Summary of the 2016 International Consensus Conference from DBAF's Research Director, Steven Ellis, PhD:

SYNERGY– the interaction or cooperation of two or more organizations, substances, or other agents to produce a combined effect greater than the sum of their separate parts.

The word synergy therefore provides me with a ready means to summarize the Diamond Blackfan Anemia International Consensus Conference held in Atlanta Georgia, March 5-7, 2016. This was the 14th ICC meeting where experts from around the globe were brought together for 42 hours to exchange information and ideas pertaining to all facets of Diamond Blackfan anemia.

One of the most exciting elements of helping to organize this year's meeting was the number and quality of abstracts submitted in the area of emerging therapies. As you can see below there are a number of potential new therapies on the horizon for DBA. Some of these involve reversing the genetic change that causes DBA, while others involve more traditional small-molecule based drugs. With so many different pans in the fire (so to speak) it seems likely that we will have new therapies reaching DBA patients in the not-too-distant future.

EMERGING THERAPIES

Gene Therapy/Editing

This year's meeting began with the keynote address by Dr. Mark Osborn of the University of Minnesota. The title of Dr. Osborn's talk was "Gene editing

and cellular engineering for inherited anemia." Dr. Osborn has considerable experience using various gene-editing technologies to correct genetic lesions causing human disease. Dr. Osborn gave an overview of the various gene-editing technologies available for potential use in treating genetic diseases, highlighting both strengths and weaknesses of each approach. Of particular importance in selecting Dr. Osborn for this year's keynote address was his ongoing work in developing CRISPR technology as a potential therapy for Fanconi anemia. The strategy for using CRISPR to treat an inherited bone marrow failure involves isolating hematopoietic stem cells from a patient, using CRISPR to change the mutant sequence back to the sequence found in healthy individuals, and then using the corrected hematopoietic stem cells to repopulate the patient's bone marrow using standard bone marrow transplant methodologies.

While his work was directed toward a cure for Fanconi anemia and not DBA, there is every reason to believe that the approaches developed for Fanconi anemia could be directly applied to DBA once the field matures. In fact, there was considerable discussion that using CRISPR technology to treat DBA might actually be easier than treating Fanconi anemia, given that the genetic defect in Fanconi anemia involves some of the same cellular machinery than CRISPR technology relies upon to work its magic.

We will be anxiously awaiting the work in Dr. Osborn's laboratory and many other laboratories around the world as CRISPR technology moves closer and closer to the clinic.

Lest you wonder, there are individuals applying CRISPR technology to DBA. Dr. Erik Westin working with Dr. Fredrick Goldman at the University of Alabama, Birmingham presented their pre-clinical studies using CRISPR technology to correct a mutation in *RPS19* in patient-derived induced pluripotent stem cells (IPSC). Briefly, IPSC are created from cells isolated from the skin of a patient. These cells are then engineered to behave more like embryonic stem cells, which could then be induced to differentiate into potentially any cellular lineage in the body. In theory, one could potentially create IPSC cells from a patient, correct the genetic lesion, induce these cells to differentiate into hematopoietic stem cells, and then use these corrected cells to transplant back into the patient. The advantage of using CRISPR to correct IPSCs as opposed to hematopoietic stem cells isolated from a patient is that IPSCs could potentially be easier to manipulate in culture making them easier to more thoroughly characterize before transplant. Unfortunately, IPSCs currently have significant safety concerns relating to their use therapeutically. Nevertheless, these cells serve as an

excellent pre-clinical model for studying how CRISPR technology could be used to treat DBA.

The talks on gene therapy/editing finished with Dr. Stefan Karlsson from Lund University in Sweden. Dr. Karlsson's work is well known to readers of this column as I have discussed his work on using classic gene therapy to treat his mouse model of DBA several times. You might think that a person who spent much of his career developing classic approaches to gene therapy might feel a little threatened or frustrated by all the hype given to CRISPR technology these days, but Dr. Karlsson's talk was an outstanding example of the type of synergy that occurred at this year's meeting. In the previous two talks on gene editing, a major issue that was raised related to how well the cells manipulated outside of the body during the gene editing process would be able to compete with endogenous cells when transplanted back into a patient. At issue, was the extent to which transplanters might have to ablate the current marrow to maximize successful reconstitution with the gene-edited cells. Dr. Karlsson's pioneering work on correcting mouse hematopoietic stem cells by introducing normal copies of genes like *RPS19* has provided some of the answers to these questions and importantly showed these the corrected cells actually compete very well with endogenous cells in successfully reconstituting the marrow after gene therapy.

Thus, rather than having a raucous debate on whether CRISPR or other forms of gene editing were better than classic gene therapy, the different technologies fed off of one another with one informing the other. This type of synergistic interaction will certainly help move these technologies forward into clinical application.

Small Molecule Therapies

Another example of synergism at work in the DBA field arose from the next two talks by Drs. Johan Flygare from Lund University and Ross Hannan at the Australian National University. Both of these investigators have initiated high-throughput screens for small molecules that reverse DBA-like phenotypes in their respective model systems. Both have also developed large screens for genes that when disrupted, reverse these DBA-like phenotypes. Both of these screens work on a very large scale and involve considerable resources and personnel. While both investigators reported progress in their respective screens, what was most encouraging is that these two investigators, separated by almost 10,000 miles, decided to join forces rather than compete, and work together toward a common goal.

The next talk in this session was by Dr. Anu Narla, filling in for her father Dr. Mohandas Narla who was unable to attend the meeting. This talk emphasized some of the differences in erythropoiesis between mice and humans. This was an important talk at this juncture in the meeting, in that several of the drugs screens currently being carried out are at least initially performed in mice before being tested in human cell culture models. The differences between mice and humans may explain how drugs that look encouraging in one system may eventually fail in another. We were all reminded that no matter how efficacious a drug may be in model systems, it is ultimately how well they perform in clinical trials with patients that will dictate whether they will successfully compete with current therapies for use in the broader DBA patient population.

While the screens being carried out by Drs. Flygare and Hannan were still in relatively early stages, other screens carried out on a more limited scale have nevertheless yielded exciting new drug prospects, which were the subject of the next three talks. The first of these talks was from Dr. Harvey Lodish at the Whitehead Institute in Boston. In studying the genes expressed at a critical juncture in the development of red blood cells, the Lodish group discovered a signaling pathway that appears to suppress red cell production at this stage. Interfering with this signaling pathway was shown to increase the production of red blood cells. Importantly, a drug that interferes with this pathway has already been tested clinically as an anticancer drug and so there is already information pertaining to the pharmacological parameters of this drug in humans. Having this information in hand could in principle shorten the time needed to get this drug into clinical trials for DBA patients should further studies warrant.

If the previous talk sounded familiar, it was. Dr. Lodish has recently published on another drug currently used clinically that synergizes with glucocorticoids to stimulate red blood cells production. In an ongoing collaborative effort with the Lodish lab these initial studies have been followed up and extended by Dr. Sandra Martinez-Morilla in the laboratory of Dr. Shilpa Hattangadi at Yale University. Discussions are currently underway with Drs. Vlachos and Lipton to set up a clinical trail to test this drug in DBA patients.

Finally, Dr. Elizabeth Macari from the laboratory of Lenoard Zon at Harvard Medical School presented a drug screen begun in zebrafish models of DBA that yielded yet another repurposed drug that may be of potential benefit to DBA patients. This drug, quite distinct from the drugs identified in the Lodish screen, also looks very promising in human cellular models of

DBA. Here again, discussions are underway to see if this drug ready to be tested in a clinical trail with DBA patients.

I hope you can appreciate what an exciting session this was. The latter three drugs have already been used in humans for other purposes means that we already have information pertaining to their adverse effects, which will allow physicians, patients, and families a much better understanding of the risks and benefits associated with participating a clinical trial.

GENOTYPE/PHENOTYPE RELATIONSHIPS

In this session on genotype/phenotype relationships we heard from DBA registries in Canada, Europe, and the US.

The session began with a presentation by Dr. Omri Arbiv, working with Dr. Yigal Dror at the Hospital for Sick Children in Toronto, on information from the Canadian Inherited Marrow Failure Registry. The focus of this talk was on the frequencies of different genes affected in DBA and whether there was an association between genes affected and any of the clinical parameters measured in DBA patients. The two major finding from this analysis were that patients with mutations in *RPL11* tended to have milder hematological phenotypes than patients with mutations in *RPS19* and that only patients with mutations in *RPL5* and *RPS26* appeared to be associated with certain types of congenital anomalies. It was somewhat surprising that patients with *RPL11* mutations did not show an association with congenital anomalies since this has been reported previously. Whether this difference reflects relatively small numbers in the Canadian Registry or unique characteristic of the Canadian DBA population remains to be determined.

The second talk in this session was from Dr. Marcin Wlodarski at the University Children's Hospital in Freiburg Germany. Dr. Wlodarski presented data on behalf of the EuroDBA Consortium. The focus of this talk was on the prevalence of microdeletions in the EuroDBA patient cohort that had originally tested negative for mutations in known ribosomal protein genes. Over 20% of these patients were shown to have small deletion mutations in known DBA genes that were missed by DNA sequence analysis. The inability of DNA sequence analysis to identify deletion mutations is well documented and has required the development of alternative technologies to better cover the complete range of mutation types that could be encountered when trying to pinpoint a causative gene for human diseases. Many of the patients with these deletions in the European cohort had one or more congenital anomaly including some rarely

observed in DBA patients. Some of these more unusual traits may be associated with genes found near the ribosomal protein gene affected by these deletions and so may be related to the loss of these contiguous genes. These studies re-enforce that deletion mutations can represent a significant fraction of mutations in DBA patient cohorts and that deletions should be tested for when trying to identify a causative mutation in DBA patients.

This session finished with a talk by Dr. Adrianna Vlachos at the Cohen Children's Medical Center (now Northwell Health) in New York. Dr. Vlachos spoke on the incidence of cancer in the DBA Registry of North America. This was a follow up on a recently published paper by Drs. Vlachos, Lipton, Alter and others on the cancer risk in DBA patients. New data as the DBA patient cohort in the DBA Registry of North America ages has been gathered and is currently being evaluated and will hopefully soon be ready for publication. These new data should provide additional insights into the links between DBA and cancer and be of immense importance in counseling patients on cancer risk and surveillance.

PATHOGENIC MECHANISMS I

Over the years there have been several mechanisms proposed whereby defects in the production of ribosomal proteins could give rise to the clinical features of DBA. One of the most difficult aspects of these mechanisms to reconcile is the seemingly selective effect of mutations in genes encoding ribosomal proteins on the production of red blood cells in DBA patients.

The first talk in this session was from Dr. Lydie Da Costa at the hospital Robert Debre in Paris. Dr. Da Costa provided data that appeared to tie together the ribosomal protein and GATA1 worlds of DBA. She showed that mutations in certain ribosomal proteins interfered with the synthesis of a cellular chaperone for the GATA1 transcription factor. Without this chaperone, levels of GATA1 fall. Since GATA1 is a critical transcription factor required for erythropoiesis, a decrease in its levels could potentially explain how a defect in something as ubiquitous as a ribosomal protein could have a selective effect of production of red blood cells. Dr. Da Costa also showed that globin synthesis was affected in cells haploinsufficient for certain known DBA ribosomal proteins, consistent with models where excess heme could contribute premature death of red cell progenitors.

Dr. Da Costa's talk was followed by Dr. Nicholas Watkins from Newcastle University in the United Kingdom. Dr. Watkins spoke from the perspective of an alternative model for DBA pathogenesis caused by nucleolar stress linked to abortive ribosome assembly. His talk focused on the mechanism by which abortive ribosome assembly activates p53, thereby promoting apoptotic cell death. The central player in this pathway is a subcomplex of 5S rRNA with two ribosomal proteins Rpl5 and Rpl11. His talk centered on how this subcomplex is regulated and how these regulatory properties might be exploited as targets for the development of novel drugs to treat DBA.

The next talk by Marie-Francoise O'Donohue in the laboratory of Pierre-Emmanuel Gleizes at the University of Toulouse seemed to bridge the gap between the translation-centric and ribosome stress-centric mechanisms of DBA pathogenesis by reporting on new DBA gene that had both a nuanced effected on ribosome synthesis and also appeared to influence ribosome function. Haploinsufficiency for this new ribosomal protein leads to the production of slightly altered ribosomes with distinct functional properties. These novel ribosomes may have distinct ways that mRNAs are selected for translation, potentially disrupting erythropoiesis. Therefore, identifying transcripts whose translation is influenced by these novel ribosomes may provide new insight into DBA pathogenesis.

The session wrapped up with a talk by Dr. Rajiv Khajuria from Dr. Vijay Sankaran's lab at the Dana Farber Institute. The Sankaran lab was the first to show a connection between ribosomal proteins and GATA1 in DBA pathogenesis by showing that GATA1 expression is selectively decreased in cells with reduced amounts of ribosomal proteins. The current study was focused on whether the translational defect in DBA is because of a reduction in the amount of presumably ribosomes or, if as suggested by the previous talk from the Gleizes lab, that the ribosomes themselves may be functionally altered. While they could not rule out the latter possibility, the work from the Sankaran lab seemed most consistent with a reduction in the number of otherwise normally functioning ribosomes. The two studies could be potentially reconciled, if there were one or more rare ribosomal proteins that affected both ribosome number and function. It will be very interesting to see if patients with mutations in these new genes have unique clinical phenotypes because of their combined effects on ribosome synthesis and function.

Dr. Pakaj Qasba, Program Director of the Blood Diseases Branch of the National Heart, Lung, and Blood Institute began Day 2 with a talk on ongoing programs at the NHLBI of relevance to meeting attendees.

PATHOGENIC MECHANISMS II

This last session was a bit more eclectic, covering a range of topics related to the potential mechanisms underlying DBA pathogenesis.

The session began with a presentation by Dr. Barry Paw from Harvard Medical School. Dr. Paw's work is focused on the regulation of protein synthesis in erythroid progenitors by amino acids. This work is relevant to DBA because one of these amino acids is leucine, which has been studied as a potential treatment for DBA through its ability to stimulate ribosome synthesis. This up-regulation is mediated through a critical sensor by the name of mTOR. Dr. Paw hopes that these studies may lead to more effective strategies to exploit this regulatory network as a therapeutic target for DBA.

The next talk in the session shifted away from ribosomes and protein synthesis to the relationship between two transcription factors GATA1 and GATA2. This talk was by Dr. Shai Izraeli from the Sheba Medical Center and Tel Aviv University. Dr. Izraeli is testing the hypothesis that the balance between the amount of GATA1 and GATA2 plays a critical role in the pathogenesis of DBA. His studies reveal that the increased ratio of GATA2 to GATA1 in patients with mutations in GATA1 disrupts erythropoiesis. These data would suggest that one or more genes regulated by GATA2 may be contributing to the effect on erythropoiesis and by identifying these genes it might be possible to target them in future drug development.

We turned back to the ribosome with a talk by Dr. Fabrizio Loreni from the University Tor Vergata in Rome. Dr. Loreni is interested in the signaling pathways involved in nucleolar stress, particularly the role of a protein kinase called Pim1 in the process. Protein kinases are a huge family of cellular signaling enzymes involved in controlling a wide range of processes within cells. The signaling pathway regulated by Pim1 may converge on p53 together with the pathway involving the 5S subcomplex and so provide an alternative target for therapeutic intervention.

The next talk by Dr. Jason Farrar from the University of Arkansas used an *in vitro* culture system to study the effects of ribosomal protein haploinsufficiency and the reduction of GATA1 on the ability of $CD34^+$ progenitor cells from DBA patients to differentiate along the erythroid lineage. This model system should closely mimic what is going on within

the marrow of DBA patients. One of the surprises from Dr. Farrar's presentation was the view that GATA1 functions upstream of ribosomal protein, perhaps in regulating ribosome synthesis during erythroid differentiation.

The final talk of the meeting was by Dr. Colin Sieff from Boston Children's Hospital. Dr. Sieff presented work on a new mouse model of DBA. This mouse is haploinsufficient for Rpl11. This mouse is unusual because these mice more closely resemble the clinical picture of DBA seen in humans than some of the other mouse models that have been developed over the years. While there is still much to learn about these mice, they could be a valuable resource for the DBA community.

POSTER SESSIONS

In addition to the oral sessions there were two poster sessions at this year's meeting. These sessions followed a format introduced at the previous ICC meeting where presenters gave a brief overview of each poster to all attendees prior to individualized viewing. There were a range of topics presented in the poster session and I will groups posters together under a limited number of topic headings for discussion.

Clinical

There were several posters relating to clinical features of DBA. Drs. Alyson MacInnes and Ina Hainmann presented data on characteristics patients registered with the European Diamond Blackfan Anemia Consortium including an analysis of patients with still unidentified genes. These patients showed similar clinical characteristics with patients that have identified genes suggested shared pathogenic mechanisms.

There were also two posters from Dr. Blanche Alter's group at the National Cancer Institute, one of which dealt with pregnancy outcomes among unaffected mothers with children with DBA. The second poster outlined Dr. Alter's efforts in developing a strategy to mathematically model potential outcomes of bone marrow transplantation for DBA patients taking into consideration data available on risks and benefits. This model is in its early stages of development but could of tremendous importance in weighing decisions as to if or when a DBA patient should go to transplant.

Dr. Irma Dianzani also had two posters that dealt with new methodologies to help diagnose DBA. One of the methods involved monitoring pre-rRNA processing as a means to monitor ribosome biogenesis in a blood sample

from suspected DBA patients. The other approach involved the analysis of small pieces of cells that break off red cell progenitors, with the rationale being that these cellular fragments may be more prevalent in patients where the progenitors are suffering stresses linked to ribosomal protein haploinsufficiency.

Drs. Anu Narla and Dagmar Pospisilova presented patients with mutations in known DBA genes that had unusual clinical presentations. These patients may create new classes of genotype/phenotype relationships and demonstrated the scope of DBA continues to broaden, presenting in adults and with markedly different severities.

Dr. Takuya Kamio from Hirosaki University in Japan also presented a poster identifying a new ribosomal protein gene responsible for DBA.

Jimmy Hom working in the laboratory of Dr. Lionel Blanc described a mouse model of DBA where they are studying the effects of ribosomal protein haploinsufficiency on skeletal development and the induction of osteosarcoma, part of the extended clinical phenotypes in DBA that have not been extensively studied in the past.

The mechanisms by which glucocorticoids stimulate erythropoiesis remain a mystery. Consequently studies on the mechanisms of glucocorticoid action are relevant to the DBA world. Dr. Eliza Geer from the laboratory of Dr. Anna Migliaccio presented a poster on the mechanisms whereby glucocorticoids stimulate erythropoiesis in patients with Cushing's disease.

New Targets for Drug Development

Brian Dulmovits, Dr. Elizabeth Macari, and Dr. Kathleen Sakamoto each presented posters identifying new targets for developing drugs to treat DBA. These studies, together with the ongoing drug screens discussed above, have opened up a number of new avenues for therapeutic development. Complementing these ongoing drug screens, Dr. Michal Hetman outlined a structural approach he plans to take to identify small molecules that disrupt the signaling pathway linking ribosome stress to p53 activation. Whereas, Anna Aspesi outlined a novel form of gene therapy that she intends to use to up-regulate the synthesis of ribosomal proteins affected in DBA as a way to offset ribosomal protein haploinsufficiency. These results add to the growing enthusiasm that there should be new drugs available for DBA patients in the foreseeable future.

Along these same lines, Shuo Lin had a poster outlining his work on identifying the mechanism of action of RAP-011, the generic form of Sotatercept, which has already been in clinical trials for DBA.

Pathogenic Mechanisms

Dr. Johnson Liu presented a poster on his work using cord blood from DBA patients as a source of hematopoietic stem cells to study the pathogenic mechanisms involved in DBA. One of these pathogenic mechanisms involves p53 activation, which Dr. Sharon Singh showed can also be induced by oxidative stress observed in cellular models of DBA. This represents yet another mechanism converging on p53 activation that may be involved in DBA pathogenesis.

Dr. Tamayo Uechi presented data derived from their zebrafish model of DBA analyzing mRNA selection by defective ribosomes. These studies are aimed at identifying critical mRNAs required for erythropoiesis that may be adversely affected by reductions in ribosome number in DBA patients.

In closing, I would like to thank everyone who attended this year's Diamond Blackfan Anemia International Consensus Conference for your willingness to share ideas and unpublished data as the field continues to progress toward our common goal of improving the lives of patients and families affected by Diamond Blackfan anemia. I would also like to thank the meeting organizers, with a particular shout out to Dr. Adrianna Vlachos who did a yeoman's job in putting together this year's agenda from the list of selected abstracts. Individuals involved in the abstract review process included Dawn Baumgardner, Drs. Vlachos, Lipton, Dianzani, Flygare, Blanc, Nathan, Bodine, and myself. Finally, I would like to thank the various foundations, agencies, and organizations mentioned for their generous financial support for this year's meeting: The DBA Foundation, DBA Canada, the Daniella Maria Arturi Foundation, DBA-UK, Captain Courageous (Australia) and the Resource Capital Funds Foundation. The meeting was supported by the National Institute Of Diabetes And Digestive And Kidney Diseases of the National Institutes of Health under Award Number R13DK109587. (Dislaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.)