Thalassemia: the continued challenge

This is a time of therapeutic hope and promise in the management of thalassemia. I began my efforts in this fascinating field 45 years ago when I saw my first patient with thalassemia intermedia. No one on the house or attending staffs believed the diagnosis. They thought this near 70 year old man, with jaundice, anemia, and intrathoracic and para spinous masses must have metastatic cancer. But his red cells were incredibly misshapen. Later, I found the telltale inclusions in them and became convinced that he had thalassemia. His case and others like his brought me to the realization that the pathophysiology of $β$ and $α$ thalassemia is directly related to the degree of imbalance of the synthesis of $α$ and $β$ chains of hemoglobin. I wish I had been the first to make that suggestion. I was preempted by that great thalassemia scholar, Phaedon Fessas, and John Clegg and David Weatherall were hot on the scent as well. But at least, I am in great company, and my admiration for my competitors is as strong now as it was then.

When I began my efforts in the early 1960s, the only effective treatment of thalassemia was blood transfusion and that one weak reed was handled poorly. Only the efforts of Wolman in Philadelphia and later Piomelli in New York convinced us to maintain the patient's hemoglobin higher than the usual 7 to 8 grams per 100 mL. The simple act of suppression of erythropoiesis with red cell transfusion would at least blunt the massive ineffective erythropoiesis, and attendant bony destruction, heart failure and organ infiltration that characterize $β$ thalassemia. Later Richard Propper and I proposed that maintenance of hemoglobin at a level no less than 12 would improve the patients status without increased iron loading. More experience suggests that a goal of 12 grams per 100 mL is neither necessary nor effective. A target of 9-10 grams per 100 mL is more realistic.

In the 1970s we also learned that filtration of blood to remove leukocytes that cause severe febrile reactions and increase alloimmunization was a vital part of transfusion management. The use of frozen blood probably increases iron load because a small fraction of frozen cells is immediately lost after transfusion. But freezing has an advantage. It demands copious red cell washing and that tends to remove leukocytes and infectious agents. Indeed thalassemia centers that used frozen red cells in the dangerous 1980s had a lower incidence of HIV and hepatitis C infection in their recipients. Today we restrict red cell washing to the 60 percent of patients who have allergic reactions to leukoreduced red cell preparations because the washing procedure is not without slight risk of bacterial contamination.

We have also learned to start transfusions early in life and to sustain them without interruption. This creates immune tolerance and greatly reduces the frequency of alloimmunization and its infrequent but very troublesome concomitant, autoimmune hemolytic anemia, the latter so often seen in intermittently transfused patients with sickle cell anemia and thalassemia intermedia.

Despite advances in transfusion techniques, the 1960s and 1970s were periods of desperation. Our brave patients faced certain death from iron overload and attendant heart failure. Almost all were carried away before the age of 20. The heart failure had little direct relationship to cardiac iron load. Death occurred whether hearts were pink or black. Only the extent of liver iron load related directly to cardiac disease. This phenomenon — well reported in the Southeast Asian literature and predicted from the studies of hemochromatosis by Bothwell, Charlton, Cook, and Finch — was explained in the 1980s by Hershko and his colleagues in Israel, who clearly demonstrated the toxic role of non-transferrin bound iron in the induction of cardiac failure. An iron loaded liver releases iron to the circulation; the flow of iron from the huge hepatic and other storage pools vastly exceeds the capacity of transferrin to bind and neutralize it. The result is free plasma iron and direct oxida-
tion of the fragile membranes of myocytes. Hence the heart is damaged by excess iron whether or not the toxic metal accumulates in the myocyte cytoplasm. For this reason we must always assess the risk of iron overload by measuring liver iron as a surrogate of total body iron. A low level of cardiac iron should not reassure any patient whose liver iron is beyond a dangerous level.

In the past two or three years emphasis has been placed on an as yet unvalidated MRI-based method of estimate of cardiac iron. The technology is admirable, but it is critical not to be deflected by it. We absolutely must focus on reduction of hepatic iron and on the development of chelators that have a much longer half life in the plasma than do either deferoxamine or deferiprone. A circulating chelator soaks up non-transferrin bound iron and thereby protects the fragile myocyte. On the other hand measurement of hepatic iron can be misleading. Biopsy, the gold standard, may be erroneous because hepatic fibrosis and cirrhosis can obfuscate the results. SQUID technology provides an integrated assay of liver iron, but it is far too expensive, unavailable, and, it turns out, not very reproducible from one SQUID center to another. MRI also offers an integrated measurement, and is very promising. But it is also expensive, the signal depends on the chemical state of iron, and the method requires a great deal of patient cooperation. CAT scanning is fast, very available, relatively cheap, and simple because it performs as an ordinary X-ray densitometer. If successfully developed it could be broadly applied worldwide. Though the most reliable method of hepatic iron measurement is not yet determined, and the MRI method of cardiac iron measurement is not independently validated, we already have experience of patients with high cardiac iron levels (as estimated by MRI) and modest liver iron burdens. These are patients who may have gotten religion about chelation after years of non-compliance. They do reduce their hepatic iron stores but cardiac iron depletion lags behind. The myocyte, like the pituitary, may have very slow kinetics of iron unloading. Chelation therapy of formerly non-compliant patients can therefore disrupt the linear relationship between liver iron and the threat to the heart. But I move ahead of the story. In the early 1970s Richard Propper and his colleagues developed continuous subcutaneous infusion of deferoxamine by means of a portable pump. The schedule for the optimal employment of the pump was modified in a sensible direction by Martin Pippard and David Weatherall at Oxford. The long term results were impressive. In the early 1990s three independent groups headed by Nienhuis and Brittenham, Borgna Pignatti, and Olivieri, Cohen and Wolf demonstrated markedly prolonged cardiac disease free survival in patients who faithfully followed the Propoer and Pippard regime. But what of those who could not or would not comply? The answer was bleak. They comprised half of the patients and they fell prey far more rapidly to cardiac failure or arrhythmia. Clearly the field needed a new paradigm — an effective orally active iron chelator. I will return to that saga after a brief flashback to the 1970s and early 1980s.

Those long days of the 1970s remained discouraging. Yes we had developed subcutaneous deferoxamine, but most of the patients were too old and too damaged to benefit from it. They continued to develop heart disease because they had been exposed to toxic levels of iron for far too long. Only patients who had begun on the pump at very young ages actually benefited. We see this in the present longevity of our patients who are currently beginning to live well into their forties if they have excellent compliance records. This of course explains why the lifespan of thalassemia patients has improved with the passing decades since the 1970s. Some in merry England would like to ascribe that change to the introduction and widespread use of deferiprone in the 1990s. That, I must say, with all due respect to my distinguished colleagues, is an obfuscatedory conclusion based on incorrect mortality analysis, and one best ignored by the unwaried. Those of us who endured the 1960s and 1970s were not at all certain that the deferoxamine pump would turn the tide of cardiac disease in our patients. We began to turn our attention to the molecular basis of the disease and to prenatal diagnosis and disease prevention.

I will not dwell on the molecular story other than to point out that it had many marvelous contributors. Enormously helped by the basic contributions of Tom Maniatis, David Baltimore, Phil Leder and the late Daniel Nathans to mention just a few, the clinical scientists rolled into action. We are forever indebted to Y.W. Kan, Stuart Orkin, Doug Higgs and David Weatherall, Haig Kazazian, Bernie Forget and many others who documented the usual deletions and the common point mutations that characterize the thalassemias. Their work proved that thalassemia had arisen independently many times in the old world presumably because of the pressure exerted by malaria. And they explained β zero and β plus thalassemia on a molecular basis. However they did not explain all of the facets of the disease. Unexplained genotype/phenotype variations persist today. Y.W Kan’s efforts documented Hermann Lehmann’s conviction that human cells contain 4 α-globin genes providing a molecular explanation of the various α thalassemia syndromes. Kan also discovered the first stop codon mutation and the SNP phenomenon, the latter used so productively by Orkin and Kazazian to clone and sequence many of the thalassemia genes.

Just before the molecular biology revolution virtually replaced hemoglobin analysis in the diagnosis of thalassemia's haematologica reports 2005; 1(issue 8):October 2005
lassemia at the gene level, our group, including Y.W. Kan, Blanche Alter, and Henry Chang decided to pursue prenatal diagnosis using the analytic techniques provided by John Clegg and David Weatherall. Ernie Huehns, George Stamatoyannopoulos, and Haig Kazazian, who had shown that the β chain of human hemoglobin could be detected in the first trimester, had paved the way. Kan was actually the first to make a successful prenatal diagnosis shortly after he moved to California. Blanche and I worked on the separation of fetal and adult cells with the help of Marie and John Crookston and then Henry Chang and I worked with John Hobbins at Yale to introduce the fetoscope for fetal red cell acquisition. Dimitris Loukopoulos took a sabbatical in our lab to learn the methods and Antonio Cao worked with Kan. As a result, new cases of thalassemia became rarities in Sardinia and Greece. We had proven our point. Given sufficient laboratory and obstetrical resources, thalassemia can be prevented.

Interestingly, while there was fierce political and clerical opposition to our approaches in Boston, the religious and political authorities in Italy and Greece looked the other way. They knew the emotional and financial cost of this disease and would not allow religious ideas to stand in the way of a program that could reduce its ravages.

Whether there is a moral here for those who hope to pursue stem cell research, I do not know. A great deal depends on proving that human embryonic stem cells can actually lead to disease amelioration. The work on the prenatal diagnosis of the hemoglobinopathies was aided by the fact that we had a method that would work and everyone involved in medicine, the church and politics knew it except of course in Boston where the learning curve has been slow for centuries. Today, of course the laborious techniques that we utilized have been supplanted by DNA based methods following chorionic villous biopsy. Thousands of couples have had children without fear. Though some might disagree, I believe that prenatal diagnosis was the most important contribution to thalassemia research in the three decades that followed the mid 1970s. The mid 70s also ushered intensive research on the regulation of fetal hemoglobin synthesis. It was clear that the unbalanced hemoglobin chain synthesis that characterizes β thalassemia could be ameliorated either by concomitant α thalassemia or by increased gamma chain synthesis. George Stamatoyannopoulos and Arthur Niemhuis provided leadership to an expanding club of physician and basic scientists who hoped to find a way to reverse the fetal switch as a physiologic approach to treatment.

Though much has been learned in the past thirty years, we still do not know the basic rules of the switch nor do we understand all of the involved proteins. But that has not stopped us from trying to stimulate γ-chain synthesis.

A practical approach began in Chicago where Joseph DeSimone and the late Paul Heller conceived of the idea that the putative methylation of gamma promoters could be inhibited by 5-azacytidine, an S phase inhibitor of the cell cycle. They observed marked increases of fetal hemoglobin synthesis in 5-aza treated and bled baboons. To their great credit, Tim Ley and Arthur Nienhuis at the National Heart Lung and Blood Institute in Bethesda, MD tested 5-azacytidine in patients with thalassemia and sickle cell disease and demonstrated an increase in fetal hemoglobin in most of them. «Molecular biology has come to the bedside», editorially exulted Edward Benz, my former student and now my boss.

I wondered about that conclusion and suggested that the real basis of the fetal hemoglobin response lay in S phase inhibition; holding the erythroblast in an early stage of development and permitting the normal gamma to β switch that occurs during normal erythroblast development to be prolonged. For once (and I think it was the only time) George Stamatoyannopoulos agreed with me (Thalia immediately concluded that we were both wrong).

I decided to prove my point by employing hydroxyurea, an S phase inhibitor with no capacity to affect methylation, in bled cynomolgous monkeys. To do so my laboratory established a collaboration with Norman Letvin at the New England Primate Center. The responses were dramatic — so profound that Orah Platt and I immediately treated two patients with sickle cell anemia who also responded.

With that, George Dover and Sam Charache launched well-designed clinical trials that eventually established that at least 50 percent of children and adults with sickle cell anemia gain benefit from hydroxyurea treatment. Whether the benefit is due solely to the increase in fetal hemoglobin is, however, less than clear. Many changes occur in the red cells of hydroxyurea treated patients that might inhibit sickling.

Despite the success of S phase specific agents and, in some reports, derivatives of butyrate, in sickle cell disease, there has been little or no benefit of these agents on the clinical course of patients with thalassemia except in individuals who have the XmnI polymorphism at –158 in the γG promoter. Patients with that polymorphism make large amounts of fetal hemoglobin during stress hematoipoiesis. The polymorphism is particularly common in the Eastern Oasis of Saudi Arabia, in Iran and in the Orissa pro vince of India but it is important to search for the polymorphism in any study of a putative fetal hemoglobin stimulating agent or in patients with unexpectedly high levels of fetal hemoglobin and reticulocytosis. (The polymorphism has no or very minimal effect on fetal hemoglobin in individuals with normal erythropoiesis). Given the lack of success
of therapies other than transfusion and chelation, and the obvious problem of non-compliance with pump-administered deferoxamine, efforts in the past two decades have been properly directed toward three different areas; the development of orally active chelators, bone marrow transplantation (a form of cellular gene therapy) and actual gene therapy involving autotransplantation of genetically corrected stem cells and progenitors. All three of these approaches are controversial but they are moving forward at variable paces.

Deferiprone was the first oral iron chelator to become available. It’s history may be more controversial than the Communist Manifesto. Leaving aside its putative toxicity, the drug has never been demonstrated to be equal or superior to defersal in an adequate prospective clinical trial. This is probably why the FDA has declined to license the drug for any purpose in the United States. It is licensed for use in Europe for those who cannot tolerate or comply with defersal, but the definition of inability to take defersal is so vague that the regulation is virtually useless.

Deferiprone fails effectively to remove or even prevent accumulation of body iron in most patients. This fact was shown by its most vociferous advocates in London, and the result is not surprising. The drug is a bidentate chelator. Three molecules are required to bind one molecule of iron. It is rapidly glucuronidated and rendered functionless, and it has a very short half life in the circulation. To be effective, it would have to be taken nearly continuously, but the necessary doses would surely be toxic. This why CIBA/GEIGY (now Novartis) abandoned the drug two decades or more ago. Now some of the drug’s advocates recommend that deferiprone should be taken with defersal. What that accomplishes for the non-compliance problem is entirely obscure. Others, including the deferiprone cheerleaders in London, hold that the failure of deferiprone to lower or prevent accumulation of hepatic iron is of no importance because deferiprone has an entirely unproven and magical capacity to reduce the iron content of the heart even though it has no utility in depletion of body iron. Indeed, vans of MRI machines have trundled ponderously over the Alps like Hannibal’s elephants on their way to the formerly malarious plains of Italy to create nebulae of T2 stars. In my opinion (and I have been wrong before), that concept will prove to be a fantasy that rivals Midsummer Night’s Dream. John Porter and his colleagues in the NHLBI supported Thalassemia Clinical Research Network are preparing a serious study of deferiprone and defersal on cardiac function that should settle the debate. The Porter study is of extreme importance. Rapid patient accrual will be highly desirable.

Meanwhile Novartis, studiously avoiding polemics about deferiprone, has constructed ICL670 a very interesting hydroxyphenyltriazole related in some respects to the desferithiocin family. The drug is very well absorbed and has a long plasma half life. One dose protects the heart from circulating non-transferrin bound iron for twenty four hours. In careful studies Novartis has shown that the drug is also as effective as standard dose defersal in removal of iron. Thus far the drug has not shown evidence of renal toxicity, but this complication has been seen in the desferithiocin family and needs to be watched very carefully. Despite that hazard, ICL670 is surely the most promising oral iron chelator as we convene in Bologna. Bone marrow or stem cell transplantation for thalassemia has also engendered controversy because no group can come close to the results claimed by Lucarelli and his associates. Some of us, and I am one, have argued that the criteria for patient assignment to risk groups offered by Lucarelli are simply non-reproducible. I have publicly stated that the risk of failure of marrow grafting for thalassemia must be given as 25 percent to all patients. Any rosier statement is simply not ethical. But recently Dr. Lucarelli has shown me his results with unrelated matched donors and his failure rate is only 25 percent. This is a group that would have a 50 percent failure rate in my hands and in the hands of most of my colleagues. I have to conclude that Dr. Lucarelli has a superb technical approach and we should emulate it. Stem cell transplant is surely a curative approach and one that is way ahead of gene therapy. If Lucarelli’s results can be widely duplicated, we are well on the way to a management system that makes sense certainly for youngsters with matched family donors and, with clear understanding of risks for patients who require matched unrelated donors.

Gene therapy has been discussed for decades at meetings such as these. Obviously the field was nearly shattered by the untoward events that occurred in Philadelphia and by the leukemia that emerged in children with X linked immunodeficiency successfully treated in France. But there are reasons to believe that gene therapy for thalassemia may reemerge from purgatory. First of all, mice with thalassemia have been almost correctly completed in two independent laboratories. Second, the vectors are improving, and third, some parents of very young children with thalassemia have preserved cord blood as a source of stem cells. Now I am one who has far less respect for cord blood banks than some of their advocates. I do not, for example believe that cord blood allo-transplants produce less GVHD because of some magical effect of cord blood cells. I think recipients of cord blood allo-transplants have less GVHD because they get much lower doses of cells than do patients who receive marrow stem cells. Cord blood recipients also suffer very slow engraftment because the doses are so low.
My opinion of cord banks notwithstanding, current data do support the idea that presently available vectors transfer functioning genes into the DNA of cord blood cells far better than into adult cells. So transfected autologous cord cells delivered in infancy may be the best way to achieve effective gene therapy for thalassemia. The problem will turn out, in my view, to relate to the successful acquisition of a niche in the marrow by the transfected autologous stem cells. That last problem will not be easy to resolve. Corrected stem or progenitor cells do not have a survival advantage in thalassemia. Only their progeny, the erythroid precursors, have a survival advantage. But if the stem cells do not find a niche in which to develop, the advantageous precursors will never appear to win the space war in the marrow. This means that an infant about to receive his or her transfected cord blood cells would require either a huge dose of transfected cells that could physically compete for niches or near ablation to provide enough empty niches to insure success. Ablation in infancy is not a happy thought. Brave investigators and unusual parents who are highly-informed and willing risk takers will need to be taken on the first step. My conclusion is that the therapy of thalassemia, which was in its infancy when I began in this field, is rapidly growing into childhood and adolescence. Transfusion, chelation, stem cell transplantation and gene replacement treatments are clearly advancing. We are making major progress, and the patients who have waited so long for a better management system have reason to be optimistic. I congratulate my colleagues who are assembled here and urge them to devote their energies to this disease—one that formed the basis of the molecular biology revolution. Patients with thalassemia changed the face of medicine. They deserve our best efforts.

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