentrations of C61-LNP formulation 25A or the control formulation 25B at 37°C/5% CO_2 for 24-72 hours. In each of the 18 ALL cases whose primary leukemia cells were tested, there was at least one untreated control, one 25B-treated control and at least one test sample treated with 25A at 3, 10 or 30 μg/ml. In 9 cases, all 3 concentrations were tested, in 5 cases only 30 μg/ml was tested, in 2 cases only 10 μg/ml was tested, in 1 case 10 and 30 μg/ml concentrations were tested and in one case 3 and 30 μg/ml concentration was tested. Thus, cumulatively, 0 μg/ml was tested in 18, 3 μg/ml in 11, 10 μg/ml in 12, and 30 μg/ml in 15 cases. Cells were incubated with 25A or 25B for 24 h in 2 cases, 48 h in 13 cases, 72 h in 3 cases. In each of the 11 B-precursor ALL xenograft cases, there was at least one untreated control (in 9 cases there also was a 25B-treated control) and at least one test sample treated with 25A. In 5 xenograft cases, all 3 concentrations were tested while in the remaining 6 cases only 30 μg/ml was tested. Thus, cumulatively, 0 μg/ml was tested in 11, 3 μg/ml in 5, 10 μg/ml in 5, and 30 μg/ml in 11 cases. Xenograft cells were incubated with 25A or 25B for 24 h in 1 case, 48 h in 9 cases, 72 h in 1 case. In these experiments, primary cells as well as xenograft cells were cultured small flasks or tissue culture plates in RPMI 1640 cell culture medium supplemented with 10% fetal bovine serum. No cytokines or feeder cells were used. Controls included cells irradiated with 200 rads γ-rays in a single exposure using a ^{137}Cs irradiator. Ionizing radiation was tested in 9 cases where the primary leukemia cells were used and 5 xenograft cases based availability of the irradiator. Apoptotic death was monitored

using multiparameter flow cytometry, as previously reported.[1,2] While the viability was >90% at the start of the treatments, it varied after the extended culture period. The percent apoptosis was calculated using the formula: 100- 100x(Percentage of non-apoptotic lymphoid cells in test sample/Percentage of non-apoptotic lymphoid cells in untreated control sample). We further used the following cell lines: ALL-1 (Ph^+ adult ALL, B-lineage, SYK^+), LOUCY (adult T-lineage ALL; ATCC® CRL-2629™), DAUDI (Burkitt's leukemia/lymphoma; ATCC®, CCL-213), HT-1080 (human fibrosarcoma; ATCC CRL-12012™), and DAOY (human desmoplasmic cerebellar medulloblastoma; ATCC HTB-186™). Dose dependent relationships were determined from the median effect equation (JMP 10, SAS, Cary, NC) describing percentage apoptosis at increasing concentrations of C-61 nanoparticle (5 concentrations ranging from 0.1-30 µg/ml for 2 experiments with ALL-1 and one with DAUDI cell lines). The Median Effect equation describes the non-linear relationship of percentage Apoptosis solving for the EC_{50} and Hill Number parameters (% Apoptosis = 100 x (C-61 concentration/EC_{50})^{Hill}/(1+(C-61 concentration/EC_{50})^{Hill}). Curve fits were performed for each cell line and combined data from ALL-1 and DAUDI cell lines. The curve fitting procedure utilizes the unweighted, non-linear least squares method to calculate the EC_{50} parameter (Gauss-Newton method with step-halving). Standard error of the fitted parameter will be determined using a method analogous to linear methods derived from the root mean square of the model and the diagonals of the derivative cross products matrix.

Biochemical assays and procedures. Immunoprecipitations, kinase assays, and immunoblotting were performed using previously published procedures, methods, and materials.[3,4].

Monoclonal antibodies used for flow cytometric immunophenotyping. The antibodies were obtained from BD Bosciences (SanJose, CA) and included: CD19 Phycoerythrin (PE): cat. #:555413, CD7 Fluorescein isothiocyanate (FITC): cat. #:555360, CD10 PE: cat. #:555375,

HLA/DR/DP/DQ FITC: cat. #:555558, CD45 PE: cat. #:555483, CD5 PE: cat. #:555354, CD34 FITC: cat. #:555821, CD33 FITC: cat. #:340533, and HLA-A,B,C Phycoerythrin: cat. #:555553. In other experiments, the following set of antibodies from BD Biosciences was used: CD10 (APC) BD catalog #: 340923, CD19 (APC-H7) clone:SJ25C1 BD catalog #: 560177, CD34 (Per CP-Cy5.5) BD catalog #: 347203, and CD45 (V450) clone:H130 BD catalog #: 560367. The SYK expression level of leukemic cells was examined by intracytoplasmic flow cytometry using BD Phospholow Fix Buffer 1 (Cat. #: 557870, BD Biosciences, San Jose, CA) for permeabilization of the cell membrane and SYK-FITC antibody (Cat. #: 5524726, BD Biosciences, San Jose, CA).

Confocal Laser Scanning Microscopy. Subcellular SYK localization using immunofluorescence and spinning disk confocal microscopy were performed as previously described.[3,4] Slides were imaged using the PerkinElmer Spinning Disc Confocal Microscope and the PerkinElmer UltraView ERS software (Shelton, CT) or the Volocity V5.4 imaging software (PerkinElmer, Shelton, CT). The coverslips were fixed with ice-cold MeOH at -20°C for 10 minutes. The fixed cells were permeabilized and their non-specific antibody binding sites blocked with 0.1% Triton X-100 and 10% goat serum in PBS for 30 minutes, respectively. In order to detect and localize the SYK protein, cells were stained with a mouse monoclonal anti-SYK antibody (sc-1240, Santa Cruz CA) for 1 hour at room temperature. Cells were washed with PBS and incubated with Alexa Fluor 488 goat anti-mouse IgG (secondary Ab) (Cat #: A11001, Invitrogen, Carlsbad, CA) for 1 hour. Cells were then washed with PBS and counterstained with the blue fluorescent DNA-specific nuclear dye 4',6-diamidino-2-phenylindole (DAPI). The coverslips were inverted, mounted onto slides in Vectashield (Vector Labs, Burlinghame, CA) to prevent photobleaching, and sealed with nail varnish. UltraCruz Mounting Medium containing 1.5 μg/ml of DAPI was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Rodent Toxicity Studies. The toxicity of C61-LNP formulation 25A (as well as the drug-free control formulation 25B) was examined at the Faculty of Veterinary Science of the Firat University in Elazig, Turkey with approval of the Animal Care and Use Committee (IACUC) at the Firat University (Approval No. 38, Meeting 2011/02, Approval date: 02-04-2011). We used 8-week old Wistar albino rats (Groups of 6-10 male rats per treatment group) and 8-week old BALB/c mice (groups of 5 female and 5 male mice per treatment except for the 100 mg/kg/day dose level at which 3 female and 2 male mice were treated). Rats were treated with a single intravenous bolus dose of 25A at 10 mg/kg or 25 mg/kg dose levels or D_5W. Rats were electively sacrificed on day 15 or day 30 to determine the toxicity of 25A by examining their blood chemistry profiles, blood counts, and evaluating multiple organs for the presence of toxic lesions. Blood was collected by intracardiac puncture following anesthesia with ketamine: xylazine and immediately heparinized. The blood chemistry profiles were examined using an Olympus AU-660 Chemical Analyzer, (Tokyo, Japan). Blood counts (red blood cells [RBC], white blood cells [WBC] and platelets [Plt]) were determined using an Abbott Cell-Dyn CD-1800 Hematology Analyzer (Illinois, U.S.A). At the time of necropsy, multiple tissues (bone, bone marrow, brain, coagulating gland, epididymis, heart, large intestine, small intestine, kidney, liver, lung, pancreas, peripheral nerve/spinal nerve, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, urinary bladder) were collected within 15 min after sacrifice for gross pathological and histopathological examinations. Organs were preserved in 10% neutral phosphate buffered formalin, and processed for histologic sectioning. For histopathologic studies, formalin fixed tissues were dehydrated and embedded in paraffin by routine methods. Glass slides with affixed 4-5 micron tissue sections were prepared and stained with Hemotoxylin and Eosin (H&E). Sections were examined by light microscopy with an Olympus BX-50 microscope (Center Valley, PA). The acute toxicity profiles of 25A and 25B in BALB/c mice were examined as previously reported for C61-salt formulation [C61-$(H_2SO_4)_4$][1]. In brief, mice were

administered an intraperitoneal (i.p) bolus injection of 25A at dose levels ranging from 1 mg/kg to 100 mg/kg or 25B daily for 5 days in a total volume of 0.2 mL D_5W or with D_5W alone. The hematology and blood chemistry profiles were examined as described for rats. Mice were electively killed on day 6 and several tissues were immediately collected for histopathologic examination as described for rats.

Pharmacokinetics Studies. In pharmacokinetics studies in BALB/c mice (8-10 weeks of age, female), 25A was injected as a single 80 mg/kg iv bolus dose. BALB/c mice (4-6 weeks of age at the time of purchase, female) were obtained from the Jackson Laboratory (Sacramento, CA). The research was conducted according to Institutional Animal Care and Use Committee (IACUC) Protocol #293-10 entitled "Evaluation of Tyrosine Kinase Inhibitors in BALB/c Mice", that was approved by the IACUC of CHLA on June 24, 2010 and in amended form on May 17, 2012. All animal care procedures conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington DC 1996, USA). Groups of mice were electively sacrificed at the indicated time points by CO_2 inhalation and blood was collected by cardiac puncture after euthanasia. Aliquots of plasma were used for extraction and HPLC analysis with fluorescence detection, as described[1]. Pharmacokinetic modeling and pharmacokinetic parameter estimations were carried out by non-linear fitting of plasma concentrations-time profiles (JMP 10 Software (SAS, Cary, NC)[1].

SCID Mouse Xenograft Model of Human B-Precursor ALL. We used an NOD/SCID mouse model of human B-precursor ALL. NOD/SCID mice (NOD.CB17-*Prkdc*^{scid}/J; 4-6 weeks of age at the time of purchase, female) were obtained from the Jackson Laboratory (Sacramento, CA). The research was conducted according to Institutional Animal Care and Use Committee (IACUC) Protocol #280-09 entitled "Evaluation of Tyrosine Kinase Inhibitors Against Childhood Leukemia in Immunodeficient Mice, that was approved by the IACUC of CHLA on 11-24-2009

and its 3-year rewrite application 280-12 that was approved on 7-10-2012. All animal care procedures conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington DC 1996, USA). NOD/SCID mice (6-8 week old, female, same age in all cohorts in each independent experiment) were inoculated with primary leukemic cells from patients with B-precursor ALL by injecting 0.5-1x10^6 leukemia cells in 0.2 mL PBS i.v. via tail vein injection with a 27-gauge needle. Mice were monitored daily and electively euthanized at the indicated time points by CO_2 asphyxia. At the time of their death or elective sacrifice, mice were necropsied to confirm leukemia-associated marked splenomegaly. Spleens of mice were removed, sized, and cell suspensions were prepared for determination of mononuclear cell counts and immunophenotype. Brain, liver, kidney, lymph nodes, and bone marrow were subjected to standard histopathologic examinations to assess their leukemic involvement. In some experiments, leukemia cells isolated from spleens of xenografted mice were treated with 25A (Lipid concentration: 162 µg/ml; C61 concentration: 30 µg/ml), 25B (Lipid concentration: 162 µg/ml; C61 concentration: 0 µg/ml; N=9), or left untreated for 48 hrs at 37°C and then reinjected into NOD/SCID mice in order to evaluate the *in vitro* potency of 25A against their *in vivo* clonogenic cell fractions capable of engrafting and causing overt leukemia in NOD/SCID mice. In other experiments, leukemic cells isolated from spleens of xenografted mice ("xenograft cells") were injected into NOD/SCID mice and mice were then treated with iv injections of 25A vs. 25B or D5W starting on day 1 in order to evaluate the *in vivo* anti-leukemic potency of 25A against *in vivo* clonogenic xenograft cells. Mice injected with xenograft cells were monitored in the same fashion as described above for mice injected with primary fresh leukemic cells taken directly from ALL patients. For the analysis of the *in vitro* potency of 25A against *in vivo* clonogenic cells in xenograft specimens, two-tailed T-tests with correction for unequal variance (Microsoft, Excel) were performed comparing the mean spleen size and cellularity for the various treatments. In addition, 2-Tailed Fisher's Exact test was used to compare the extent of leukemic multi-organ involvement in mice challenged with control

xenograft cells vs. xenograft cells treated with 25A. For the analysis of the SCID mouse xenograft data on the *in vivo* potency of 25A, event-free survival (EFS) times were measured from the day of inoculation of xenograft cells to the day of death or killing for test mice treated with C61-LNP 25A (N = 26) vs. control mice treated with drug-free control LNP 25B (N = 13), D_5W (N = 12) and untreated control mice (N = 9). The probability of survival was determined and the event-free interval curves were generated using the Kaplan-Meier product limit method.[5] Log-rank tests were performed to compare differences in median survival estimates between all groups and pairwise comparison of pooled controls (N = 34) vs. 25A-treated mice (N=26).

References for supplemental Methods

1. Uckun FM, Ek RO, Jan ST, Chen CL, Qazi S. Targeting SYK Kinase-Dependent Anti-Apoptotic Resistance Pathway in B-lineage Acute Lymphoblastic Leukemia (ALL) Cells with a Potent SYK Inhibitory Pentapeptide Mimic. *British Journal of Haematology* 2010; 149(4):508-17.
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3. Uckun FM, Qazi S, Ma H, Tuel-Ahlgren L, Ozer Z. (2010a) STAT3 is a substrate of SYK tyrosine kinase in B-lineage leukemia/lymphoma cells exposed to oxidative stress. *Proc. Natl. Acad. Sci.* USA 107(7):2902-7.
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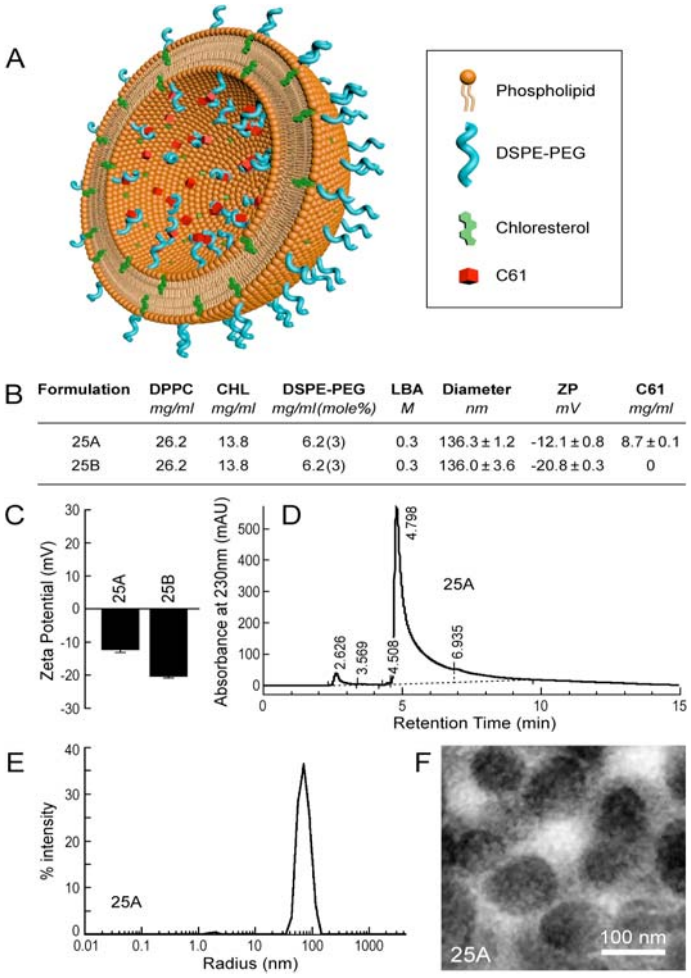
5. Uckun FM, Qazi S, Dibirdik I, Myers DE. (2012b) Rational Design of an Immunoconjugate for Selective Knock-Down of Leukemia-Specific E2A-PBX1 Fusion Gene Expression in Human Pre-B Leukemia. *Integr Biol (Camb).* 2013; 5(1):122-32.

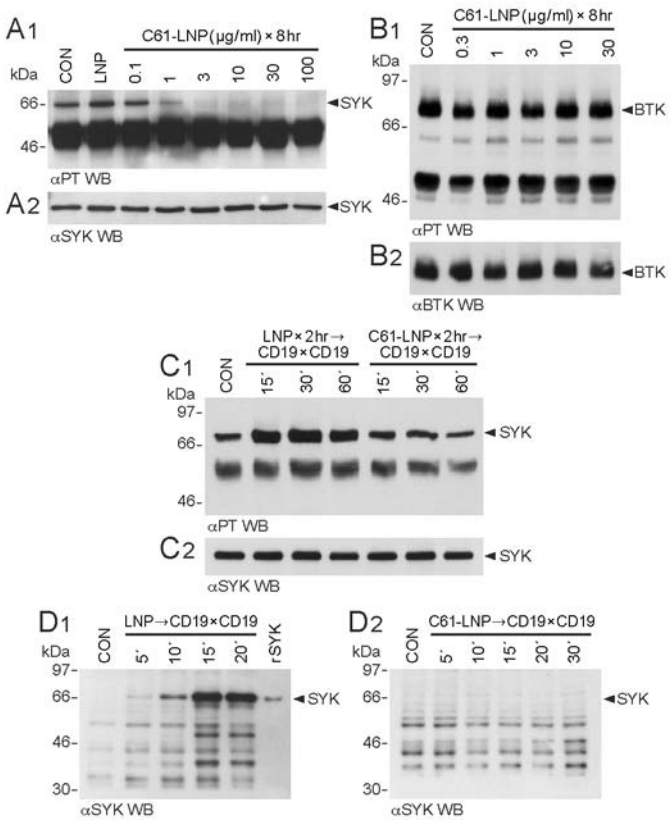
Supplemental Data - Figures and Tables



Formulation	DPPC *mg/ml*	CHL *mg/ml*	DSPE-PEG *mg/ml(mole%)*	LBA *M*	Diameter *nm*	ZP *mV*	C61 *mg/ml*
25A	26.2	13.8	6.2(3)	0.3	136.3 ± 1.2	-12.1 ± 0.8	8.7 ± 0.1
25B	26.2	13.8	6.2(3)	0.3	136.0 ± 3.6	-20.8 ± 0.3	0

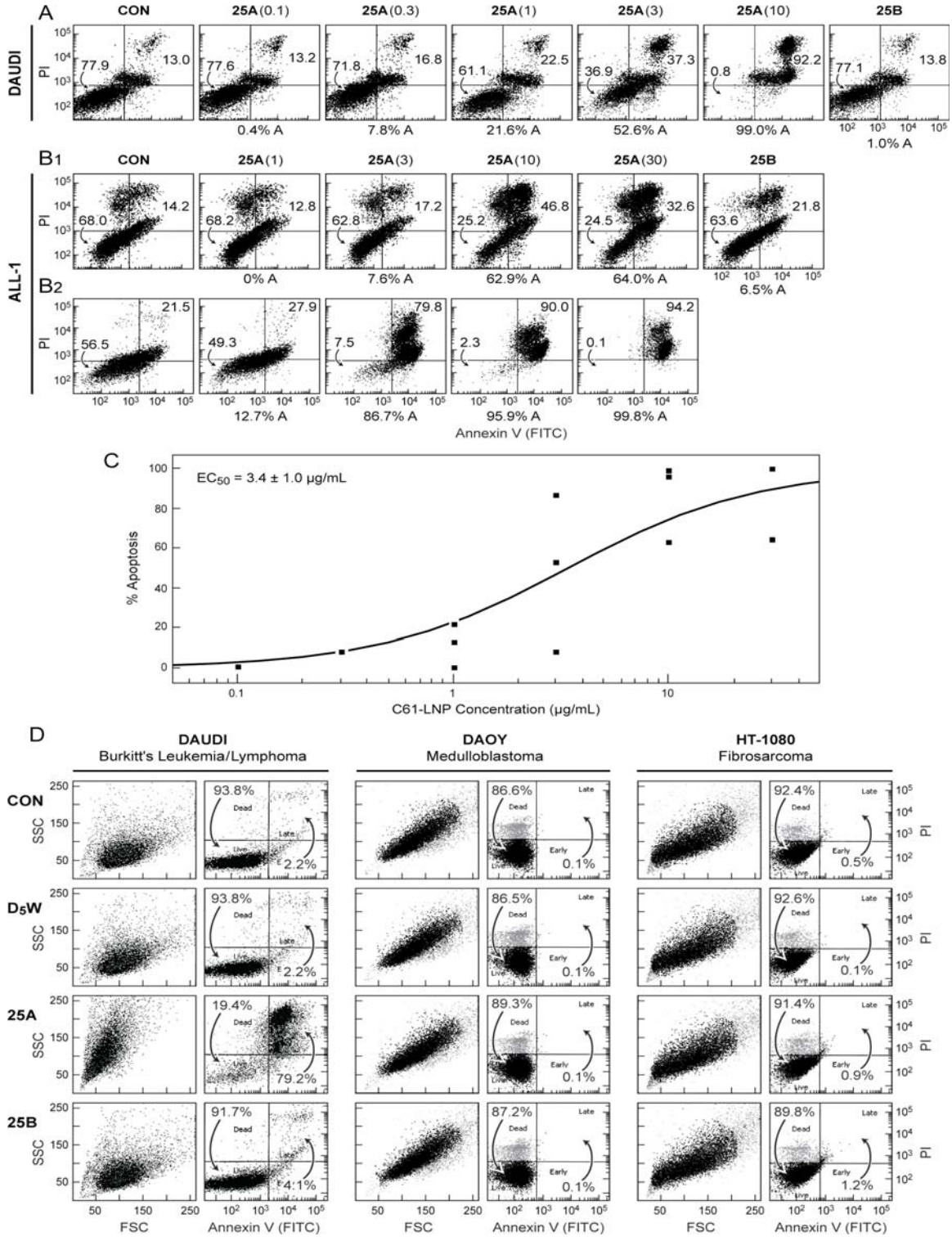
Supplemental Figure 1. Characterization of the liposomal nanoparticle (LNP) formulation of C61. [A] Cartoon illustration of the components of 25A. 25A is a PEGylated LPN formulation of the SYK P-site inhibitor C61. It was prepared by using the thin film evaporation method with the use of dipalmitoyl phosphatidylcholine (DPPC), cholesterol (CHOL), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000) and the entrapment of C61 within the interior space of LNP was achieved using a pH gradient procedure that employs lactobionic acid (LBA) [B]. Physicochemical features of C61-NP formulation 25A and its drug-free control. The particle size data represents the mean±SE value

from 15 independent measurements performed on 11 independently prepared batches. The C61 concentration represents the mean±SE value from 11 independent measurements performed on 9 independently prepared batches. The Zeta potential was measured only once and represents the mean from 3 replicate samples. [C] Zeta potential of 25A and 25B. [D] Detection of C61 in 25A using HPLC. [E] Particle size (radius) measurement of 25A LNP using dynamic light scattering (DLS). [F] Transmission electron microscopy (TEM) images of C61-loaded nanoscale 25A liposomes.



Supplemental Figure 2. SYK-inhibitory activity of C61 LNP. [A] ALL-1 cells were treated with increasing concentrations of C61-LNP for 8 hr at 37°C. SYK immune complexes were subjected to anti-phosphotyrosine (αPT) (A.1) and anti-SYK Western blot analysis (A.2). C61-LNP inhibited the activity of SYK in a concentration-dependent fashion, as evidenced by decreased autophosphorylation of the immunoprecipitated SYK protein at ≥1 µg/mL concentrations, but it did not affect the SYK protein level. [B] ALL-1 cells were treated with increasing concentrations of C61-LNP for 8 hr at 37°C. BTK immune complexes were subjected to anti-phosphotyrosine (αPT) (B.1) and anti-BTK Western blot analysis (B.2). C61-LNP did not inhibit the activity of BTK, as evidenced by unchanged autophosphorylation level of the immunoprecipitated BTK protein even at 30 µg/mL. [C] ALL-1 cells were treated for 2 hr at 37°C with C61-LNP or drug-free LNP (control sample) and then stimulated with an anti-CD19 monoclonal antibody homoconjugate (CD19xCD19, 1 µg/ml) to activate native SYK[4]. SYK

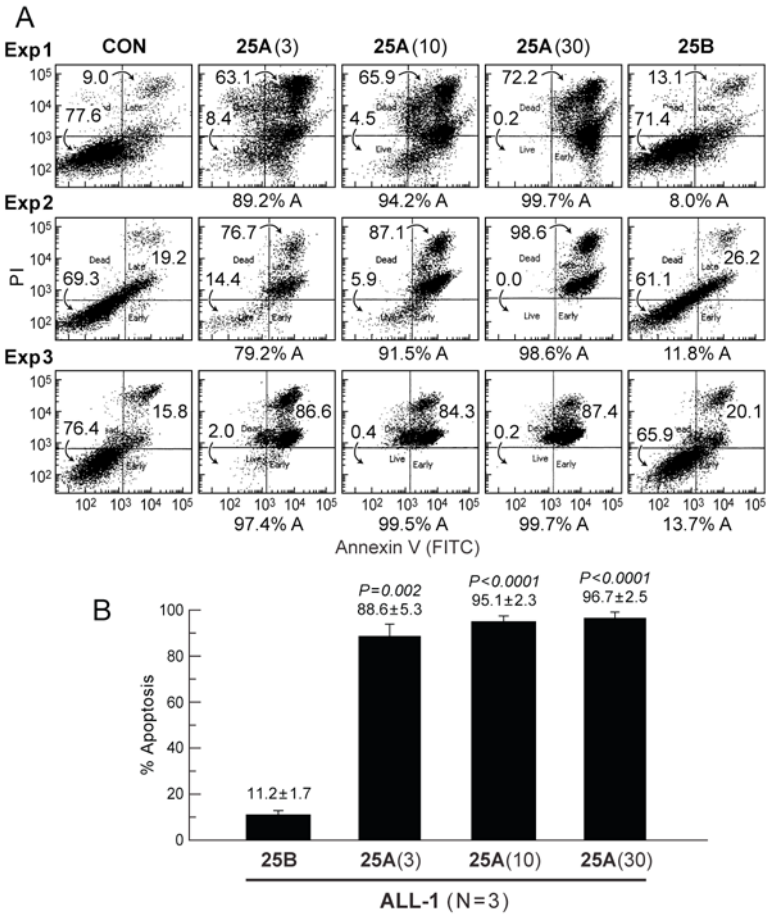
immune complexes obtained after the indicated exposure times to CD19xCD19 were subjected to αPT and αSYK Western blot analysis. In control samples treated with LNP, engagement of the CD19 receptor resulted in SYK activation, as measured by increased tyrosine phosphorylation of the immunoprecipitated SYK protein. Pretreatment with C61-LNP prevented CD19xCD19-induced activation of SYK. [D] As in C, ALL-1 cells were treated for 2 hr at 37°C with C61-LNP or drug-free LNP (control sample) and then stimulated with CD19xCD19 (1 µg/ml) to activate native SYK[4]. αPT immune complexes obtained after indicated times of exposure to CD19xCD19 were subjected to αSYK Western blot analysis. In control samples (D.1), engagement of the CD19 receptor resulted in SYK activation, as measured by increased amount of SYK protein among tyrosine phosphorylated proteins immunoprecipitated with αPT with peak activation occurring at 15-20 min. As a control sample, recombinant SYK[4] was included (Lane 6) for validation of the αSYK Western blot results. D.2: Pretreatment with C61-LNP prevented CD19xCD19-induced activation of SYK.



Supplemental Figure 3. C-61 LNP formulation 25A induces apoptosis in ALL cell lines in a concentration-dependent fashion. [A&B] DAUDI (Burkitt's leukemia/B-ALL) (A) and ALL-1 (Pre-Pre-B ALL) (B1 and B2) cells were treated for 48 h at 37°C with increasing concentrations

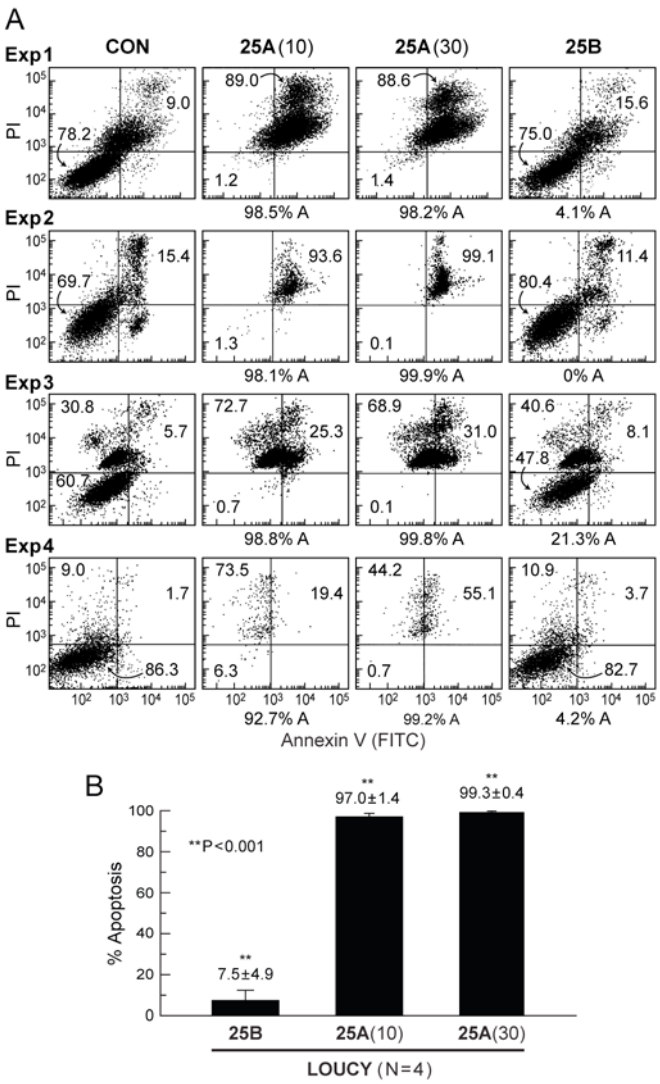
(in µg/ml based on C61 content; highest concentration 30 µg/ml based on C61 content = 162 µg/ml based on lipid content) of C61-LNP 25A or drug-free LNP formulation 25B (Concentration based on lipid content: 162 µg/ml) in D_5W. Controls included untreated cells (CON). Cells were analyzed for apoptosis using the standard quantitative flow cytometric apoptosis assay using the Annexin V-FITC Apoptosis Detection Kit from Sigma. The labeled cells were analyzed on a LSR II flow cytometer. C61-LNP 25A (but not drug-free control LNP 25B) caused apoptosis in the vast majority of treated ALL cells. The anti-leukemic potency of 25A is evidenced by the significantly lower percentages of Annexin V-FITC$^-$PI$^-$ live cells located in the left lower quadrant of the corresponding two-color FACS histogram as well as substantially higher percentage of Annexin V-FITC$^+$PI$^+$ advanced stage apoptotic cells located in the right upper quadrant. The percentages for Annexin V$^-$FITC-PI$^-$ viable cells and Annexin V-FITC$^+$PI$^+$ advanced apoptotic cells for each sample are indicated in the respective quadrants of the histograms. The percent apoptosis (%A) was calculated using the formula: 100- 100x (Viable cells[Test sample]/Viable cells[Control sample]) and the results are shown under each histogram. [C] Concentration-response curve of 25A for the combined data sets on DAUDI and ALL-1 cell lines depicted in A&B. The Median Effects equation described the non-linear relationship of percentage Apoptosis versus C-61 nano-particle concentration for ALL-1 (EC_{50} = 3.9 ± 1.7 µg/ml), DAUDI (EC_{50} = 2.5 ± 0.3 µg/ml) and combined data from the 2 cell lines (EC_{50} = 3.4 ± 1.0 µg/ml). Concentration required for 50% increase in Apoptosis is reported as the EC_{50} value and standard error calculated from root mean square of the model. [D] DAUDI, DAOY (Medulloblastoma) and HT-1080 (Fibrosarcoma) cells were treated for 48 h at 37°C with C61-LNP 25A (Concentration: 30 µg/ml based on C61 content and 162 µg/ml based on lipid content) or drug-free LNP formulation 25B (Concentration based on lipid content: 162 µg/ml) in D5W. Controls included cells treated with D_5W as well as untreated cells (CON). Cells were analyzed for apoptosis using the standard quantitative flow cytometric apoptosis assay using

the Annexin V-FITC Apoptosis Detection Kit from Sigma. The labeled cells were analyzed on a LSR II flow cytometer. C61-LNP 25A (but not drug-free control LNP 25B) caused apoptosis in the vast majority of treated ALL cells. The anti-leukemic potency of 25A against DAUDI cells is evidenced by the significantly lower percentages of Annexin V-FITC$^-$PI$^-$ live cells located in the left lower quadrant of the corresponding two-color FACS histogram as well as substantially higher percentage of Annexin V-FITC$^+$PI$^+$ apoptotic cells located in the right upper quadrant with marked shrinkage and altered SSC in the corresponding FSC/SSC light scatter plot. 25A did not cause apoptosis in control cell lines DAOY or HT-1080.



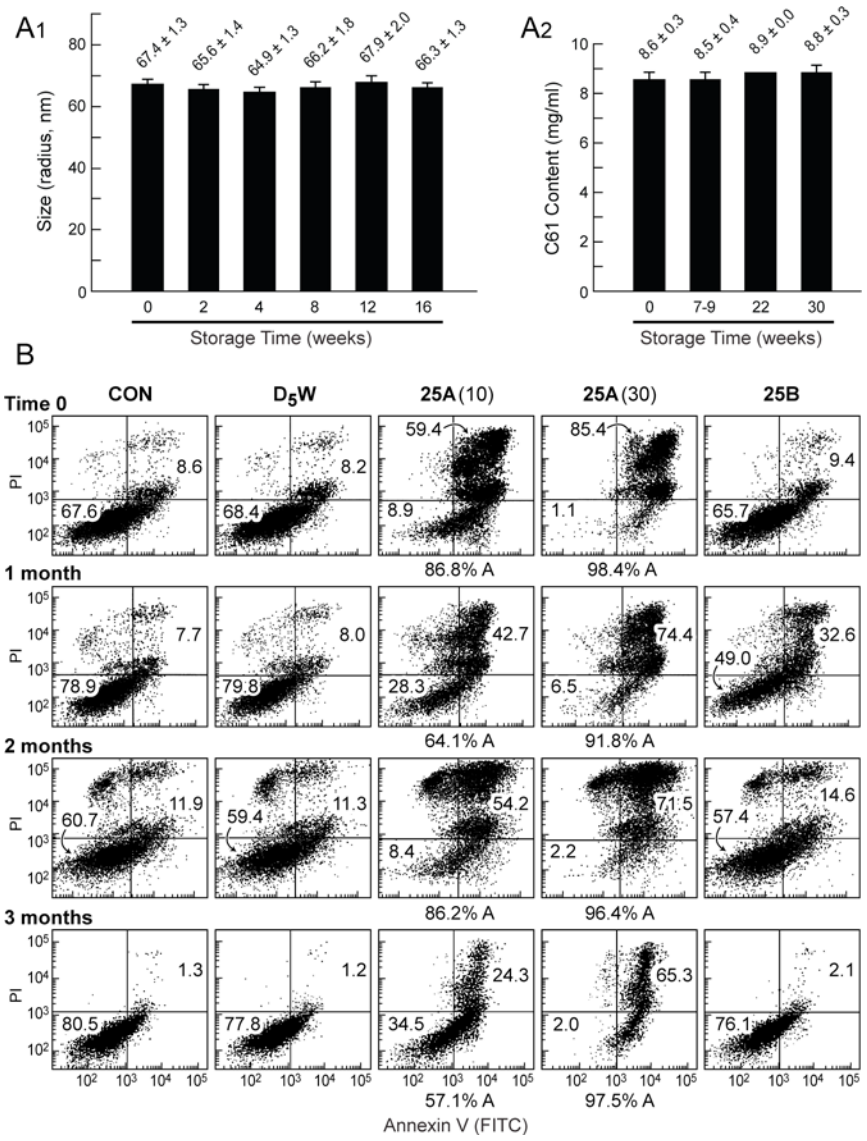
Supplemental Figure 4. C-61 LNP formulation 25A consistently induces apoptosis in BCR-ABL$^+$ B-precursor ALL cell line ALL-1. The ability of 25A to consistently cause apoptosis of the SYK$^+$ B-precursor ALL cell line ALL-1 was confirmed in 3 additional independent validation experiments (A, Exp1-Exp3). In each of the 3 experiments, ALL-1 cells were treated for 48 h at 37°C with C61-LNP 25A (Concentration range: 3-30 µg/ml based on C61 content and 16.2-162 µg/ml based on lipid content) or drug-free LNP formulation 25B (Concentration based on lipid content: 162 µg/ml) in D_5W. Cells were analyzed for apoptosis using the standard quantitative flow cytometric apoptosis assay using the Annexin V-FITC Apoptosis Detection Kit from Sigma. The labeled cells were analyzed on a LSR II flow cytometer. In each of the 3 experiments, C61-LNP 25A (but not drug-free control LNP 25B)

caused apoptosis in the vast majority of treated ALL cells. The anti-leukemic potency of 25A is evidenced by the significantly lower percentages of Annexin V-FITC$^-$PI$^-$ live cells located in the left lower quadrant of the corresponding two-color FACS histogram as well as substantially higher percentage of Annexin V-FITC$^+$PI$^+$ advanced stage apoptotic cells located in the right upper quadrant The percentages for Annexin V$^-$FITC-PI$^-$ viable cells and Annexin V-FITC$^+$PI$^+$ advanced apoptotic cells for each sample are indicated in the respective quadrants of the histograms. The percent apoptosis (%A) was calculated using the formula: 100- 100x (Viable cells[Test sample]/Viable cells[Control sample]) and the results are shown under each histogram. [B] Bar graphs depicting the mean ± SE values for the percentage of apoptotic ALL-1 cells after treatment with the drug-free LNP formulation 25B or increasing concentrations of 25A for the combined data set obtained in the 3 experiments shown in A.



Supplemental Figure 5. C-61 LNP formulation 25A consistently induces apoptosis in T-lineage ALL cell line LOUCY. The ability of 25A to consistently cause apoptosis of the SYK^+ T-lineage ALL cell line LOUCY was confirmed in 4 independent experiments (A, Exp1-Exp4). In each of the 4 experiments, LOUCY cells were treated for 48 h at 37°C with C61-LNP 25A (Concentration range: 10-30 µg/ml based on C61 content and 54-162 µg/ml based on lipid content) or drug-free LNP formulation 25B (Concentration based on lipid content: 162 µg/ml) in D_5W. Cells were analyzed for apoptosis using the standard quantitative flow cytometric apoptosis assay using the Annexin V-FITC Apoptosis Detection Kit from Sigma. The labeled cells were analyzed on a LSR II flow cytometer. In each of the 4 experiments, C61-LNP 25A

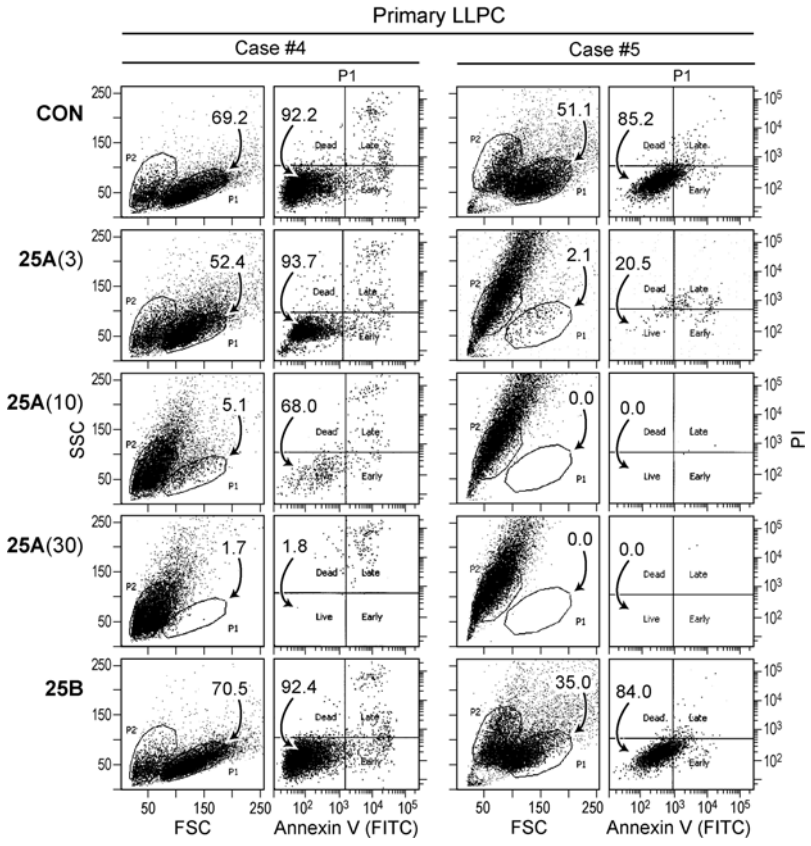
(but not drug-free control LNP 25B) caused apoptosis in the vast majority of treated LOUCY cells. The anti-leukemic potency of 25A is evidenced by the significantly lower percentages of Annexin V-FITC$^-$PI$^-$ live cells located in the left lower quadrant of the corresponding two-color FACS histogram as well as substantially higher percentage of Annexin V-FITC$^+$PI$^+$ advanced stage apoptotic cells located in the right upper quadrant The percentages for Annexin V$^-$ FITC-PI$^-$ viable cells and Annexin V-FITC$^+$PI$^+$ advanced apoptotic cells for each sample are indicated in the respective quadrants of the histograms. The percent apoptosis (%A) was calculated using the formula: 100- 100x(Viable cells[Test sample]/Viable cells[Control sample]) and the results are shown under each histogram. [B] Bar graphs depicting the mean ± SE values for the percentage of apoptotic LOUCY cells after treatment with the drug-free LNP formulation 25B or increasing concentrations of 25A for the combined data set obtained in the 4 experiments shown in A.



Supplemental Figure 6. Preliminary Stability Testing of the C-61 LNP formulation 25A.

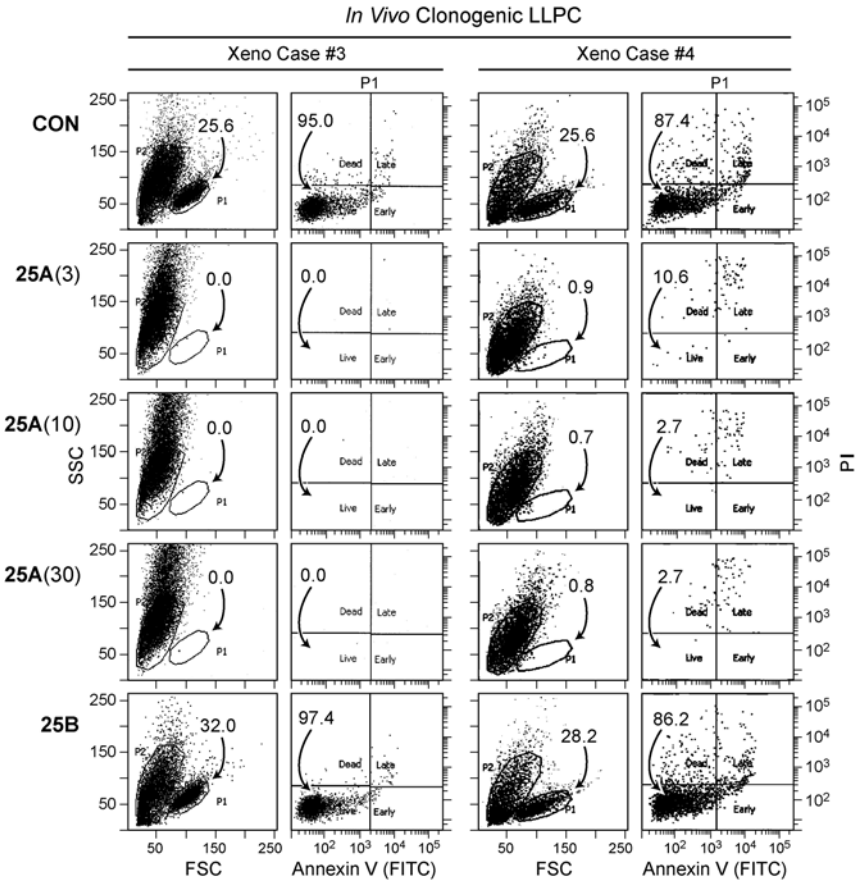
[A.1] In a preliminary stability study using extended storage at 4°C, the DLS-measured average radius values of the C61-LNP did not show significant changes up to 16 weeks to suggest emergence of aggregates or alteration of the size distribution profile. Bar graphs depicting the mean ± SE values for the measured radius of the C61-LNP after various times of storage. [A.2] The HPLC-measured C-61 content of 25A LNP showed no detectable decrease after extended storage to suggest leakage or burst release from the LNP. Bar graphs depicting the mean ± SE values for the measured C61 content of the C61-LNP after various times of storage.

[B] The ability of 25 LNP to cause apoptosis of the B-precursor ALL cell line ALL-1 was sustained over a 3-month storage time. The anti-leukemic potency of 25A is evidenced by the significantly lower percentages of Annexin V-FITC^{-}PI^{-} live cells located in the left lower quadrant of the corresponding two-color FACS histogram as well as substantially higher percentage of Annexin V-FITC^{+}PI^{+} advanced stage apoptotic cells located in the right upper quadrant The percentages for Annexin V-FITC^{-}PI^{-} viable cells and Annexin V-FITC^{+}PI^{+} advanced apoptotic cells for each sample are indicated in the respective quadrants of the histograms. The percent apoptosis (%A) was calculated using the formula: 100- 100 x(Viable cells[Test sample]/Viable cells[Control sample]) and the results are shown under each histogram.



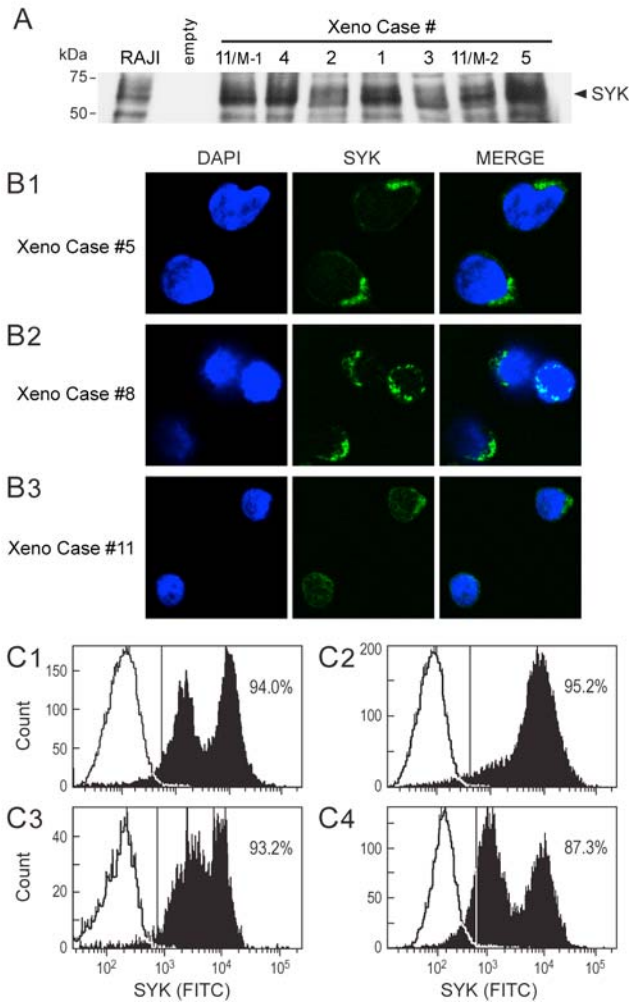
Supplemental Figure 7. C-61 LNP formulation 25A induces apoptosis in primary leukemic lymphocyte precursor cells (LLPC) taken directly from pediatric B-precursor ALL patients. Representative FACS histograms of primary leukemic cells from two B-precursor ALL patients. Cells were treated for 48 h at 37°C with C61-LNP 25A (Concentration range: 3-30 µg/ml based on C61 content and 16.2-162 µg/ml based on lipid content) or drug-free LNP formulation 25B (Concentration based on lipid content: 162 µg/ml) in D_5W. Controls included untreated cells (CON). Cells were analyzed for apoptosis using the standard quantitative flow cytometric apoptosis assay using the Annexin V-FITC Apoptosis Detection Kit from Sigma. The labeled cells were analyzed on a LSR II flow cytometer. C61-LNP 25A (but not drug-free control LNP 25B) caused apoptosis in the vast majority of treated ALL cells. The anti-leukemic potency of 25A is evidenced by the significantly lower percentages of Annexin V-FITC$^-$PI$^-$ live cells located in the left lower quadrant of the corresponding two-color FACS histogram as well as substantially higher percentage of Annexin V-FITC$^+$PI$^+$ apoptotic

cells located in the right upper quadrant with marked shrinkage and altered SSC in the corresponding FSC/SSC light scatter plot. Consequently, 25A treatment resulted in depletion of viable leukemic cells in the lymphoid-blast window P1. Similar results were obtained in a total of 18 B-precursor ALL cases were analyzed and the cumulative data is shown in Figure 1 of the main manuscript.



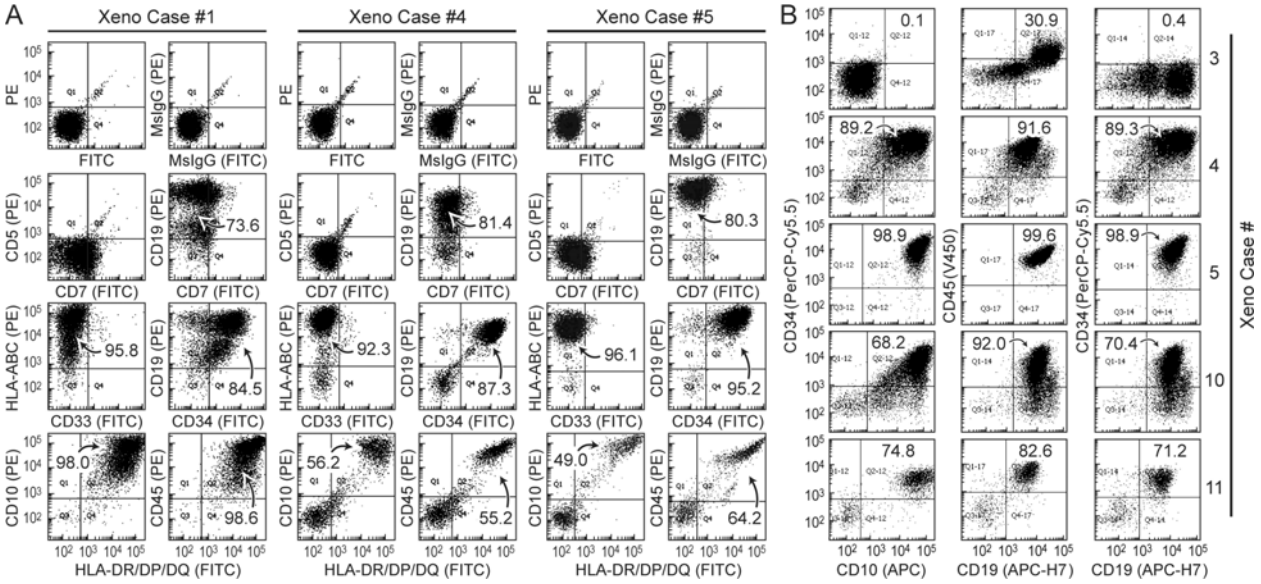
Supplemental Figure 8. C-61 LNP formulation 25A induces apoptosis in B-precursor ALL xenograft cells. Representative FACS histograms of B-precursor ALL xenograft cells from 2 separate patients. These xenograft cells represent the *in vivo* clonogenic leukemic lymphocyte precursor cells (LLPC) obtained from spleens of xenografted NOD/SCID mice challenged with primary LLPC taken directly from pediatric B-precursor ALL patients. Cells were treated for 48 h at 37°C with C61-LNP 25A (Concentration range: 3-30 µg/ml based on C61 content and 16.2-162 µg/ml based on lipid content) or drug-free LNP formulation 25B (Concentration based on lipid content: 162 µg/ml) in D_5W. Controls included untreated cells (CON). Cells were analyzed for apoptosis using the standard quantitative flow cytometric apoptosis assay using the Annexin V-FITC Apoptosis Detection Kit from Sigma. The labeled cells were analyzed on a LSR II flow cytometer. C61-LNP 25A (but not drug-free control LNP 25B) caused apoptosis in the vast majority of treated xenograft cells. The anti-leukemic potency of 25A is evidenced by the

significantly lower percentages of Annexin V-FITC^{-}PI^{-} live cells located in the left lower quadrant of the corresponding two-color FACS histogram as well as substantially higher percentage of Annexin V-FITC^{+}PI^{+} apoptotic cells located in the right upper quadrant with marked shrinkage and altered SSC in the corresponding FSC/SSC light scatter plot. Consequently, 25A treatment resulted in depletion of viable leukemic cells in the lymphoid-blast window P1. Similar results were obtained in a total of 11 B-precursor ALL xenograft cases and the cumulative data is shown in Figure 1 of the main manuscript.



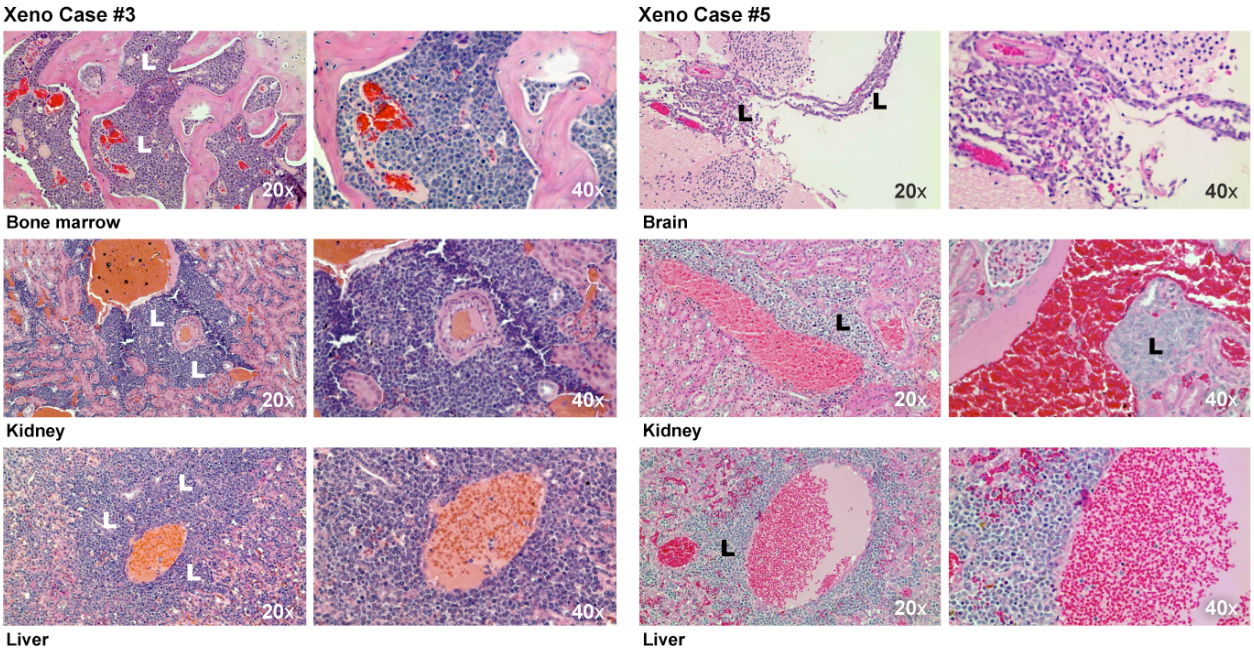
Supplemental Figure 9. Human B-precursor ALL xenograft cells are characterized by high-level SYK expression. [A] Western blot analysis was performed to confirm SYK expression in whole cell lysates from 7 randomly selected B-precursor ALL xenograft samples (2 separate clones, 11/M-1 and 11/M-2, were included in xenograft case#11). [B] B-precursor ALL xenograft cells from 3 randomly picked xenograft cases were fixed and stained with mouse anti-SYK MoAb (primary Ab)/ Alexa Fluor 488 goat anti-mouse IgG (secondary Ab) (green). Nuclei were stained with blue fluorescent dye 4',6-diamidino-2-phenylindole (DAPI). MERGE panels depict the merge two-color confocal image showing predominantly cytoplasmic/peri-nuclear localization of green-fluorescent SYK around DAPI-stained blue nucleus. System Magnification: 630 (Magnification[objective]: 63 x Magnification[Eyepiece]: 10). [C] SYK

expression levels of xenograft ALL cells was examined by intracellular flow cytometry using BD Phosphlow Fix Buffer 1 for permeabilization of the cell membrane and SYK-FITC antibody (Cat. #: 5524726, BD Biosciences). The labeled cells were analyzed on a LSR II flow cytometer (Becton Dickinson, Lakes, NJ). Similar results were obtained in 11 of 11 independent experiments. Depicted are the FACS histograms for the first 4 xenograft cases (Xenograft case#'s 1-4). See supplemental Table 1 for the data on all 11 xenograft cases.

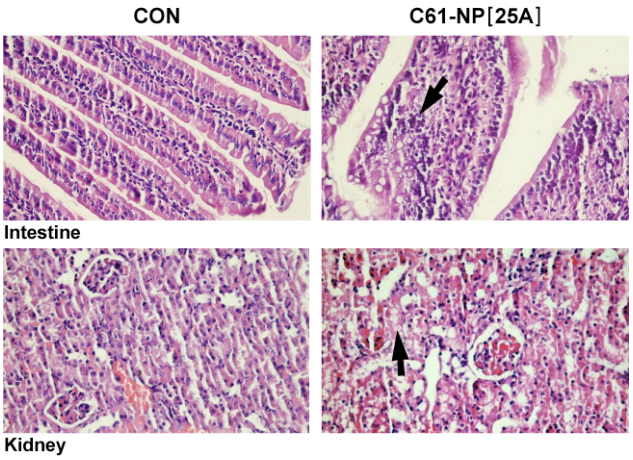


Supplemental Figure 10. Surface antigen profiles of B-precursor ALL xenograft cells. [A] FACS-correlated two-parameter displays of B-precursor ALL xenograft cells isolated from spleens of NOD/SCID mice that developed overt leukemia after inoculation with primary ALL cells from 3 B-precursor ALL patients. Cells were stained by direct immunofluorescence for human lymphoid differentiation antigens CD5, CD7, CD10, CD19, CD33, CD34, CD45, HLA-DR/DP/DQ, and HLA-A,B,C. The labeled cells were analyzed on a LSR II flow cytometer. Xenograft cells co-expressed CD45, HLA-DR/DP/DQ and HLA-A,B,C antigens and had an immature B-cell precursor immunophenotype characterized by absence of T-lineage antigens CD5 and CD7, absence of the myeloid antigen CD33, and co-expression of B-lineage antigens CD10/CALLA, CD19, and CD34. [B] FACS-correlated two-parameter displays of B-precursor ALL xenograft cells isolated from spleens of NOD/SCID mice that developed overt leukemia after inoculation with primary ALL cells from 5 B-precursor ALL patients. Cells were stained by direct immunofluorescence for human lymphoid differentiation antigens CD10, CD19, CD34, and CD45 using a different set of fluorescently labeled monoclonal antibodies from BD Biosciences (San Jose, CA) than in B (viz: CD10 (APC)/BD catalog #: 340923, CD19 (APC-H7) clone:SJ25C1/BD catalog #: 560177, CD34 (Per CP-Cy5.5)/BD catalog #: 347203, CD45

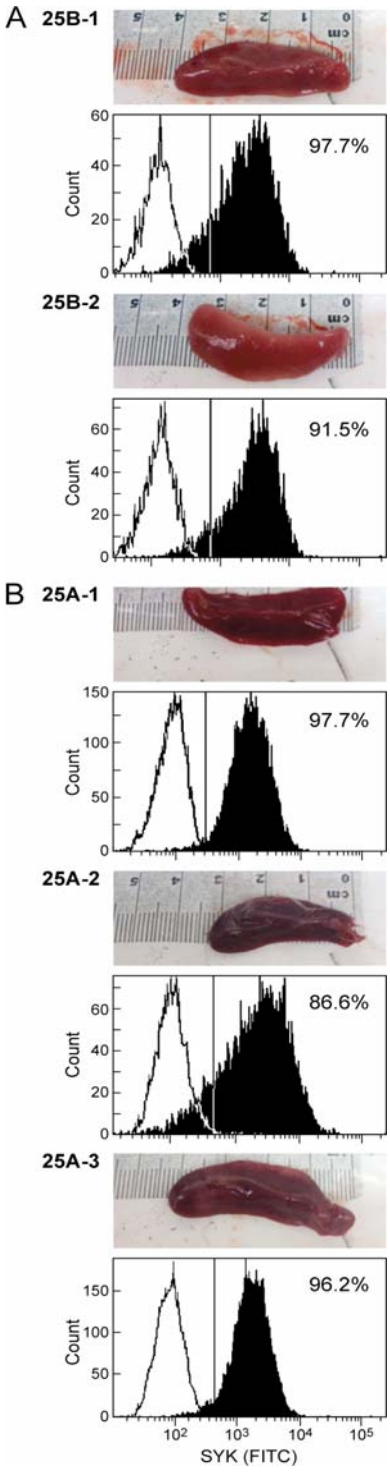
(V450) clone:H130/BD catalog #: 560367). In 4 of the 5 cases, xenograft cells were $CD10^{+}CD19^{+}CD34^{+}CD45^{+}$ and in one case (Xeno Case #3) they were $CD10^{-}CD19^{+}CD34^{-}CD45^{+}$. These 5 B-precursor ALL xenograft cases were used for evaluation of the *in vitro* anti-leukemic activity of 25A against *in vivo* clonogenic leukemic B-cell precursors capable of initiating disseminated leukemia after reinjection into NOD/SCID mice. Xeno Case #'s 3, 5, and 10 were used for evaluation of the *in vivo* anti-leukemic activity of 25A in NOD/SCID mice.



Supplemental Figure 11. Histopathological evidence of disseminated leukemia in NOD/SCID Mice challenged with unpassaged human B-precursor ALL xenograft cells. In 5 independent experiments, NOD/SCID mice challenged with untreated or 25B-treated unpassaged xenograft ALL cells rapidly developed disseminated leukemia. Depicted are the histopathological results for select organs in control mice from the 2 representative cases shown in Figure 2 of the main manuscript. The cumulative organ infiltration data is shown in Figure 2, Panel E. Bone marrow involvement was manifested as replacement of normal tissue elements by diffuse sheets of densely packed leukemic cells. Infiltrated kidneys showed cortical, interstitial and perivascular accumulations of leukemic cells. Livers showed leukemic infiltrates in the portal spaces and sinusoids. The leptimeninges of the brain contained rafts of leukemic cells. Images were taken with an EVOS XL Core Light Microscope (AMG Bothel, WA) using 20X and 40X objectives.



Supplemental Figure 12. Intestinal inflammatory response and tubular epitheilal degeneration in C61-LNP treated mice. C61-LNP 25A was overall very well tolerated by mice. Mice did not develop any clinical or laboratory evidence of moderate-severe acute toxicity at cumulative dose levels ranging from 5 mg/kg to 500 mg/kg. The cumulative data is shown in supplemental Table 2. At the highest dose level, histopathological examination revealed a few small clusters of inflammatory cells in the intestinal mucosa and a mild-moderate segmental hydropic degeneration/intracellular edema in renal tubular epithelium in 2 of 5 mice. Depicted are H&E-stained sections from paraffin blocks of normal intestine and kidney from a healthy control mouse and H&E-stained sections from paraffin blocks of intestine and kidney from a 25A treated mouse showing the aforementioned changes (indicated by arrows). Sections were examined by light microscopy with an Olympus BX-50 microscope. System magnification: 200X.



Supplemental Figure 13. SYK expression profile of B-precursor ALL xenograft cells that escaped 25A treatments and caused leukemia in NOD/SCID mice. [A] All of the 25B treated NOD/SCID mice challenged with B-precursor ALL xenograft cells rapidly developed leukemia

(MST: 50 days, see Figure 3 of the main manuscript). At the time of necropsy, all mice were found to have massive splenomegaly with very high cell counts. SYK expression levels of xenograft ALL cells was examined by intracellular flow cytometry using BD Phosphlow Fix Buffer 1 for permeabilization of the cell membrane and SYK-FITC antibody (Cat. #: 5524726, BD Biosciences). The labeled cells were analyzed on a LSR II flow cytometer (Becton Dickinson, Lakes, NJ). Depicted are the spleen images and FACS histograms from two representative mice challenged with xenograft cells from B-precursor ALL Xenograft Case#3 and treated with 25B: 25B-1 (Sacrificed moribund on day 24, Spleen size: 4.0 cm, Spleen cell count: $525x10^6$ cells) and 25B-2 (Sacrificed moribund on day 24, Spleen size: 3.8 cm, Spleen cell count: $534x10^6$ cells). [B] 25A improved the EFS outcome of NOD/SCID mice challenged with B-precursor All xenograft cells (MST: 226 days, see Figure 3 of the main manuscript). Mice developing leukemia despite 25A treatments also had massive splenomegaly with very high cell counts. Depicted are the spleen images and FACS histograms from three representative mice challenged with xenograft cells from B-precursor ALL Xenograft Case#3 and developed leukemia despite 25A treatments: 25A-1 (Sacrificed moribund on day 57, Spleen size: 4.0 cm, Spleen cell count: $114x10^6$ cells), 25A-2 (Sacrificed moribund on day 233, Spleen size: 3.3 cm, Spleen cell count: $537x10^6$ cells), and 25A-3 (Sacrificed moribund on day 240, Spleen size: 4.2 cm, Spleen cell count: $134x10^6$ cells). In [A] and [B], the spleen images were obtained using an iPhone 4S equipped with an 8-megapixel iSight camera.

Supplemental Table 1. Potency of C61-LNP Formulation 25A Against *In Vivo* Clonogenic Human B-precursor ALL Cells From Xenografted NOD/SCID Mice

Xenograft Case No.	*Patient Diagnosis*	*% SYK Expression*	*Maximum Apoptotic Death (%)*		
			25A	*25B*	*γ-rays*
1	B-lineage ALL	94.0	93.5	7.2	ND
2	B-lineage ALL	95.2	97.9	0.0	ND
3	B-lineage ALL	93.2	>99.99	0.0	15.1
4	B-lineage ALL	87.3	99.9	0.0	12.3
5	B-lineage ALL, Relapse	76.0	69.2	1.4	23.2
6	B-lineage ALL	83.2	99.1	8.2	ND
7	B-lineage ALL, Relapse	98.9	95.1	ND	ND
8	B-lineage ALL	99.2	99.1	ND	7.8
9	B-lineage ALL, Relapse	86.5	99.7	31.2	ND
10	B-lineage ALL, Relapse	97.1	98.1	0.0	ND
11	B-lineage ALL	94.9	98.7	2.5	49.8

In 11 independent experiments, human xenograft ALL cells isolated from spleen specimens of NOD/SCID mice that developed disseminated leukemia after iv inoculation with primary leukemia cells taken directly from 7 newly diagnosed and 4 relapsed pediatric B-lineage/B-precursor ALL patients were used to examine the expression levels of the molecular target SYK and to evaluate the potency of the C61-LNP formulation 25A. The cumulative data are shown in Figure 1. Depicted in this table are the maximum percentages for apoptotic death triggered by 25A vs. 25B in each of the 11 experiments. The percentage of apoptosis obtained with 2 Gy γ-rays is also shown for the 5 cases examined for radiation sensitivity.

Supplemental Table 2. Acute Toxicity of C61 LNP Formulation 25A in BALB/c Mice

		0	Daily 25A Treatment Dose in mg/kg (Cumulative Total Dose in mg/kg)				
Parameter	*Controls*	*25B*	*1 (5)*	*10 (50)*	*25 (125)*	*50 (250)*	*100 (500)*
Number of Mice	6	10	10	10	10	10	5
Body Weight (g), Pre	20.2 ± 0.5	20.8 ± 0.5	20.0 ± 0.6	20.1 ± 0.4	20.5 ± 0.6	20.4 ± 0.7	21.8 ± 0.7
Body Weight (g), Post	20.3 ± 0.4	20.5 ± 0.5	19.9 ± 0.4	20.0 ± 0.4	20.6 ± 0.5	20.6 ± 0.6	21.2 ± 0.6
Morbidity	0/6	0/10	0/10	0/10	0/10	0/10	0/5
Weight Loss (>10%)	0/6	0/10	0/10	0/10	0/10	0/10	0/5
Gross Lesions at Necropsy	0/6	0/10	0/10	0/10	0/10	0/10	0/5
Hematology							
Hgb (g/dl)	13.4 ± 0.9	15.1 ± 0.3	15.2 ± 0.2	15.2 ± 0.2	14.6 ± 0.3	14.2 ± 0.5	14.4 ± 0.6
RBC ($x10^6$/µL)	7.8 ± 0.7	8.4 ± 0.4	8.3 ± 0.4	8.4 ± 0.4	8.4 ± 0.6	8.2 ± 0.4	7.5 ± 0.2
WBC ($x10^3$/µL)	6.6 ± 0.4	6.9 ± 0.9	6.8 ± 0.7	7.1 ± 0.5	7.6 ± 0.7	7.3 ± 0.7	5.0 ± 0.5
ANC ($x10^3$/µL)	1.5 ± 0.2	1.8 ± 0.2	1.5 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	1.8 ± 0.2	1.4 ± 0.3
ALC ($x10^3$/µL)	5.2 ± 0.7	5.2 ± 0.6	5.5 ± 0.5	5.5 ± 0.6	5.1 ± 0.5	6.6 ± 1.0	4.1 ± 0.8
Plt ($x10^3$/µL)	697 ± 73	715 ± 66	709 ± 32	714 ± 51	707 ± 33	703 ± 23	667 ± 54
Liver Function							
ALT (IU/liter)	93 ± 6	115 ± 5	103 ± 6	102 ± 7	98 ± 5	99 ± 6	93 ± 4
AST (IU/liter)	160 ± 16	154 ± 15	157 ± 11	148 ± 8	144 ± 9	145 ± 7	312 ± 25
Total Bilirubin (mg/dl)	ND	ND	ND	ND	ND	ND	0.1 ± 0.0
Albumin (g/dl)	ND	ND	ND	ND	ND	ND	2.5 ± 0.1
Total Protein (g/dl)	ND	ND	ND	ND	ND	ND	6.2 ± 0.3
Renal Function							
BUN (mg/dl)	41 ± 2	42 ± 2	42 ± 1	42 ± 2	42 ± 2	42 ± 2	66 ± 12
Creatinine (mg/dl)	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.7 ± 0.1
Pancreas Function							
Amylase (units/liter)	ND	ND	ND	ND	ND	ND	303 ± 45
Histopathological Lesions							
Lung	0/6	0/10	0/10	0/10	0/10	0/10	0/4
Heart	0/6	0/10	0/10	0/10	0/10	0/10	0/4
Kidney	0/6	0/10	0/10	0/10	0/10	0/10	2/4
Brain	0/6	0/10	0/10	0/10	0/10	0/10	0/4
Liver	0/6	0/10	0/10	0/10	0/10	0/10	0/4
Pancreas	0/6	0/10	0/10	0/10	0/10	0/10	0/4
Intestine	0/6	0/10	0/10	0/10	0/10	0/10	1/4
Testes	0/3	0/5	0/5	0/5	0/5	0/5	0/2
Ovary	0/3	0/5	0/5	0/5	0/5	0/5	0/2
Muscle	0/6	0/10	0/10	0/10	0/10	0/10	0/4
Bone / Bone Marrow	0/6	0/10	0/10	0/10	0/10	0/10	0/4

25A was well tolerated by female and male BALB/c mice when administered intraperitoneally at daily dose levels ranging from 1 to 100 mg/kg and cumulative dose levels ranging from 5 mg/kg to 500 mg/kg (N=45). Mice were monitored and examined for clinical, laboratory, and histopathological evidence of 25A-induced toxicity using standard procedures.

Supplemental Table 3. Acute Toxicity of C61 LNP Formulation 25A in Wistar Albino Rats

		Day 15			*Day 30*		
Parameter	*Controls*	*D_5W*	*10 mg/kg*	*25 mg/kg*	*D_5W*	*10 mg/kg*	*25 mg/kg*
Number of Rats	10	4	5	2	5	4	3
Morbidity	—	0/4	0/5	0/2	0/5	0/4	0/3
Weight Loss (>10%)	—	0/4	0/5	0/2	0/5	0/4	0/3
Histopathologic Lesions at Necropsy	—	0/4	0/5	0/2	0/5	0/4	0/3
Hematology							
Hgb (g/dl)	15.9 ± 0.3	15.2 ± 0.2	15.2 ± 0.2	15.9 ± 0.2	15.7 ± 0.2	15.8 ± 0.3	15.5 ± 0.7
RBC ($x10^6/\mu L$)	8.7 ± 0.2	7.8 ± 0.1	8.2 ± 0.2	8.5 ± 0.2	8.3 ± 0.1	8.2 ± 0.1	8.3 ± 0.2
WBC ($x10^3/\mu L$)	9.6 ± 1.0	10.0 ± 1.7	8.4 ± 0.6	7.6 ± 2.0	14.0 ± 1.6	12.6 ± 0.9	8.1 ± 1.2
ANC ($x10^3/\mu L$)	2.3 ± 0.2	2.5 ± 0.2	2.0 ± 0.4	1.7 ± 0.7	2.9 ± 0.4	1.7 ± 0.5	1.0 ± 0.2
ALC ($x10^3/\mu L$)	6.2 ± 0.5	5.1 ± 0.4	6.4 ± 0.9	4.2 ± 0.1	8.7 ± 1.0	8.3 ± 1.6	6.1 ± 1.1
Plt ($x10^3/\mu L$)	542 ± 79	587 ± 19	628 ± 39	630 ± 51	332 ± 105	344 ± 60	406 ± 70
Liver Function							
ALT (IU/liter)	70 ± 5	71 ± 6	70 ± 4	80 ± 8	74 ± 1	70 ± 7	80 ± 5
AST (IU/liter)	223 ± 19	257 ± 29	258 ± 41	287 ± 49	234 ± 22	256 ± 20	187 ± 14
Total Bilirubin (mg/dl)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Albumin (g/dl)	3.6 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.2	3.7 ± 0.2	3.9 ± 0.1	3.4 ± 0.2
Total Protein (g/dl)	6.8 ± 0.1	6.9 ± 0.2	6.8 ± 0.1	6.4 ± 0.1	7.0 ± 0.2	7.0 ± 0.1	6.8 ± 0.4
Renal Function							
BUN (mg/dl)	43 ± 1	44 ± 2	43 ± 1	45 ± 6	39 ± 2	47 ± 7	41 ± 2
Creatinine (mg/dl)	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1
Pancreas Function							
Amylase (units/liter)	2337 ± 92	2350 ± 90	2549 ± 191	2304 ± 28	2331 ± 113	2421 ± 221	2415 ± 204
Cardiac Function							
Creatine Kinase (IU/liter)	8548 ± 789	9694 ± 1523	9674 ± 1882	12102 ± 2579	8695 ± 837	9258 ± 935	8978 ± 871

25A was well tolerated by male Wistar Albino rats (N=14) at 10 mg/kg and 25 mg/kg intravenous bolus dose levels with no clinical or laboratory evidence of acute or subacute toxicity. Rats were monitored and examined for clinical, laboratory, and histopathological evidence of 25A-induced toxicity.