

Supplementary materials

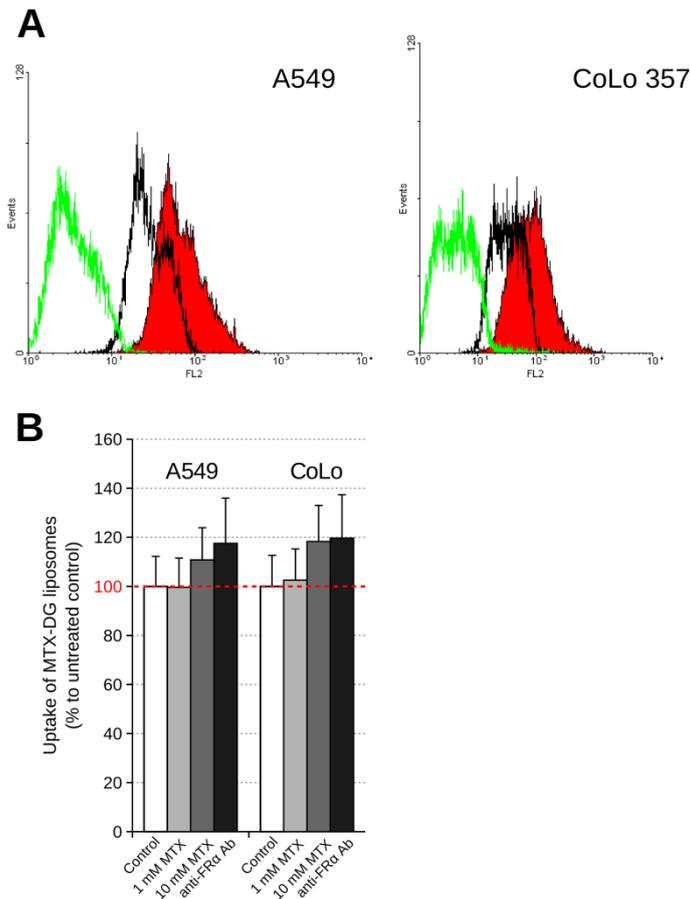


Figure S1. A. Flow cytometry histograms of folate receptor alpha (FR α) expression by A549 and CoLo357 cells. Green, control (untreated) cells; black, non-specific secondary antibody staining; red, specific immunostaining with anti-FR α antibody. Prior to immunostaining, cells were fixed with PFA and permeabilized with 0.1% Tritox X-100. **B.** Competitive inhibition of MTX-DG liposomes binding to A549 and CoLo357 cells with excess of free MTX (100- and 1000-fold excess over MTX-DG concentration, 1 mM and 10 mM, respectively) or anti-FR α antibody (10 μ g/mL). Cell monolayers on 24-well plates were incubated with free MTX or anti-FR α antibody for 1 h at 37°C. Then, with or without washing with PBS, MTX-DG liposomes were added (100 μ M total lipids, 10 μ M MTX-DG). After a 1-h incubation, cells were rinsed with PBS, detached with 0.02% EDTA solution, and analyzed by flow cytometry.

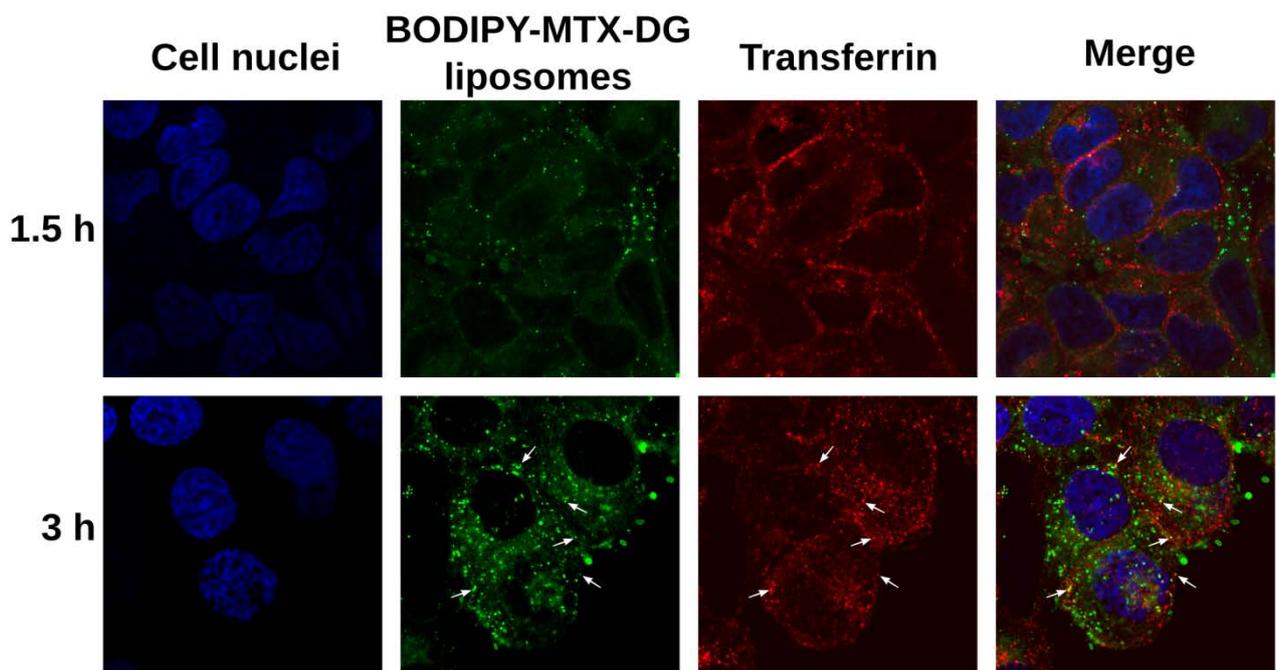


Figure S2. Confocal microscopy images of intracellular delivery of MTX-DG liposomes in A549 cells. The liposome membrane is labelled with BODIPY-MTX-DG (green); early endosomes are visualized with Transferrin (red). A549 cells were pre-stained with Hoechst for nuclei (blue), incubated with MTX-DG liposomes (100 μ M total lipid) for 1 h or 3 h at 37°C, co-incubated with Transferrin for 15 min and then fixed with 1% PFA for 15min at 37°C. White arrows indicate areas of co-localization of liposome with the trackers.

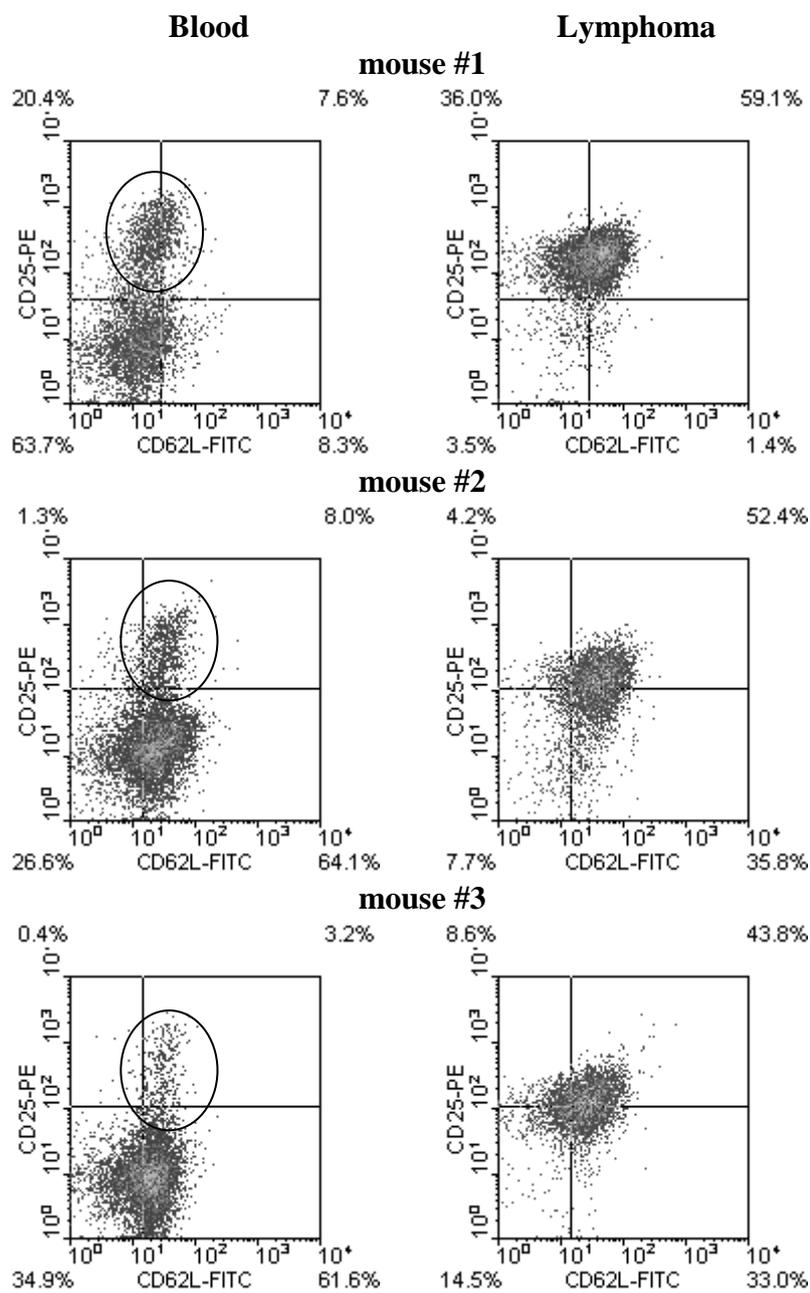


Figure S3. Flow cytometry expression patterns showing *in vivo* lymphoma ASF-LL spreading. Blood leukocytes and lymphoma cells were collected from transplanted lymphoma bearing mice, day 13 *post* transplantation.

The references in Figures S4 and S5 are given to demonstrate distinct similarities between ASF-TLL mouse model and human adult T-cell leukemia/lymphoma in terms of morphology of tumor cells and histopathological profile of leukemic manifestation along with an aggressive clinical course and immunological nature (CD4+CD25+ phenotype, Figure S3).

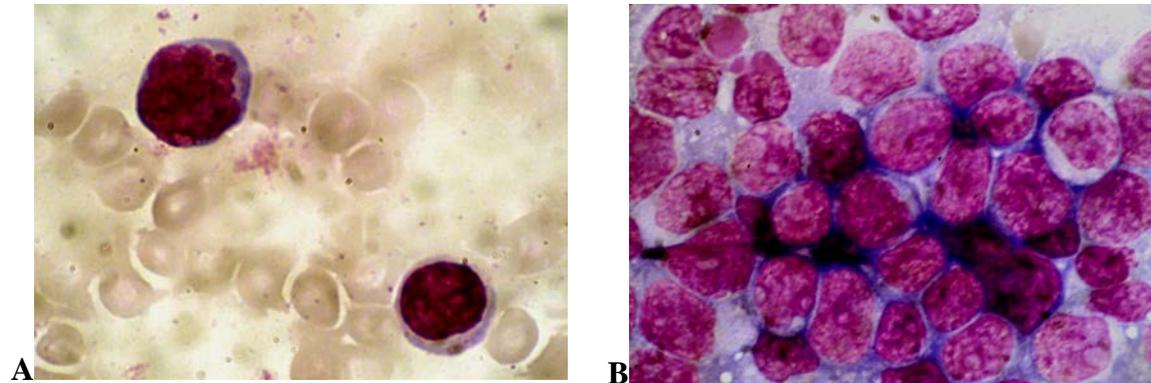
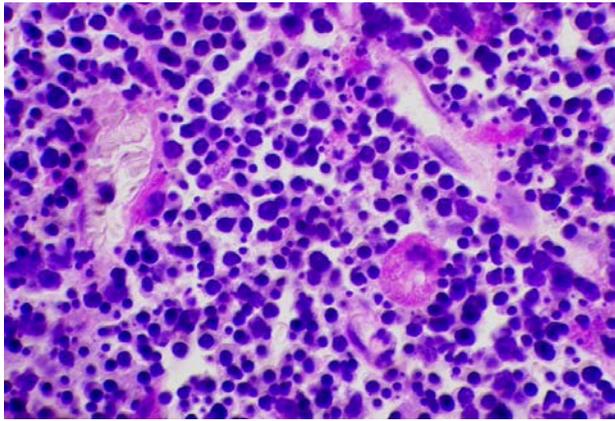


Figure S4. Cytology of ASF-LL T-cell lymphoma cells in the blood (flower appearance of tumor cell, smear) (A) and in the *s.c.* tumor (tumor imprint) (B) showing some similarities to human adult T-cell leukemia/lymphoma morphology [1, 2]; oil immersion, true Gimsa staining. Image made by Dr. Ekaterina Moiseeva, PhD.

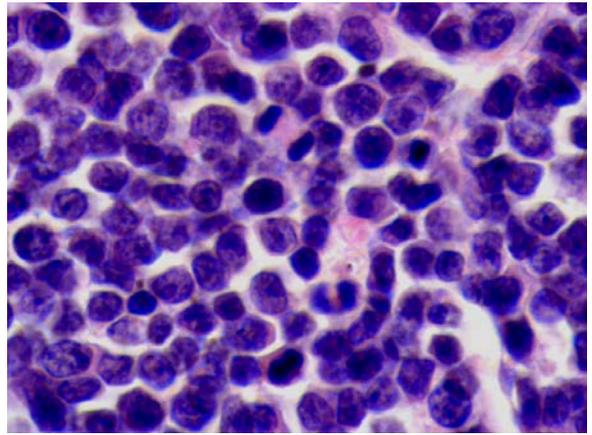
References

1. Ohshima K. Pathological features of diseases associated with human T-cell leukemia virus type I. *Cancer Sci* 2007;98:772-778.
2. Tsukasaki K., Hermine O., Bazarbachi A., Ratner L., Ramos J.C., Harrington W.Jr., O'Mahony D., Janik J.E., Bittencourt A.L., Taylor G.P., Yamaguchi K., Utsunomiya A., Tobinai K., Watanabe T. Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a proposal from an international consensus meeting. *J Clin Oncol* 2009;27:453-459.

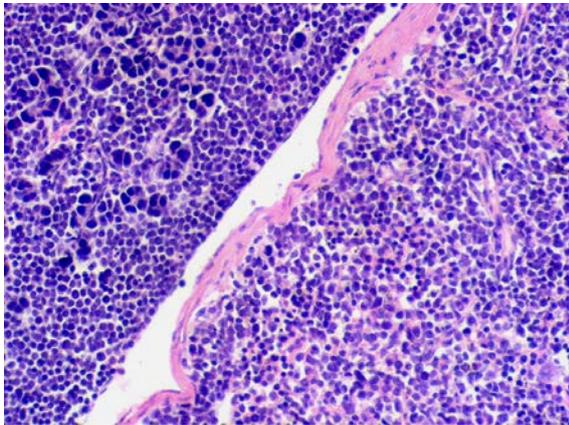
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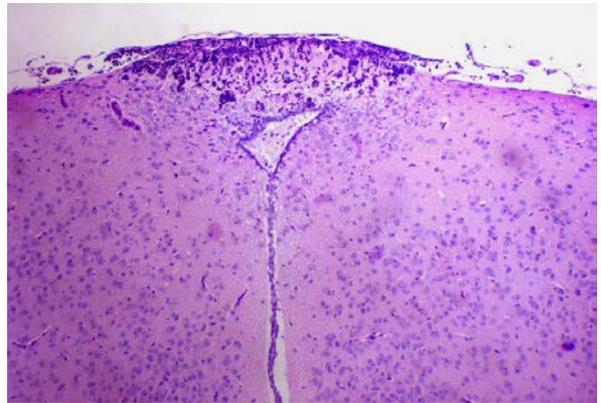
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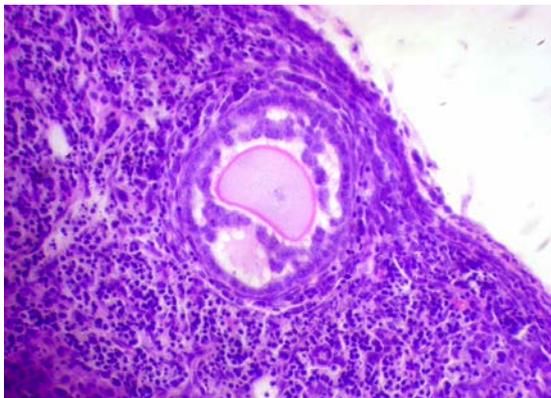
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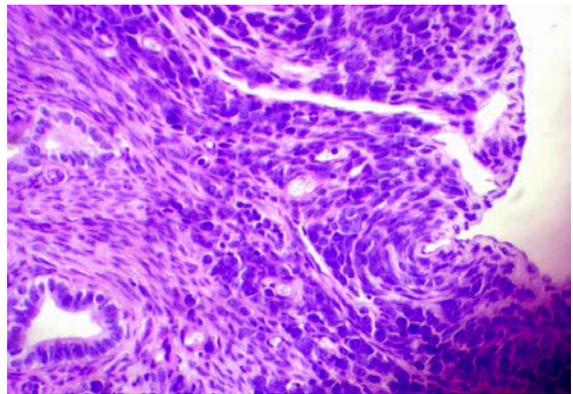
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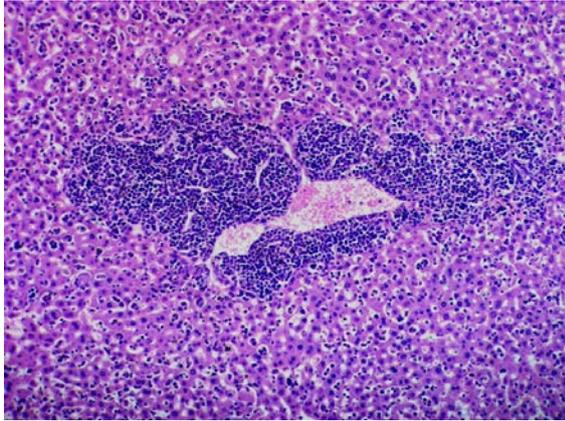
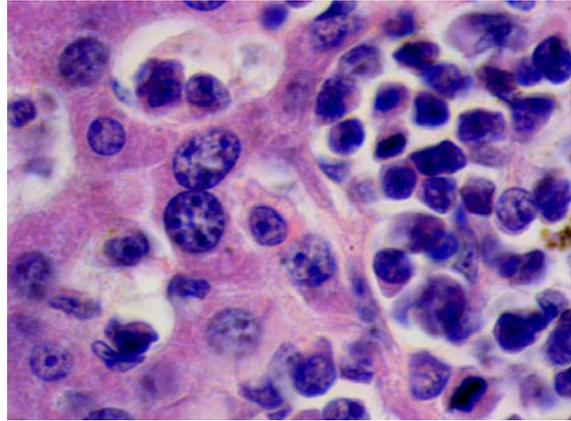
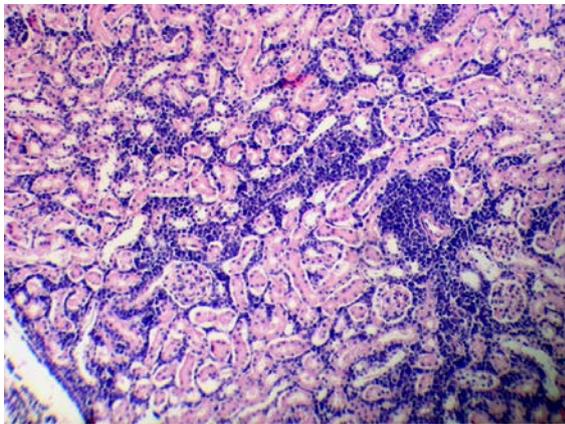
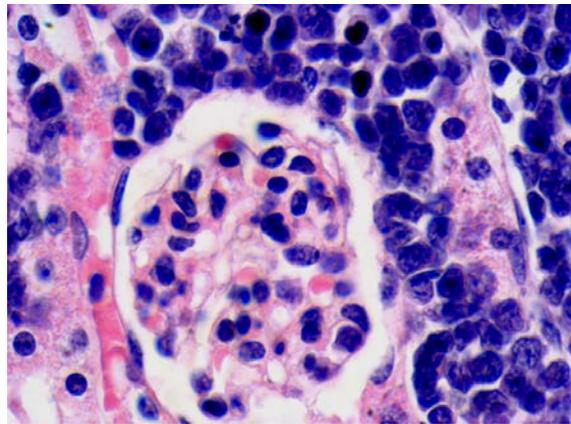
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Figure S5. Histopathology of ASF-LL T-cell lymphoma *in vivo* showing lymphoma growth patterns (A, B) and diffuse patterns of leukemic spread to several murine organs (C–J). Initial spontaneous lymphoma growth in the murine lymph node showed karyorrhexis patterns similarly to human T-cell lymphoma [1], infiltrating macrophages contained purple material, oil immersion, PAS staining (A); advanced transplanted lymphoma in the lymph node showing several mitotic figures, oil immersion, HE staining (B); advanced transplanted lymphoma in the spleen (right), pancreas is full of lymphoma cells (left), $\times 200$, HE staining (C); tumor growth in the pituitary gland, $\times 100$, PAS staining (D); the ovary is full of lymphoma cells, $\times 400$, PAS staining (E); lymphoma growth in the uterus, $\times 400$, PAS staining (F); lymphoma growth in the liver, $\times 100$, HE staining (G); details of C showing several mitotic figures, oil immersion, HE staining (H); lymphoma growth in the kidney, $\times 100$, HE staining (I); details of E, oil immersion, HE staining (J). Image made by Dr. Ekaterina Moiseeva, PhD.

References

1. Erter J., Alinari L., Darabi K., Gurcan M., Garzon R., Marcucci G., Bechtel M.A., Wong H., Porcu P. New targets of therapy in T-cell lymphomas. *Curr Drug Targets* 2010;4:482–493.