GENE THERAPY FOR SICKLE CELL DISEASE

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knowledge changing life

MEDICAL COLLEGE OF WISCONSIN

Conflict of Interest

- None

- Funding
  - National Institutes of Health U24 CA76518
  - National Heart Lung and Blood Institute OT3 HL147741

What is Gene Therapy?

- Gene therapy modifies the expression of a gene
  - With the intent to cure a disease
- Works by several mechanism
  - Replace a disease-causing gene with a healthy copy of the gene
  - Inactivate a disease-causing gene that is not functioning properly
  - Introduce a new or modified gene to treat a disease

Role of the FDA

- Gene therapy products are biological products
- Regulated by the FDA's Center for Biologics Evaluation & Research (CBER)
- All clinical studies must apply for an IND (Investigational New Drug application) prior to initiating trial
- Marketing a gene therapy product requires submission and approval of a BLA (Biologic License Application)

Role of the FDA

- Required to establish a long-term follow up protocol
  - Duration (total): 15 years
  - Establish outcomes, timepoints for assessment, monitoring tests
- Long-term Assessments:
  - Clinical efficacy
  - Durability of the product
  - Complications from exposure
    - Morbidity from chemotherapy/radiation/organ function/malignancy
  - Survival

What is Genotoxicity?

- Genotoxicity is damage to DNA or chromosomal material by an agent
  - If a germ cell is damaged it can cause germline mutation
  - If a somatic cell is damaged it can cause somatic mutation
  - Mutations can lead to cancer – a concern after gene therapy
- Two important follow up testing after gene therapy
    - Integration site analysis – safety
    - Vector copy number – durability
  - Off the shelf gene therapy products are known to trigger immune response in recipients that require immune suppression

Genotoxicity: Efficiency, Durability, Safety

- Efficiency is improved by delivering higher doses of gene-modified hematopoietic stem cells
  - Use of mobilized peripheral blood instead of bone marrow
  - Improve viral vector transduction (e.g., higher titer vector, use serum-free media that support ex vivo HSC survival)
  - Avoid using retroviral vectors with strong enhancer elements that lead to insertional oncogenesis and leukoproliferative complications
    - This has led to use of lentiviral vectors that lack strong enhance elements (self-inactivate during reverse transcription - “SIN” vectors)
    - Significantly reduced risks for causing transformations

Diseases

- Primary immune deficiencies: (T cells, B cells, NK cells)
- Bleeding disorders: Severe Hemophilia
- **Hemoglobinopathy** (RBC): Thalassemia major, **Sickle cell disease**
- Monocytes: X-ALD, MLD, MPS
- Platelets: Wiskott Aldrich syndrome
- Neutrophils: CGD, LAD-1
- Cancer
  - Suicide gene therapy, therapeutic vaccines, anti-angiogenesis

Sickle cell disease

- Single point mutation in the gene coding *β*-globin(*HBB*) leading to production of sickle *HB* and impaired *RBC* function
- Clinical manifestations leading to early death
  - Vaso-occlusive events (pain, acute chest syndrome)
  - Stroke
  - Progressive vasculopathy
  - Chronic hemolytic anemia
- Current treatment option(s): disease modifying without halting disease progression *OR* curative but with severe challenges

Disease Modifying Treatment(s)

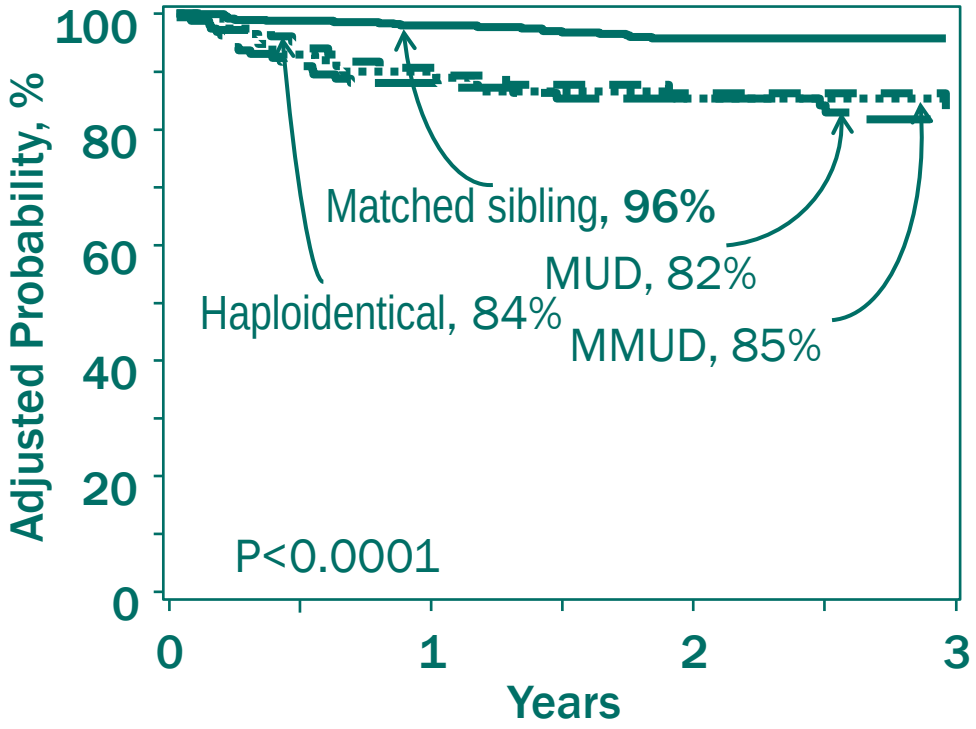
Disease modifying treatments

- Newborn screening, careful management of fever, penicillin prophylaxis, pneumococcal vaccination and other supportive measures = survival exceeds 90% through age 18 years
  - HU approved in 1998; ↓frequency of VOC, transfusion and hospitalization
  - L-glutamine approved in 2017; ↓pain over 48 weeks, 25% reduction vs. placebo (p=0.005)
  - Crizanlizumab approved in 2019; ↓annual rate of pain crisis, 45% lower rate vs. placebo (p=0.001)
  - Voxelotor approved in 2019; ↑Hemoglobin –increase > 1 g/dL, ↑percentage of participants (51%) vs. placebo

Charache S, N Engl J Med 1995; Niihara Y, N Engl J Med 2018; Wang W, Lancet 2011; Ataga K N, Engl J Med 2017; Vichinsky E, N Engl J Med 2019

Allogeneic Transplant

Overall Survival



Higher mortality

Age 13 – 48 years

HR 3.15, p<0.0001

Reduced intensity regimen

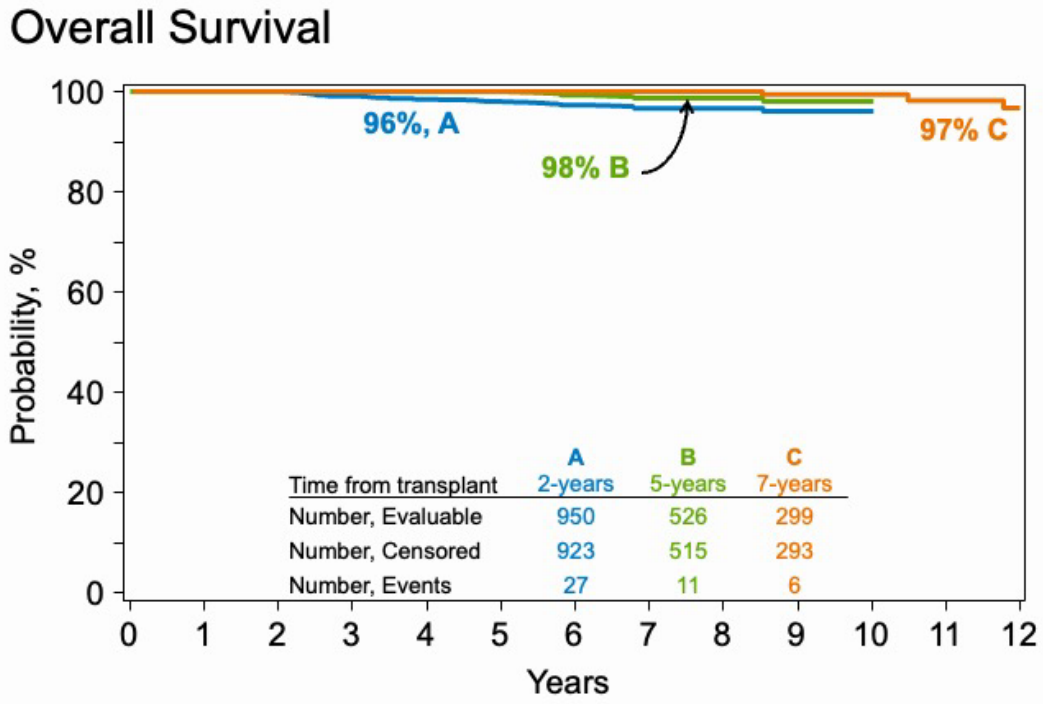
HR 3.79, p=0.006

Myeloablative regimen

HR 4.62, p=0.001

Eapen M, Lancet Haematol 2019

Overall Survival



- Risk factors for late death
  - Age
    - HR 1.75, p=0.0004
  - For every 10-year increment in age, an older patients is 1.75 times to die than a younger patient
  - Alternative donor
    - HR 3.49, p=0.003
  - No difference between alternative donors
  - Chronic GVHD
    - HR 2.18, p=0.08

St. Martin A Transplant Cell Therapy 2022

Causes of Late Death

	Number
Total number	27
Chronic graft vs. host disease	12
Infection without graft vs. host disease	2
Recurrent sickle cell disease	6
Pulmonary hypertension*	1
Intracranial hemorrhage*	1
Multi-organ failure*	1
Acute myeloid leukemia*	2
Not reported	2

*Graft failure with recurrence of sickle cell

Graft failure: loss of donor chimerism to <5% or second transplant

Incidence beyond 2-years:
7% (95% CI 5 – 9)
Higher risk after alternative donor HCT
HR 2.59, p<0.0001

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Considerations for Transplantation

- Deciding whether to recommend HCT for sickle cell anemia is not straight forward
  - CNS (stroke)
  - Abnormal TCD
  - Recurrent vaso-occlusive episodes
- Anxieties regarding age (adults) and suitable donor (*alternative* donor)
    - Does transplantation offer a survival advantage compared to disease-modifying agents?

Survival after HCT – better or worse?

- Recent report of unselected adult Hb SS/Sβ^0/SD from 2 sites
  - 09/2003 – 01/2016 and 08/2004 – 05/2012 *(DeBaun MR Blood 2019)*
  - N=225, median survival, adjusted for left truncation was **48 (95% CI 44 – 58) years**
- Our population of adults, Hb SS/Sβ^0
  - N=216, median survival, adjusted for left truncation was **44 (95% CI 38 – 54) years**
  - *Survival after HCT is at least as good as an unselected population*
  - *Ideal comparator are those eligible for HCT but did not receive HCT*

Gene Therapy

Gene therapy: *BBB HGB 205 and 206*

- First 2 phase I/II trials with BB305 vector (*BBB*)
  - HGB-205 in France; HGB-206 in US
  - Unsatisfactory outcomes with HGB-205 (Grp A) led to HGB-206 (Grp B)
- Newer generation lentivirus (HIV-derived) carrying a β-globin-like gene has allowed effective HSC transduction
- Switch from bone marrow to peripheral blood collection has allowed for higher CD34 cell dose for transduction
- These changes led to HGB-206 (Grp C)

Ribeil JA N Engl J Med 2017; Margin E Blood 2019

BB305 vector: Grp C

- Grp C established for pivotal evaluation of LentiGlobin
  - BB305 lentiviral vector encodes a modified *β*-globin gene, which produces an anti-sickling hemoglobin, HbA^{T87Q}
  - To-date 43 patients enrolled and collected, 35 infused
    - Age 12 – 50 years, Sickle cell genotypes: β^s/β^s, β^s/β^0, β^s/β^+
    - ≥ 4 vaso-occlusive events in the 24 months before enrollment
  - Myeloablative dosing Busulfan (IV), target AUC 5000 µmol x minute
  - Pre-specified follow-up: 24 months
    - *Efficacy endpoint*: complete resolution of severe VOE between months 6-18 post infusion

Kanter J, Walters MC N Engl J Med 2021

BB305 vector: Grp C

- Interim analysis (not prespecified; ITT population)
  - Median follow-up 17 months (range 4 – 38)
  - Achieved stable vector copy number in all patients
  - Median VCN was at least 1.1 copies/diploid genome
  - Median HbA^{T87Q} level was at least 5.1 g/dL
  - Median total Hb level increased from 8.5 to ≥11 g/dL (unsupported)
  - No evidence of hemolysis
  - 3 (12%) patients had severe vaso-occlusive events
  - 1 death (sickle related cardio-pulmonary disease)
  - Anemia/abnormal erythroid precursors in bone marrow (N=2)

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BB305 vector: Grp C

- Conclusion
  - One-time treatment with LentiGlobin led to stable durable production of HbA^{T87Q}
  - HbA^{T87Q} was expressed in ~85% of red cells
  - Reduction in levels of HbS
  - Reduction in key hemolysis markers
  - Minimal fetal Hb production
- Limitations
  - Modest patient number, median follow-up for efficacy ~12 months

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Gene therapy: BCH-BB694 vector

- BCL11A is a repressor of HbF
- BCL11A is knocked down through RNA interference, using a microRNA-adapted short hairpin RNA (shRNAmiR)
- shRNAmiR is inserted into a lentiviral backbone to optimize vector titre
  - Self-inactivating 3rd generation BCH-BB694 lentiviral vector
- Objective: increase fetal hemoglobin (*HbF*) levels in RBC
  - Improved oxygen carrying capacity and prevent sickling

Esrick E N Engl J Med 2021

Gene therapy: BCH-BB694 vector

- Autologous CD34 cells are transduced with BCH-BB694 and infused into the subject
  - At least 4 million CD34 cells/kg; VCN 1.8-6.9 copies/diploid gene
  - Myeloablative condition with IV busulfan
- At publication 6 patients were treated; None recorded VOE post infusion
  - VCN 0.42 – 1.49 copies/diploid gene at 6-months

Esrick E N Engl J Med 2021

Phase II Study of Hematopoietic Stem Cell Transfer Inducing Fetal Hemoglobin in Sickle Cell Disease

PI Dr. David A. Williams
IND 17660
NCT 05353647

GRASP

Gene therapy to Reduce All Sickle Pain

Funded in part by the National Institutes of Health and the California Institute of Regenerative Medicine

Inclusion Criteria

- Sickle genotype: β^s/β^s, β^s/β^0
- Age 13 – 40 years
- Severe disease, defined as:
  - ≥4 VOEs within the past 24 months (VOE defined as acute chest syndrome or pain (with no other medically determined cause VOE)
    - Require a ≥24-hour hospital or ER observation unit visit or at ≥2 visits to a day unit or ER over 72 hours with both visits requiring parenteral opioids
- Must not have an HLA-matched sibling donor

Inclusion Criteria

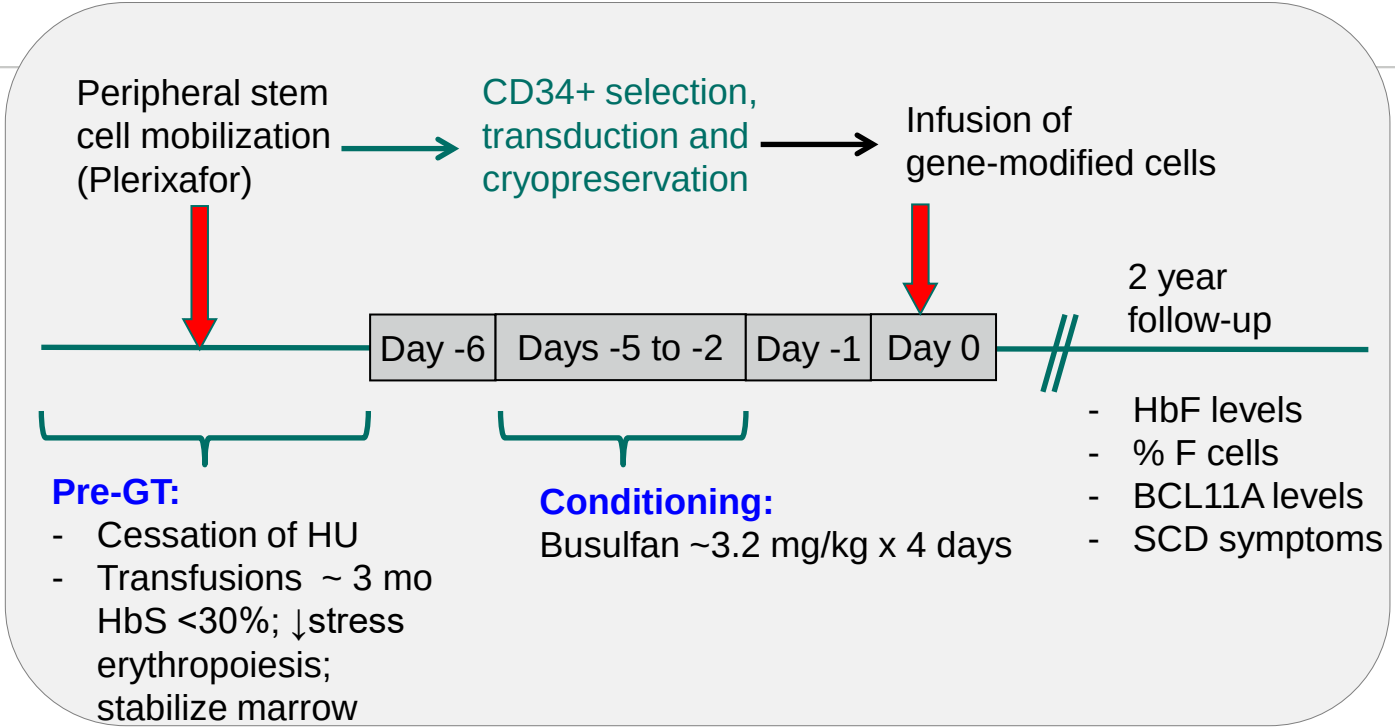
- WBC 2.5 – 25 x 10^9/L, Hb 5 – 11 g/dL, platelets >150 x 10^9/L
- Adequate organ function
  - Renal (calculated creatinine clearance ≥ 60 mL/min/1.73 m^2
  - Hepatic (AST, ALT, Bilirubin <3 times ULN)
  - Pulmonary (DLCO corrected for Hb, FEV1, FVC >50% predicted), cardiac (LVEF >40%)
- Performance score ≥ 80
- Access to EMR

Exclusion Criteria

- Chronic sickle-related pain
- Receiving chronic transfusion for primary or secondary stroke prophylaxis
- Past history of stroke
- Past history of abnormal TCD and were ever on transfusion and subsequently transitioned to hydroxyurea
- Vasculopathy (Moya-moya, occlusion or stenosis of circle of Willis)
- Myelodysplasia or abnormal bone marrow cytogenetics
- Active infection, malignancy
- Hepatic iron overload – with bridging fibrosis/cirrhosis
- Medical or psychiatric illness that would limit compliance with study

Study Design

- Open-label, single arm study
- Plerixafor mobilized CD34 collection
  - CD34 collection dose 10 x 10^6/kg
  - CD34 dose after purification for transduction ≥4 x 10^6/kg
- Infusion of autologous CD34+ HSC cells transduced with the lentiviral vector containing a shRNA targeting BCL11a
- Myeloablative conditioning regimen
  - Busulfan IV x 4 days, target AUC 5000 µmol x minute



CD34 10 X 10^6 CD34+/kg, ≥4 X 10^6 CD34+/kg after CD34 purification for transduction

Primary Endpoint

- Complete absence of VOE (defining VOE as ACS or VOC requiring parenteral opioids) in the period from *month* 6 to 24 after GT as compared to the 24 months prior to consent
  - First 6 months after infusion is excluded from VOE count
- Failure of primary endpoint
  - ≥ 1 severe VOE between month 6 and month 24
  - Failure to engraft (primary graft failure)
  - Initiation of disease modifying agent(s) for prevention/management VOEs
  - Loss to follow up within 24-month period

Secondary Endpoints

- Efficiency of BCL11A knockdown, by estimating the amount of BCL11A protein on peripheral whole blood and sorted erythroid precursors at baseline, 6-, 12-, and 24-months post infusion
- Changes in organ function: *renal and cardiopulmonary*
- Association between baseline socioeconomic status as measured by household material hardship (HMH) and reduction in VOE, and changes in HbA and HbF
- Impact of the BCL11A shmiR gene therapy as measured by HRQoL *domains for* fatigue, pain interference, and sleep

Secondary Endpoints

- Describe the proportion of patients who are absent all the following events at month 24 post-infusion:
  - Death
  - Cerebral vascular events, including strokes
  - Graft failure (requiring rescue with back-up CD34 cells)
  - Lack or loss of engraftment of gene-modified cells as determined by VCN <0.1 copies per cell in peripheral blood MNC at 6 months
  - MDS, AML or other leukemia
  - Presence of replication competent lentivirus

Follow up Beyond 2 years

Long-term Assessment: Measuring Cure

- *Endpoint*: Assessment of Gene Therapy
  - *Measures*: VCN in infused product measured at 0.6, 1, 2, 5, 10 and 15 years
  - *Grades of cure*
    - 100% genetically corrected RBC (**1**), 75-99%% (**2**), 50-74%% (**3**), 25-49% (**4**) improvement and 0-24% (**5**)
- *Endpoint*: Engraftment of Genetically modified RBC
  - *Measures*: Chimerism
    - >50% grade 1
    - >20% grade 2

Farrell A et al. Blood Adv 2019

Long-term Assessment: Red Blood Cell

- *Endpoint*: Anemia and Hemolysis
  - *Measures*: Hb, Retic count, Epo, LDH, sTIR, haptoglobin, hemopexin, cell free Hb, RBC microparticles
- *Endpoint*: Erythropoiesis and Genetically modified RBC
  - Measures: RBC count, Epo, soluble transferrin receptor, Hb electrophoresis, % anti-sickling Hb, cellular distribution of % anti-sickling Hb (e.g., Hb F)
- *Grades of cure*
  - Complete (**1**), 75% (**2**), 50% (**3**), 25% (**4**) improvement and none (**5**)

Farrell A et al. Blood Adv 2019

Long-term Assessment: Organ function

- *Renal*:
  - *GFR, albuminuria, serum creatinine, initiation of renal replacement treatment (dialysis or renal transplant)*
- *Cardiac*:
  - *Systolic/diastolic BP (change in 5 mmHg), pulmonary hypertension (composite: TRV, NT-pro-BNP, 6 minute walk distance)*
- *Pulmonary*
  - *ACS, asthma, FEV1, oxygen requirement*
- *Thromboembolism*
  - *Pulmonary or VTE*

Farrell A et al. Blood Adv 2019

Long-term Assessment

- *Secondary neoplasm*:
  - MDS, AML, other leukemia, solid tumor
- *Survival*:
  - *Alive/dead; cause of death*

Curative Treatment: Sickle Cell Disease

Morbidity and mortality with any treatment
Economic burden
Does any treatment offer survival advantage?