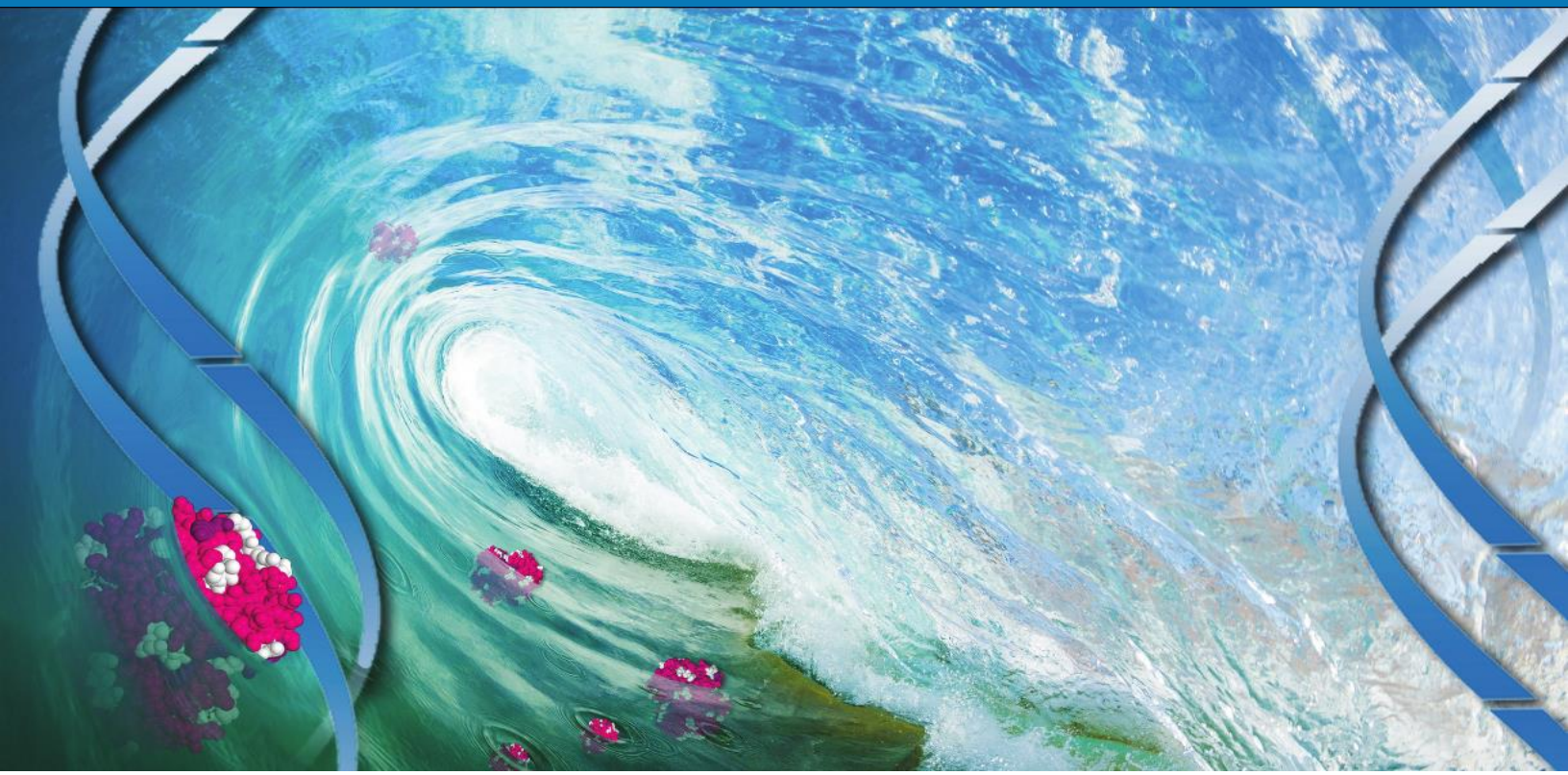
TENTH COOLEY'S ANEMIA SYMPOSIUM



Poster Session Abstract Booklet

October 18 – 22, 2015
Loews Chicago O'Hare Hotel
Rosemont, Illinois

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Poster Sessions

October 20th, 5:00 PM – 6:00 PM
October 21st, 12:30 PM – 1:30 PM

The New York Academy of Sciences

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Leading the Fight Against Thalassemia

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Tenth Cooley's Anemia Symposium Travel Awards

The following awardees were chosen for travel fellowships, made possible by generous support from the Cooley's Anemia Foundation and National Institutes of Health Award Number 1R13HL129737-01, on a competitive basis by the Scientific Organizing Committee from submitted abstracts:

Sachith Mettananda, MD
Weatherall Institute of Molecular Medicine, University of Oxford

Faisal Reza, PhD
Yale University, School of Medicine

Orapan Sripichai, PhD
Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University

Andrew Wilber, PhD
Southern Illinois University School of Medicine and Simmons Cancer Center

Tenth Cooley's Anemia Symposium Short Talk Recipients

The following presenters were chosen for oral presentation on a competitive basis by the Scientific Organizing Committee from submitted abstracts:

Suean Fontenard, BS
University of Alabama at Birmingham

Fyodor D. Urnov, PhD
Sangamo Biosciences

Andrew Wilber, PhD
Southern Illinois University School of Medicine and Simmons Cancer Center

Tenth Cooley's Anemia Symposium Poster Abstracts

1. *Implementation of an Innovative Transition Navigator Role to Support Adolescents and Young Adults with Thalassemia*

Brooke Allemang, MSW, RSW[1,2], Colleen Johnson, MN[2], Richard Ward, MD[2], Melina Cheong, MN[1], Suzan Williams, MD[1]
[1]The Hospital for Sick Children, Toronto, Ontario, Canada
[2]Toronto General Hospital, Toronto, Ontario, Canada

Transition from pediatric to adult healthcare coincides with various life changes that adolescents and young adults (AYA) face alongside maintaining a chronic health condition. It has previously been demonstrated that AYA with red blood cell disorders, such as thalassemia, have poor health and social outcomes when transition is poorly coordinated. Lack of engagement with adult providers, an increase in complications following transfer of care, and a loss of follow-up have all been identified as factors which lead to poor transition outcomes. In an attempt to mitigate these risks, improve patient satisfaction and provide continuous care to AYA with thalassemia, an innovative transition navigator (TN) role was implemented. This position is the first of its kind to be established amongst this population. Reflective of the collaborative partnership between the pediatric and adult programs, the TN has staff appointments at both SickKids and Toronto General Hospital. The TN provides support to the largest cohort of patients with thalassemia in Canada, having administered transition readiness surveys to 49 patients between 12–18 years and creating tailored transition plans to meet their needs. An overview of the various components of the transition program, including transition preparation, a monthly transition clinic, and post-transfer follow-up will be presented. Interim analysis of patient satisfaction data will be shared along with discussion about lessons learned in forming and implementing this quality improvement project. The next step in evaluating this project involves a retrospective chart review assessing the impact of the transition program in its entirety on health care utilization.

2. *Screening Prevalence of β-thalassemia in Medical Students from Karachi, Pakistan through NESTROF Test*

Afsheen Arif, PhD[1], Sitwat Zehra, PhD[1], Akbar Agha, MD, MPhil[2], Abid Azhar, PhD[1]
[1]The Karachi Institute of Biotechnology and Genetic Engineering, KIBGE, University of Karachi, Karachi, Pakistan
[2]Director, Dow Institute of Hematology (DIH), Dow University of Health Sciences, Karachi, Pakistan

β-thalassemia is one of the most commonly inherited disorders in Pakistan, according to Thalassemia Federation of Pakistan (TFP), its prevalence rate is 6%. β-thalassemia is a group of hereditary blood disorders characterized by defect in the synthesis of the beta globulin chain. Realizing the fact that the only way to control β-thalassemia is prevention, the present study was carried out to screen β-thalassemia trait among medical students under Dow-Thalassemia Awareness Program (DOW-TAP). In Pakistan every year 5,000 babies are born with thalassemia; where 5/100 people are thalassemia patients and around 8 million populations is carrier for thalassemia. Ahmed et al (2010) has reported that approx. 5–7% carrier rate, with 9.8 million carriers in the total Pakistani population. This is an observational study and the sampling technique was random. An awareness camp was designed in medical colleges under DOW-TAP. Total 915 medical students from DMC and SMC voluntarily gave their blood samples which were analyzed by NESTROFT test, CBC and confirmed by HPLC. A total of 42.6% samples were found positive with NESTROFT test. The CBC showed 39.2% subjects were positive for IDA remaining 27.6% were selected for HPLC for the confirmation of β-thalassemia trait. Only 2.4% subjects were confirmed for β-thalassemia trait. The overall prevalence for β-thalassemia is 2.4% compared to 1.48%–3.64% in previous studies. The study also demonstrates 17.59% IDA cases in normal population. It also suggests that primarily NESTROF can be used to rule out the healthy population more than 50% for further screening of β-thalassemia.

3. *Thalassemia Transition Program "Step-Up": Seven-Year Outcomes*

Sherif M. Badawy, MD, MBBCh[1], Alexandra L. Batts, MPH[1], Janice Beatty, BSN[1], Diane Calamaras, MSN, CPNP[1], Stephanie A. Pelligra, MPH, A. Kyle Mack, MD[1], Alexis A. Thompson, MD, MPH[1]
[1]Department of Pediatrics, Division of Hematology, Oncology and Stem Cell Transplantation, Ann & Robert H. Lurie Children's Hospital of Chicago, Feinberg School of Medicine at Northwestern University, Chicago, Illinois, United States

Background: The lifespan of patients with thalassemia has increased due to advances in monitoring and treatment. Adolescents with thalassemia face challenges with transitioning from child-centered to adult-oriented care. Lurie Children's Hospital developed "Step-Up", a thalassemia transition program in 2008, to prepare adolescents to manage thalassemia as adults. Step-Up is a 4-year multidisciplinary-program that includes: pre/post knowledge tests, annual checklists, education handbooks and individualized care plans.

Objective: To report the uptake, progress and clinical outcomes of the Step-Up program.

Methods: Retrospective-prospective data collection from program assessments and participants' electronic medical records. Data were compared to a cohort of non-participants.

Results: Step-Up had 23 participants: 14 currently enrolled and 9 graduates. Participant mean age was 18.8(3.24) years, 61% female and 74% Asian. Frequent knowledge deficits included: inheritance, pre-transfusion hemoglobin, ferritin results and complications; overall knowledge improved in post-test in all patients. Adherence to recommended annual MRI R2/T2* and bone scan was 100%. Mean appointment "no-show" rate was 3.5% for current enrollees, 6.6% for non-participants. Enrollees, graduates and non-participants had a mean serum ferritin level of 1676, 1920 and 3368ng/mL, and mean liver iron contents (MRI-R2*) of 8.9, 6.7 and 15.5 mg/gdw, respectively. Average Cardiac-T2* values were normal for all subsets. All Step-Up graduates are current students or college graduates with full-time employment; 56% have private insurance compared to 29% for non-participants.

Conclusions: Early results show improved iron control, better adherence with high vocational achievement in Step-Up participants. This suggests that enhanced education, surveillance and individualized plans may contribute to improved outcomes in thalassemia.

4. *The Next Step for STEP-UP, the Individualized Care Plan*

Janice R. Beatty, BSN, Diane M. Calamaras, MSN, CPNP, Alexandra L. Batts, MPH, Crystal Roach, MSW, LCSW, A. Kyle Mack, MD, Alexis A. Thompson, MD, MPH
Division of Hematology/Oncology/SCT, Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, United States

The transition process to adult-centered care is a difficult challenge facing pediatric providers caring for adolescents with complex diseases like thalassemia. Thalassemia is a severe inherited blood disorder, often requiring life-long blood transfusions with subsequent transfusional iron overload and multi-system complications.

In 2008, Lurie Children's Hospital developed STEP-UP, a 4 year program teaching adolescents to develop independent adult skills and self-efficacy in managing healthcare needs. Results from the initial years of the program showed knowledge gaps and adherence to treatment worsened during adolescence continuing into young adulthood.

To improve treatment adherence and address knowledge gaps, a thalassemia-specific Individualized Care Plan (ICP) was developed. The ICP addresses patient-determined goals/barriers in 6 key areas: Transfusion, Iron Management. Specialty Care, Social, Educational/Vocational and Access to Care. During a transfusion or clinic appointment, the nurse practitioner, social worker or program coordinator meets with an individual to develop an ICP specific to that individual's disease manifestations and unique needs. Actions to address barriers for each of the areas are defined with input from the patient.

ICPs were implemented in 2013 as an additional STEP-UP program tool and are also utilized to facilitate comprehensive care coordination for our young adults (ages 18 – 30 years). Plans are reviewed annually and updated as needed.

This self-management tool has been implemented with 25 individuals; all STEP-UP enrollees and 75% of our young adults.

We believe the ICP is a novel addition to the STEP-UP program and it will be an effective, individualized way to improve treatment adherence and self-efficacy.

5. *Transfusion and Transplantation Therapies in Humanized Mouse Models of Cooley's Anemia*

Michael Berlett, BS[1], Yongliang Huo, PhD[1,2], Jonathan Lockhart, BS[1], Shanrun Liu, PhD[1], Suean Fontenard, BS[1], and Thomas M. Ryan, PhD[1]
[1]University of Alabama at Birmingham, Birmingham, Alabama, United States
[2]Vanderbilt University, Nashville, Tennessee, United States

Humanized mouse models of Cooley's Anemia (CA) were generated by targeted gene replacement of the adult mouse α- and β-globin genes with human α-, γ-, and nonfunctional β^0-globin genes. Homozygous CA mice synthesize solely human fetal hemoglobin (Hb F) at birth and succumb to lethal anemia before weaning with a mean postnatal survival of around two weeks of age.

The lifespan of humanized CA mice can be extended into adulthood with regular red blood cell (RBC) transfusion therapy. Transfusions are initiated during the first few days of life by either intraperitoneal or facial vein injection of packed RBCs. Weekly retro-orbital RBC transfusions are used to maintain CA mice into adulthood. Regularly transfused male and female CA mice are healthy enough to breed and produce entire litters of homozygous CA mice for study.

Homozygous CA mice are cured by myeloablative bone marrow (BM) transplantation. The transplantation of donor BM cells isolated from GFP transgenic mice results in >99% GFP positive RBCs for the remainder of the life of the animal; however, the animals are no longer fecund. We have developed a novel allogenic transplantation strategy that can cure CA mice by establishing low-level hematopoietic chimeras in the absence of any cyto-reductive conditioning. A single injection of GFP transgenic BM cells into CA pups results in long-term hematopoietic chimerism that is capable of reconstituting greater than 90% of the erythron without graft rejection, graft versus host disease, or the loss of fecundity.

6. *Transitioning Care of Adult Patients with Thalassemia*

Vanessa Nixon Carrion, RN, CPN and Lisa A. Bjorkelo, MSN, RN, CPON, BMT-CN
The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States

Over 150 patients are followed at the Thalassemia Program at The Children's Hospital of Philadelphia (CHOP). Advances in transfusion medicine and chelation have contributed to the increased lifespan of this patient population. Age-appropriate care for adults can be challenging in a pediatric facility. All thalassemia care, including blood transfusions, chelation, clinic visits, specialized MRI testing, dexascan, audiology and ophthalmology, remained at CHOP until a partnership with the Hospital of the University of Pennsylvania (HUP) was established in 2012. The idea of transitioning to an adult care facility initially was viewed negatively by adult patients. Issues identified included: Concerns about adult providers; level of expertise in thalassemia care; abandonment issues; transfusion, insurance and intravenous access concerns. In this poster we will discuss the processes we developed to insure a smooth transition to an adult institution for our patients with thalassemia. We will review our transition program's progress thus far, including our challenges, as well as our vision for the future.

7. *Retinal Abnormalities in Beta-Thalassemia Major*

Devang L. Bhoiwala, BS[1,2] and Joshua L. Dunaief, MD, PhD[1]
[1]Scheie Eye Institute, Philadelphia, Pennsylvania, United States
[2]Albany Medical College, Albany, New York, United States

Patients with beta (β)-thalassemia have a variety of complications that may affect all organs, including the eye. Ocular abnormalities include retinal pigment epithelium degeneration, angioid streaks, venous tortuosity, night blindness, visual field defects, decreased visual acuity, color vision abnormalities, and acute visual loss. Patients with beta- thalassemia major are transfusion dependent and require iron chelation therapy (ICT) in order to survive. Retinal degeneration may result from either retinal iron accumulation due to transfusion induced iron overload or retinal toxicity induced by ICT; patients who were never treated with ICT exhibited retinopathy, and other patients receiving ICT had chelator-induced retinopathy. This literature review will focus on retinal abnormalities present in individuals with beta-thalassemia major, viewed in light of new findings on the mechanisms and manifestations of retinal iron toxicity.

8. *A Case Report of Patients Affected by Dominant Beta Thalassemia Mutation*

Susan M. Carson, MSN, CPNP, Anne Nord, BSN, RN, Thomas C. Hofstra, MD, and Thomas D. Coates, MD
Children's Hospital Los Angeles, Los Angeles, California, United States

Beta thalassemia is a red cell defect caused by an autosomal recessive mutation located on chromosome 11, position 6. People who are heterozygous usually have mild to no anemia, microcytosis and do not require treatment except for genetic counseling. Patients who are homozygous, or compound heterozygous have varying degrees of anemia ranging from moderate to life threatening requiring life-long chronic blood transfusions.

We have identified two beta zero thalassemia mutations in several of our patients of Hispanic and Italian descent, which are causing anemia despite being inherited in the single heterozygous fashion. Alpha globin gene triplication was done in the patients to rule out excessive alpha chains as a cause of the anemia.
We have collected more than 10 patients with Codon 39(C-T) and the IVSI-1(G-A) mutation whose clinical picture ranges from hemoglobins < 10 g/dl to hemoglobins of 6 g/dl – requiring blood transfusions. We will describe the case of 2 sisters who were stable until pregnancy when they developed life threatening anemia, splenomegaly and required transfusions. We will analyze their iron status and report on level of iron overload and chelation. We are following the younger patients regularly to monitor for signs of iron overload and worsening anemia. The more severe patients are being managed with transfusions (if indicated) and chelation. The natural history of these patients is not currently known. Long term follow up of the younger patients will hopefully provide more insight.

9. *Microparticles Induced Hemostatic Changes in Thalassemia*

Pornthip Chaichompoo, PhD[1], Wasinee Kheansaard, MSc[2], Kunwadee Phongpao, MSc[2,4], Phatchanat Klaihmon, MSc[3], Suthat Fucharoen, MD[2], Kovit Pattanapayasat, PhD[3], and Saovaros Svasti, PhD[2,4]
[1]Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok, Thailand
[2]Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhon Pathom, Thailand
[3]Office for Research and Development and Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[4]Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand

Thromboembolic complications have been documented in β-thalassemia patients. The mechanisms of thromboembolic events in patients are associated with chronic platelet activation and endothelial dysfunction. Damaged RBCs and activated platelets are reportedly released microparticles (MPs), which enhance procoagulant activity and vascular dysfunction in several diseases, into circulation. Splenectomized β-

thalassemia/HbE patients had increased levels of MPs compared with normal individuals. Herein, the effects of MPs on hemostatic changes including *ex vivo* study on platelets and in vitro study on endothelial cells were investigated.

Co-incubated MPs with whole blood showed increased platelet activation in MPs origin- and dose-dependent manner. Splenectomized-platelets treated with splenectomized-MPs at pathophysiological conditions (platelet:MP ratio 1:10) had significant increased %P-selectin+ platelets than normal-MP-treated platelets (9.5±3% and 5.2±2.7%, respectively) ($P<0.05$). Splenectomized-MPs had also increased platelet-neutrophil and platelet-monocyte aggregation when normalized with individual spontaneously aggregation (3.9±1-fold and 5.7±0.2-fold increases, respectively) compared to normal-MPs treated blood samples (1.9±0.1-fold and 1.4±1-fold increases, respectively) ($P<0.05$). Moreover, 2-fold increases in platelet aggregation compared to 1.5 µM ADP-treated platelets were observed by aggegrometer. Interestingly, HUVEC treated with splenectomized-MPs resulted in dose dependent increased expression of adhesion molecules; ICAM-1, E-selectin and VCAM-1. At 5x106 particles/mL splenectomized-MP-treated HUVEC had showed 9.5±1.7-fold increases of ICAM-1 expression ($P<0.001$). Additionally, the ICAM-1, E-selectin and VCAM-1 mRNA levels were increased in splenectomized-MP-treated HUVEC compared to untreated HUVEC ($P<0.05$). In conclusion, splenectomized-MPs could enhance activation and aggregation of platelets and adhesion molecule expression of endothelial cells, consequently thrombus formation. This suggested that MPs play important roles on thrombosis and vascular biology in thalassemia.

10. *A Long Noncoding RNA Expressed in Maturing Erythroid Cells Mediates Alternative Splicing of Fas Receptor Pre-mRNA and Resistance to Apoptosis*

Christopher B. Chambers, PhD[1], Olga Villamizar, PhD[1], Yin-Yuan Mo, PhD[2], Janice M. Riberdy, PhD[3], Derek A. Persons, MD, PhD[3], and Andrew Wilber, PhD[1,4]
[1]Southern Illinois University School of Medicine, Springfield, Illinois, United States
[2]University of Mississippi Medical Center, Jackson, Mississippi, United States
[3]St. Jude Children's Research Hospital, Memphis, Tennessee, United States
[4]Simmons Cancer Institute, Springfield, Illinois, United States

Erythropoiesis is tightly controlled to ensure red blood cell production and oxygen transport to tissues. Thus, homeostasis is critical and maintained by competitive outcomes of pro- and anti-apoptotic pathways including Fas receptor (Fas)-Fas ligand (FasL) interaction. This program is skirted through production of soluble Fas (sFas), a splice product of Fas pre-mRNA created by exclusion of exon 6, which can bind FasL to inhibit apoptosis. We isolated RNA from erythroid cells derived from fetal liver, umbilical cord blood and adult bone marrow hematopoietic progenitors and screened a focused array of long noncoding RNAs (lncRNAs); sequences >200-bp that interact with other RNAs, DNA and proteins to regulate gene expression. We found the antisense Fas transcript (termed Saf) was induced during maturation and transcriptionally regulated by GATA-1 and KLF1. Biochemical studies revealed that Saf is localized in the nucleus and directly interacts with: i) Fas pre-mRNA forming RNaseA-resistant dsRNA at regions that flank exon 6 and ii) human splicing factor 45 (SPF45). Knockdown of SPF45 impaired production of sFas (37 + 4 pg/mL, SPF45 kd) versus (123 + 3 pg/mL, control) and increased sensitivity to Fas-mediated apoptosis (52 + 2%, SPF45 kd) versus (37 + 3%, control) when cells were exposed to the Fas activating antibody CH11. Overexpression of Saf in SPF45 knockdown cells failed to rescue sFas production supporting the hypothesis that Saf and SPF45 co-participate in modulating Fas pre-mRNA splicing. These studies reveal a novel mechanism to modulate this critical cell death program by an lncRNA and its protein partner.

11. *An Exploratory Analysis of the Complement System in β-Thalassemia Major*

John Chapin, MD[1], Hunter Terry[1], Dorothy Kleinert, NP[2], Sujit Sheth, MD[2], Patricia-Jane Giardina, MD[2], and Jeffrey Laurence, MD[1]
[1]Weill Cornell Medical Center-New York Presbyterian Hospital Department of Medicine, Division of Hematology-Oncology, New York, New York, United States
[2]Weill Cornell Medical Center-New York Presbyterian Hospital Department of Pediatrics, Division of Hematology-Oncology, New York, New York, United States

Homozygous β-thalassemia major and intermedia are characterized by multiple vascular co-morbidities as a consequence of chronic hemolysis and surgical asplenia, which result in circulating free heme/hemoglobin and red cell fragments, and thrombocytosis. Manifestations include pulmonary hypertension, thrombosis, and leg ulcer formation, all of which are more severe in non-transfusion-dependent-thalassemia (NTDT) patients. Complement proteins cause red cell hemolysis, and deficiencies in complement regulatory proteins have been described in β-thalassemia. Free heme has also been identified as a C5 convertase. We sought to characterize complement activation in transfusion-dependent-β-thalassemia (TDT). Adult patients (>18 years old) with TDT were enrolled. Peripheral blood was collected prior to transfusion and 30–60 minutes post-transfusion. Complement antigens (C5b-9; MAC, C5a, MASP-2), activity (CH50), and free heme were measured. The electronic medical record was reviewed for related clinical and laboratory parameters. MAC was significantly elevated in pre-transfusion TDT compared to controls. CH50, C5a, and MASP-2 were not significantly elevated. Both C5a and MAC significantly decreased by 20% post-transfusion. Free heme increased post-transfusion. An inverse correlation was observed between thalassemia severity score and CH50 activity. As a clinical correlation, two patients with CH50 activity of 0 died of cirrhosis during the study period. The complement system is activated in TDT, and transfusion reduces complement activity. It is likely that NTDT patients will have more marked abnormalities in complement. CH50 may be a useful marker to identify TDT patients with advanced disease severity. More complement studies are needed in thalassemia in the era of anti-complement therapies.

12. *New Hepatitis C Treatments Are Safe and Effective in Transfusion-Dependent Thalassemia*

John Chapin, MD[1], Dorothy Kleinert, NP[2], Sujit Sheth, MD[2], and Patricia-Jane Giardina, MD[2]
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Hepatitis C virus (HCV) is a significant source of morbidity and mortality in transfusion-dependent thalassemia (TDT), especially since iron overload may potentiate progression to hepatic fibrosis. Prior therapies included interferon and ribavirin, which had significant side effects including hemolysis. Furthermore, these treatments required prolonged duration of 24–48 months. New combinations of oral nucleotide transferase inhibitors have been developed, although their efficacy and safety have not been reported in TDT. A retrospective chart review was conducted of 6 TDT patients with chronic HCV who were treated with these agents, 3 of whom had previously failed interferon-based therapy. Demographics, average annual transfusion units, ferritin, hemoglobin, liver function tests, liver pathology, fibroscan results, cardiac T2* MRI, liver iron content, and chelation agents were collected. Patients received non-interferon-based anti-HCV medications at the discretion of the physician. HCV viral load was measured pre-, during and post-treatment. Time from treatment initiation to negative viral load was tracked. Charts were reviewed for adverse events that occurred during treatment. The median time to negative viral load was 26 days (range 11–29). One patient receiving ribavirin with HCV treatments developed thrombocytopenia and neutropenia that resolved. None had any increase in transfusion requirements while on treatment. All 6 patients had undetectable viral loads at the end of treatment, and remain HCV negative after a median follow up of 128 days. In this small case series, we found that new HCV treatments were well tolerated in TDT, and rapidly reduced HCV viral loads. Larger studies are needed to confirm prolonged efficacy of treatment.

13. *A Pilot Study of PROMIS and Patient Reported Outcomes in Thalassemia*

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Adult patients with transfusion-dependent thalassemia (TDT) can expect to live into their 5th decade as a result of safer transfusion practices and improved management of iron overload. However living with a chronic condition also contributes to TDT patients developing multiple co-morbidities which contribute to poor health as they age. Patient reported outcomes (PROs) are important in understanding disease impact, and are key measures of efficacy for new therapeutics. Other PRO instruments like SF-36v2, TranQoL and BPI have indicated impairment in multiple domains of life in TDT. The NIH's Patient Reported Outcome Measurement Information System (PROMIS) utilizes item response theory, a psychometric measurement method that is administered electronically via Computer Adaptive Testing (CAT). This allows greater measurement precision, while at the same time decreasing responder burden. We hypothesize that PROMIS is feasible and acceptable to adult patients with TDT. Patients will be enrolled from the New York Comprehensive Thalassemia Center. CATs will be administered at 2 scheduled transfusions. Domains will be compared to similar domains from legacy instruments using Spearman correlations. An anchor question and CATs will be re-administered at 7 days, and test-retest reliability assessed by intra-class correlations on patients who are clinically unchanged. Primary outcomes will be the ability to complete PROMIS, and the comparison with domains of other instruments. Secondary outcomes will be the impact of co-morbidities on scores and qualitative patient experiences. Upon completion, PROMIS use will be expanded to other Thalassemia Centers in North America to prospectively measure the impact of new therapeutics on quality of life.

14. *Spectrum of Hemoglobinopathies from Antenatal Women Screening Data Reveals Frequencies of Various Hemoglobin Disorders in North India*

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Our hospital is a referral centre in north India for Comprehensive Thalassemia Care for over three decades. We retrospectively analyzed the universal antenatal screening program data from January 2009 to June 2015. All cases underwent complete automated blood cell counts (LH 750 Beckman Coulter) and high performance liquid chromatography (HPLC, Variant II instrument, Bio-Rad Laboratories, Hercules, USA using beta-thal Short™ programme). Cases with abnormal peaks underwent alkaline pH cellulose acetate electrophoresis (Genio-S system, InterLab, Italy). Genomic DNA analysis by PCR-RFLP (HbD/E/S), GAP-PCR for HPFH and δβ thalassemia and automated DNA sequencing of globin genes were done as indicated. We screened 12,200 individuals (92% antenatal women) and detected βTT prevalence as 4.2% and HbD Punjab as 1.5%. HbE and sickle cell trait were 0.2% and 0.5% respectively. Gene sequencing revealed two cases of Hb Fontainebleau [α2 21(B2) Ala→Pro; HBA2:c.64G>C] and one case each of heterozygous Hb Lepore-Boston-Washington (g.63632_71046del) and Hb Lepore-Baltimore (g.63564_70978), Hb Matsue-Oki [α2 75 (EF4) Asp→Asn; HBA2:c.226G>A]; Hb Burke [β 107 (G9) Gly→Arg; HBB:c.322G>C]; Hb Pyrgos [β 83 (EF7) Gly→Arg; HBB:c.251G>A] was found. Three cases had HbH disease which was first diagnosed during pregnancy. Three cases of variant hemoglobins remain to be characterized. Appropriate genetic counseling is offered depending on the diagnosis and prenatal diagnosis is offered whenever indicated. Apart from generating a population based database on asymptomatic individuals, this program has contributed to significantly increasing thalassemia awareness. The number of prenatal diagnosis at first pregnancy, in families with no history of abnormal hemoglobinopathies is also progressively increasing.

15. *Paving the Way for Prevention of Thalassemia in Egypt: National Research Centre Experience*

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Objectives: In Egypt thalassemia is the most common hereditary chronic hemolytic anemia; carrier rate 5.3 – 9 % and more than 1000 patients born yearly. Still there is no national prevention program. The disease creates a social and financial burden for the patient, family and government with an average estimated financial burden of 20 million US dollars/year. Even in carefully managed patients complications may develop due to iron overload, blood related viral transmission and organ failure.
We aim at reviewing cases frequenting Hereditary Blood Disorders (HBD) clinic and progress achieved as a first step in prevention.

Methods: Cases referred were subjected to;
- Clinical examination
- Defining haplotype map for beta-thalassemia
- Mutations characterization, bioinformatics analysis & defining the spectrum of mutations
- Genotype/phenotype correlation
- Prenatal diagnosis for pregnant mothers
- Assessment of quality of life

Results & Conclusion: This is a prospective cross-sectional study performed from 2005 till 2014 in the HBD Clinic.
Egyptian beta-thalassemia haplotype map was established and up to 30 beta thalassemia mutations could be characterized, 80% are Mediterranean. Comparing our spectrum to previous reports, novel & rare mutations were characterized.
Mostly rare mutations persistently correlated with the phenotypic outcome.
No intra-familial heterogeneity was detected helping genetic & prenatal counseling.
Quality of life studies mostly correlated to educational level & frequency of transfusion.
In conclusion, the study helped in providing better counseling and set a challenge to continuity of research for better screening and management.

16. *Rescue of Humanized Cooley's Anemia Mice from Lethal Anemia by a Single Point Mutation in the γ-Globin Gene Promoter*

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Humanized Cooley's Anemia (CA) mice have human α-, γ-, and nonfunctional β^0-globin genes inserted directly into the endogenous mouse α- and β-globin loci replacing the mouse adult α- and β-globin genes. When homozygous for these human globin gene knock-in alleles, the mice synthesize 100% human fetal hemoglobin (Hb F) in their definitive red blood cells. Humanized CA mice survive solely upon Hb F at birth, but become progressively anemic during the postnatal period as the fetal-to-adult hemoglobin switch is completed. Survival to weaning age and into adulthood requires weekly transfusion therapy.

We tested whether the incorporation of non-deletional hereditary persistence of fetal hemoglobin (HPFH) mutations into the promoters of the human γ-globin knock-in alleles in humanized CA mice could increase the expression of Hb F and extend their postnatal survival. Insertion of the Greek HPFH allele, a G to A mutation at position -117 of the γ-globin gene promoter, results in increased γ-globin gene expression that is heterocellularly distributed. The Greek HPFH mutation increases the mean survival of homozygous CA mice to 15 days postnatally. Incorporation of a Black HPFH allele, a T to C transition at position -175 of the γ-globin gene promoter, results in much greater γ-globin gene expression levels that are also pancellularly distributed. Homozygous CA mice with -175 HPFH alleles survive into adulthood in the absence of any transfusion therapy.

These results suggests that increasing γ-globin expression levels by promoter modification via gene editing in patient's hematopoietic stem cells may be a future therapeutic option.

17. *Nutritional Attitudes and Beliefs Held by Patients with Thalassemia*

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Given the essential role nutrition can play in the overall health and quality of life of patients with thalassemia (Thal), we explored the question, "Do patients with Thal believe that nutrition is important for their overall health?" *Objective*: to describe nutritional attitudes and beliefs held by patients with Thal. *Methods:* An anonymous online survey was developed to gather information on what patients understand about nutrition, where they obtain their nutritional information from and who influences their decision making. The survey was emailed to ~370 patients through the Cooley's Anemia Foundation listserve. The survey was closed after 3 weeks, results downloaded into excel and compared to a 2012 US Food & Health Beliefs Survey conducted in 1,057 Americans (Non-Thal). One hundred Thal participants completed the survey (67% female, 94% transfused, 18–60 yrs) of which 88% believed their health was good or excellent, while 21% believed their diet was unhealthy compared to only 11% of the Non-Thal population. Similar to Non-Thal, patients believe that nutrition information is confusing, and choose foods based more on taste rather than health claims. Though Thal patients appear to be receiving nutritional messaging regarding calorie contribution to weight gain and the importance of vitamin D for bone health, the majority (74%) strongly believe dietary iron affects their total body iron stores. Overall most believe nutrition is important for overall health (80%), but lack key dietary insights that may enhance their well-being. Information gained from this survey can be used to cultivate and enrich future nutrition intervention protocols.

18. *Patient Outreach Methods Used to Locate Patients with Thalassemia in the United States*

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The Cooley's Anemia Foundation (CAF), in collaboration with the Centers for Disease Control (CDC), developed new methods to update and expand its patient database. Methods to locate thalassemia patients included: (1) Partnering with Thalassemia Treatment Centers (TTCs); (2) Conducting site visits to TTCs; (3) Creating patient registration materials, (4) Conducting outreach to new patients appearing on the CAF Facebook page, an online adoption group, or in the news media; (5) Registering patients who call CAF for assistance; and (6) Reaching out to affected relatives of registered patients. These various outreach methods have enabled us to identify a total of 59 new patients. The majority of these patients (25/59, or 42%) were identified through partnerships with TTCs. Another 10/59 (17%) of patients were discovered when they called CAF for assistance. In addition, CAF had lost contact with 185 thalassemia patients over the years due to relocation. We have pursued several strategies to re-establish contact with these "lost patients", including use of online search methods. These searches helped us to re-establish contact with 46/185, or 25% of the "lost patients". In total, these methods have enabled us to add 105 patients to our database, bringing the number of thalassemia patients registered with CAF to 905. Our communications with hematology clinics indicate that there may be more than 800 additional patients not yet registered with CAF. This suggests that the total number of U.S. patients with thalassemia may exceed 1,700.

19. *Atypical Fractures of the Femur and Bisphosphonate Therapy in Patients with Beta Thalassemia-associated Osteoporosis: Report of Three Cases*

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Atypical subtrochanteric/femoral shaft (ST/FS) fractures are increasingly reported in patients on long-term treatment with bisphosphonates (BPs). These fractures have a low prevalence (<0,5% of all femoral fractures). However, concerns have been raised due increased risk in patients on long-term BPs treatment. BPs are the mainstay of therapy in patients presenting with thalassemia-associated osteoporosis (Thal-Op), as five randomized-controlled trials demonstrated their efficacy and safety on the short term (2-year). The optimal duration of BPs treatment in Thal-Op is still uncertain. We report on three women who sustained bilateral spontaneous atypical ST/FS incomplete fractures while on BPs therapy for Thal-Op. The clinical and radiological presentation, and the course of fracture healing (delayed) of these atypical fractures were similar to that described in the literature. However, we found a number of features that distinguish these fractures from those described in postmenopausal women receiving BPs: the duration of BPs therapy in two women was relatively shorter (4-6 years) compared to what reported in observational studies; the bone mineral density T-scores were below the diagnostic threshold for osteoporosis (-2,5) defined by the WHO, indicating the

presence of osteoporosis; the markers of bone turnover at the time of fracture were within the reference range, indicating no suppression of bone turnover. This is the first report of atypical ST/FS fractures in patients receiving BPs for Thal-Op. Our observations emphasize the need for a better definition of the optimal treatment duration, and for increased awareness for this potential adverse event while using BPs in Thal-Op.

20. *Blood Transfusion among Thalassemia Patients: A Single Egyptian Center Experience*

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Background: although red cell transfusions are lifesavers for patients with thalassemia, they are responsible for a series of complications and expose the patients to a variety of risks.

Material and methods: This cross sectional study included 464 Egyptian thalassemia major patients whose age ranged between 10 months and 31 years (mean 10.2 ± 6.6 years). All patients were subjected to thorough history taking with special emphasis on blood transfusions regarding rate of blood transfusion, type of received blood and history of previous transfusion reactions in addition to type of chelation and compliance to iron chelation therapy and history of Diabetes Mellitus. Serum ferritin, and pretransfusion hemoglobin assessment were done for all patients.

Results: the mean pretransfusion hemoglobin level was 5.7 ± 1.16 g/dl. Allergic reactions were observed in 3.9% of the patients during the period of the study, while the history of previous allergic reaction was given by 72% of the patients. Deferiprone showed better compliance (58.6%) than deferoxamine (26.3%). The prevalence of diabetes was 10.1% among the studied group. On comparing diabetics to non diabetics, serum ferritin, transfusion intervals and age were statistically higher among diabetics (P<0.001).

Conclusion: Lower pretransfusion hemoglobin and high rate of prevalence of diabetes, in addition to better compliance to deferiprone than deferoxamine were detected among the patients.

21. *Chronic HCV Infection and Hemosiderosis among Egyptian Thalassemia Patients: The Role of Homa Index*

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Background: Hepatitis C virus (HCV) is a major cause of chronic hepatitis C (CHC) which increases the risk for insulin resistance, glucose intolerance and type 2 diabetes. Egypt has the highest reported rates of HCV infection especially among chronically transfused patients. Abnormal glucose tolerance is common in multitransfused thalassemia and is attributed to early impaired beta cells function with increasing insulin resistance (IR). The HOMA index (homeostasis model assessment) has been extensively used to investigate insulin resistance in CHC.

Aims: To detect insulin resistance in transfusion dependent thalassemia major patients and to evaluate its possible link to chronic hepatitis and iron overload

Methods: Fifty nine Egyptian thalassemia major patients were divided into hepatitis C (HCV) positive (group A; n= 39) and HCV negative thalassemics (group B; n=20) by HCV antibody and RNA. Thalassemia patients were compared to 20 age and sex matched HCV positive (non thalassemic) patients (group C). Blood samples were withdrawn from all patients for assessment of HOMA index, serum ferritin, AST and ALT.

Results: Abnormal HOMA test was evident in 30.8% (n=12) of group A and 40% (n=8) of the control group while none of group B had abnormal test. Significant results of HOMA-IR was observed between the three

groups (p=0.029) and between group B and each of groups A and C (p= 0.012, 0.043 respectively) while no difference was observed between HCV positive thalassemics and control group (p=0.99) Positive correlation (p=0.038) was observed between HOMA- IR and AST but no correlation to age or serum ferritin level.

Summary/Conclusion: Chronic hepatitis in transfusion dependent thalassemia major patients is a major risk factor for insulin resistance in these patients.

22. *Vitamin E and Selenium Concentration in Egyptian Pediatric Patients Chronic Hemolytic Anemia: Are They Deficient?*

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Background: Increased oxidative damage is well known in chronic hemoglobinopathies. But it is still unknown whether the levels of antioxidants differ in transfusion dependent β-thalassemia (TDT) and sickle cell anemia (SCA).

Objective: To investigate antioxidants namely vitamin E and selenium and lipid profile in Egyptian children with TDT and SCA and to examine whether these variables differ in these two diseases or correlate with iron overload status or transfusion requirements.

Materials and Methods: A case-control study where the serum levels of vitamin E, selenium and lipid profile were measured in 60 transfusion dependent β-thalassemia and SCA children in a steady state aged from 6 to 18 years and 30 apparently healthy age/sex matched controls.

Results: All β-Thalassemia and SCA cases had below normal selenium level versus 11 (36.7%) of the control group, and mean selenium level was comparable between β-Thalassemia and SCA groups (p>0.05) and both groups showed significantly lower levels when compared to the control group (p<0.05). Similarly, all β-Thalassemia and SCA cases had below normal vitamin E level vs. none of the control group, and mean vitamin E level was comparable between β-Thalassemia and SCA cases (p>0.05) and both groups had significantly lower levels when compared to the control group (p<0.05). Total cholesterol, LDL-cholesterol, as well as TG were comparable in patients with β-Thalassemia and SCA (p>0.05) and all were significantly lower than relevant controls (p<0.05). However, there was no significant differences between mean HDL- cholesterol levels in the three groups (p>0.05). Among SCA cases, serum ferritin, selenium and vitamin E levels didn't correlate with any of the tested variables. Among β-Thalassemia group; serum ferritin and selenium levels didn't correlate with any of the tested variables including other antioxidants. But, vitamin E levels were proportionally correlated with ALT values *(r* = 0.4; *P* = 0.049) and AST values *(r* = 4; *P* = 0.039). Transfusion rate correlated positively with CRP (r=0.341, p=0.065) and AST correlated inversely with TG (r=-0.389, p=0.034).

Conclusion: These results demonstrate that children with β-Thalassemia and SCA have increased oxidative stress and depleted antioxidants relative to healthy controls. However, levels of these antioxidants did not correlate with indices of iron overload, hemolysis, or inflammation in chronically transfused β-Thalassemia and SCA patients.

23. *Dietary Iron in Thalassemia: A New Look at Old Ideas*

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Transfusion-independent patients with thalassemia intermedia (TI) develop fatal iron overload from excessive intestinal iron absorption. Better understanding of non-heme iron pharmacokinetics will further optimize management. The objective of this clinical pilot study was to compare intestinal iron absorption of different forms of non-heme iron with and without tannins in TI and healthy control subjects. We serially measured serum iron concentrations over 24 hours in 4 TI and 6 control subjects who consumed 1 mg/kg of elemental iron as ferritin or ferrous sulfate with or without black tea (~500mg tannins); we also measured serum iron concentrations in 1 TI and 1 control subject after 2 mg/kg of elemental iron as ferritin or ferrous sulfate without tea. Baseline findings confirm ineffective erythropoiesis and concomitant inflammation in TI subjects. Serum iron concentrations peaked within 4 hours after consumption of ferritin and ferrous sulfate in both populations. The different forms of non-heme dietary iron yielded similar serum iron concentrations over 24 hours, even with the increased dose dietary iron, in TI and control subjects. The addition of black tea with ferrous sulfate in a single serving had no effect on serum iron concentrations in either population. Results of this pilot demonstrate bioequivalence of two different forms of non-heme iron, as there was no significant difference in intestinal absorption of ferritin or ferrous iron. Findings suggest that patients with TI may liberalize dietary restrictions and safely consume a low iron diet. Larger studies re-evaluating dietary management in this subpopulation are warranted.

24. *A Lentiviral Vector Conferring Coregulated, Erythroid-Specific Expression of Gamma-globin and BCL11A Knockdown for the Treatment of Severe Hemoglobin Disorders*

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Expression of γ-globin, which forms fetal hemoglobin (HbF), lessens the severity of β-thalassemia and sickle cell anemia. The benefits of HbF may be achieved through ectopic expression of γ-globin or reactivation of endogenous γ-globin genes. We previously achieved potentially therapeutic levels of HbF in cultured β-thalassemia cells using lentiviral vectors encoding a human γ-globin gene with an insulator (termed V5m3-400) or a short-hairpin RNA (shRNA) to knockdown BCL11A; a significant repressor of fetal globin gene expression in maturing adult red blood cells. Our goal was to combine these approaches into a single vector to achieve co-regulated, erythroid-specific expression and augmented levels of HbF. We modified V5m3-400 to include microRNA (miR)-adapted shRNAs targeting BCL11A (based on miR-30A and miR-E architectures) in the first and second introns of γ-globin genomic sequences. Inclusion of miR30A-shRNAs had no effect on integrity of the integrated provirus or vector titer. Vector performance was tested using K562 human erythroleukemia cells expressing a flag-tagged version of the BCL11A XL-isoform. Both γ-globin and shRNA expression were induced when K562 cells were differentiated with hemin. BCL11A knockdown was significantly improved using miR-E-adapted shRNAs due to a 200-fold increase in processing of mature shRNA sequences. Erythroid-specific expression of the γ-globin transgene and mature shRNA sequences was confirmed in maturing erythroid cells derived from transduced CD34+ cells of healthy donors. This compound vector has the potential to maximize γ-globin expression and promote levels of HbF that are unlikely to be safely and effectively achieved by conventional globin gene addition approaches alone.

25. *Pregnancy in Thalassemia: A Decade of Experience at the New York Presbyterian Hospital-Weill Cornell Medical Center*

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Pregnancies in women with thalassemia have been considered high risk owing to iron overload related co-morbidities including liver disease, endocrinopathies, osteoporosis and cardiac disease. We conducted a retrospective review of the electronic medical record of female patients of reproductive age followed at this institution's Comprehensive Thalassemia Program between 2005 and 2015. Patient records were reviewed for diagnosis, transfusion history, chelation, pre-conception iron burden (cardiac/abdominal MRIs, ferritin), cardiac function by echocardiogram, hypercoagulable status, use of reproduction assistance and perinatal outcomes. Nine women were identified who conceived; all carried pregnancies to term delivering a total of 12 healthy infants, including a set of dizygotic twins. Pre-pregnancy, all of these women had extensive counseling and cardiac clearance. Four of the nine women had beta thalassemia major, two had transfusion dependent beta thalassemia intermedia, two had transfusion dependent E Beta thalassemia and one had non-transfusion dependent E Beta thalassemia intermedia. One woman developed pre-eclampsia and one eclampsia; these same two women developed gestational diabetes. Another had a catastrophic outcome with heart failure during a second pregnancy. Two additional women with thalassemia major underwent uneventful ovarian stimulation with embryo harvesting for a surrogate to carry to term. Our experience has been women with thalassemia who require reproductive assistance may have a higher risk of complications despite being well chelated and having normal cardiac function. However, the advances in iron chelation, in the monitoring of non-invasive measurements of tissue iron and in reproductive assistance provides safer options for pregnancies in women with thalassemia.

26. *Adult Life Expectations, Fulfilment, and Depression in Greek Thalassemia Major Patients*

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In Greece, chelation therapy was introduced 40 years ago; as a result, thalassemia major patients (TMPs) survive into maturity and beyond. However, very little information is available on their life expectations (Exp) and fulfilment (Ful). We studied 303 adult TMPs (mean age 33.3±5.8) from September 2014–January 2015 to investigate life expectations, fulfilment and depression. Participants completed our Multidimensional Expectation/Fulfillment Questionnaire for Thalassemia Major Patients* and the BDI. We used paired t-test, and squared semi-partial correlation (sr2). TMPs seemed to fulfil overall expectations (mean(Ful)=3.05±0.02; mean(Exp)=3.02±0.52; t=1.40; p=0.162); they achieved more in their career (mean(Ful)=2.87±0.86; mean(Exp)=2.78±0.81; t=-2.13; p=0.034). Higher education level and seamless blood provision helped them overcome obstacles (sr2(Ful)=0.028 and sr2(Ful)=0.022, respectively) to pursue career advancement (sr2(Exp)=0.032 and sr2(Exp)=0.026, respectively). Participants fell short of marital expectations (mean(Ful)=2.88±0.88; mean(Exp)=3.08±0.74; t=4.48; p<0.001). Fearing TM complications prevent them from planning their own family (sr2(Exp)=0.062) unless gaining more awareness of TM challenges (sr2(Ful)=0.011). Transfusion reactions affected TMPs daily life functioning (sr2(Exp)=0.028, sr2(Ful)=0.027). Finding themselves blood was correlated with fulfilled expectations on maintaining a large number of social ties (sr2(Exp)=0.020, sr2(Ful)=0.017). Most participants (220/303; 72.6%) had minimal levels of depression that positively influenced their overall expectations (sr2(Exp)=0.116) and fulfilment (sr2(Ful)=0.155), made them

anticipate supportive social network (sr2(Exp)=0.146) and cope with everyday activities (sr2(Ful)=0.133). Greek adult TMPs accomplished tasks normative for their age; though living in a country with organized blood supply, they are still haunted by the spectre of blood supply shortage.
*Koutelekos et al. GJHS 2015; http://dx.doi.org/10.5539/gjhs.v8n2p77)

27. In Utero *Transplantation in Patients with Alpha Thalassemia Major — the Birth of a New Therapy*

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In utero transplantation (IUT) is a promising strategy to treat congenital hematological disorders and induce tolerance to donor cells without using pre-transplant conditioning. Although previous attempts have had limited success, recent studies in animal models indicate that IUT of maternal cells leads to levels of engraftment that are sufficient to establish immunological tolerance. The biological basis of this strategy is that the presence of maternal cells in the fetal circulation leads to fetal tolerance of non-inherited maternal antigens. Alpha thalassemia major is a particularly attractive disease to test the safety and efficacy of this approach: affected fetuses typically die but can be survive to birth by receiving *in utero* red blood cell (RBC) transfusions, which we have successfully performed in our multidisciplinary center. Thus, maternal hematopoietic cells could be transplanted concurrently with RBC into the fetus, avoiding the risk of an additional fetal procedure. Our group has applied for an Investigational New Drug approval to use this strategy in a Phase 1 clinical trial. Additional considerations include optimal gestational age for transplantation, given the ontogeny of fetal immune development, and potential changes in the fetal immune system after fetal intervention, and these may require alterations to the protocol such as transplantation earlier in gestation or minimal conditioning. Identification of eligible patients and dissemination of information about this new therapy to the obstetric and hematology communities will be critical for its success. In the future, IUT for other hemoglobinopathies such as beta thalassemia major or sickle cell disease may be considered.

28. *Ameliorating β-thalassemia by Manipulating Expression of the α-globin Gene*

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β-Thalassemia is a disorder of hemoglobin production characterised by severe anemia requiring life-long blood transfusions. The genetic defects are predominantly based in and around the β-globin gene resulting in reduced or absent β-globin chain synthesis. The resultant excess of free α-globin chains, which precipitates in red blood cells and their precursors and causes ineffective erythropoiesis, is now believed to be the main pathophysiological mechanism of anemia in these patients. Indeed, co-inheritance of α-thalassemia (single or two α-globin gene deletion) ameliorates disease severity in almost all patients with HbE/β-thalassemia and many patients with β-thalassemia major. Therefore, reduction of α-globin levels by 25%-50% has the potential to ameliorate disease phenotype from severe (thalassemia major) to mild or moderate (thalassemia intermedia) and render patients transfusion independent. To date, pharmacological studies in β-thalassemia have focused on increasing expression of the γ-globin genes (the fetal β-like globin genes) and have rarely investigated the alternative strategy of reducing expression of the α-globin genes. Here we aim to identify target pathways that would down regulate α-globin gene expression without affecting expression of the β-like globin genes which could lead to greater balanced globin chains and potential clinical application.

We first developed and validated an *in-vitro* miniature erythroid differentiation system suitable for small-scale and single-cell experiments. Using this assay we identified a few genetic and epigenetic pathways which down regulate α-globin expression without affecting β-globin expression in human primary erythroid cells. In this way, we have demonstrated selective down-regulation of α-globin expression is therapeutically feasible.

29. *Effect of Testosterone on Short-Term Transfusion Requirement and Quality Of Life in β Thalassemia Major*

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Background: In developing countries, Thalassemia Major (TM) patients are often treated with transfusions but irregular chelation. Many develop endocrinopathies, commonly hypogonadism, in their second decade of life. Testosterone is known to have to enhance erythropoiesis. This study was done to assess the effect of testosterone on short-term transfusion requirement & quality of life (QOL) in male hypogonad β thalassemia major patients.

Methods: This prospective study included 20 male TM patients with hypogonadism (cases) compared with 30 non-hypogonad male TM patients (controls). The transfusion requirement & mean pre-transfusion hemoglobin levels were assessed for a period of six months in both cases and controls. QOL by SF-36v2 questionnaire & anthropometry were measured at baseline, 3 & 6 months of therapy.

Results: Out of 347 total TM subjects, 77 males were ≥18 years of age. Of these, 28 males had hypogonadism and twenty TM fulfilling inclusion criteria were supplemented testosterone. The mean pre-transfusion hemoglobin increased from 8.0g/L at baseline, to 8.24 g/L at 3 months and 8.46 g/L at 6 months of therapy ($p<0.01$). Similarly mean blood requirement during 6 months decreased from 17.8 ml/kg/month to 16.7 ml/kg/month ($p<0.01$). QOL also showed significant improvement in physical & mental well-being. Adverse events noted with testosterone therapy were acne vulgaris, gynecomastia, pedal edema and mild elevation in liver transaminases.

Conclusion: Testosterone has been shown to increase the mean hemoglobin and decrease the transfusion requirement when replaced in hypogonadal men with Beta thalassemia major.

30. *Precisely Treating Beta Hemoglobinopathic Health Disparities by Mutagenic and Recombinagenic Small Molecules Mediated Genome Engineering*

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Mutagens, such as triplex-forming small molecules, precisely target a genomic locus and co-localize the cellular endogenous genome repair machinery. Upon co-localization, this repair machinery is co-opted by recombinagens, such as recombinagenic donor DNA small molecules, as homology-dependent templates to edit a genomic locus safely and efficaciously. This genome engineering as applied to hematopoietic progenitor cells enables a regenerative treatment modality for hematological disorders, such as those where the pathophysiology involves a defective adult β-globin subunit that causes β-thalassemia or sickle cell disease.

On human chromosome 11, triplex-forming PNA and recombinagenic donor DNA small molecules are designed to, respectively, target and edit the β-globin gene, in order to repair the defective adult β-globin subunit, or the γ-globin gene to order to replace the defective adult β-globin subunit with an induced, higher-oxygen affinity, fetal γ-globin subunit. The triplex-forming PNA designs demonstrate sequence-specific and dose-dependent binding affinity to their intended human β-globin and γ-globin gene targets cloned into episomes in vitro. Translating this technology to human chromosomes, further molecular designs place this induction under exquisite regulation of a hypoxia response element *in vivo*. Cells are transfected with designed mutagens and recombinagens, and quantified for modified mRNA expression and repair frequency with quantitative reverse transcription-PCR (qRT-PCR) and allele-specific quantitative PCR (qPCR), respectively.

Findings indicate that designed targeting mutagens and editing recombinagens successfully treat the genomic basis of β-thalassemia and sickle cell disease in regenerative progenitor cells. As opposed to treatment modalities that ameliorate symptoms and demand patient compliance, this therapeutic genome engineering innovation may permanently address these hematological disorders in disparate populations.

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31. *Serum Ferritin Values between 300 and 800 ng/mL in Non-transfusion-dependent Thalassemia: A Probability Curve to Guide Clinical Decision Making When MRI Is Unavailable*

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The assessment of liver iron concentration (LIC) by magnetic resonance imaging (MRI) remains the recommended means of quantifying iron overload in patients with non-transfusion-dependent thalassemia (NTDT) to guide iron chelation decisions. With the high prevalence of NTDT in resource-poor countries, MRI technology may still not be readily available to a significant proportion of patients with NTDT. Serum ferritin, a simple and more affordable tool, has a significant positive correlation with LIC from observational studies and may be used in countries were MRI is not available or affordable. In earlier studies, serum ferritin levels of >800 ng/mL were shown to most certainly predict an LIC ≥5 mg/g dry weight (dw), the level that is association with iron-related morbidity at which iron chelation therapy is recommended. However, close to 50% of patients with levels <800 ng/mL also continued to have LIC levels ≥5 mg/g dw; and thus remain at risk of being denied iron chelation therapy although they need it. To this aim, we evaluated 71 patients with serum ferritin levels ranging between 800 and 300 ng/mL (300 being the upper level of normal serum ferritin) attending centers in Italy, Lebanon, Oman, and Thailand. Their mean age was 33.3 ± 13.9 years. 53.5% of the patients were female and 45.1% were splenectomized. Using a logistic regression analysis with LIC ≥5 mg/g dw as the desired outcome, we were able to construct a probability curve for serum ferritin levels in predicting an LIC ≥5 mg/g dw (Figure 1). Instead of categorically assuming patients with serum ferritin levels <800 ng/mL are not at morbidity risk and eligible for chelation, while a good majority in fact will be; our probability curve helps assign a percentage chance of still having LIC ≥5 mg/g dw to be taken into consideration with the patients general clinical picture and better guide decision making towards introduction of iron chelation therapy.

32. *Downregulation of microRNAs and Their Possible Functions in Fetal Erythropoiesis*

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Hematopoietic stem cells (HSCs) are responsible for lifelong production of all blood cells. HSCs are generated once in the lifetime during embryogenic development and production of mature hematopoietic cells starts in

the fetal liver and continues in the bone marrow. However, fetal HSCs are markedly different from adult HSCs, respecting to their cell cycle status and proliferation capacity. HSCs expand rapidly in the fetal liver, whereas in the adult bone marrow, HSCs are generally quiescent and have greatly reduced proliferation in comparison to fetal HSCs.

In order to understand the differences in gene regulation between fetal and adult erythropoiesis, we have conducted a whole-genome microarray analysis comparing gene expression profiles of fetal liver derived- and adult peripheral blood derived-erythroblasts. We found that a group of newly discovered class of genetic material, termed microRNAs is downregulated in fetal liver derived erythroblasts compared to adult peripheral blood derived erythroblasts. 12 out of 1,063 pri-miRNAs were downregulated with more than 2-folds. Of these, two are known erythroid specific miRNAs, 3 are known tumor suppressive miRNAs and 7 are unknown function miRNAs. In silico miRNA target analysis incorporating all binding sites produced by 4 prediction programs; miRWalk, miRanda, RNAhybrid, and TargetScan, revealed that the downregulated miRNAs shared a common set of target mRNAs. We found that 92 of these common targets were upregulated by more than 2-fold in fetal liver derived erythroblasts and were predominantly involved in signal transduction and cancer-related pathways.

33. *Transition of Adult Thalassemia Patients from a Pediatric to Adult Health Care Setting: The Philadelphia Experience*

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Background: In the USA, adult thalassemia patients are commonly managed in pediatric health-care settings due to the lack of adult-specific infrastructure. Programmatic models for transitioning patients with thalassemia do not exist. Here we describe The Children's Hospital of Philadelphia (CHOP) to the Hospital of the University of Pennsylvania (HUP) thalassemia transition program, initiated in 2014, that utilizes standard processes to enhance communication and coordination, provide for individualized care, and deliver psychosocial support throughout transition.

Methods: Key features of the program include: (i) patient preparedness at CHOP (ii) two pre-transition HUP nurse visits at CHOP; (iii) program-specific information exchange; and (iv) a pre-transition provider meeting. Program success was evaluated by clinical surveys (six months pre- and post-transition), and surveys targeting patient retention, psychological outcomes, and patient satisfaction.

Results: From August 2014-July 2015, 10 patients were transitioned. Preliminary analysis of seven patients with post-transition follow-up longer than 6 months reveals; mean age of 47 years (range 32-62); serum ferritin 1192±1898 ng/mL (pre-transition) and 1842±2405 (post-transition); and equivalent hemoglobin values. Compliance was 100% for scheduled physician and transfusion visits. Surveys of transition readiness indicate patient comfort managing medications, scheduling appointments, interacting with providers, and managing daily activities, but a need for help tracking their health issues. Psychological scores indicating fear and anxiety improved significantly post-transition.

Conclusion: The CHOP-HUP thalassemia program has designed and implemented a transition plan that — while still in development — evidences success in several areas including visit adherence, maintenance of transfusion and iron-chelation goals, and improved metrics for patient anxiety and satisfaction.

34. *Endothelial Dysfunction in Thalassemia and Amelioration by Deferiprone*

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Nitric oxide (NO) is synthesized by the vascular endothelial cells. NO levels in blood represent the endothelial function. In blood, NO is oxidized to be nitrite anion (NO2-) which serves as a bioactive form stored in erythrocytes. NO has a variety of physiologic functions such as vasodilation and platelet inhibition. Decreased NO has been shown to be related with cardiovascular disorders; for example, atherosclerosis, systemic hypertension, stroke, and thrombosis. Thalassemia has decreased circulating NO which may be associated with pulmonary hypertension and thrombosis. In this study, we investigated the plasma levels of L-arginine (substrate for NO production), asymmetric dimethylarginine (ADMA, NO synthase inhibitor), and symmetric dimethylarginine (SDMA, L-arginine uptake inhibitor) as markers of endothelial dysfunction in 31 splenectomized β-thalassemia/HbE patients. In addition, the effect of deferiprone, an iron chelator, on the nitrite levels was examined. We found the decreased levels of L-arginine but increased levels of ADMA and SDMA in thalassemia. However, the baseline nitrite levels in thalassemia were not different from those in healthy subjects (N = 10). The nitrite levels in plasma and whole blood increased at 3 hours after a single oral administration of deferiprone (25 mg/kg) while the blood pressure was unchanged. We conclude that thalassemia patients have endothelial dysfunction which was ameliorated by deferiprone.

35. *On the Development of a Functional Simulation of Bone Marrow Using Fuzzy Logic Approach and Implementation with Nanotechnology*

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Recently some interest has been expressed on the development of bone marrow on a chip by a group at Boston. Such a chip demonstrates the complex functions of the bone marrow. Some studies have been made on implementation of bone marrow on some live animals. The purpose of this paper is to suggest an alternative engineering approach to simulate a bone marrow. We suggest here fuzzy logic approach for such a simulation. In this work, an algorithm is suggested to obtain the functional simulation of bone marrow. For the last four decades there has been an increasing interest in the use of fuzzy logic approach for a variety of engineering applications. This approach has not much been studied in the area of hematology. This will offer an alternative approach to what has been suggested by the Boston group. The approach consists of writing down fuzzy if then rules which will simulate the behavior of bone marrow. The rules will then be implemented in the form of Verilog language. The algorithm thus developed will be tested using field programmable gate arrays (FPGA). The procedure can also be implemented using Engineering software such as Cadence. Such a simulation and its implementation will pave a way to the development of nano circuits in the broad area of hematology in particular and medicine in general.

36. *Association between Blood Transfusion and Platelet Activation in Thalassemia Patients*

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Regular red blood cells transfusion is the mainstay treatment of thalassemia. Chronic transfusion does not only reverse hypercoagulable state but also decreases pulmonary artery pressure in splenectomized thalassemia. In this study, we investigated the effect of blood transfusion on platelet activity in thalassemia. Twenty-seven β-thalassemia/hemoglobin E patients were divided into non-transfusion-dependent (n = 8) and transfusion-dependent (n = 19). P-selectin, activated glycoprotein (aGP) IIb/IIIa and platelet-leukocytes aggregates (platelet-neutrophil, platelet-monocyte and platelet-lymphocyte aggregates) were measured by flow cytometry. Platelet activation markers (P-selectin, aGPIIb/IIIa and platelet-leukocyte aggregates) were not different between non-transfusion-dependent and transfusion-dependent patients. However, duration after last transfusion showed positive correlation with P-selectin ($r = 0.67$; $P < 0.01$) and aGPIIb/IIIa ($r = 0.46$; $P = 0.04$) expression. Transfusion may reduce platelet activation due to normalized ineffective hematopoiesis and decrease levels of abnormal red blood cells.

37. *The Influence of HBS1L, MYB and HBS1L-MYB Intergenic Region on Clinical Severity and Fetal Hemoglobin Level in Beta-Thalassemia/HbE*

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Genetic polymorphisms on chromosome 6q23; HBS1L, MYB and HBS1L-MYB intergenic region (HMIR), have been shown significantly associated with fetal hemoglobin (HbF) levels in β-hemoglobinopathies. As a therapeutic target for genetic manipulation, investigation for its role is required. In this study, the association of frequency of polymorphisms with disease severity and HbF level was performed in a cohort of 1,100 Thai β-thalassemia/HbE patients. The cohort displayed a large clinical heterogeneity and normal distribution of HbF level (baseline %HbF = 10.5 to 77.7, mean = 33.1%). The particular variants on this region were associated with disease severity (odd ratio > 2) and account for 12% of the variation in HbF levels. The relative expression levels of HBS1L, MYB and HMIR transcripts were investigated in CD34+-derived erythroblasts. The HbF levels in cultured erythrocytes were 4.4±0.2% and 19.1±6.0% in normal- and β0-thalassemia/HbE-derived cells, respectively (n=5). qRT-PCR was performed in CD71-high/GPA-positive erythroblasts, greater than 2-fold upregulation of HBS1L and MYB transcripts were observed in β0-thalassemia/HbE erythroblasts. Four potential active transcription regions in HMIR were indentified from the ENCODE dataset. Three intergenic transcripts were detected in CD71-high/GPA-positive erythroblasts or K562 cells by qRT-PCR. While one HMIR transcript was existed in both normal and β0-thalassemia/HbE erythroblasts and K562 cells, the other two HMIR transcripts were detected in only β0-thalassemia/HbE erythroblasts and K562 cells. Additional loss-of-function studies are underway to explore the roles of HBS1L, MYB and HMIR in regulating erythropoiesis and globin gene expression in β-thalassemia/HbE.

38. *Patient Involvement in Building a Thalassemia Self-Management Smartphone Application Using Consensus Methodology with the Aim of Improving Long-term Care*

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Background and Purpose: Thalassemia Major necessitates chronic blood transfusions with consequent iron overload complications. Survival has improved dramatically over the past decade, but consistent, lifelong iron chelation medication adherence remains a major challenge for patients. This study assesses the impact of a novel patient education initiative to improve outcomes, through the development of a smartphone application ("thalTracker") designed by and for patients.

Methods: 25 patients consented to participate in a modified Delphi method to reach consensus on the tools and features to include in a patient-centered app. We generated a ranked list of features including: medication reminders, healthcare provider contacts, connectivity to other patients, and visually appealing interface. "thalTracker" was built based on this list and launched to iTunes for free download globally in January of 2015.

Results: "thalTracker" aims to improve adherence to treatment by allowing users to record values from blood tests and imaging results into the app. An adherence function lets you set a chelation goal and then tracks how well you achieve your target. It encourages positive behaviors in disease self-management and greater engagement in health care through improved health literacy. To date, "thalTracker" has been downloaded 196 times, with 131 of these from North America.

Conclusions: Patients, as experts in their disease, were successfully engaged in a needs assessment and development of an app to assist with thalassemia self-management. Further work through surveys built into the app will assess usability of the app, impact of the app on clinical outcomes, and overall quality of life.

39. *Clinical Efficacy of Combined Deferasirox (DFX) and Deferoxamine (DFO) in Transfusion Dependent Thalassemia (TDT) Patients who Resist to Standard Iron Chelation Regimens*

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Background: Recently, Aydinok *et al.** have reported a robust efficacy of combined DFX and DFO in TDT patients who had moderately-severe cardiac and severe liver iron overload. However, there was a limited data on the role of this regimen in TDT patients who had resistance to current standard iron chelation therapy.

Methods: Four β-thalassemia major patients (3 males) who were unresponsive to standard iron chelation for >6 months based on increasing serum ferritin (SF) and MRI-liver iron concentration (LIC); three were on DFX (~40 mg/kg/d) and 1 with combined DFP (94 mg/kg/d) and DFO (30 mg/kg/d x5 days/wk), have been enrolled. All were treated with 12-mths period of DFX (mean actual dose, 39 mg/kg/d) and DFO 30 mg/kg for 3-5 days/week. Iron status and side effects were evaluated and monitored every 3-4 weekly.

Results: The median age was 17.25 years (range; 14-21). Two patients were splenectomized and all received the average of 0.4 mg/kg/d of transfusion iron loading. All, but one, showed a significant reduction of SF from baseline compared to 6 and 12 mths (mean SF; 6531.2, 4445.2 and 4074.6 ng/ml, respectively). However all had reduced LIC from baseline at 12 mths (average 33.77 vs. 24.67 mg/g dry wt.). During study period, none have experienced any treatment-related adverse events.

Conclusion: Desirable clinical efficacy and acceptable tolerability of iron chelation in TDT patients can be achieved through optimizing regimens. Our data demonstrated that a combined DFX-DFO could be an effective measure for TDT patients who resist to current standard iron chelation therapies.

*Aydinok Y, et al. Effects of Deferasirox-Deferoxamine on Myocardial and Liver Iron in Patients with Severe Transfusional Iron Overload. *Blood* 2015 Jun 18;125(25):3868-77.

40. *Clinical-Scale Genome Editing of the Human BCL11A Erythroid Enhancer for Treatment of β-Thalassemia*

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Reversal of HbF silencing in patients is an appealing therapeutic strategy in β-thalassemia. Mutations in the erythroid-specific enhancer of the fetal globin repressor, BCL11A, elevate HbF, and individuals carrying a monoallelic knockout of BCL11A have up to 30% circulating HbF. We find that a knockout of BCL11A using genome editing with engineered zinc finger nucleases (ZFNs) yields up to 40% HbF in erythroid progeny of edited human CD34 cells in vitro. Further, targeted ablation of a single, specific GATAA motif in the BCL11A intronic enhancer reproducibly (n=6) activates fetal globin transcription in erythroid progeny of modified CD34 cells; at similar levels of on-target marking in CD34+ cells, these effects on fetal globin mRNA are comparable to those resulting from ZFN-driven coding knockout of BCL11A itself. We demonstrate reproducible (n=8), high-efficiency (up to 82%; average, 69%) ZFN-driven marking at the enhancer in mobilized human CD34 cells at clinical production scale (>1e8 cells) in a GMP-compliant setting. Up to 70% of the cells in the resulting population are biallelically modified at the target locus, and comparably high levels of marking are seen in CD34 cells from patients with β-thalassemia. We observe robust long-term (18-24 week) engraftment of genome-edited cells in immunodeficient mice, similar to control cells, and equivalent modification at the targeted enhancer locus at all timepoints in both differentiated (CD19+, CD3+, CD33+) and more primitive progenitor (CD34+CD38low) cells of human origin. Our findings support clinical development of enhancer editing as a treatment of β thalassemia with autologous hematopoietic stem cell transplant.

41. *Analysis of the Mechanisms Underlying the Developmental Regulation of Embryonic and Fetal β-Like Globin Genes*

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Improved understanding of the γ- to β-globin switching mechanism holds the key to devising targeted therapies for β-hemoglobinopathies. To further investigate this clinically important developmental switch a novel fluorescent-based cellular reporter assay system was developed. Two fluorescent reporter genes, DsRed and eGFP, were inserted into an intact 183 kb intact human β-globin locus, replacing the coding regions of the ^{G}γ- and β-globin genes, respectively and was stably transfected into adult murine erythroleukemic (MEL). Following RNA interference (RNAi)-mediated knockdown of two key transcriptional regulators, Myb and BCL11A, we observed a derepression of γ-globin, measured by DsRed fluorescence and RT-qPCR. Interestingly, double knockdown of Myb and DNA methyltransferase 1 (DNMT1) resulted in a robust induction of ε-globin, (up to 20% of total β-like globin species) compared to single-knockdowns. Conversely, double-knockdowns of BCL11A and DNMT1 enhanced γ-globin expression (up to 90% of total β-like globin species) compared to single-knockdowns. Moreover, following RNAi treatment, expression of human β-like globin genes mirrored the expression levels of their endogenous murine counterparts. These results demonstrate that Myb and BCL11A cooperate with DNMT1 to achieve developmental repression of embryonic and fetal β-like globin genes in the adult erythroid environment.

42. *Analysis of β-Thalassemia Mice to Understand Innate Immune Abnormalities in β-Thalassemia Patients*

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β-Thalassemia is associated with several abnormalities in the immune system, including defective neutrophil functions, predisposing patients to infections with both Gram-positive and Gram-negative bacterial pathogens. The molecular mechanisms involved in impaired neutrophil function during bacterial infection in thalassemia patients are not completely understood. To better understand the impact of thalassemia on the innate immune system, we investigated susceptibility to bacterial infection and neutrophil function in the $Hbb^{th3/+}$ β-thalassemia mouse model. We demonstrate that $Hbb^{th3/+}$ β-thalassemia mice are highly susceptible to infection with *Streptococcus pneumoniae*, a Gram-positive extracellular pathogen that causes sepsis, pneumonia and meningitis. Our results showed that blood and splenic neutrophils from $Hbb^{th3/+}$ β-thalassemia mice show defective chemotaxis, reduced opsonophagocytosis and decreased reactive oxygen species (ROS) production compared with neutrophils from normal mice. In addition, we used gene expression studies and quantitative RT-PCR to demonstrate that genes that regulate neutrophil chemotaxis, CXCR2 and CD11b expression, opsonophagocytosis, and ROS production (p22phox, p67phox and p91phox) are significantly repressed during infection with *S.pneumoniae*. The outcome of this study provides direct molecular evidence that changes in gene expression in $Hbb^{th3/+}$ β-thalassemia mice contribute to the deficiencies in neutrophil antimicrobial functions observed in β-thalassemia.

43. *Amelioration of Murine Sickle Cell Disease by Non-Ablative Conditioning and Gamma-globin Gene-Corrected Bone Marrow Cells*

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Patients with severe sickle cell disease (SCD) are candidates for gene therapy using autologous hematopoietic stem cells (HSCs), but concomitant multi-organ disease may contraindicate pre-transplant conditioning with full myeloablation. We tested whether non-myeloablative conditioning, a regimen used successfully for allogeneic bone marrow transplantation of adult SCD patients, allows engraftment of γ-globin gene corrected cells to a therapeutic level in the Berkeley mouse model of SCD. Animals transplanted according to this regimen averaged 35% engraftment of transduced hematopoietic stem cells with an average vector copy < 2.0. Fetal hemoglobin (HbF) levels ranged from 20 to 44% of total hemoglobin and approximately two-thirds of circulating red blood cells expressed HbF detected by immunofluorescence (F-cells). Gene therapy-treatment of SCD mice ameliorated anemia, reduced hyperleukocytosis, improved renal function and reduced iron accumulation in liver, spleen, and kidneys. Thus, modest levels of chimerism with donor cells expressing high levels of HbF from an insulated γ-globin lentiviral vector can improve the pathology of SCD in mice, thereby illustrating a potentially safe and effective strategy for gene therapy in humans.