Hydroxyurea therapy requires HbF induction for clinical benefit in a sickle cell mouse model

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Online Supplementary Table S1. Hydroxyurea at 100 mg/kg after four weeks produced critical pancytopenia.

	Vehicle (n=8)	100 mg/kg Hydroxyurea(n=8)
White Blood Cell Count $\times 10^9$/L	30.9±6.0	10.0±4.8[a]
Absolute Neutrophils $\times 10^9$/L	5.0±0.3	2.9±1.2[a]
Hemoglobin g/L	90±3	53±23.5[a]
Platelets $\times 10^9$/L	421±75	248±43[a]
Absolute Reticulocyte Count $\times 10^9$/L	500±33	293±123[a]
Death/Compassionate removal	0	4

Values represent the mean and SEM o 4 weeks after initiation of intraperitoneal injections.
[a]P value < 0.001 for vehicle vs. 100mg/kg of hydroxyurea

Online Supplementary Table S2. Hydroxyurea treatment does not improve sickle cell organ pathology. (A) Individual mouse histology scores based on treatment with hydroxyurea, vehicle, gene therapy or control C57Bl/6. Hydroxyurea and vehicle treated mice demonstrate marked organ pathology in comparison to gene therapy and control mice. (B) Histology scoring system developed by veterinary pathologist (KB).

(A) Histology Scores for Individual Mice

Animal ID	LIVER PATHOLOGY	RENAL PATHOLOGY	SPLEEN HEMATOPOIESIS
Hydroxyurea Tx	2	2	3+
Hydroxyurea Tx	2	2	3+
Hydroxyurea Tx	3	2	3+
Hydroxyurea Tx	3	2	3+
Hydroxyurea Tx	3	3	3+
Hydroxyurea Tx	4	3	3+
Vehicle Control	4	4	3+
Vehicle Control	3	2	3+
Vehicle Control	3	2	3+
Vehicle Control	3	2	3+
Vehicle Control	4	2	3+
Vehicle Control	3	2	3+
Gene Therapy	0	0	1
Gene Therapy	0	0	1
Gene Therapy	0	0	0
Gene Therapy	1	0	1
Gene Therapy	0	0	0
BL6 control	0	0	0
BL6 control	0	0	0
BL6 control	0	0	0
BL6 control	0	0	0

(B) Histology Scoring System

Liver

0- minimal periportal, centrilobular to random lymphocytes and plasma cells

1- mild infiltrates with few siderophages in peri-portal areas

2- moderate infiltrates, siderophages are prominent in sinusoids and periportally, central lobular and random distribution; minimal hepatocelluar necrosis

3- moderate to marked inflammation prominent siderophages throughout liver, large clusters common, multifocal small infarctions and hepatocellular necrosis

4- marked inflammation, siderophages, fibrosis, multiple infarctions often large

KIDNEY

0- no change

1- hemosiderin rarely observed in tubular epithelium

2- hemosiderin prominent in tubular epithelium, glomerular capillary thickening, proteinaceous casts in tubules, multifocal mild tubular necrosis

3- hemosiderin prominent with epithelial degeneration, glomerular obsolescence, protenaceous casts and tubular necrosis wide spread

4- grade 3 + fibrosis

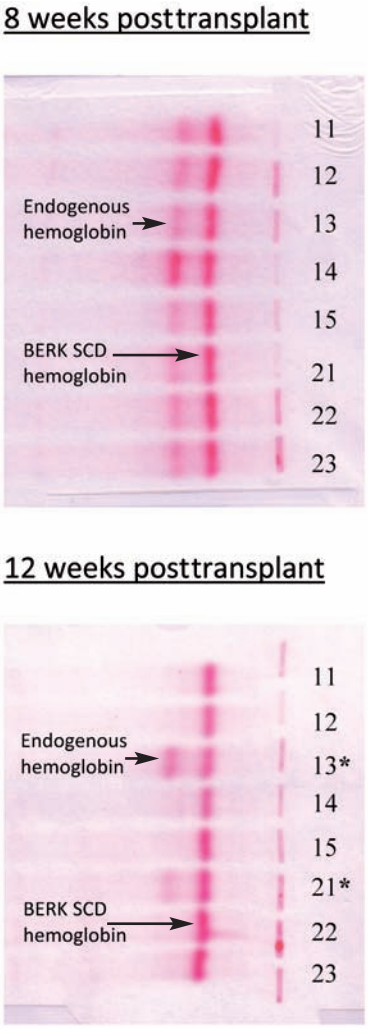
SPLEEN

0 = minimal or absent extramedullary hematopoiesis

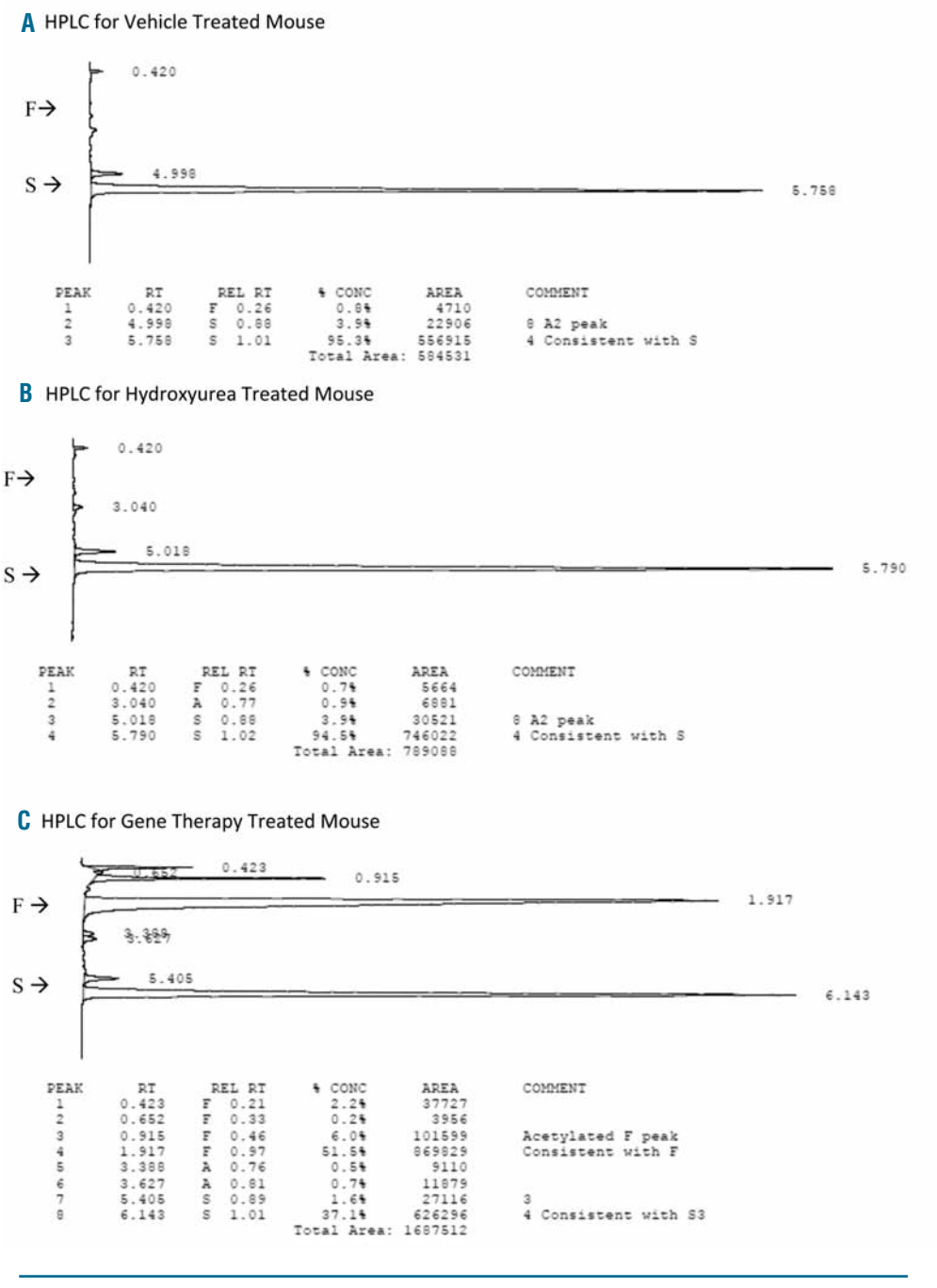
1= minimal increase in extramedullary hematopoiesis with a shift toward erythropoiesis

2= moderate increase in extramedullary hematopoiesis erythropoiesis

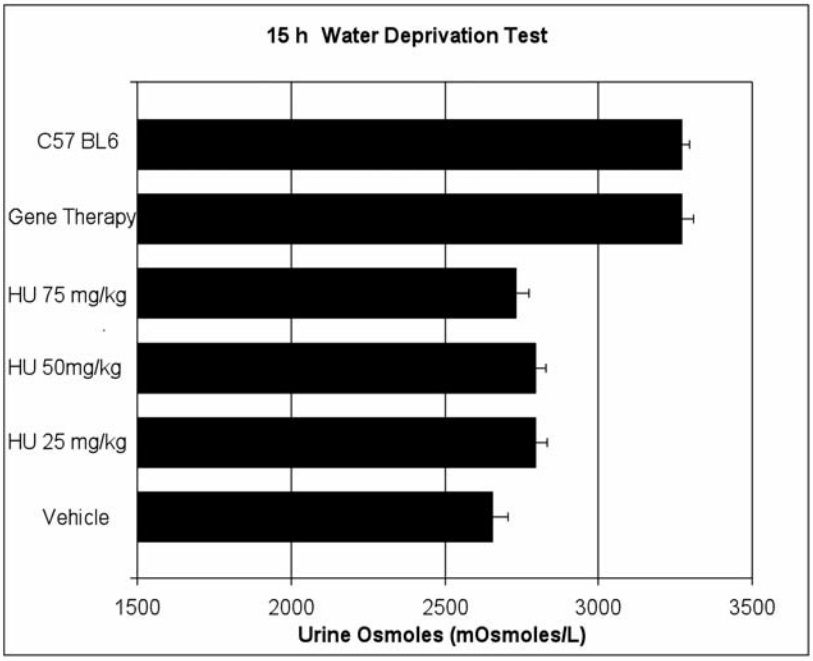
3= Marked increase in hematopoiesis almost purely erythropoiesis and grossly enlarged spleen



Online Supplementary Figure S1. C57BL6 mice transplanted with BERK bone marrow. Eight weeks after transplantation, mice were not fully engrafted. Hydroxyurea therapy or vehicle was initiated at week 9, prior to full engraftment. Twelve weeks after transplantation, hemoglobin electropheresis demonstrates full sickle cell engraftment (>99% HbS) without fetal hemoglobin induction. Any mice not fully engrafted (*) were removed from the study.



Online Supplementary Figure S2. HPLC of red cell hemolysates from vehicle-, hydroxyurea-, and gene therapy-treated SCD mice. (A) Representative HPLC profiles of hemolysates from a vehicle-treated SCD mouse (HbF: 0%), (B) a hydroxyurea-treated mouse (HbF: 0%), and a (C) gene therapy treated mouse (HbF: 52%). Below each trace is the retention time (RT), relative retention time (REL RT), and the percentage concentration (CONC).



Online Supplementary Figure S3. Hydroxyurea therapy does not protect against urine concentration defects. Sickle cell mice treated with vehicle, hydroxyurea (25, 50, 75 mg/kg), gene therapy, as well as control C57/Bl6 mice underwent a 15 h water deprivation test. Urine was collected after 15 h in urine osmolality was compared with standard deviation shown for each group. Sickle cell mice receiving chronic hydroxyurea therapy or vehicle developed a urine concentration defect, demonstrated by a reduced ability to concentrate urine after water deprivation. Sickle cell mice with high levels of fetal hemoglobin (Gene therapy) demonstrated the same urine concentrating ability as control C57/Bl6 mice. Data obtained 2-3 months after engraftment. *P* value < 0.05 for Gene Therapy *vs.* Vehicle-treated sickle cell mice. *P* value <0.05 for Gene therapy *vs.* HU-treated sickle cell mice at 25 mg/kg, 50 mg/kg or 75 mg/kg. *P*=0.99 for gene therapy *vs.* control C57BL6 mice