



Plan to Cure or Ameliorate Sickle Cell Disease (SCD), Parkinson's Disease (PD), and other diseases by Gene Regulation Therapy and Iron Management



Research steps

Stage 1: Basic Research

Goal: Elucidate the cellular & molecular controls mediating developmental hemoglobin switching, *in vitro*, *in vivo* in human & primate cell models, and *in vivo* in transgenic mice.

Goal achieved.

Funding: NIH, Res.Corp. PHF, AHA, other (OMRF, 1999&2005)

Time-frame: 1970-2005 **Funds expended:** ca. \$2.5M

Publications: #s 1 – 27 (attached)



Stage 2: Pre-clinical Research

Goals: (1) Use HbSw factors (esp. Ferritin-H [FtH]) to repress the Sickle Cell gene, i.e., the human adult β -globin gene, **and** to activate fetal γ -globin genes in its place, in SCD red cell precursors.

Goal achieved.

(2) Use FtH and/or inducers of the *FtH* gene to restore normal iron balance in SCD & models of PD and cancer.

In progress.

Funding: OMRF/OUHSC, NIH/NCI ca. \$615K

Time-frame: 2005- 2010

Publications: #s28-32 (below)



Stage 3: Patents:

(1) “*Gene Regulation Therapy Involving Ferritin*” (**issued** Europe, Australia, 2006; U.S., 2009)

Priority date: 1 Nov. 2000

(2) “*Abscissic Acid and Derivatives Thereof for the Treatment of Diseases*” (**issued** U.S., 2010)

Priority date: 4 March 2005

(3) “*Method for Regulating Production of Hemoglobin Beta Chains*” (CIP (1), pending, U.S.)

Funding: OMRF, 2000-08, \$87,000; SCCF, \$49,000



Stage 4: Pre-Clinical Safety & Efficacy Trials in Animals
(for SCD and PD, simultaneously if possible)

Goals: (1) Determine if FtH is safe in mice, using a 4-log range of doses of the pure protein.
(2) Determine the efficacy of FtH for **HbSw** in mice when FtH is delivered as a protein.
(3) Determine if ABA is safe in mice, using a 4-log range of doses.
(4) Determine the efficacy of ABA as an inducer of FtH expression, especially in BM, spleen & brain.

Main method: *Gene arrays* to measure expression of all genes in 10 tissues for different doses & times; qRT-PCR to quantify human and mouse ferritins and mouse Hb genes.

Addtn. methods: Motor activity by rotorod &/or exercise wheel.

Toxicity measures: Gene arrays, motor activity, death

Funding needed: \$1,600,000

Probable funding sources: *Foundations* + *Matching by* OARS, NIH (incl. SBIR/STTR grants, RAID); for-profit partners will also be considered at this stage, as well as stages 5 & 6.



Stages 5 & 6: Phase I, II, and III Clinical Trials in Human SCD Patients (efficacy & safety)

Req'd: Source of pharmaceutically pure FtH and ABA in large quantities.

Adequate clinical facilities & personal for in-patient trials (Phase I).

Funding: NGOs, SBIR/STTRs, Orphan Drug, pharmaceutical collaborations, totalling \$17.3M

II. References cited – Publications leading to and describing the discovery

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- (16) Broyles RH, Barker-Harrel J, Ramseyer LTH, McBride KA, Sexton DL. Erythroid heterokaryons: a system for investigating the functional role of trans-acting factors in developmental hemoglobin switching. **Prog Clin Biol Res** **316B**: 83-96 (1989).
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- (18) Kurien BT, Broyles RH. Plasmid DNA preparation by heat treatment of *Escherichia coli* lysates. **Anal Biochem** **213**: 174-176 (1993).
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- (30) **Broyles RH, Belegu V, Roth AC, Clarkson EJ, Williamson KS, Stewart CA, Pye QN, Floyd RA, Jani K, Trudel M, Santambrogio P, Levi S, Arosio P, Cain JP. Ferritin heavy chain stimulates HbS-to-HbF switching in erythroid precursor cells from sickle cell patients. Blood 108: 790a (2006).**
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- (32) Broyles RH, Belegu V, Roth AC, Clarkson EJ, Williamson KS, Stewart CA, Pye QN, Floyd RA, Jani K, Trudel M, Santambrogio P, Levi S, Arosio P, Cain JP. Ferritin heavy chain stimulates HbS-to-HbF switching in erythroid precursor cells from sickle cell patients. **Blood [In preparation]**

International presentations:

1. Oxford University, Oxford, England, UK; International Hemoglobin Switching Conference, October, 2004.
2. Prague, The Czech Republic; International Biolron World Congress, May, 2005.
3. Memphis, Tennessee, USA; SCDA/NIH Sickle Cell Centers 30th Anniversary Meeting, April, 2006.
4. Orlando, Florida, USA; American Society of Hematology, December, 2006.
5. Kyoto, Japan; International Biolron World Congress, April, 2007.
6. New Orleans, Louisiana, USA; Sickle Cell Disease Association of America, September, 2008.
7. Porto, Portugal; International Biolron World Congress, June, 2009.