Gene Therapy

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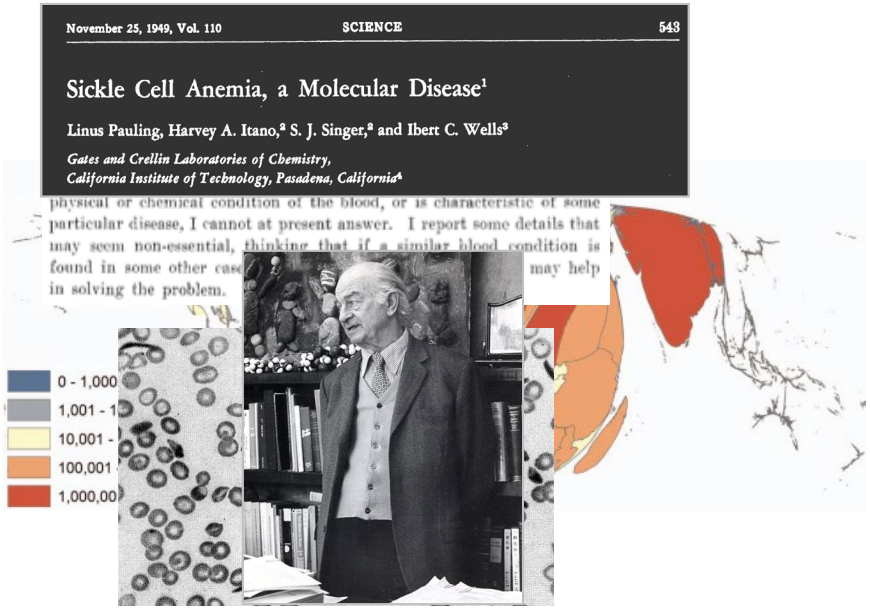
The rationale for the development of gene therapy in sickle cell disease

- Sickle cell disease first described >100 years ago in a dental student from the West Indies
  (Herrick, Arch Intern Med. 1910)
- First "Molecular Disease"
  (Pauling et al, Science, 1949)
- Single substitution at position 6 of ß-globin chain
  (Ingram, et al, Nature, 1957)
- Abnormal red blood cells with Hb prone polymerization upon deoxygenation
  - Severe anemia, frequent severe pain, end organ damage, early mortality
- A rare disease in the US, but not in the world
  (Piel F B et al, PLoS Medicine 2013)
- Current therapies limited, largely supportive



Herrick, *Arch Intern Med.* 1910; 5, 517

Sickle cell disease affects all organ systems and shortens lifespan

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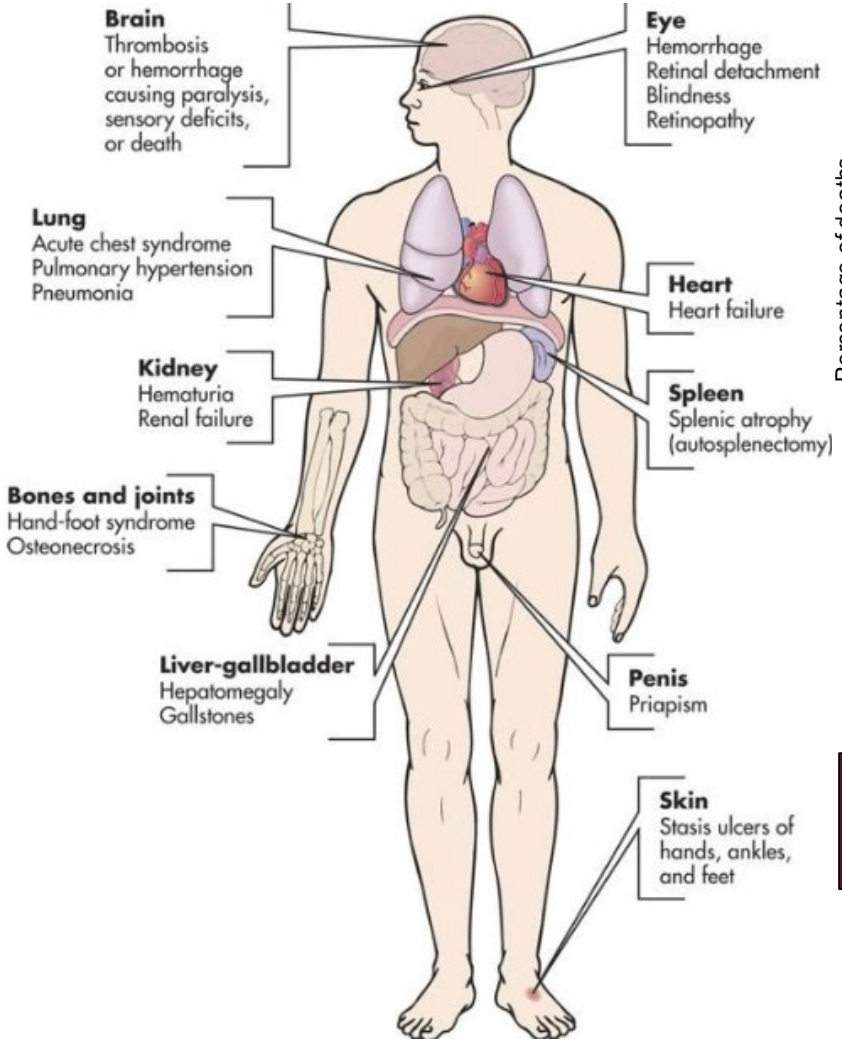
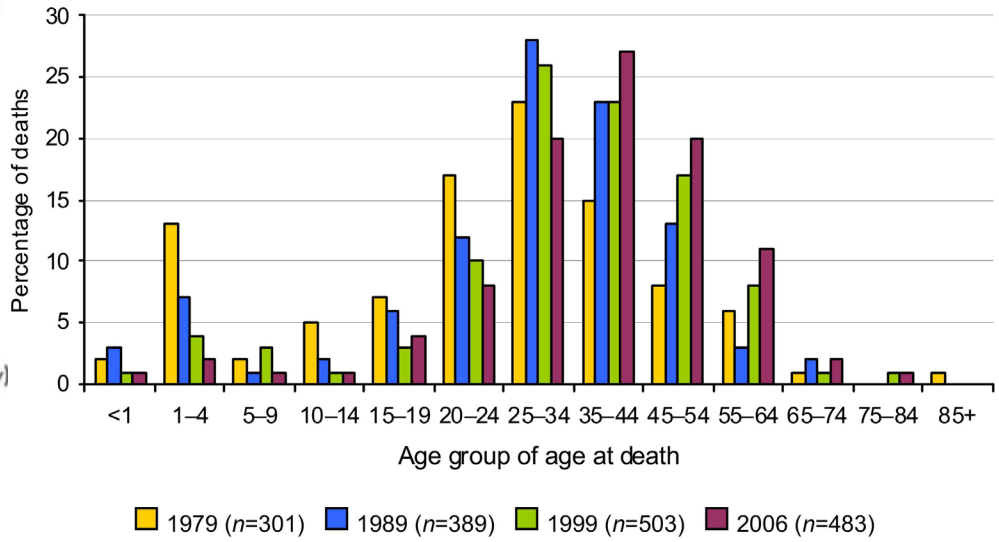




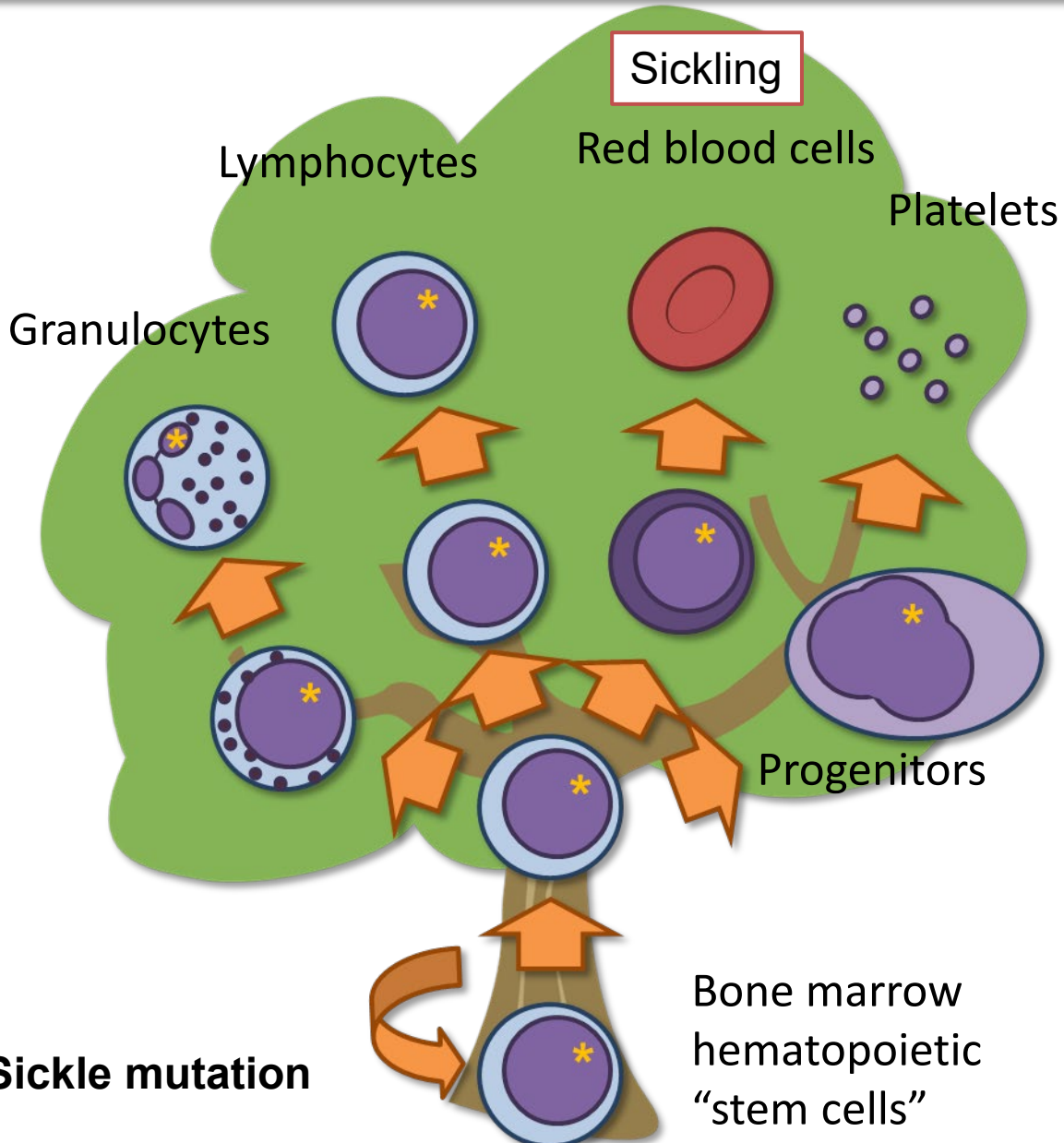
Mean age at death was 39 in 2006,
only 35% alive at age >45 years

Hassell / Am J Prev Med 2010;38(4S):S512–S521

“There is a cure for sickle cell disease”

Bone marrow transplantation

Bone marrow transplants are the seeds of the blood

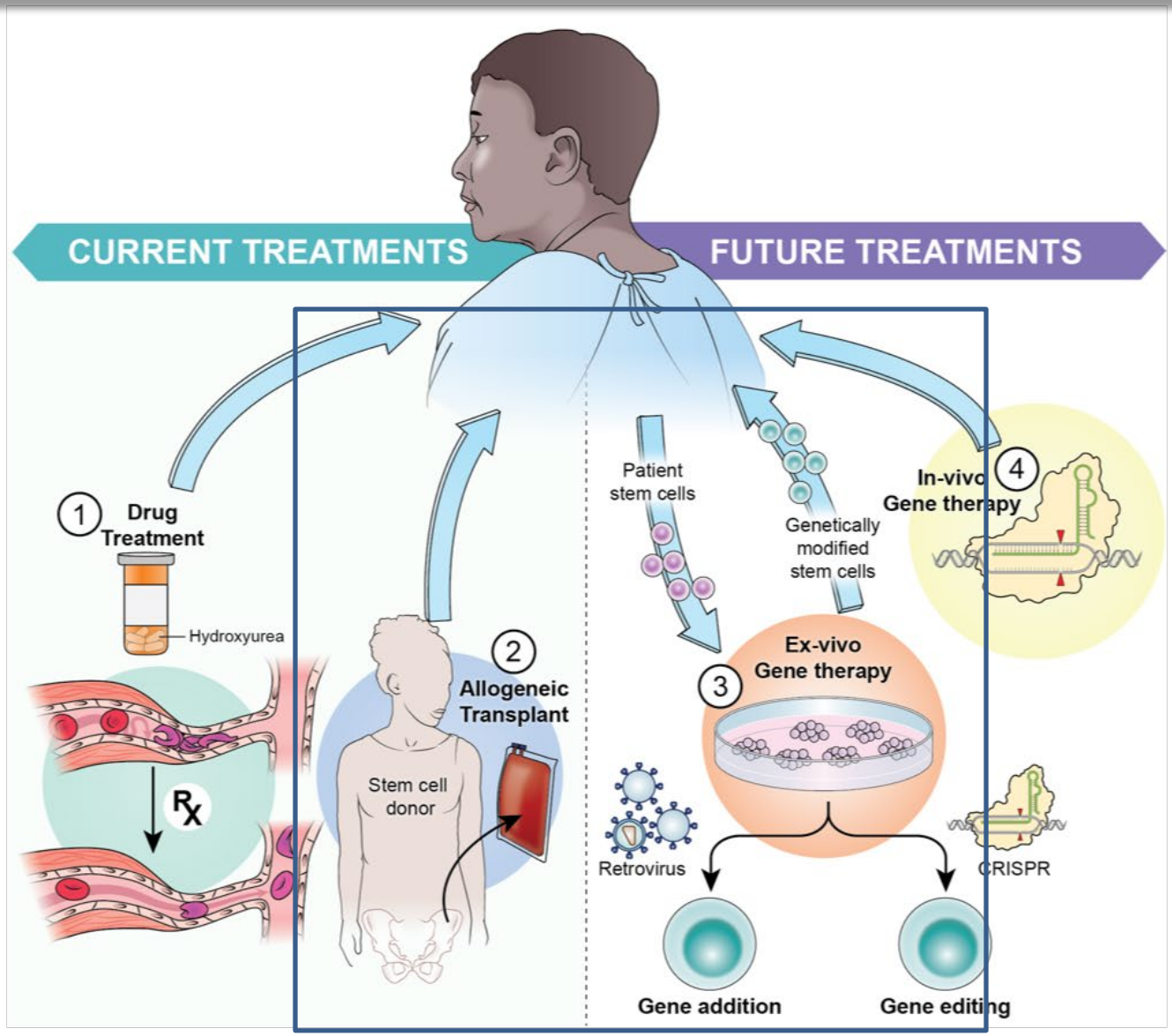


***Sickle mutation**

Bone marrow stem cells produce all types of blood cells for the life of a patient.

We have sought to develop curative strategies based upon replacing or repairing bone marrow stem cells.

Strategies for the treatment of SCD



Tisdale, Thein and Eaton, Science, 13 Mar, 2020

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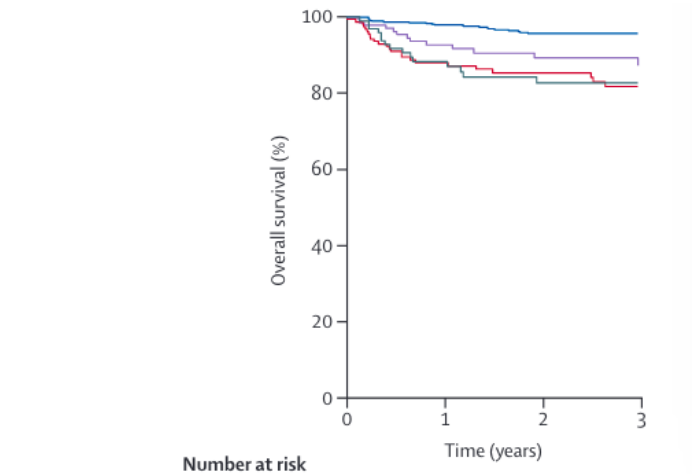
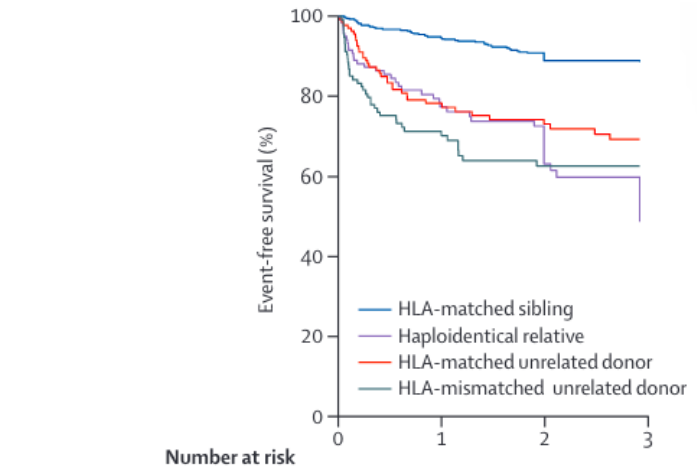
Retrospective Cohort Study From 90 US Centers Reporting to the Center for International Blood and Marrow Transplant Research

- Identified 996 SCD patients who underwent transplant from 2008-2017
- Event free survival
  - highest in those < or = 12 yo and recipients of HLA matched sibling grafts
  - worse in those conditioned with reduced intensity
  - equivalent between myeloablative and nonmyeloablative regimens



Number at risk				
HLA-matched sibling	557 (0)	460 (86)	362 (175)	276 (261)
Haploidentical relative	137 (0)	97 (31)	71 (54)	50 (74)
HLA-matched unrelated donor	111 (0)	79 (14)	63 (27)	45 (42)
HLA-mismatched unrelated donor	104 (0)	82 (11)	64 (25)	52 (37)



Number at risk				
HLA-matched sibling	557 (0)	444 (86)	349 (165)	261 (245)
Haploidentical relative	137 (0)	85 (28)	61 (47)	33 (60)
HLA-matched unrelated donor	111 (0)	72 (11)	57 (22)	40 (35)
HLA-mismatched unrelated donor	104 (0)	61 (11)	42 (23)	36 (29)

Eapen, et al, Lancet Haematol. 2019 Nov;6(11):585-596.

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How much corrected bone marrow is enough to fix SCD?

- Nonmyeloablative allogeneic bone marrow transplant (matched and haplo) performed in over 100 patients with SCD, 67 patients with long-term follow up available
- Donor percentage of white blood cells, red blood cells, and symptoms monitored at day 100 and at least every 6-12 months post-transplant thereafter
- Identified 3 patients who experienced declining donor levels over prolonged follow up despite robust initial engraftment
  - All 3 patients' donors had sickle cell trait with sickle hemoglobin $\leq$50%
- All 3 patient developed a rise in sickle hemoglobin >50%, severe anemia and recurrent SCD symptoms when donor white blood cells fell <20%

Fitzhugh, Cordes et al, Blood, 2017 Oct 26;130(17):1946-1948

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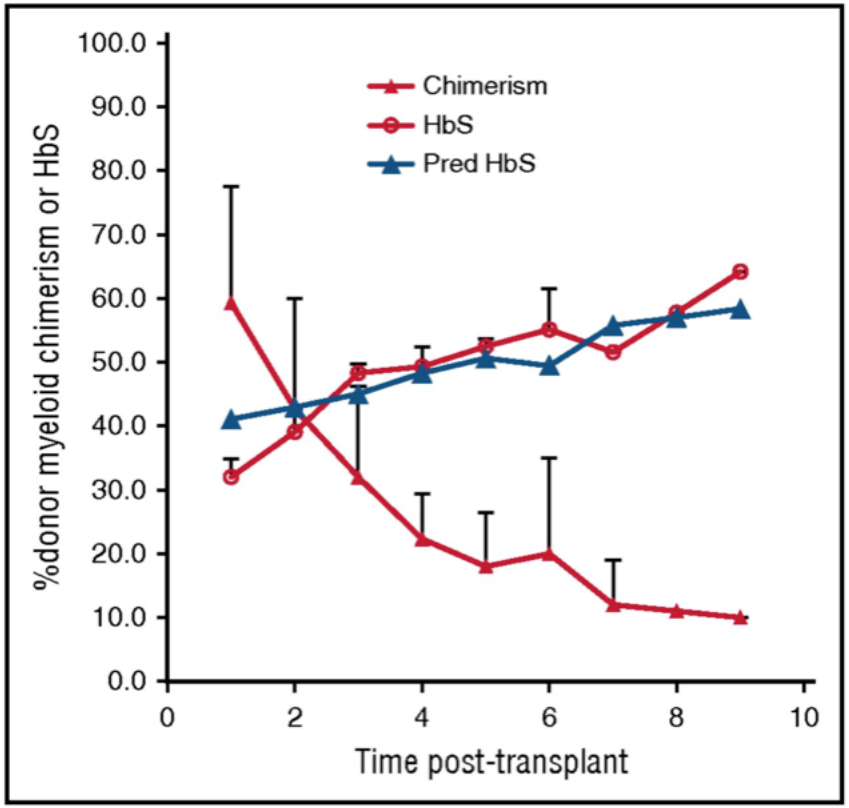
Comparison between NIH transplant results and mathematical modeling demonstrates that only 20% donor level needed and is dependent only on red blood cell life span differences

$$f_M = \frac{f_P t^D}{f_P t^D + (1 - f_P) t^H}$$

In our model the fraction of mature donor erythrocytes in the periphery (f_M) is a function of

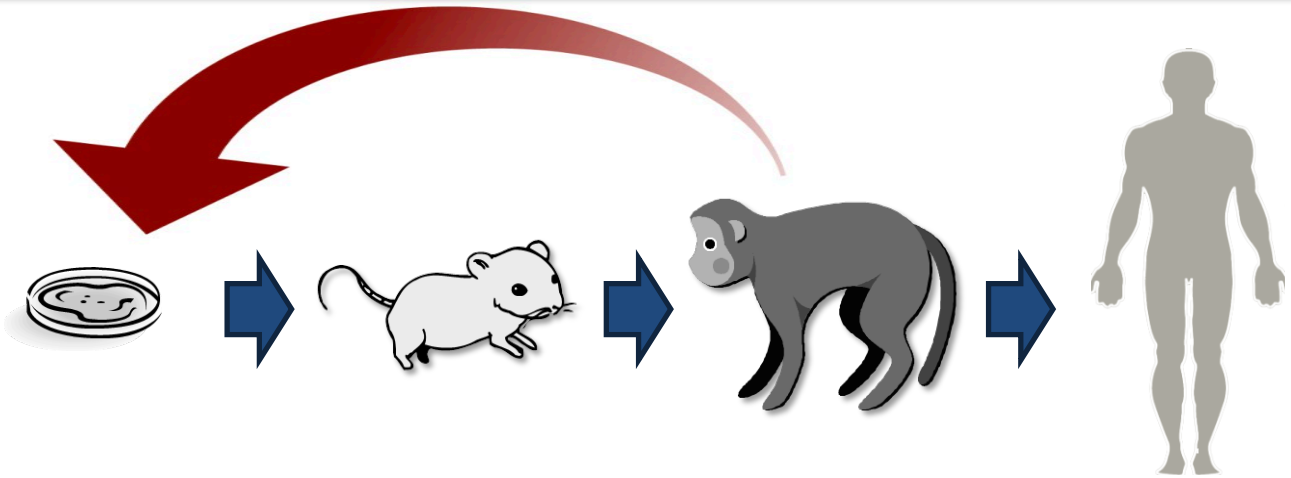
- Progenitor chimerism, f_P
- Donor and recipient erythrocyte half-lives, t^D and t^H, respectively.



Can we achieve this modest 20% correction level with gene therapy with the patients' own bone marrow HSCs?

Fitzhugh, et al, Blood, 2017 Oct 26;130(17):1946-1948

Translational research milestones to develop gene therapy for sickle cell disease



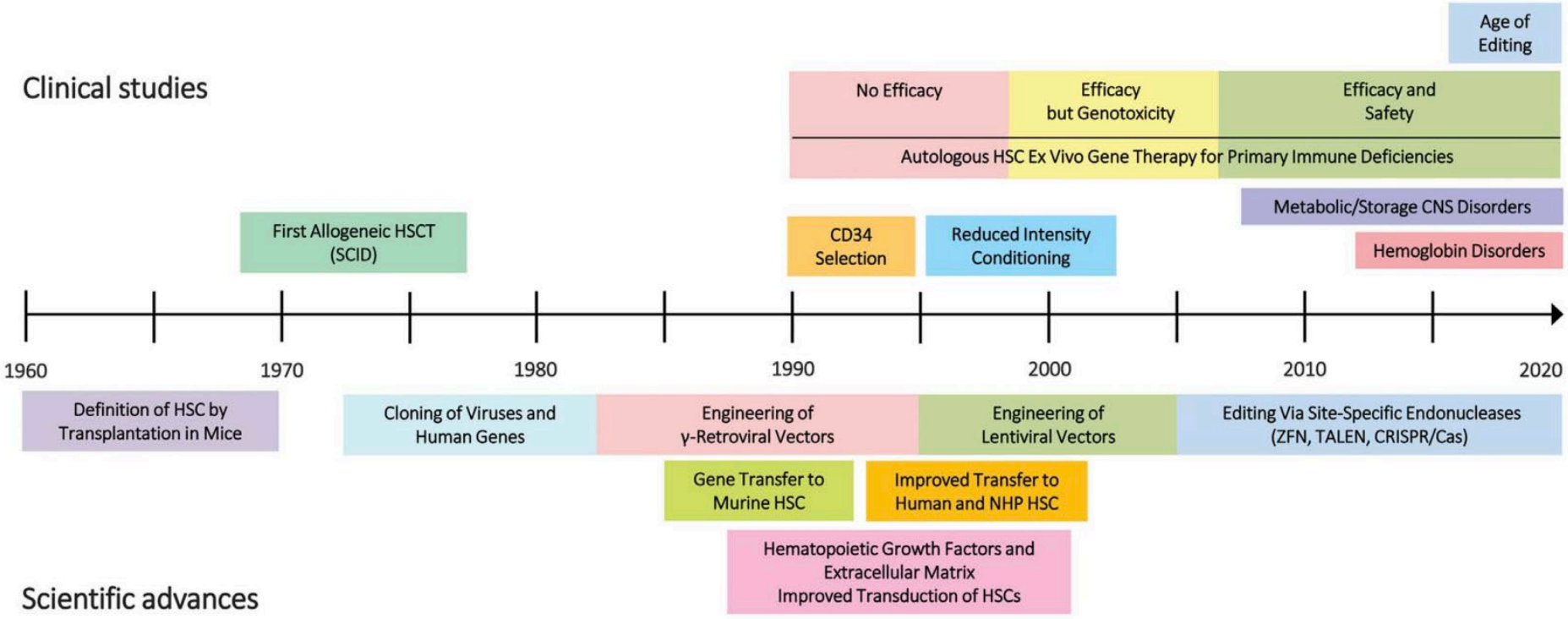
Cell culture	Small animal	Large animal	Clinical trial
Cell lines iPS cells	Mice Disease model mice Humanized mice	Non-human primates	Phase I Phase II Phase III Phase IV

Efficiency

Cell lines > Mouse HSCs >> Rhesus HSCs ≈ Human HSCs

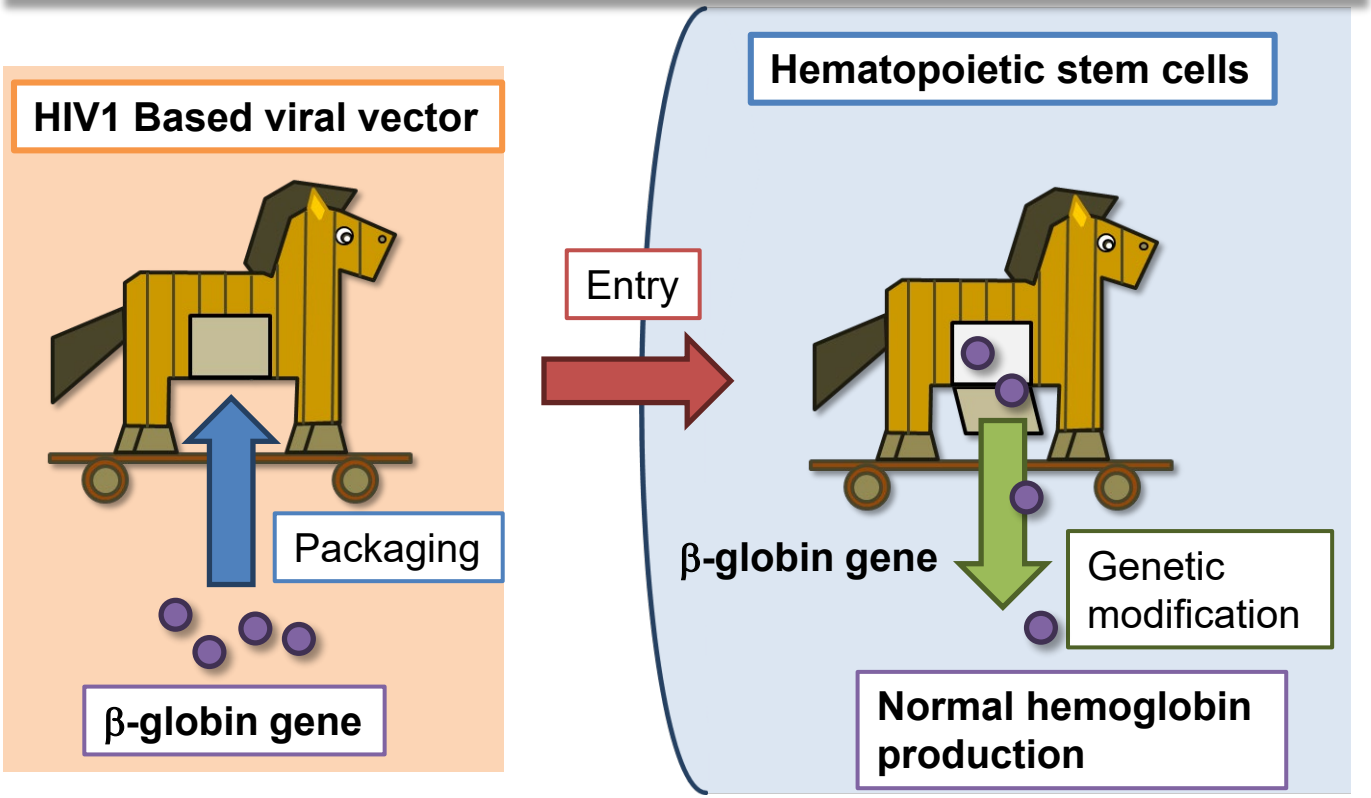
Maximize benefit/minimize risks

Gene therapy comes of age



Dunbar *et al.*, *Science* **359**, 175 (2018) 12 January 2018

Gene transfer for “gene addition” gene therapy



Stringent requirements for the hemoglobinopathies including integration to allow sustained, high-level, lineage restricted expression of therapeutic globin sufficient to overcome HbS, self inactivating to abolish promoter activity, tissue specificity to further reduce promoter activity

Lentiviral vectors based upon HIV1 allow for therapeutic gene transfer in the hemoglobinopathies

letters to nature

Therapeutic haemoglobin synthesis in β-thalassaemic mice expre lentivirus-encoded human β-

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Karen M. L. Gaensler‖, Lucio Luzzatto*¶ & Michel Sadelai

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‖ *Department of Medicine, University of California, San Francis California 94143, USA*

Correction of Sickle Cell Disease in Transgenic Mouse Models by Gene Therapy

Robert Pawliuk,[1,2] Karen A. Westerman,[1,2] Mary E. Fabry,[3] Emmanuel Payen,[4] Robert Tighe,[1,2] Eric E. Bouhassira,[3] Seetharama A. Acharya,[3] James Ellis,[5] Irving M. London,[1,6] Connie J. Eaves,[7] R. Keith Humphries,[7] Yves Beuzard,[4] Ronald L. Nagel,[3] Philippe Leboulch,[1,2,4,8*]

Sickle cell disease (SCD) is caused by a single point mutation in the human β^A globin gene that results in the formation of an abnormal hemoglobin [HbS ($\alpha_2\beta^S_2$)]. We designed a β^A globin gene variant that prevents HbS polymerization and introduced it into a lentiviral vector we optimized for transfer to hematopoietic stem cells and gene expression in the adult red blood cell lineage. Long-term expression (up to 10 months) was achieved, without preselection, in all transplanted mice with erythroid-specific accumulation of the antisickling protein in up to 52% of total hemoglobin and 99% of circulating red blood cells. In two mouse SCD models, Berkeley and SAD, inhibition of red blood cell dehydration and sickling was achieved with correction of hematological parameters, splenomegaly, and prevention of the characteristic urine concentration defect.

14 DECEMBER 2001 VOL 294 SCIENCE

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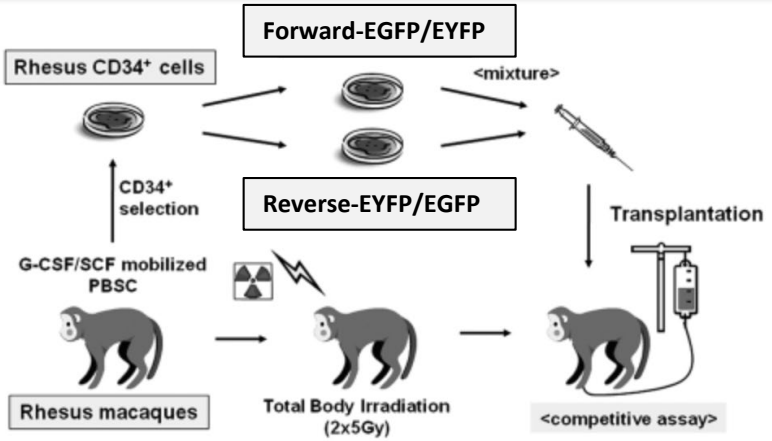
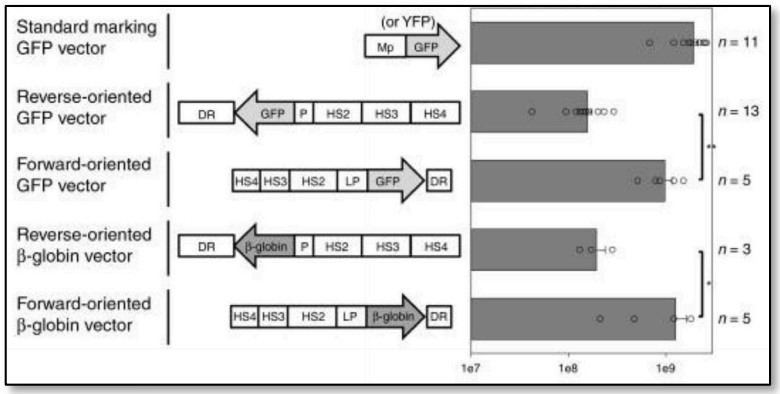
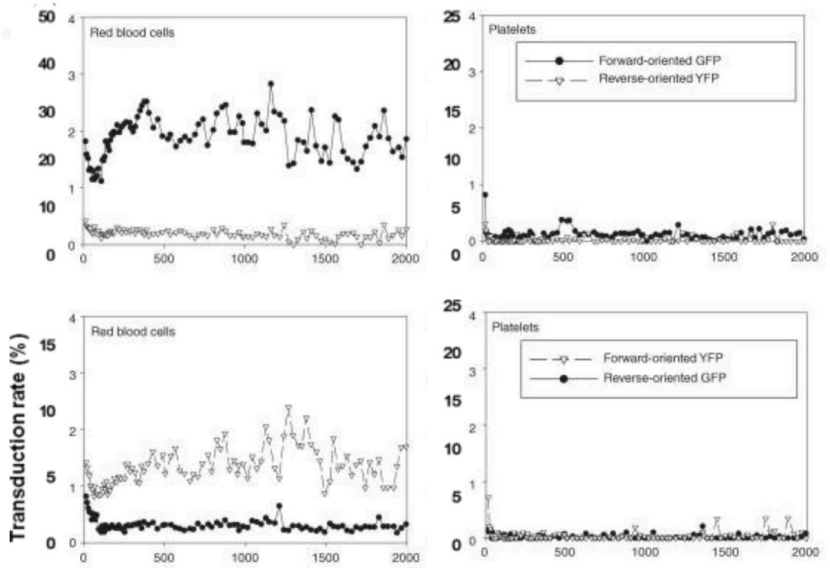
Chimeric HIV1-based lentiviral vectors utilizing the SIV capsid circumvent the species specific restriction

- TRIM5alpha targets CA for degradation in species mismatch
- Chimeric vector with simian CA constructed
- Allows comprehensive testing of HIV-based vectors in the rhesus







Uchida et al. J Virol, 2009 Oct;83(19):9854-62.

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HGB-206: study of HIV-based vector gene therapy for severe sickle cell disease

HSC collection
Bone marrow harvest or mobilization with plerixafor & apheresis

Busulfan myeloablative conditioning

DP infusion

Transduced HSCs engraft and contribute to reconstitution of functional RBCs

β^{T87Q} α α β^{T87Q} Modified RBCs express gene therapy-derived, anti-sickling HbA^{T87Q}

2-yr follow-up

Long-Term Follow-Up Study

	Group A	Group B	Group C
Pre-collection transfusion regimen	Optional	Required	Required
HSC source	Bone marrow	Bone marrow	Mobilized PB
Manufacturing process	Original	Orig ➔ Refined	Refined

LentiGlobin DP centralized manufacturing

Select CD34+ cells → *Transduce with BB305 lentiviral vector* → *Cryopreserve, test, release DP*

Key Enrollment Criteria

- 18+ years of age (12+ in group C)
- History of symptomatic SCD
- Adequate organ function
- No previous HSCT or gene therapy

Study Objectives

- Primary objective: Safety
- Key Secondary Objectives:
  - Frequency of VOCs and ACS
  - Total Hb and Hb fractions

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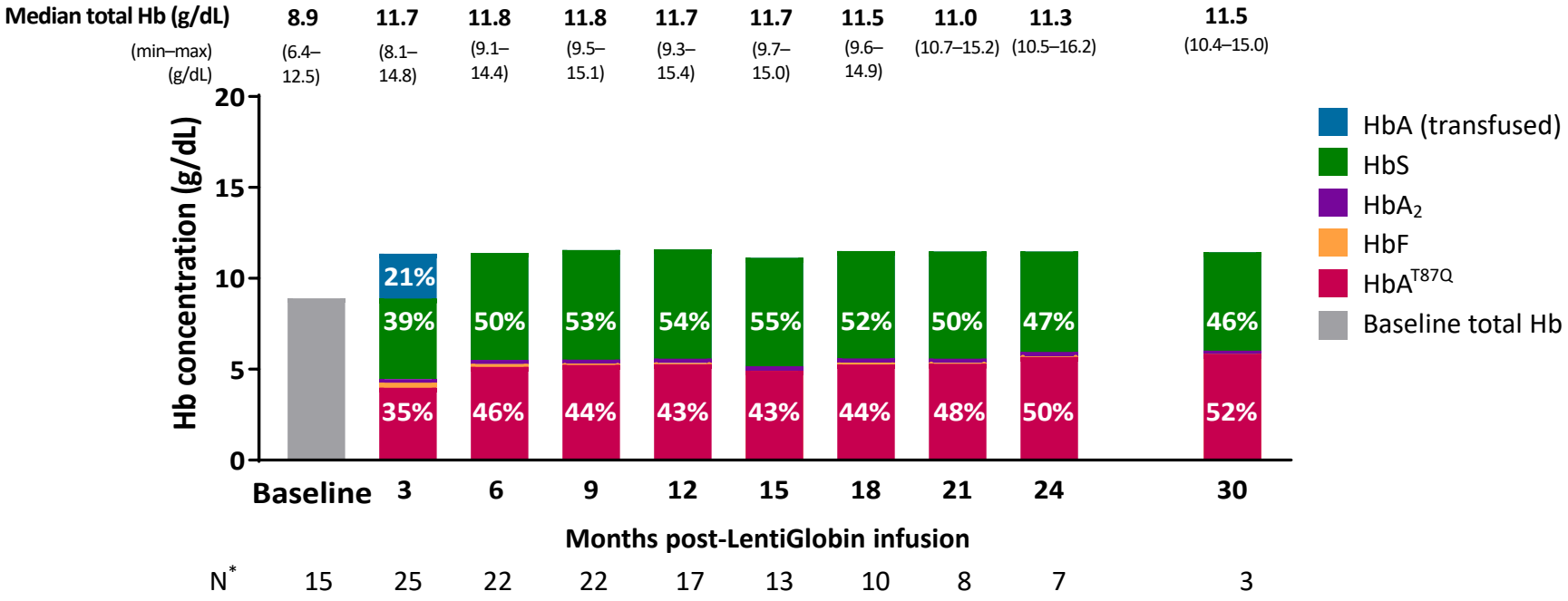
HGB-206 Group C: Median HbS ≤ 60% and HbA^{T87Q} ≥ 40% at ≥ 6 Months Post-Treatment



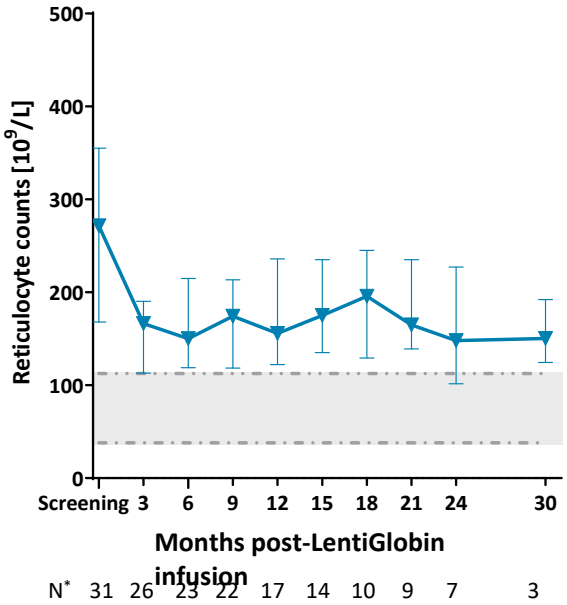
- In patients with ≥ 6 months of follow-up, median total Hb increased from 8.9 g/dL at baseline to ≥ 11.8 g/dL at Month 6
- At last visit in adolescents with ≥ 6 months of follow-up (n=6), median total Hb and HbA^{T87Q} were 13.5 g/dL and 6.1 g/dL, respectively

% represents median Hb fraction as % of total Hb; *Number of patients with data available. Hb, hemoglobin; max, maximum; min, minimum.

Data as of 20 August 2020

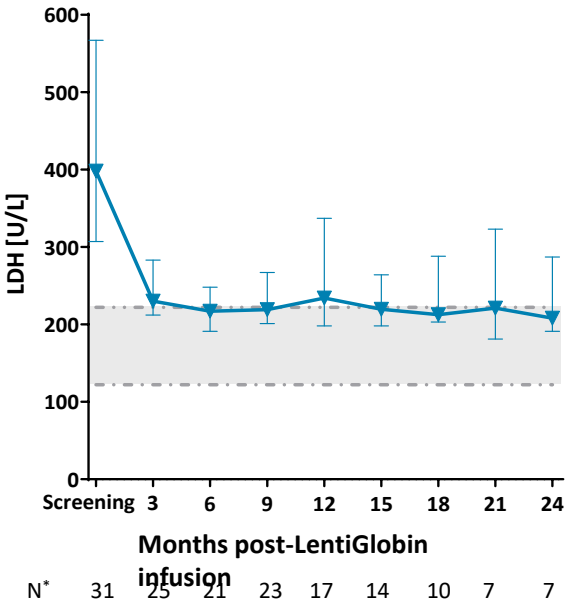
HGB-206 Group C: Hemolysis markers approaching near-normal levels post-LentiGlobin treatment

Reticulocyte counts



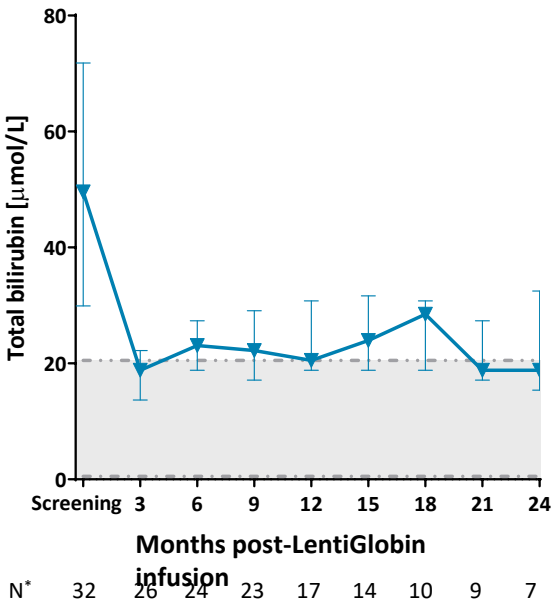
N*	31	26	23	22	17	14	10	9	7	3

Lactate dehydrogenase



N*	31	25	21	23	17	14	10	7	7

Total bilirubin

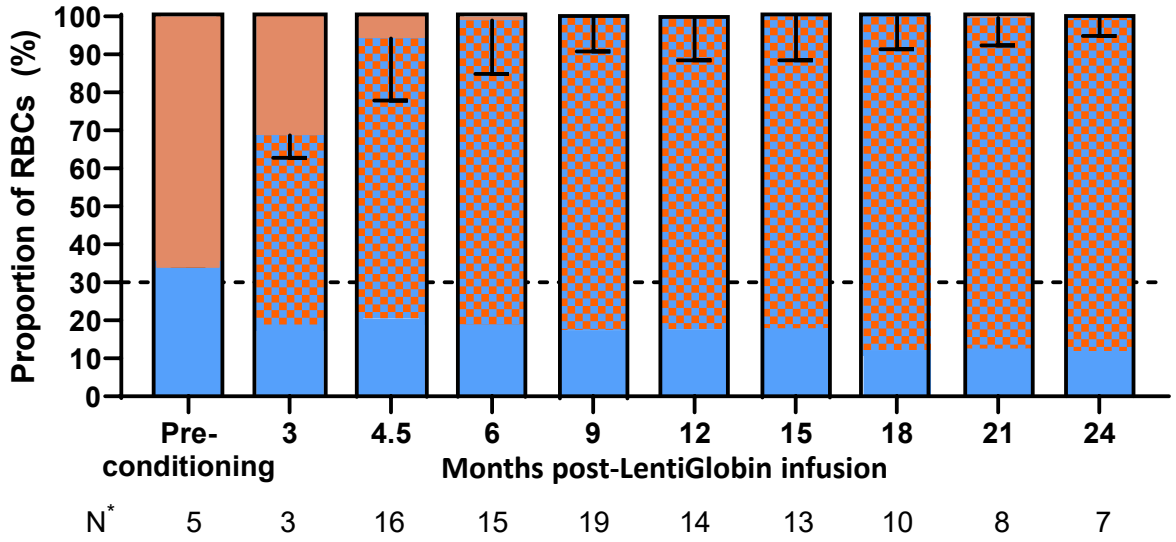


N*	32	26	24	23	17	14	10	9	7

Data as of 20 August 2020

Median (Q1, Q3) depicted; Dot-dash lines denote lower and upper limits of normal values; *Number of patients with data available; Q1, quartile 1; Q3, quartile 3.

HGB-206 Group C: Near pancellular expression of HbA^{T87Q} ≥ 6 months post-LentiGlobin treatment



RBCs with β^A only
(Source: transfused blood)

RBCs positive for $\beta^{A\text{-}T87Q}$ by single cell Western (RBCs with detectable $\beta^{A\text{-}T87Q}$ in addition to β^S)

RBCs with β^S only

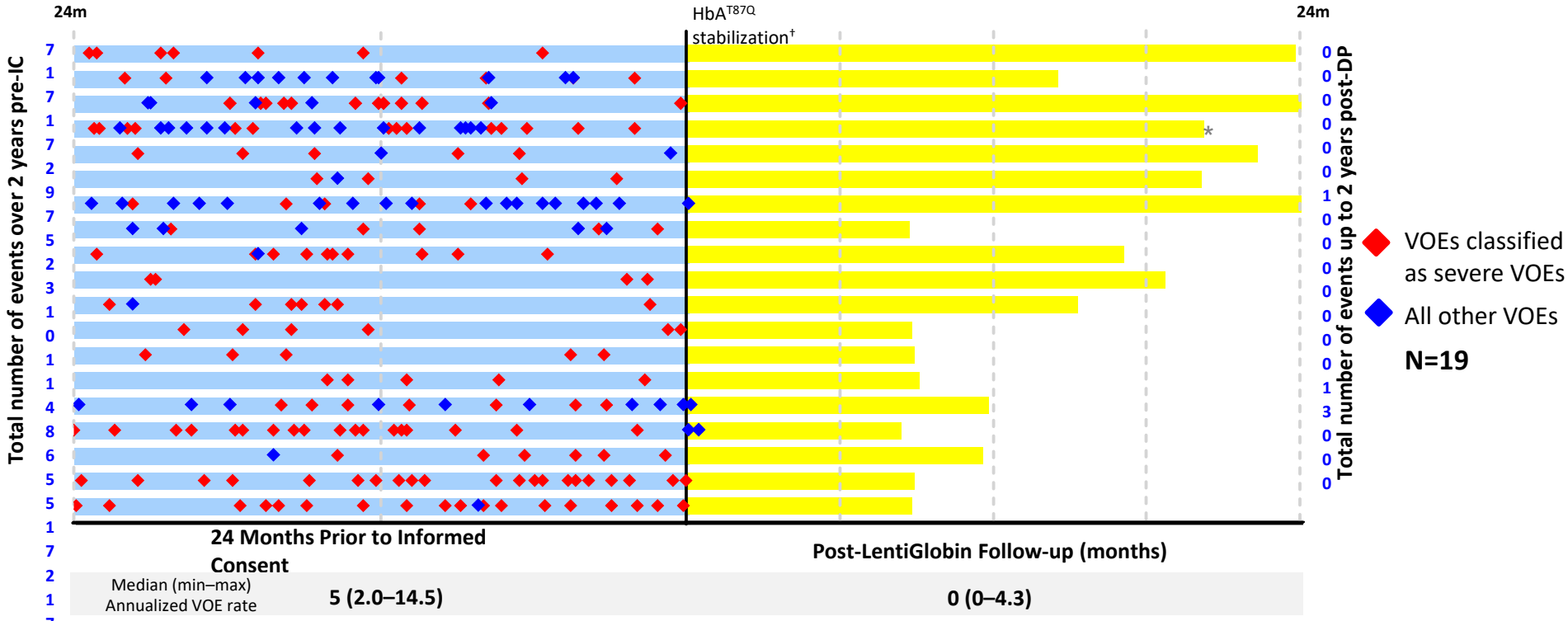
- Median (min–max) HbA^{T87Q}/RBC was 15.3 (11.7–20)† pg in patients with ≥ 6 months follow-up, which is comparable to the 13–18 pg of HbA/RBC in individuals with sickle cell trait‡ and higher than 10 pg of HbF/RBC in those with HPFH§

Mean & SD are depicted; Reducing HbS to < 30% is recommended by guidelines for exchange RBC transfusions for patients with SCD (indicated by dashed line); *Number of patients with data available; †Calculated as (% HbA^{T87Q} of total Hb/% RBCs containing $\beta^{A\text{-}T87Q}$) x MCH; ‡Calculated to 13–18 pg HbA/RBC using 50% HbA/RBC for the lower end of the range and 60% HbA/RBC for the upper end of the range; §Estimated in Steinberg MH et al., Blood 2014.

Data as of 20 August 2020

Hb, hemoglobin; HPFH, hereditary persistence of fetal hemoglobin; max, maximum; MCH, mean corpuscular hemoglobin; min, minimum; pg, picogram; RBCs, red blood cells; SD, standard deviation.

HGB-206 Group C: Complete resolution of severe and other VOEs ≥6 months post-LentiGlobin treatment



Protocol VOE are shown; Patients with ≥ 4 sVOE at baseline before IC and with ≥ 6 months of follow-up post-DP infusion are included. A VOE includes episodes of acute pain with no medically determined cause other than a vaso-occlusion, lasting more than 2 hours and severe enough to require care at a medical facility, a VOE includes acute episodes of pain, acute chest syndrome, acute hepatic sequestration, and acute splenic sequestration; †HbA^{T87Q} expression stabilizes within 6 months; *One death, unlikely related to LentiGlobin, > 18 months post treatment in a patient with significant baseline SCD-related cardiopulmonary disease.
Note: In the last datacut, one patient had a non-serious VOC at Day 107. The event is recorded as an investigator reported VOE but does not meet the definition of a protocol VOE

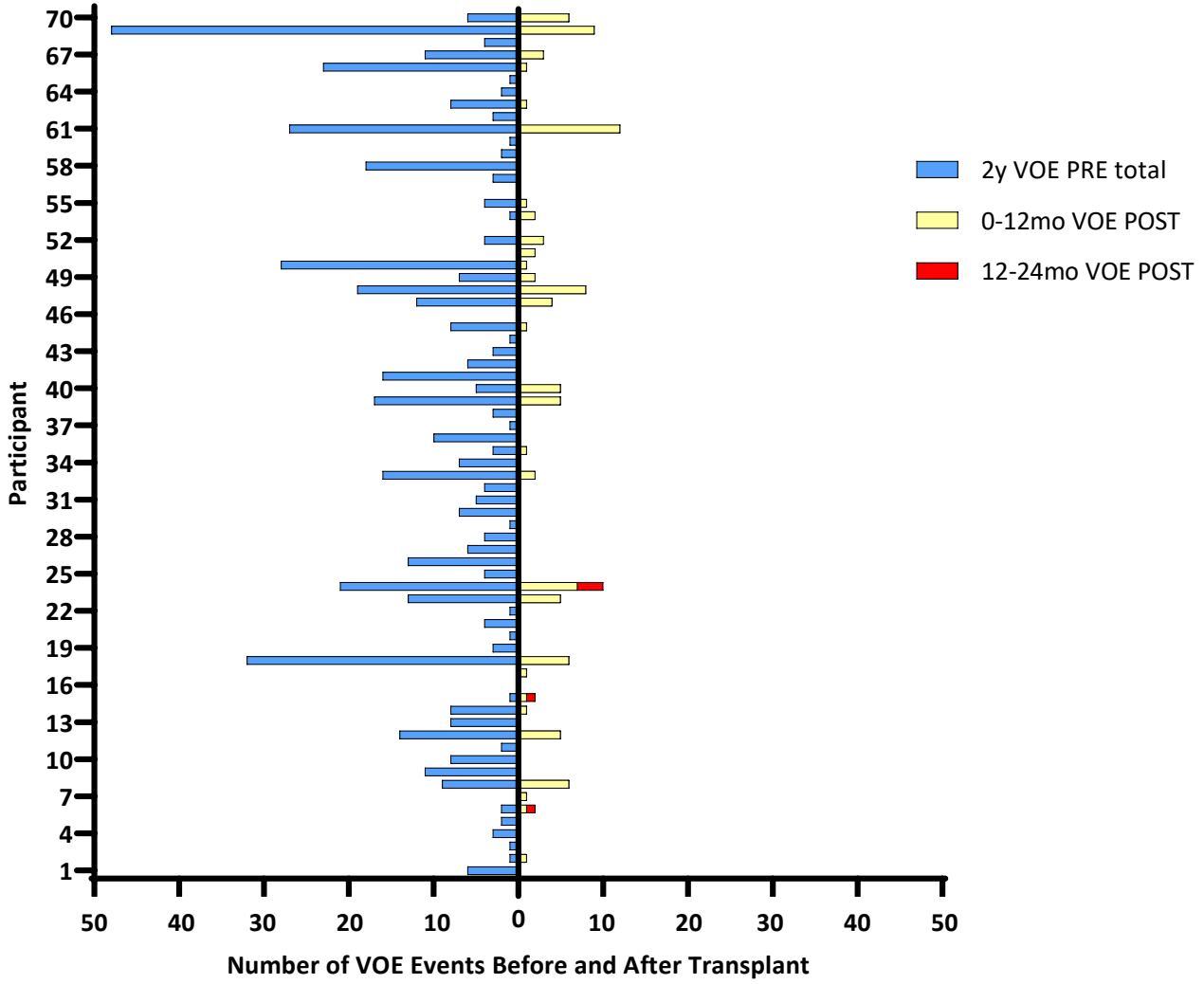
Data as of 20 August 2020

DP, drug product; IC, informed consent; max, maximum; min, minimum; sVOEs, severe VOEs; VOE, vaso-occlusive event; VOC, vaso-occlusive crisis.

Allogeneic transplantation experience at NIH:

Near complete resolution of severe and other VOEs

1 year post-transplantation



American Society of Hematology 2021 guidelines for sickle cell disease: stem cell transplantation

Julie Kanter,[1] Robert I. Liem,[2] Françoise Bernaudin,[3,4] Javier Bolaños-Meade,[5] Courtney D. Fitzhugh,[6] Jane S. Hankins,[7] M. Hassan Murad,[8] Julie A. Panepinto,[9] Damiano Rondelli,[10] Shalini Shenoy,[11] John Wagner,[12] Mark C. Walters,[13] Teonna Woolford,[14] Joerg J. Meerpohl,[15,16] and John Tisdale[6]

Table 2. Outcomes prioritized by the guideline panel

Question	Secondary outcome of interest
Q1: Should individuals with SCD and neurologic injury (overt stroke, SCI, or abnormal TCD) undergo MSD transplantation?	1. Improvement/normalization of TCD velocity 2. Primary ischemic stroke 3. Secondary ischemic stroke 4. New or progressive SCI 5. New or progressive CNS vasculopathy 6. HRQOL 7. Engraftment kinetics
Q2: Should individuals with frequent pain requiring interventions by a health care provider undergo MSD HSCT vs standard of care?	1. Change in frequency of acute pain episodes requiring acute care 2. Change in hospitalization frequency 3. HRQOL 4. Engraftment kinetics
Q3: Should individuals with recurrent episodes of ACS undergo MSD HSCT vs standard of care?	1. Change in frequency of ACS 2. Change in hospitalization frequency 3. Resolution or improvement in chronic lung disease 4. HRQOL 5. Engraftment kinetics
Q4: Should individuals with SCD with an indication for HSCT (as above) who do not have an MSD undergo nonmyeloablative transplantation from alternative donor vs standard supportive care?	Same as Q1-3
Q5: Should individuals with SCD undergoing allogeneic transplantation receive a TBI-based regimen (low-dose TBI ≤400 cGy) or chemotherapy-based regimen?	Same as Q1-3 Additional outcome: potential for fertility post-HSCT
Q6: What is the optimal conditioning regimen for individuals with SCD who have an indication for HSCT and a matched sibling donor (myeloablative transplantation vs reduced intensity or nonmyeloablative transplantation)?	Same as Q 1-3 Additional outcome: Potential for fertility post-HSCT
Q7: Should age be a determining factor for HSCT with MSD for individuals with SCD with the above indication?	Same as above (Q6)
Q8: In pediatric patients with SCD undergoing matched related donor HSCT with available cryopreserved matched sibling cord blood use the cord blood or BM as donor source?	Same as above (Q6)

CNS, central nervous system; HRQOL, health-related quality of life.

Long-term studies are needed to assess the reversibility of disabilities after curative transplantation approaches in sickle cell disease

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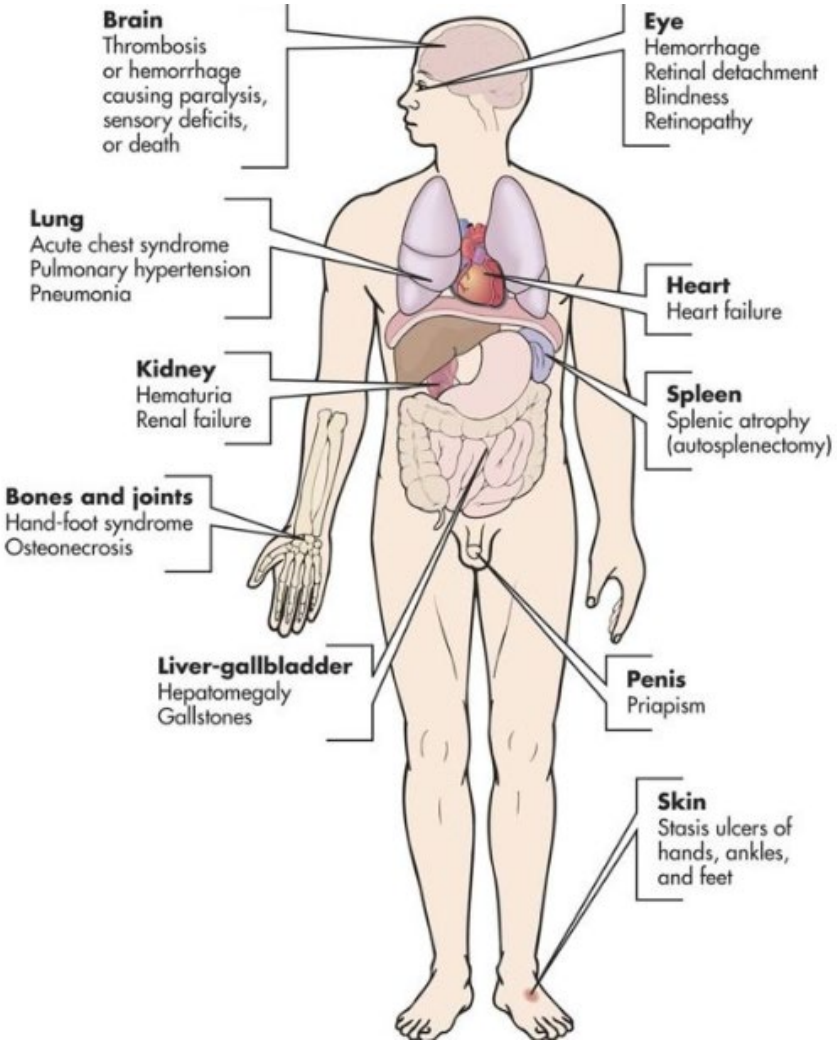

Thoughts

1. Gene therapy trials are now demonstrating efficacy in SCD.
   - Reduction in pain similar to that achieved with allogeneic transplantation.
   - Long-term follow up underway

2. Much of the accumulated organ damage in SCD persists after curative approaches.

3. Application of curative approaches before the onset of organ damage should improve outcomes.

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