Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

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1. Supplementary Methods

1.1. Flow FISH Telomere Length Measurements of Leukocytes

Flow FISH (flow cytometry and fluorescent *in situ* hybridization) telomere length measurements[1] were performed by Repeat Diagnostics (Vancouver, BC, Canada) using either the whole blood method or previously cryopreserved peripheral blood mononuclear cell (PBMC) samples prepared at Stanford University. Red-cell lysed whole blood or rapidly thawed PBMC samples were washed and then mixed with bovine thymocytes of known telomere length, which served as an internal control. Samples were next denatured with formamide at 87°C and hybridized with telomere-specific fluorescein-labeled (CCCTAA)3 peptide nucleic acid (PNA) probes. The samples were then stained with monoclonal antibodies (mAbs) specific for CD20, CD45RA, and CD57, and the DNA was counterstained with LDS751 DNA dye.[1] For whole blood assays, lymphocytes and granulocytes were distinguished by flow cytometry based on LDS751 fluorescence intensity and light scatter signals. Additional method details are described elsewhere.[2]

1.2. Fludarabine Pharmacokinetics

In order to identify the optimal AUC for fludarabine while on intermittent hemodialysis dialysis for Patients 1 and 3, and with a CKD stage 3 renal function for Patient 2, pharmacokinetics were determined after the first dose for all three patients. Blood samples were taken on day 1, 5 minutes and 3, 4, 5, 6, and 10 hours after the end of the 1-hour infusion of fludarabine. For patients on hemodialysis, dialysis was performed approximately 12 hours after each fludarabine dose with additional blood samples drawn immediately prior to dialysis, 1 and 2 hours following dialysis completion, and immediately prior to the second dose of fludarabine. Samples were centrifuged, frozen and shipped on dry ice to the Laboratory of the Clinical Pharmacy of the University Medical Center Utrecht, the Netherlands.

Fludarabine plasma concentrations were determined using a validated liquid chromatography–tandem mass spectrometry assay.[3] The fludarabine AUC0-∞ was calculated using the population

pharmacokinetic model with InsightRx dose optimization software.[3] A cumulative AUC0-∞ of 17.5-22.5 mg · hour/liter was considered adequate exposure.[4]

1.3. One-Way Mixed Lymphocyte Cultures

The method we employed was similar to that published by Tanaka *et al.*[5] except that irradiated Epstein-Barr virus transformed B lymphoblastoid cell lines (EBV-LCL) were used in place of peripheral blood mononuclear cells (PBMC) as simulators and Cell Trace Violet dye was used in place of CFSE for the vital cell membrane staining of the responder PBMC. In brief, PBMC responder cells were isolated by Ficoll Hypaque separation and labelled with a CellTrace Violet Proliferation Kit (Invitrogen). The assays were performed in round-bottom microtiter plates with 100,000 labelled PBMC as responder cells and 30,000 irradiated (30 Gy) EBV-LCL as stimulator cells. Individual EBV-LCL lines were previously derived from the three patients, their parents, and from a healthy adult unrelated control by standard methods in which PBMCs were cultured with PHA in the presence of EBV producer cell culture supernatant.[6] The responder and stimulator cells were cultured in RPMI-1640 medium with 10% fetal calf serum. Cells were harvested on Day 6, stained with CD3 monoclonal antibodies and T-cell proliferation determined by the loss of Cell Trace Violet expression by CD3+CD19- T cells that had divided using a Novocyte Penteon flow cytometer (Agilent).

Supplementary Table 1: Pre-transplantation Patient Characteristics

Characteristic	Patient 1	Patient 2	Patient 3
Age at SIOD diagnosis	3 years	20 months	6 years
SMARCAL1 mutation	c.[395_411del;416dup] p.[Glu132Alafs*5;Leu139Phefs*4] (inherited from father); c.2459G.A p,Arg820His (inherited from mother)	c.[395_411del;416dup] p.[Glu132Alafs*5;Leu139Phefs*4] (inherited from father); c.2459G.A p,Arg820His (inherited from mother)	c.35delA; p.Lys12Argfs*53 (homozygous)
Pre-HSCT lymphocyte telomere length*	<1st percentile	<1st percentile	<1st percentile
Pre-HSCT recipient bone marrow cellularity (percentage)	Hypocellular (5-10%)	Hypocellular (20-30%)	Hypocellular (40%)
Pre-HSCT CD3+(LLNA)/CD4+(LLNA)/CD8+ (LLNA) T cells (cells/μl)	449 (1400)/229(392)/ 160(224)	876(2100)/267(672)/ 82(294)	59(1200)/38(372)/ 16(216)
Pre-HSCT T-cell proliferative responses	Normal PHA response; absent response to tetanus toxoid, low response to candida	Normal response to CD3/CD28 mAb ± cytokines	Low responses to PHA, ConA and PWM; absent responses to candida
Pre-HSCT antibody responses	Hep-A positive	CMV, VZV, Hep-A positive; 12/23 serotype-specific protective responses after Pneumovax	VZV, Hep-A positive

*Telomere length was determined by flow-FISH analysis of peripheral blood leukocytes and the values obtained are expressed as their normal percentile for age (see Supplementary Methods). CMV denotes cytomegalovirus, ConA concanavalin A, Hep-A hepatitis A, HSCT hematopoietic stem cell transplantation, KT kidney transplant, LLNA lower limit of normal for age, mAb monoclonal antibody, ND not done, PHA phytohemagglutinin, PWM pokeweed mitogen, VZV varicella zoster virus.

Supplementary Figures

Supplementary Figure 1: Average Telomere Length of Lymphocytes by Flow FISH

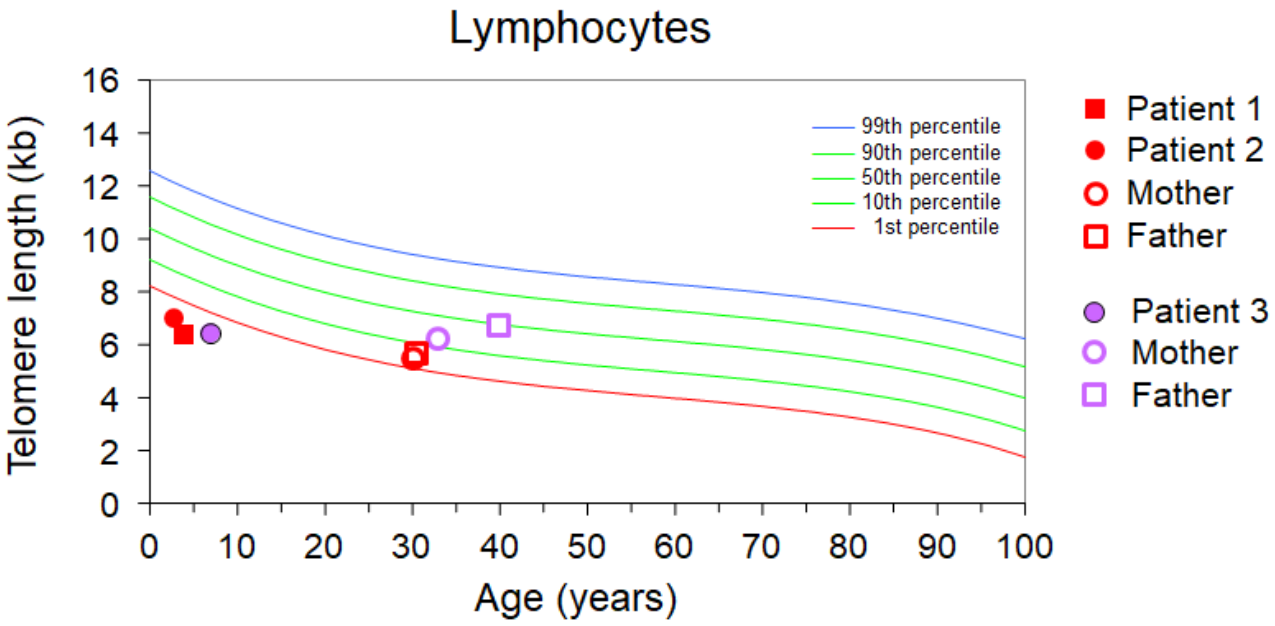

Figure S1. Telomere length measurements of lymphocytes from the three patients with SIOD and their parents. Measurements are displayed for age with normal percentile distribution for age derived from 835 healthy subjects as previously described.[1] Results for lymphocytes are displayed and demonstrate that the telomere length was less than the first percentile for age for all of the SIOD patients. Not shown, similar short telomere lengths were also observed for granulocytes and for the various lymphocyte subsets defined by expression of CD20, CD45RA, and CD57. This very short telomere length indicates that SIOD is a telomeropathy that involved hematopoietic stem cells and their lymphoid and myeloid derivatives. This is similar to what has been observed in classic dyskeratosis congenita disorders due to specific defects in telomere template RNA, telomerase, or shelterin proteins.[7]

Supplementary Figure 2: Fludarabine Pharmacokinetics: Bayesian Modeling Following Single-dose Sampling

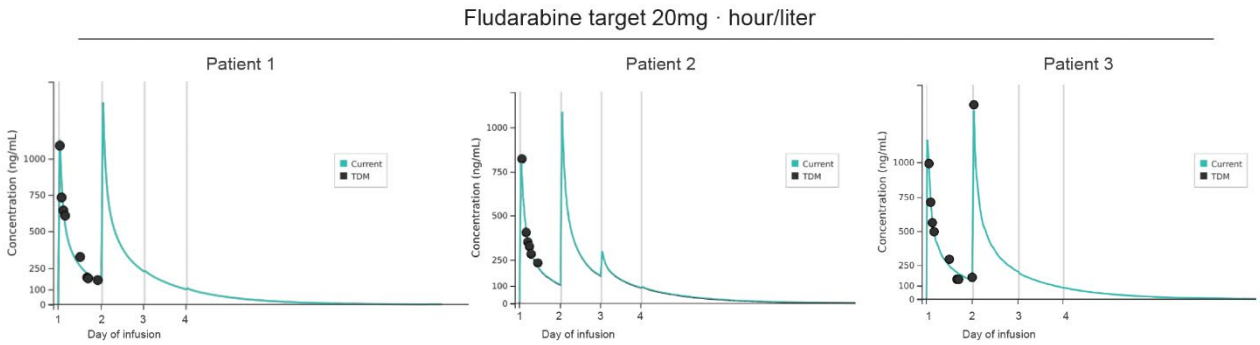

Figure S2. Fludarabine plasma concentrations of the patients used for pharmacokinetic modeling and dosing with a goal of an $AUC_{0-\infty}$ 17.5-22.520 mg · h/liter. Patient 1: based on the administering the first two doses of 13mg, the expected cumulative AUC was predicted to be 26 mg*h/L. Therefore, no additional doses were administered. Patient 2: simulation was performed based on a serum creatinine of 1.7 mg/dL (leading to an estimated GFR of 30 mL/min/1.73m2) after 2 doses of 13.3 mg. To reach a cumulative target AUC of 20 mg*hr/L only one dose of 2 mg was administered.
Patient 3: based on administering the first two doses of 15mg, the expected cumulative AUC was predicted to be 24 mg*h/L. Therefore, no additional doses were administered.
Note: As the concentration of fludarabine is decreased by 30-40% after each dialysis session, it was predicted that the continued use of daily hemodialysis in patients 1 and 3 throughout the remainder of conditioning would result in acceptable fludarabine concentrations of <25ng/mL at the time of stem cell infusion.

3. Supplementary References

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