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GENE REGULATION THERAPY FOR SICKLE CELL DISEASE UTILIZING FERRITIN HEAVY CHAIN

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We have found that ferritin heavy chain (FtH), an antioxidant/stress response/iron-storage protein, localizes to the nucleus in K562 cells and represses the human adult beta-globin promoter in transient assays in primate cells (Broyles et al., *PNAS* **98**: 9145, 2001). The nuclear localization of FtH (but not ferritin light chain, FtL) has been confirmed in several laboratories for several cell types, and at least two groups are investigating the nuclear transport mechanisms. During development of all vertebrates including humans, FtH is expressed in high amounts in embryonic erythroid cells but at only transient, very low levels in adult erythroid cells where FtH disappears at the beginning of erythroid differentiation. Since other work indicates FtH is a gene activator of fetal-globin genes and we have found that FtH repressed adult beta-globin, we hypothesize that FtH is a long-sought developmental hemoglobin (Hb) switching factor and that delivery of FtH to human adult erythroid cell precursors will reverse the phenotype to HbF, preventing sickling and offering a phenotypic cure for sickle cell disease (SCD). Chromatin immunoprecipitation (ChIP) assays show that FtH is bound *in vivo* to the previously mapped

-153/-148 CAGTGC promoter site of the adult beta-globin gene, in K562 cells in which the beta-globin gene is repressed. Conversely, treatment of K562 cells with an antisense oligonucleotide to FtH relieves the beta-globin repression. Interestingly, the FtH antisense, which knocks down FtH expression by 90%, also knocks down fetal gamma-globin expression by 90%, confirming the previous suggestion that FtH is an inducer of fetal globin expression. Construction of FtH transgenic mice, in which the regulated FtH transgene is expressed in adult Hb-producing definitive erythroid cells, yield founder mice born with reduced beta-globin expression and a mild beta-thalassemia, confirming that FtH acts as a beta-globin gene repressor protein *in vivo*. Mice are born with as many as 50% target cells, an indicator of beta-chain reduction and alpha chain excess. With erythroid precursor cells from pediatric SCD patients, under an IRB-approved protocol, we have used a two-phase culture system for *in vitro* maturation of erythroid cells in the presence of FtH, delivered to the cells as pure protein, as an expression plasmid, or as a priority inducer compound that activates the endogenous FtH gene. HPLC with a PolyCAT A column was used to separate and quantify human Hbs. With each mode of delivery, FtH stimulated a complete switch from HbS to HbF. This result was repeatable in five experiments using erythroid precursor cells from three different SCD donors, as well as in experiments using erythroid precursors from normal donors. FITC-labeled recombinant human FtH protein was taken into red cell precursors in culture, suggesting that the purified protein can be directly delivered without gene therapy. This method of producing a phenotypic cure in SCD patients should be easy and inexpensive to deliver *in vivo*. Funded in part by *The Sickle Cell Cure Foundation, Inc.*

Certification for Human Subjects: I certify that this study abides by the rules of the appropriate internal review board and the tenets of the Helsinki protocol

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