GLOBAL BRIDGES 2024

MADRID 11-12th JUNE

In person & online hybrid meeting

BOOK OF ABSTRACTS

10th June Business meeting dinner
Meeting with the patients representatives

Global Bridges 2024

Madrid June 11-12

ORGANIZING COMMITTEE:
Pierre-Emmanuel Gleizes, Marije Bartels and Susana Navarro

euro DBA

EUROPEAN JOINT PROGRAMME RARE DISEASES

Index

Global Bridges 2024

Madrid June 11-12

The European Diamond Blackfan anemia syndrome symposium

"Individually, we are one drop. Together, we are an ocean."

Ryunosuke Satoro

Welcome to Madrid!

Dear participants,

A warm welcome to you all on the 5th edition of the EuroDBAS **"Global Bridges"** symposium hosted this year at **CIEMAT (Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas) in Madrid.**

This international meeting is intended to bring together researchers, physicians and patient organizations and aims to explore the mechanisms underlying DBAS, to discuss the latest scientific and medical advances on this pathology and especially, to share resources and ideas. As Japanese poet Ryunosuke Satoro said, **"Individually, we are one drop. Together, we are an ocean".** The collaboration and coordination between patients, investigators and clinicians is the key to writing the present and future of the investigation into Blackfan-Diamond anemia syndrome. Therefore, let´s take advantage of these days and keep on looking for new collaborations and projects.

Preparing this meeting has been a great opportunity to foster the collaboration with and between patient advocacy organizations (PAOs) throughout Europe, and we are very glad that this year's program was built together with them. Therefore, in addition to a session especially dedicated to the patients, we have now included panels of discussion for several sessions in which we intend to address some of the topics that concern the families and patients. We want to thank all of them for their involvement, support and participation.

Most of the costs of this meeting are being covered with the support of several PAOs, **DBA España, AFMBD (DBA France), DBA UK, DBA Germany and DBA Italia** as well as funds from a **European Joint Program on Rare Disease**s grant.

We thank you for your attendance, welcome you to the meeting and hope that you'll enjoy it and have a great time in Madrid!

The organizers

Marije Bartels, Pierre-Emmanuel Gleizes and Susana Navarro

Acknowledgement

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Diamond-Blackfan Anemia Report

EUROPEAN JOINT PROGRAMME RARE DISEASES

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Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas

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With the help of DBA Spain

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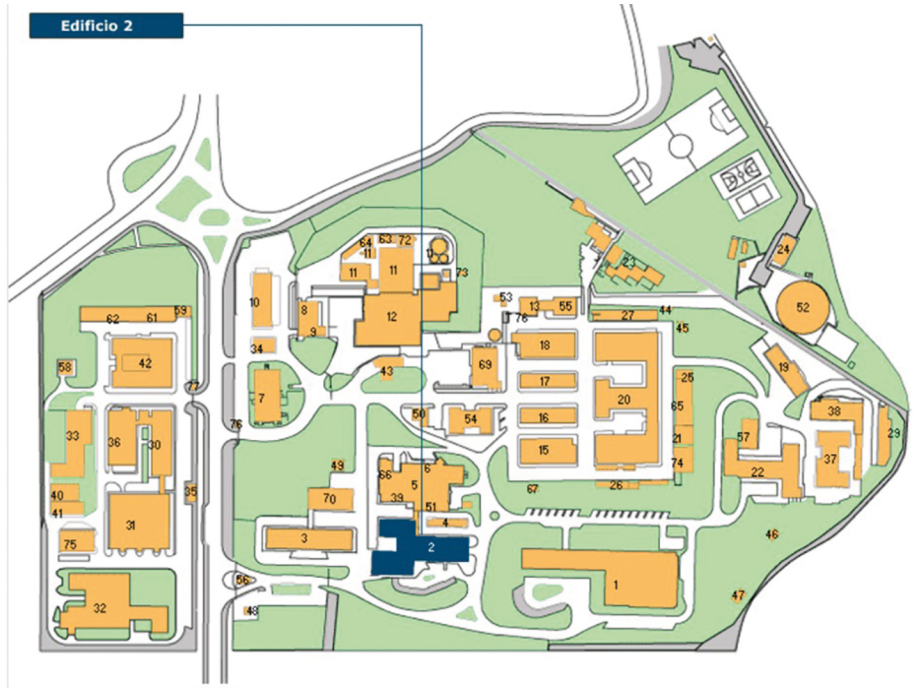
CONGRESOS

Meeting venue

Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT)

Av. Complutense, 40, Moncloa - Aravaca, 28040 Madrid

Ball room Building 1



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Program: Daily Agenda

JUNE - MONDAY 10th

CIEMAT BUILDING 70. BIOMEDICAL INNOVATION UNIT (room 11 (Building70)
and ALBERT EISTEIN (Building 3)

17:00 - 19:30	**Business Meeting & PAOS: Business Meeting Meeting with the patient representatives RiboEurope EJPRD consortium & PAOs** Visit to Biomedical Innovation Unit labs and CliniStem Manufacturing facility (If someone is interested)
20:30 - 22:30	**Dinner with the patient representatives and the invited speakers** **(Hotel Indigo Madrid Princesa)**

JUNE - TUESDAY 11th

CIEMAT BUIDING 1 MAIN BALLROOM

8:45 - 8:55	**Welcome & Introduction** *Marije Bartels / Pierre-Emmanuel Gleizes / Susana Navarro*
08:55 - 10:15	**Basic & translational reseach I (Ribosome biology):** (**Chairs**: *Denis Lafontaine & Marie-Françoise O'Donohue)*
08:55 - 09:10	**Introduction:** **Probing the ribosomal RNA methylation repertoire in Diamond-Blackfan anemia** *Denis Lafontaine*
09:10 - 09:30	**Ribosomes as drivers and targets in colorectal cancer** *John Knight*
09:30 - 09:45	**Investigating the function of HEATR3 in ribosome biogenesis and the nucleolar surveillance pathway** *Amee J. George*
09:45 - 10:00	**SURF2 is a new player in nucleolar stress response** *Simon Lebaron*
10:00 - 10:15	**Detecting pre-rRNA processing defects by RNA capture and capillary electrophoresis for DBA study and diagnosis** *Pierre-Emmanuel Gleizes*
10:15 - 12:00	**Basic & translational reseach II (Hematopoiesis):** (**Chairs**: Lydie Da Costa, Emile van den Akker)
10:15 - 10:30	**Deciphering disease mechanisms in DBA, one cell at a time** *Deena Iskander*
10:30 - 10:45	**Diamond Blackfan Anemia: Impact of the arginine-polyamine-hypusine axis on erythroidprogenitor metabolism and differentiation** *Sandrina Kinet*
10:45 - 11:00	**Transfusion-ready red blood cell generation from induced pluripotent stem cells** *Emile van den Akker*
11:00 - 11:30	**Morning Coffee break**
11:30 - 11:45	**Investigating Novel Erythropoietic Factors in Diamond-Blackfan Anemia Syndrome via Erythroblast Differentiation Assays** *Devon Germain*
11:45 - 12:00	**Response to anaemic stress in Diamond-Blackfan anaemia reveals metabolic decompensation** *Helen Brown*
12:00 - 13:15	**Mechanisms of treatment independence/hematopoietic evolution** (**Chairs**: *Allan Warren, Deena Iskander)*
12:00 - 12:15	**Mechanisms of somatic genetic rescue in germline ribosomopathy** *Alan Warren*
12:15 - 12:30	**Natural gene therapy in DBA syndrome explored using single cell genomics** *Marcin Wlodarski*
12:30 - 12:45	**Treatment-independence due to self-reverting mutation in DBA patients** *Paola Quarello*
12:45 - 13:15	**PANNEL DISCUSSION: somatic mosaicis and clonal slection in DBA** *Alan Warren, Marcin Wlodarski, Paola Quarello*
13:15 - 14:30	**LUNCH and poster viewing**
14:30 - 16:15	**Novel & Advanced therapies** (**Chairs**: *Charlotte Niemeyer, Paula Río)*
14:30 - 15:00	**"KEYNOTE LECTURE: Advances in Hematopoietic Stem Cell Gene Therapy"** *Juan Bueren*
15:00 - 15:15	**Development of gene therapy for RPS19 deficient Diamond Blackfan anemia** *Stepahn Karlsson*
15:15 - 15:30	**Development of a gene editing approach for the treatment of RPL5-deficient Diamond-Blackfan anemia patients** *Manuel Palacios*
15:30 - 15:45	**Preclinical development of RPS19 lentiviral vector for gene therapy** **ON LINE PRESENTATION** *Senthil Bhoopalan*

15:45 - 16:00 **Single-cell-multiomics of gene therapy treated ribosomal protein S19-deficient Diamond-Blackfan Anemia patient cells demonstrates molecular efficacy and uncover new pathogenic mechanisms**
Johan Flygare

16:00 - 16:15 **Exploring Tyrosine Kinase Inhibitors as Potential Therapeutics for Diamond-Blackfan Anemia**
Victoriano Mulero

16:15 - 16:45 **Coffe break**

16:45 - 17:15 **PANNEL DISCUSSION & PATIENT PERSPECTIVE**
Stefan Karlsson, Apriligen, Johan Flygare
Marcin Wlodarski, Senthil Bhoopalan (on line)
Susana Navarro, Juan Bueren,
Bas Reijnen

Topics of interest expressed by the PAOs

- Conditioning regimen
- Who can be enrolled?
- Inclusion criteria/Exclusion criteria

17:15 - 17:50 **Poster session I**
(**Chairs**: Susana Navarro & Marien Van der Stel)

SHORT TALKS: selected posters

17:15 - 17:30 **Elucidating the Role of RPL5/uL18 Mutations in the Pathogenesis of Diamond-Blackfan Anemia**
Federica Marchesini

17:30 - 17:35 **iPSC disease models to study and treat Diamond-Blackfan Anemia Syndrome (DBAS)**
Chantal Clark

17:35 - 17:40 **Towards an improved erythroid production from iPSCs**
Marien Van der Stel

17:40 - 17:45 **Elucidating glucocorticoid-responsiveness in Diamond-Blackfan anemia syndrome using iPSC-derived erythroblasts**
Teun Slijkerman

17:45 - 17:50 **The biology of RPS19-R62W: Interacting proteins and their roles in red blood cell differentiation**
Stefan Weitzer

19:30 **TOURISTIC TOUR & TAPAS DINNER Mesón Rincón de la Cava**

Meeting point Plaza España at 19:30

JUNE - WEDNESDAY 12th

09:00 - 10:30 **Allogeneic Hematopoietic Stem Cell Transplantation**
(**Chairs**: Cristina Beléndez, Marije Bartels)

09:00 - 09:30 **KEYNOTE LECTURE: From Transfusion to Stem Cell Transplantation**
Charlotte Niemeyer

09:30 - 09:45 **Predicting outcome of hematopoietic stem cell transplantation in pediatric DBAS: does age matter?**
Marije Bartels

09:45 - 10:00 **Hematopoietic stem cell transplantation in Diamond Blackfan Anemia Syndromes**
Carlo Dufour

10:00 - 10:30 **PANNEL DISCUSSION:**
Elective HSCT in DBA patients: Is reduced conditioning an option?
Brigitte Strahm
Carlo Dufour
Jeffrey Lipton
Cristina Belendez
Marco Zecca

Topics of interest expressed by the PAOs

- Is there a relation between chronic transfusions and cognitive decline (more severe than lack of concentration/forgetfulness)? Do psychological tests show abnormalities in some patients due to chronic transfusions or can they be explained by other co-factors like premature birth?
- When/how should patients react, if they suffer from unspecific symptoms (e.g. bone pain)? Which “symptoms“ are relevant to be reported?

10:30 - 11:00 **Coffee break**

11:00 - 12:30 **The adult patient with DBA syndrome**
(**Chairs**: Brigitte Strahm & Hannah Tamary)

11:00 - 11:30 **KEYNOTE LECTURE:**
Hematological and systemic features of DBA in adults in Germany
Alexander Puzik

11:30 - 11:45 **Pregnancy and genetic counseling**
Lydie Da Costa

11:45 - 12:00 **Women’s Health in Diamond Blackfan Anemia Syndrome: Data from the Diamond Blackfan Anemia Registry**
Adriana Vlachos

12:00 - 12:15 **How do I transition DBA patients?**
Cristina Belendez

12:15 - 12:30 **The Global Diamond Blackfan Anemia Cancer Consortium**
Jeffrey Lipton

Topics of interest expressed by the PAOs

- DBAS patients and the use of growth hormones
- Aging: is there a relation between low hb levels in childhood and a fast(er) aging process? Are there specific consequences of faster aging like being cold, fatigue, lower physical/mental performance, psychological/social restrictions,..?
- Are there specialists for adult dba patients available?
- Influence of puberty/pregnancy/menopause on changes of being in remission or the need of more/less transfusions
- Cancer risk compared to patients without DBA (especially bone/colon cancer and mds), recommendations for preventive actions

12:30 - 14:00 **LUNCH + poster viewing**

14:00 - 14:50 **The patient perspective**
(**Chairs**: Bas Reijnen & Colin Noel)

14:00 - 14:20 Living with DBAS
Beate Buchholz, Boris Marte

14:20 - 14:50 **PANNEL DISCUSSION:**
What needs to be done to improve the health status of adults with DBA?
(Chair: Alex Puzik)
Beate Buchholz
Boris Marte
Carlos Carrión
Alexander Puzik
Adriana Vlachos
Dagmar Pospisilova

14:50 - 15:05 **Late breaking presentation session**
(**Chairs**:Irma Diazani & Eva-Maria Kornemann)

Clonal hematopoiesis in long-term survivors of pediatric hematopoietic stem cell transplantation
Mirjam Belderbos

15:05 - 15:30 **Poster Session II and Award**
(**Chairs**: Irma Diazani & Eva-Maria Kornemann)

15:05 - 15:10 **De novo heterozygous RPL8 missense variant in a patient from Yakutia with Diamond-Blackfan anemia**
Anna V Pavlova

15:10 - 15:15 **Learning through registries, a desired and demanded need by patient associations, clinicians and investigators. Preliminary data from the Spanish Registry of Blackfan Diamond Anemia Syndrome (DBA).**
Alba González-Guerrero

15:15 - 15:20 **Implementing Human Phenotype Ontology into the German DBA registry – what's the benefit?**
Eva-Maria Kornemann

15:20 - 15:30 **Poster award**
(**Chairs**: Marije Bartels, Susana Navarro, Pierre-Emmanuel Gleizes)

15:30 - 16:00 Coffee break

16:00 - 17:00 **How can we implement DBA Guidelines efficiently?**
(**Chairs**: Paola Quarello & Marcel Hibert)

16:00 - 16:20 **1. Stepwise approach from diagnosis to treatment: Overcoming practical challenges**
Thierry Leblanc

16:20 - 16:40 **2. Online implementation and future updates**
Marcin Wlodarski

16:40 - 17:00 **PANNEL DISCUSSION**
Thierry Leblanc & Marcin Wlodarski

- Are there different laboratory reference ranges available between a healthy and a DBA patient (e.g. for vitamin D)?
- Recommendations for food supplements or diets (e.g. vitamin D, leucine,..)
- Any new evidence for the influence of epo on hb in dba patients available?
- DBA patients who are not transfusion-dependent: influence of exjade on hematopoiesis (is hb (temporarily) lower than without exjade?), ferritin and organ iron overload in comparison to transfusion-dependent patients
- Describing the phenotype(s) of dba patients: is it possible to classify/describe different phenotypes and to adapt treatment recommendations for each phenotype?
- Covid: is evidence about the course/duration of a covid disease in dba patients available? Vaccination recommendation?

17:00 - 17:15 **Closing remarks**
Marije Bartels / Pierre-Emmanuel Gleizes / Susana Navarro

Abstracts Oral Sessions

01

RIBOSOMES AS DRIVERS AND TARGETS IN COLORECTAL CANCER

Zornitsa Vasileva Stoichkova[1], Zijian Zhang[1], Nicole Simms[1], Nikola Vlahov[2], David Gay[2], Rachel Ridgway[2], William Faller[2], Anne Willis[3], Owen Sansom[2], David Thornton[4,5], Michael Braun[6], Mark Saunders[6], John Knight[1]

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[2] *CRUK Scotland Institute, Glasgow*

[3] *MRC Toxicology Unit, University of Cambridge*

[4] *Wellcome Trust Centre for Cell-Matrix Research, University of Manchester*

[5] *Lydia Becker Institute of Immunology and Inflammation, University of Manchester*

[6] *The Christie Hospital, Manchester*

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Colorectal cancers (CRCs) rapidly synthesise both RNA and protein leading to their description as addicted to RNA and its translation. Using mouse models and human cell lines we demonstrate CRC's reliance on ribosomes to drive these addictions and reveal how oxaliplatin, a standard of care chemotherapy agent for CRC, directly targets ribosomes.

The *Rpl24*^{Bst} mutant mouse reduces the expression of the ribosomal protein RPL24 and has been used as a tool to analyse ribosome function in various mouse models of disease. Consistent with previous studies, we show that *Rpl24Bst* suppresses tumorigenesis and proliferation in a model of CRC. However, in contrast to previous reports, *Rpl24*^{Bst} mutation has no effect on ribosome subunit abundance but instead suppresses translation elongation through phosphorylation of eEF2, reducing protein synthesis by 40% in tumour cells. Ablating eEF2 phosphorylation in *Rpl24*^{Bst} mutant mice by inactivating its kinase, eEF2K, completely restores the rates of elongation and protein synthesis while also restoring tumour cell proliferation and tumorigenesis. Furthermore, we show that eEF2K activity is responsible for some of the congenital abnormalities associated with the *Rpl24*^{Bst} mutant. This work defines a role for RPL24 in regulating cellular signalling to translation elongation which is important for normal and disease biology.

Oxaliplatin is a platinum-based chemotherapy used to treat CRC with emerging evidence implicating RNA as oxaliplatin's major target. Using a qPCR-based method we analyse platinum adducts present in specific abundant RNAs, observing RNA damage to 18S rRNA in oxaliplatin treated cells. Using the sa me method, we observe damage signatures in RNA from oxaliplatin-treated mice from CRC models, but not normal intestinal tissues. Confirming this molecular mechanism, we have developed and applied AquIRE (Aqueous Identification of RNA Elements) to demonstrate oxaliplatin adduct formation on total RNA. Modifying this AquIRE platform, we have identified a previously unknown RNA damage modality for oxaliplatin, whereby the drug induces RNA-protein crosslinks. RNA damage, in particular at the ribosome, is therefore a significant molecular consequence of oxaliplatin treatment, adding further to the observations of the disease being addicted to RNA and its translation.

02

INVESTIGATING THE FUNCTION OF HEATR3 IN RIBOSOME BIOGENESIS AND THE NUCLEOLAR SURVEILLANCE PATHWAY

Jasmina S. Frawley[1], Olivia Delfino[1], Karagh E. Loring[1], Mei S. Wong[1], Nadine Hein[1], Rita Ferreira[1], Kira D. Wysoke[1], Eric Kusnadi[3], Katherine M. Hannan[1], Jin-Shu He[2], Nicholas J. Watkins[4], Ross D. Hannan[1,3] and Amee J. George[1,2]

1. *Genome Sciences and Cancer Division, The John Curtin School of Medical Research, The Australian National University, Canberra, Australia*
2. *ANU Centre for Therapeutic Discovery, The Australian National University, Canberra, Australia*
3. *Research Division, Peter MacCallum Cancer Centre, Melbourne, Australia*
4. *Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle-Upon-Tyne, United Kingdom*

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The nucleolar surveillance pathway (NSP) monitors nucleolar integrity and responds to nucleolarstresses (i.e., perturbations in ribosome biogenesis) by mediating the inhibitory binding of ribosomalproteins (RPs) to the mouse double minute 2 homolog (MDM2), a nuclear-localised E3 ubiquitinligase, resulting in p53 accumulation. Inappropriate NSP activity is implicated in the pathogenesis of ribosomopathies, while drugs that selectively activate the NSP are in clinical trials as cancer therapies.

To further investigate the molecular basis of NSP activity, we have previously conducted several high-throughput screens (loss-of-function RNAi screens published in Hannan et al 2022 Cell Reports, andtherapeutic-based screens) to identify the proteins required for this process, and chemical modulators which either suppress or enhance p53 when ribosome biogenesis is perturbed due to RPS19 depletion. Our genome-wide loss of function screening approach demonstrates that the ribosome biogenesis (RiBi) axis is the most potent class of genes whose disruption stabilises p53. We also identified a novel suite of genes critical for the NSP due to ribosomal stress caused by RPS19 depletion, includinga mammalian protein implicated in 5S ribonucleoprotein particle (5S-RNP) biogenesis, HEATR3.

Intriguingly, *HEATR3* gene variants have also recently been associated with a DBA-like syndrome(DBAS) and is now officially recognised by the most recent DBAS International Consensus Statement as such. Our recent studies include the development of stable *in vitro* and *in vivo* models to study therole of HEATR3 in cellular growth and ribosome biogenesis, and how it impacts on the bone marrowmicroenvironment, and we will present our latest data in this area.

In summary, our collective data demonstrates that the NSP has evolved as the dominant centralintegrator of stresses that regulate nuclear p53 abundance, thus ensuring RiBi is hardwired to cellularproliferative capacity. Furthermore, our recent data identifies a suite of novel regulators of the p53-dependent NSP, in cluding HEATR3, which modulate the response of cells to perturbations in ribosome biogenesis and steps immediately downstream. Together, these findings will provide furtheravenues for the development of therapeutics for disorders where the NSP is central to thepathogenesis.

Reference:

Hannan et al 2022 Cell Reports 41, 11157 (https://doi.org/10.1016/j.celrep.2022.111571)

03

SURF2 IS A NEW PLAYER IN NUCLEOLAR STRESS RESPONSE

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Ribosome synthesis, is very energy consuming process, with more than 200 factors involved. When this process is altered due to endo- or exogenous stresses, human cells trigger so called nucleolar stress response. Indeed, nutrient deprivation, U.V/drug exposure or genetic mutations that affect one the factors involved in this mechanism, will promote ribosome synthesis alteration and stop, which will drive to the accumulation of an unassembled sub-ribosomal particle: the 5S RNP, in the nucleoplasm. In this free form, 5S RNPs are able to sequester and inhibit MDM2, thus promoting p53 stabilization, and cell cycle arrest. This regulation by 5S particles is key in cell response to most of nuclear stresses 1.

In patients suffering from ribosomopathies, this activation of p53 by free 5S RNPs plays a full part in the ethiology of the diseases 2. The 5S rRNA synthesis by the RNA polymerase III is uncoupled from the synthesis of the other rRNAs by the RNA polymerase I. This independency might drive to unwanted accumulation of free 5S in the cell even under normal conditions.

To investigate how free-5S could be regulated in the cell, we purified free-5S RNP and uncovered a new interaction partner, SURF2 and we set-out its functional characterization. During this study, we were able to show that SURF2 competes with MDM2 for 5S binding both *in vivo* and *in vitro* and that its depletion promotes cell cycle arrest and apoptosis upon stress whereas its over-expression protects cells from nucleolar stresses and impedes p53 activation following exposure to different drugs. During this presentation, I will present our last data on SURF2 characterization and why we think that SURF2 could represent an innovative therapeutic target for patients suffering from ribosomopathies, including DBA.

1. Hannan, K.M., Soo, P., Wong, M.S., Lee, J.K., Hein, N., Poh, P., Wysoke, K.D., Williams, T.D., Montellese, C., Smith, L.K., et al. (2022). Nuclear stabilization of p53 requires a functional nucleolar surveillance pathway. Cell Rep 41, 111571. 10.1016/j.celrep.2022.111571.

2. Aubert, M., O'Donohue, M.-F., Lebaron, S., and Gleizes, P.-E. (2018). Pre-Ribosomal RNA Processing in Human Cells: From Mechanisms to Congenital Diseases. Biomolecules 8, 123. 10.3390/biom8040123.

04

DETECTING PRE-rRNA PROCESSING DEFECTS BY RNA CAPTURE AND CAPILLARY ELECTROPHORESIS FOR DBA STUDY AND DIAGNOSIS

Nathalie Montel-Lehry[1,2,4], Léo Gagliardi[1,2], Marion Aguirrebengoa[2], Sarah Le Page[1,2,4], Sheila Bohler[3,4], Miriam Erlacher[3,4], Marie-Françoise O'Donohue[1,2,4], Pierre-Emmanuel Gleizes[1,2,4]

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Defective maturation of ribosomal RNAs is a hallmark of DBA patient cells. Detecting pre-rRNA processing anomalies provides important clues for confirming DBA diagnosis, characterizing new gene variants or studying the disease mechanisms. It can also help to identify unaffected gene variant carriers in a family when no gene variant has been identified, which becomes critical when searching for healthy donors for hematopoietic stem cell transplantation. This assay relies mostly on the detection of pre-rRNAs by northern blot, a highly sensitive but technically challenging technique that is performed by specialized research laboratories. To facilitate the use of this test, we have developed an alternative protocol based on the detection and quantification of pre-rRNAs by capillary electrophoresis coupled to laser-induced fluorescence (CE-LIF). Our approach combines the specific capture of pre-rRNAs on magnetic beads and their separation by CE-LIF. Due to the significant length of human pre-rRNAs (up to 13 kb), pre-rRNA separation required the development of a suitable electrophoretic gel. This method was tested on a panel of lymphoblastoid cell lines established from DBA patients with variants in *RPS19* and *RPL5*, two of the most frequently affected genes. We show that quantifying pre-rRNAs from the chromatogram clearly discriminates patients from control individuals in a statistical analysis. Principal component analysis indicates that *RPS19* variants are primarily characterized by the accumulation of 18S rRNA precursors. In contrast, *RPL5* variants significantly affect precursors of both the small and the large ribosomal subunits. Variants of other ribosomal protein genes can be discriminated as well. A model based on these data can predict the presence of *RPS19* or *RPL5* variants in LCLs with >85% accuracy. The whole procedure can be performed in 48 hours using standard laboratory reagents. We are currently adapting this approach on a new automated CE platform in collaboration with Adelis, a private company based in Toulouse, in order to provide a standardized and commercially available solution.

05

DECIPHERING DISEASE MECHANISMS IN DBA, ONE CELL AT A TIME

Dr Deena Iskander MD PhD[1,2]

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[2] Department of Paediatric Haematology, St Mary's Hospital, Imperial College Healthcare Trust.

Single-cell technologies have rapidly developed in recent years alongside large-scale analyses to measure RNA, protein, DNA and chromatin accessibility at single-cell resolution. This has had a significant impact on cancer research, but application to non-malignant disorders has been slower.

Diamond-Blackfan anemia syndrome (DBA) exemplifies a rare disorder where the clinical picture is dominated by a developmental defect in a single hematopoietic lineage, erythropoiesis. Here, I will demonstrate how single-cell approaches using primary patient samples have successfully characterized the identity of the cellular defect and provided new insights into the molecular basis of the disease.

First, using immunophenotyping combined with single-cell clonogenic assays, I showed a selective erythroid defect in DBA: both burst-forming unit erythroid (BFU-e) and colony-forming erythroid (CFU-e) colonies were severely reduced while myeloid colonies were maintained. This quantitative erythroid commitment defect was compounded by qualitative defects in proliferation and differentiation of the residual erythroid progenitors. Next, single-cell RNA-sequencing of patient CD34+Lin- stem and progenitor cells, alongside single-cell culture assays and flow cytometry, revealed differences in the site of erythroid failure in DBA bone marrow according to whether the large or small RP subunit was mutated. Patients with pathogenic variants in *RPS* genes (comprising the remaining 60-70% cases of DBA) showed a pronounced erythroid commitment defect at the HSC and MPP level, underpinned by reduced activity of the master megakaryocyte-erythroid transcription factor, GATA1. In contrast, patients with DBA-causing variants in *RPL5* or *RPL11* (comprising 30-40% of genetically diagnosed cases), exhibited relative preservation of GATA1 activity and erythroid-megakaryocyte committed progenitors. These cellular and molecular differences had clinical correlates, with later age of presentation and better steroid responses in patients with *RPL*-DBA.

The residual erythropoiesis in *RPL*-DBA had the transcriptional hallmarks of glucocorticoid deficient stress erythropoiesis: upregulation of stress erythropoiesis genes alongside downregulation of glucocorticoid receptor targets such as *ZFP36L2*. This caused accelerated differentiation, contributing to anaemia. My ongoing work is using single cell gene expression combined with chromatin accessibility to define the mechanisms underpinning steroid responsiveness in DBA.

I anticipate that future applications of single-cell technologies have the potential to inform the development of novel lines of therapy for DBA.

06

DIAMOND BLACKFAN ANEMIA: IMPACT OF THE ARGININE-POLYAMINE-HYPUSINE AXIS ON ERYTHROID PROGENITOR METABOLISM AND DIFFERENTIATION

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Erythropoiesis, resulting in the production of 200x109 red cells per day in the bone marrow, is an essential physiological process that is exquisitely sensitive to alterations in protein synthesis. This sensitivity is highlighted by the finding that mutations resulting in impaired ribosome biogenesis, including allelic variations in more than 20 different ribosomal protein (RP) genes, all result in Diamond-Blackfan Anemia (DBA). This rare congenital disease predominantly affects erythroid lineage cells and is characterized by macrocytic anemia and bone marrow failure. Nonetheless, the metabolic processes controlling protein syn-

thesis under conditions of erythropoietin-induced erythroid commitment and differentiation are not well understood. Our data show that in physiological conditions, the erythroid commitment of human hematopoietic stem and progenitor cells (HSPC) is associated with a marked increase in protein synthesis and this process is regulated by SLC7A1/CAT1-mediated arginine uptake and its catabolism to the polyamine spermidine. Mechanistically, we find that arginine/spermidine metabolism controls protein synthesis in erythroid progenitors via eukaryotic translation initiation factor 5A (eIF5A); eIF5A-driven translational programs are only active when the arginine-catabolized spermidine moiety is incorporated into lysine 50 of eIF5A, a process known as hypusination. Notably, attenuation of hypusine synthesis in erythroid progenitors – under conditions of limiting arginine, decreased arginine uptake, or direct inhibition of the enzymes catalyzing polyamine biosynthesis – abrogates erythropoiesis but not myeloid cell differentiation. Within the hypusine network, Sievert and colleagues found that the most highly enriched eIF5A-interacting proteins are associated with ribosomal function, including RPL and RPS ribosomal proteins . Indeed, we now show that the ineffective erythropoiesis linked to RP haploinsufficiency is associated with a diminished pool of hypusinated eIF5A, both in engineered progenitors and in DBA patient progenitors. Attenuated eIF5A hypusination results in a dramatic decrease in the expression of mitochondrial proteins (FDR<1e-18), as assessed by quantitative proteomics, and we reveal detectivemitochondrial metabolism to be a hallmark of RPaltered erythroid progenitors. Most importantly, pharmacological interventions augmenting mitochondrial function and polyamine metabolism partially rescue human erythropoiesis under conditions of attenuated hypusination, thereby identifying novel pathways for potential therapeutic targeting in DBA patients.

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07

TRANSFUSION-READY RED BLOOD CELL GENERATION FROM INDUCED PLURIPOTENT STEM CELLS

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In vitro red blood cell (RBC) production provides considerable benefits such as thoroughly screened products, possibility of genetic manipulation and therapeutic loading. Induced pluripotent stem cells (iPSC) is a promising cell source to derive transfusable RBCs and blood products due to their immortality, donor independency and availability in GMP-grade. However, till to date the field lacks a sufficient iPSC differentiation protocol that is capable of sufficient erythroid yield, efficient enucleation (currently 5-25%), and which can be applied in large scale turbulent environments. RBC-transfusion products contain 10^11-12 cells/unit and to be able to generate this, feasible iPSC differentiation with high enucleation and scalable, suspension culturing allowing bioreactor applications is required.

Here we spontaneously differentiate iPSC into embryoid bodies culminating in Hematopoeitic organoids (HeO) through limited directed differentiation (SCF, IL3, Epo). Within 3-4 weeks, HeO produce hematopoietic stem and progenitor cells (HSPCs) for ~3-4 weeks, allowing multiple harvests. Microcarriers were used to provide adherence surface within dynamic setups (Cytodex 1 (Cdex1, non-poreus); Cytopore 1 (CP1, poreus) or Cytopore 2 (CP2, poreus)), through supplementation during HeO formation upon orbital shaking. These improvements resulted in a 3 phase iPSC to RBC differentiation protocol that translated the static/adherent culture to dynamic/suspension culture conditions. This allowed scalability and eventual bioreactor application using specific microcarriers. Our system gives rise to ~16000 fold increase in cell number, with a constant 50-60% enucleation. The iRBC derived here, passed the necessary functional assays, including but not limited to morphological analyses, hemoglobin content, blood group phenotype, deformability, and oxygen association/dissociation. *In vivo* survival was studied by transfusing mini-transfusion products (1% if total RBC population) of cultured iPSC-derived red blood cells into phagocyte depleted MISTRG mice. The stability of iRBC was tested in mouse transfusion experiments using humanized MISTRG mice and showed similar survival compared to donor-derived erythrocytes.

In conclusion, an efficient 3 phase iPSC-RBC differentiation and their translation to dynamic culturing described here for the first time, provides a bridge from small-scale static culturing to large-scale bioreaction RBC production aiding clinical transfusion application.

08

INVESTIGATING NOVEL ERYTHROPOIETIC FACTORS IN DIAMOND-BLACKFAN ANEMIA SYNDROME VIA ERYTHROBLAST DIFFERENTIATION ASSAYS

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Diamond-Blackfan Anemia Syndrome (DBAS) is canonically the result of dominant mutations in ribosomal proteins, however some mutations are not 100% penetrant. We have recruited families where, following diagnosis of DBAS of a child, a parent has been revealed to carry the DBAS allele but not had a medical diagnosis. Our work is aimed to both uncover novel molecular mechanisms of DBAS and reveal molecular adaptations present in silent carriers that rescue the severe anemia observed in DBAS patients.

We have collected primary hematopoietic stem cells from peripheral blood and generated induced pluripotent stem cell lines from DBAS patients, and related silent carriers and healthy family members. Our initial inquiries included transcriptomic, proteomic and metabolomic analyses, as well as full genome sequencing. We observed no changes to mRNA levels of RPS19, with the healthy and mutant allele equally represented. Northern blotting of ribosomal precursor RNA also revealed that RPS19-R62W silent carriers accumulate 21S rRNA. Additionally, we have established erythroblast differentiation assays for primary hematopoietic stem cells, induced pluripotent stem cells (iPSCs) and immortalized erythroblasts (HUDEP-2). In our assays we recapitulated the patient phenotype, with a severe reduction in erythroblast generation in DBAS patient material. Intriguingly, a substantial reduction is observed in silent carrier derived cells, though they produce approximately five to ten times as many erythroblasts as the DBAS derived cells.

Through the biochemical characterization of RPS19-R62W, we have identified several novel potential erythropoietic factors that specifically bind to RPS19-R62W compared to RPS19 WT. To validate these targets, we are depleting our potential erythropoietic modulators during differentiation assays to determine what effect, if any, they have on erythropoietic capabilities in healthy, silent carrier and DBAS derived cells. So far our preliminary results have identified factors which both enhance and suppress erythropoietic capabilities. Our goal is to identify potential druggable targets which could be eventually developed into novel treatments for DBAS patients.

09

RESPONSE TO ANAEMIC STRESS IN DIAMOND-BLACKFAN ANAEMIA REVEALS METABOLIC DECOMPENSATION.

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Diamond-Blackfan Anaemia (DBA) is a pure red cell aplasia usually presenting in childhood. DBA is caused by heterozygous mutations or loss of a ribosomal protein gene, most commonly *Rps19*. To further understand DBA disease mechanisms, we developed a zebrafish model using TALENS, introducing a frameshift mutation in the 2nd coding exon of *Rps19*. Haemoglobin levels in *Rps19*$^{+/-}$ embryos showed minimal change compared to *Rps19*$^{+/+}$, determined by *O*-dianisidine staining. However, under haemolytic stress with phenylhydrazine (PHZ), *Rps19*$^{+/-}$ embryos exhibit significantly less staining compared to *Rps19*$^{+/+}$ embryos, indicating an erythropoiesis recovery failure.

We previously showed that branched chain amino acid (BCAA) L-leucine improves anaemia and growth in transient zebrafish knockdowns and primary human cells via mTOR (Payne et al., 2012). DBA patients also clinically benefit from L-leucine (Vlachos et al., 2020). We hypothesised metabolic changes drive the effects of L-leucine in *Rps19*$^{+/-}$.

To assess metabolic effects of *Rps19*$^{+/-}$, we assessed mitochondrial respiration complex components using immunoblotting. We showed UQCRC2 (Complex III) was increased in *Rps19*$^{+/-}$ compared to *Rps19*$^{+/+}$ embryos. PHZ increased UQCRC2 expression in *Rps19*$^{+/+}$ embryos, however no increase was observed in *Rps19*$^{+/-}$ embryos, indicating *Rps19*$^{+/-}$ embryos lack the capacity to respond to stress. We next measured the oxygen consumption rate (OCR) as an oxidative phosphorylation readout. We found *Rps19*$^{+/+}$ but not *Rps19*$^{+/-}$ embryos demonstrated increased OCR with PHZ, indicative of increased oxidative phosphorylation due to PHZ. We also assessed the L-leucine effects on PHZ-stressed embryos. With L-leucine, *Rps19*$^{+/+}$ embryos exhibit reduced OCR back to baseline levels, without any effect on *Rps19*$^{+/-}$. This suggests that L-leucine is protective against haemolytic stress in wildtype embryos, but a different mechanism accounts for the effects in *Rps19*$^{+/-}$ embryos.

We performed metabolomic analysis to capture changes in glycolytic pathway metabolites and amino acids. Counterintuitively, we observed BCAAs including L-leucine were upregulated in *Rps19*$^{+/-}$ compared to *Rps19*$^{+/+}$ embryos post PHZ treatment. This suggests that while total leucine levels appear increased, *Rps19*$^{+/-}$ may lead to a defect in L-leucine utilisation, or alternatively BCAAs may reside in an inaccessible subcellular compartment. Our ongoing work will test these hypotheses and define mechanisms of improved haematopoiesis and growth in L-leucine-treated *Rps19*$^{+/-}$.

011

MECHANISMS OF SOMATIC GENETIC RESCUE IN GERMLINE RIBOSOMOPATHY

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Tracking how hematopoietic stem cell (HSC) clonal dynamics change over time, assessing whether somatic genetic rescue mechanisms affect these dynamics, and mapping out when leukemic driver mutations are acquired is important to understand which individuals with germline ribosomopathy may go on to develop myelodysplastic syndrome or leukaemia.

Using whole genome sequencing of haematopoietic colonies from individuals with the inherited ribosomopathy Shwachman-Diamond syndrome (SDS), we have reconstructed haematopoietic phylogenies, identifying mutually exclusive mutations in *TP53*, *EIF6*, *RPL5*, *RPL22, PRPF8*, plus structural variants in chromosomes 7 and 15 that increase the gene dosage of *SBDS* and *EFL1*respectively. Target gene mutations arise early in life, even in utero, resulting in a profusion of clonal expansions, with only a few haematopoietic stem cell lineages contributing to around half of haematopoietic colonies by young adulthood. Rapid clonal expansion during disease transformation is associated with the acquisition of biallelic *TP53* mutations and increased mutation burden. While convergent somatic variants may offset the deleterious effects of germline ribosomopathy, they may increase the opportunity for *TP53*-mutated cancer evolution.

Determining the complete range and type of genetic rescue mechanisms in germline ribosomopathy and their relative risk of driving disease evolution to blood cancer may help inform precision medicine and facilitate the design of disease-modifying therapeutics.

013

TREATMENT-INDEPENDENCE DUE TO SELF-REVERTING MUTATION IN DBA PATIENTS

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Somatic revertant mosaicism is a rare phenomenon caused by spontaneous correction of a pathogenic allele and subsequent clonal expansion of the revertant cell. A common cause of revertant mosaicism in dominant disorders is mitotic recombination, resulting in mosaic segmental uniparental disomy(UPD).

In Diamond Blackfan Anemia (DBA), approximately 20% of patients can achieve a state of treatment independence, defined by an acceptable haemoglobin (Hb) level without any treatment, regardless of prior therapy and molecular defect.

To date, three patients with DBA reversion have been described, involving a deletion spanning the *RPS26* gene, a novel pathogenic splice site mutation in *RPL4*, and a pathogenic missense mutation in the *RPS19* gene (*Venugopal et al. 2017, Jongmans et al. 2018, Garelli et al. 2019*).

Here we describe a novel patient with a 15q25.2 deletion encompassing the *RPS17* gene.

The patient was a 35-year-old male with a clinical diagnosis of DBA. At the age of 2 months hyporegenerative anaemia was detected, requiring red blood cell transfusions (minimum Hb: 47 g/l). The erythrocyte adenosine deaminase activity (eADA) was slightly elevated. At the age of 2 years he was started on prednisone with steroid dependency for 10 years. At 12 years of age, he achieved a stable treatment independence, which was maintained at the last follow-up (age 35 years: Hb 134 g/l, MCV 98 fl, eADA 0.86 U/g Hb).

The analysis by Multiplex Probe Ligation Amplification assay and real-time PCR suggested a mosaic *RPS17* gene deletion. High-density SNP array was performed on the proband at 27 years of age and identified a mosaic segmental loss of heterozygosity on chromosome 15q, indicating the presence of multiple clones with a UPD.

The review of the reported DBA cases with UPD-related haematological rescue allows us to make same remarks on the molecular basis of independence treatment, emphasizing the relevance of revertant mosaicism to understanding the variable phenotypic expression of DBA and for justifying the absence of an expected mutation in diagnostics.

Further work is needed to understand the hidden mechanisms and the dynamics of this "natural gene therapy" phenomenon.

015

ADVANCES IN HEMATOPOIETIC STEM CELL GENE THERAPY

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Ex vivo hematopoietic stem cell (HSC) gene therapy is based on the collection of autologous HSCs, followed by their ex vivo genetic correction and reinfusion, in most cases after conditioning with cytotoxic agents. This therapeutic modality has been mainly used for the treatment of diseases that have been successfully treated by allogeneic cell transplantation, including primary immunodeficiencies or red blood cell disorders, among others. In this presentation, I will compare the results of two different ex vivo lentiviral-mediated gene therapy studies, specifically those corresponding to patients with leukocyte adhesion deficiency type I (LAD-I) and Fanconi anemia (FA). While LAD-I is caused by the defective function of mature leukocytes due to mutations in the ITGB2 gene (CD18), FA affects the very primitive HSCs and is caused by mutations in any of the 23 FA genes involved in the FA/BRCA DNA repair pathway. Two different lentiviral vectors carrying the *ITGB2* and *FANCA* genes have been developed for the treatment of these patients. In contrast to LAD-I, where corrected HSCs do not develop proliferative advantage, in the case of FA, a marked proliferative advantage occurs in corrected HSCs *in vivo*. Consequently, while in the LAD-I gene therapy protocol myeloablative conditioning was used in all patients, in FA patients transduced cells were infused without any prior conditioning to prevent cancer risks in a disease characterized by DNA repair defects. The implications of these two very different clinical trials for the design of DBA gene therapy will be discussed.

016

DEVELOPMENT OF GENE THERAPY FOR RPS19 DEFICIENT DIAMOND BLACKFAN ANEMIA.

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Diamond-Blackfan anemia is a rare genetic bone marrow failure disorder which is usually caused by mutations in ribosomal protein genes. In the present study, we generated a traceable *RPS19*-deficient cell model using CRISPR-Cas9 and homology-directed repair to investigate the therapeutic effects of a clinically applicable lentiviral vector at single-cell resolution. We developed a gentle nanostraw delivery platform to edit *RPS19* gene in primary human cord blood-derived CD34+ hematopoietic stem and progenitor cells. The edited cells showed expected impaired erythroid differentiation phenotype and a specific erythroid progenitor with abnormal cell cycle status accompanied by enrichment of TNFα/NF-κB and p53 signaling pathways was identified by single-cell RNA sequencing analysis. The therapeutic vector could rescue the abnormal erythropoiesis by activating cell cycle-related signaling pathways and promoted red blood cell production. Overall, these results establish nanostraws as a gentle option for CRISPR-Cas9-based gene editing in sensitive primary hematopoietic stem and progenitor cells, and provide support for future clinical investigations of the lentiviral gene therapy strategy.

017

DEVELOPMENT OF A GENE EDITING APPROACH FOR THE TREATMENT OF RPL5-DEFICIENT DIAMOND-BLACKFAN ANEMIA PATIENTS

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Our studies have previously laid the groundwork for future lentiviral-mediated gene therapy in Diamond-Blackfan anemia patients caused by mutations in *RPS19*. In this respect, we showedthat the reservoir of the hematopoietic stem cell (HSC) should not constitute a remarkable limitation for DBA gene therapy and that by means of a clinically applicable lentiviral vector (*PGK.CoRPS19 LV)* it is possible to restore healthy ribosomal biogenesis and improve erythroid differentiation in the long term with a safe profile, showing promise for Diamond-Blackfan anemia. These findings have paved the way for clinical gene therapy studies in patients with defects in *RPS19* gene.

However, in the case of other ribosomal proteins genes it might be important to maintain endogenous regulation of the protein and therefore more refined gene therapy strategies need to be developed. This is the case of RPL5, the second most mutated gene in DBA (11%) due to its direct interaction with MDM2, the master regulator of P53. Thus, as a proof of concept we have developed a homologous recombination (HR) gene editing strategy for the treatment of *RPL5*-deficient HSCs based on CRISPR/Cas9 system and adeno-associated viral vectors harboring a codon-optimized sequence of *RPL5*-cDNA.

Delivery optimization of the therapeutic donor either by single-stranded (ssAAV6) or self-complementary (scAAV6) configurations was initially studied. The efficacy of the HDR-mediated gene editing was optimized using healthy donor CD34+ cells from bone marrow (BM) and cord blood (CB) and the two vectors at different multiplicities of infection (MOIs). The scAAV yielded higher efficacy and lower toxicity than the ssAAV at 10-30 times lower MOI. In CB-CD34^{+} cells, HR analysis of the scAAV showed a mean of 79% edited alleles detected by ddPCR and 73% of edited colonies (CFCs) at MOI of 3x10^{3} genome-copies per cell (GC/cell).In BM-CD34+ cells, the toxicity of ssAAV was unbearable but further optimization based on the use of scAAV and variable pre-stimulation protocol allowed to achieve a mean of 50% edited alleles detected by ddPCR at MOI of 3x10^{2} GC/cell. CB-CD34+ edited cells in the RPL5 locus were transplanted in *NBSGW* immunodeficient cells showing over 80% of long term *in vivo* engraftment with a mean of 7% edited alleles by HR in different hematopoietic populations, including the erythroid lineage. Different strategies to achieve further optimization in HD and RPL5 deficient cells are under study.

018

PRECLINICAL DEVELOPMENT OF RPS19 LENTIVIRAL VECTOR FOR GENE THERAPY

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Current treatment options for Diamond-Blackfan anemia syndrome (DBAS) are suboptimal and it is difficult to obtain DBA patient hematopoietic stem and progenitor cells (HSPCs) in sufficient quantity for preclinical development of new therapies. Recently, we developed a Cas9-mediated *RPS19*-edited model using HSPCs from healthy donors (Bhoopalan et al, *JCI Insight,* 2023). Using this approach, we have developed a clinically relevant *RPS19*-encoding lentiviral vector (LV) for DBA gene therapy.

Healthy donor HSPCs were electroporated with ribonucleoprotein (RNP) complex consisting of Cas9 and guide RNAs (gRNAs) targeting *RPS19* or the *AAVS1* locus as a negative control, followed by *in vitro* hematopoietic differentiation or *in vivo* xenotransplantation into NSGW mice. These studies resulted in the following key findings:

1. *RPS19* RNP-treatment lead to insertion-deletion mutations (indels) in *RPS19*, reduced RPS19 protein level, impaired pre-rRNA processing and impaired erythropoiesis but not myelopoiesis *in vitro*.

2. Down-titration of RNP dose generated a mixed population of equal numbers of *RPS19*$^{+/-}$ and *RPS19*$^{+/+}$ HSPCs; *RPS19*$^{-/-}$ cells were not viable.

3. Xenotransplantation studies showed that *RPS19*$^{+/-}$ HSPCs were outcompeted for bone marrow (BM) repopulation by *RPS19*$^{+/+}$ HSPCs after 16 weeks.

To rescue the hematopoietic defects of *RPS19*-targeted HSPCs, we constructed a third-generation, self-inactivating LV encoding *RPS19 (RPS19* LV). *RPS19*-targeted HSPCs resulted in ~50% fewer erythroid cells *in vitro*. Transduction with *RPS19* LV with three different promoters (EF1a short, EF1a long and MND) restored correction of this *in vitro* erythroid defect similarly; the EF1a short promoter was chosen for subsequent experiments due its track record for clinical use. Similarly, transduction of *RPS19*- targeted HSPCs with *RPS19* LV allowed engraftment of *RPS19*$^{+/-}$ HSPCs in recipient mouse BM at 16 weeks post-transplant.

In summary, our studies show that our *RPS19* LV shows efficient transduction of HSPCs and rescues the *in vitro* and *in vivo* hematopoietic defects of *RPS19*-edited HSPCs, supporting its potential utility for DBA gene therapy.

019

SINGLE-CELL-MULTIOMICS OF GENE THERAPY TREATED RIBOSOMAL PROTEIN S19-DEFICIENT DIAMOND-BLACKFAN ANEMIA PATIENT CELLS DEMONSTRATES MOLECULAR EFFICACY AND UNCOVER NEW PATHOGENIC MECHANISMS.

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Diamond-Blackfan anemia (DBA) is a genetic syndrome characterized by severely impaired red blood cell production. Most DBA cases are caused by mutations in ribosomal protein genes, of which ribosomal protein S19 (RPS19) is most common. The exact mechanisms by which ribosomal protein deficiencies mainly impact erythropoiesis remain unclear. Our study explores these mechanisms using single-cell molecular analysis on hematopoietic progenitor cells from DBA patients with RPS19 mutations, before and after *in vitro* gene therapy.

We studied CD34+ cells from four DBA patients and four healthy individuals, applying a gene therapy protocol intended for clinical trials. Post-transduction, we analyzed the cells using CITE-seq, which examines transcriptomes and cell surface proteins at the single-cell level. In gene therapy groups, transduced cells were identified based on coRPS19 transgene expression.

Expression of coRPS19 in DBA erythroid progenitors lead to a significant induction of genes associated with terminal erythropoiesis and down-regulation of genes associated with apoptosis. The two most-significantly altered genes in DBA erythroid cells as well as most-significantly changed genes in coRPS19-positive erythroid cells in all DBA samples were RPL22L1 and CD70, whose contributions to DBA pathogenesis have not been previously recognized. RPL22L1 is a component of the 60S ribosomal subunit and may also play a role in the regulation of pre-mRNA splicing. CD70 mRNA and protein were exclusively expressed in erythroid progenitors from DBA samples with significant down-regulation in coRPS19-positive cells.

Interestingly, myeloid progenitor DBA cells only responded to gene therapy by up-regulation of ribosomal protein genes, suggesting RPS19-deficiency in myeloid progenitors leads to reduced ribosome biogenesis without the nucleolar stress observed in erythroid cells.

In conclusion, our approach uncovered genes such as RPL22L1 and CD70 that after further studies may improve DBA diagnosis and understanding. The normalization of gene expression post-treatment highlights the molecular success of our therapy, supporting its progression to clinical trials.

020

EXPLORING TYROSINE KINASE INHIBITORS AS POTENTIAL THERAPEUTICS FOR DIAMOND-BLACKFAN ANEMIA

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Recent findings demonstrate that the canonical inflammasome directly regulates hematopoiesis by modulating the master erythropoiesis transcription factor GATA1 in hematopoietic stem and progenitor cells (HSPCs). This study identifies the NLRP1 inflammasome as a critical regulator of the erythroid-myeloid lineage decision in HSPCs. In zebrafish larvae, genetic inhibition of Nlrp1 led to decreased neutrophil counts and increased erythrocyte numbers. Mechanistically, erythroid differentiation induced ribosomal stress, activating the ZAKα/P38 kinase axis, thereby promoting NLRP1 inflammasome assembly in both zebrafish and human systems. Inhibiting ZAKα with FDA/EMA-approved tyrosine kinase inhibitors (TKIs) mitigated neutrophilia in zebrafish models, facilitated erythroid differentiation in K562 cells, and enhanced primary human HSPCs differentiation from healthy donors and Diamond-Blackfan anemia (DBA) patients. Additionally, TKIs reduced exacerbated inflammasome activation in RPS19-edited CD34+ HSPCs, thereby rescuing defective erythropoiesis. These results underscore the critical role of the NLRP1 inflammasome in hematopoiesis regulation and suggest that TKIs commonly used in clinical settings hold promise for repurposing in treating hematopoietic disorders linked with chronic inflammation and rare diseases like DBA.

022

PREDICTING OUTCOME OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC DBAS: DOES AGE MATTER?

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Diamond-Blackfan Anemia Syndrome (DBAS) is an inherited bone marrow failure syndrome (IBMFS). Whereas the hematological manifestations of this disease can be cured with hematopoietic stem cell transplantation (HSCT), historical data have shown that the outcomes of HSCT in patients older than 10 years of age are significantly inferior compared to younger patients. This age effect is different from HSCT outcomes in other IBMFS or rare inherited anemias and has not been explained so far. Since most DBAS patients were chronically transfusion dependent prior to HSCT, and suffered from secondary iron overload, it has been suggested that the toxic effects of iron overload resulting from chronic blood transfusions play an important role in post HSCT complications and outcome.

Driven by our local experiences in teenagers with DBAS, treated with HSCT, we performed a literature study to evaluate what is currently known about the role of iron overload with respect to the complications and outcome of HSCT in DBAS patients. Our analysis of 41 reports describing more than 450 patients in total, illustrated that data on iron status were rarely reported or analyzed. In studies that have reported iron parameters in DBAS patients, a correlation between iron status and outcome was not found. Still, it remains reasonable to assume that iron overload, and transfusion burden increase the risk for adverse HSCT outcomes, especially in older patients that have been treated with blood transfusions for many years. While the age at HSCT most likely is less important than the transfusion burden in young patients with HSCT, we therefore recommend to develop an individual risk assessment tool to evaluate pre-existing toxicity (cell/organ damage) next to routine evaluation of iron overload, in order to improve the selection of DBAS patients eligible for HSCT.

023

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN DIAMOND BLACKFAN ANEMIA SYNDROMES

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Diamond Blackfan Anemia Syndromes (DBAS) is a rare disease with an estimated incidence of 5 to 7 cases/million live births. The term syndrome underlines that DBAS patients may have non classical presentation without anemia but polymalformative syndrome, immunodeficiency, hematological malignancies with no history of anemia but with an increased risk for osteosarcoma and colorectal cancer. The advent of new techniques and new diagnostic criteria enable diagnoses of DBAS also in adults.

Allogeneic HSCT is the only treatment option capable to cure anemia in steroid non-responding patients and to prevent clonal evolution. Two recent studies described the HSCT outcome of DBAS patients. In a German- French report that analyzed the outcome of 70 HSCTs, matched-sibling donor (MSD) and matched-unrelated donor (MUD) transplant showed a comparable 5-year overall survival (OS) (91% and 92% respectively) with similar rate of chronic graft-versus-host disease-free survival (89% and 83% respectively). Age below 10 years and transplant after 2000 were regarded as positive predictive factors. In an EMBT survey on 106 transplants, 53% of whom transplanted prior to year 2000, 3-year OS and event-free survival were respectively 84% and 81% with no difference between MSD and MUD HSCT. Post- transplant malignancies occurred in 6.6%of cases. Indeed the role of transplant in increasing the risk of solid tumors has not been clearly established. In spite of this some Authors suggest early systematic colonoscopies in transplanted patients. In 2024 DBAS guidelines HSCT is recommended in non-steroid responsive transfusion dependent patients. Optimal time is indicated prior to age 10 years and best time prior to age 5 years either from MSD or 10/10 HLA MUD. It is important to outline that as in all inherited bone marrow failures, the disease must be excluded in candidate MSD, a challenging task if the propositus does not carry a variant in DBAS genes. In this circumstances, workup should include clinical examination, CBC with reticulocytes, fetal Hb and eADA activity evaluation. In case of dubious results a 10/10 MUD transplant can be considered as an alternative. Another indication to HSCT is non-manageable iron overload due to chelation failure or exceedingly high toxicity.

025

PREGNANCIES AND DBA, NOT A LOVE STORY.

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DBA complications include in addition to the complications related to the treatments (steroid or iterative transfusions) the risk of malignancy estimated to 5% and the complications related to the pregnancy. Pregnancy in DBA is considered a major complication. We have performed a retrospective study in 2006 (Faivre et al., Haematologica, 2006) including 44 DBA patients >18 y-old from the German and French registries, who has experimented at least one pregnancy. Out of 66 pregnancies, only 22 DBA patients exhibited non-complicated pregnancies, while the 42 left exhibited severe complications including spontaneous abortions, preeclampsia, premature birth, in utero growth retardation, and fetal death. Only 34 births occurred from the 66 pregnancies. From this study, during pregnancy, the DBA patients should be carefully monitored in a high care level maternity. We suggested as well the use of aspirin after the second trimester since in the few placenta we observed, signs of thrombosis have been seen. We do hope that with the careful monitoring of pregnancy the rate of complications will decrease. The progress of the genetics have made possible prenatal diagnosis (even preimplantation diagnosis, not available everywhere). Non invasive prenatal diagnosis will probably be developed in the next years but for now, the usual test if requested by the parents and the consortium (pediatricians, gynecologists, radiologists, geneticist) remains the prenatal diagnosis (PND) which may lead to pregnancy interruption. We will focus on the PND and the results in the French registry of the few PND performed. We will present a project work in Europe (GT-Perigenomed), which will consist in performing whole genome sequencing as a neonatal screening in a defined number of new borns for some diseases and for some genes in order to improve the diagnosis and the care of some diseases earlier (DBA has been included). We will review all these new advances in diagnosis during or just after pregnancy.

026

WOMEN'S HEALTH IN DIAMOND BLACKFAN ANEMIA SYNDROME: DATA FROM THE DIAMOND BLACKFAN ANEMIA REGISTRY

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Diamond Blackfan anemia syndrome (DBAS) is a rare, congenital bone marrow failure syndrome characterized by red cell aplasia, birth anomalies, and a predisposition to cancer. Treatments for the anemia of DBAS include corticosteroid therapy, chronic red cell transfusions with iron chelation, and hematopoietic stem cell transplantation. DBAS and its therapies have an impact on women's health. Women enrolled in the Diamond Blackfan Anemia Registry (DBAR) report delayed menarche, irregular menstrual cycles, decreased fertility, pregnancy complications, and early menopause. We will report on these findings with regards to treatments received. Transfusion dependent females were more likely to have delayed menarche than steroid dependent females and those in remission. Transfusion dependent females also reported premature ovarian failure compared to the other groups. Of the females who reported entering menopause, 75% did so by age 40 years or younger. Pregnancy complications will also be reviewed. Thyroid disorders and other endocrine disorders were also reported more frequently in transfusion dependent women. Early and frequent monitoring for endocrinopathies, along with aggressive iron chelation, may improve the health of women with DBAS.

027

HOW DO I TRANSITION BLACKFAN DIAMOND ANEMIA SYNDROME PATIENTS?

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"Each to Their Own"

The diagnosis of a serious illness should not become a person's destiny. Despite the limitations it implies, we must help foster the development of each individual's uniqueness. Each person is much more than a Blackfan Diamond Anemia Syndrome (DBA) patient; they are unique beings who must find their place in the world.

Chronic diseases pose a challenge for patients, their families, and healthcare providers. The transition from pediatric to adult health services is a critical moment that requires a scheduled and planned handover to maintain a good quality of life and proper biopsychosocial development. Joint follow-up with psychological support is crucial. Chronological age should not automatically dictate the transition to adulthood; the ideal guide should be mental development age. Currently, there is no single model for transition. The challenge lies in moving from theory to practice and clinical reality.

028

THE GLOBAL DIAMOND BLACKFAN ANEMIA CANCER CONSORTIUM: DEFINING CANCER IN DIAMOND BLACKFAN ANEMIA SYNDROME

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The cancer risk for patients with DBA syndrome has been quantitated (Vlachos…Lipton, Blood 2012,119: 3815-3819; Vlachos…Lipton, Blood 2018,132: 2205-2208). In addition to MDS/AML, a wide array of associated solid tumors has been identified. The statistically significant predilection to early onset colorectal cancer (EOCRC) and osteogenic sarcoma (OS) predominate, with observed to expected ratios (O/E) of 45 and 42, respectively. Breast cancer will likely emerge at the next stastistical analysis. International (Czech/Slovak and Italian DBA syndrome cohorts) corroborate the cancer risk. Based upon the high risk of EOCRC and the known efficacy of screening in the general population, a screening and surveillance strategy has been strongly suggested and is being implemented for individuals with DBA syndrome. While quantitating the cancer risk in DBAS has been a major step forward, little is known about mechanisms by which germline ribosomal protein haploinsufficiency can lead to cancer. Notably, all but one of the common DBA syndrome genotypes, RPS26, are associated with an increased risk of malignancy, and patients with RPL11 and RPL35a appear overrepresented in the cancer population. Whether these findings are real or statistical anomalies need to be determined. In order to better understand the biology of cancer predilection in individuals with DBA syndrome, we propose to:

Harmonize cancer incidence data across international DBA syndrome cohorts to provide additional statistically valid cancer incidence data.

Create a robust DBA syndrome biobank to facilitate laboratory science.

Create the international DBAS Cancer Consortium of cancer biologists, ribosome biologists and other laboratory scientists, supported by incidence data and the biobank, to investigate the mechanism of cancer predilection in individuals with DBA syndrome and,

Develop screening/surveillance and treatment strategies for patients likely to be sensitive to conventional treatment regimens.

The first step in this proces has been to create a Cancer Survey that will characterize the type, stage, pathology and other biologic characteristics of solid tumors in patients with DBAS.

029

CLONAL HEMATOPOIESIS IN LONG-TERM SURVIVORS OF PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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During hematopoietic cell transplantation (HCT), donor stem cells carrying somatic mutations in leukemia-associated genes can engraft and expand in the recipient, resulting in clonal hematopoiesis (CH). Whether CH is present after pediatric HCT, and which transplant-related characteristics affect its prevalence, remains unknown. Using targeted error-corrected sequencing, we assessed CH in a cohort of 144 long-term survivors of pediatric HCT (>5 years post-transplant) and 116 healthy controls. CH was detected in 16% of HCT recipients, at variant allele frequencies (VAFs) of 0.01-0.31. These mutations were predominantly found in DNMT3A (80%) and TET2 (20%). Older hematopoietic stem cell age and the HCT procedure independently increased the risk of CH (odds ratios: 1.07 per year increase ($p<0.001$) and 2.63 for HCT ($p=0.02$)). Large clones (>0.10 VAF) were exclusively found in HCT recipients. Post-HCT CH was independent of stem cell dose. Inflammatory processes around graft infusion, including serotherapy, increased C-reactive protein and viral reactivations were associated with increased risk of CH. In conclusion, CH is prevalent in long-term survivors of pediatric HCT, potentially driven by systemic inflammation. Future studies are required to track the evolution of these clones and dissect their impact on long-term outcomes, including cardiovascular disease, second malignancies and overall survival.

030

DIAMOND BLACKFAN ANEMIA SYNDROME: STEPWISE APPROACH FROM DIAGNOSIS TO TREATMENT

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Diamond Blackfan Anemia syndrome (DBAS) comprises classic DBA associated with genes involved in ribosomal structure and biosynthesis, and related DBA-other diseases. Classic DBA, the first disease in human described as a ribosomopathy, is associated with mutations in genes encoding for ribosomal structural protein or for proteins involving in ribosomal biosynthesis (23 genes). DBA-other are associated in mutations within *GATA1*, involved in different clinical entities, and *TP53* with gain-of function mutation, and present similar phenotype and a pathology in part common to classic DBA.

The recent international guidelines1 underline that DBAS diagnosis can be confirmed in any patient harboring a pathological or likely pathological variant (AMCG class 4 and 5) in one *bona fide* DBAS gene. This allow the classification as patients of individuals previously known as "silent phenotype" who actually deserve to be followed as they may present with hematological and cancer outcomes. Additionally, DBAS diagnosis may be retained in a patient with clinical and hematological features consistent with DBA syndrome. Currently, a significant variant is identified in up to 90% of patients and more and more patients are diagnosed, including at adult sages, through systematic genetic studies, allowing identifying a new sub class of patients.

DBAS is a very heterogeneous disease and patients may face very different issues. At best, patients must be followed by expert teams.

In children, current therapeutic options are transfusions, steroids or HSCT. The 2024 guidelines modify all these options, recognizing that the transfusion threshold must be raised to at least 9 g/dL, lowering the maximum tolerable dose of steroids to 0.3 mg/kg/d, and extending transplant indications to 10/10 unrelated donors. The best option is to be discussed for each individual child.

In adult patients on steroids, return to transfusion support may be justified by a progressive loss of hematological response, or by the side effects associated with corticosteroids. HSCT indications are limited to hematological malignancies.

New therapeutic options will include gene therapy, and targeted therapies able to improve erythropoiesis.

Today's challenges remain better control of iron deficiency, which typicall implies devoted consultations, and the prevention, screening and treatment of hematological malignancies and cancers.

1: *Diagnosis, treatment, and surveillance of Diamond-Blackfan anaemia syndrome:* international consensus statement. Wlodarski MW, Vlachos A, Farrar JE, Da Costa LM, Kattamis A, Dianzani I, Belendez C, Unal S, Tamary H, Pasauliene R, Pospisilova D, de la Fuente J, Iskander D, Wolfe L, Liu JM, Shimamura A, Albrecht K, Lausen B, Bechensteen AG, Tedgard U, Puzik A, Quarello P, Ramenghi U, Bartels M, Hengartner H, Farah RA, Al Saleh M, Hamidieh AA, Yang W, Ito E, Kook H, Ovsyannikova G, Kager L, Gleizes PE, Dalle JH, Strahm B, Niemeyer CM, Lipton JM, Leblanc TM; international Diamond-Blackfan anaemia syndrome guideline panel. Lancet Haematol. 2024 May;11(5):e368-e382

P1

ELUCIDATING THE ROLE OF RPL5/UL18 MUTATIONS IN THE PATHOGENESIS OF DIAMOND-BLACKFAN ANEMIA

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Despite significant scientific efforts, the molecular pathogenesis of Diamond-Blackfan Anemia (DBA) remains largely unknown. Several hypotheses have been proposed, with two gaining substantial support. The first suggests that insufficient ribosome production impairs the translation of transcripts crucial for erythroid lineage differentiation. The second hypothesis suggests that reduced synthesis of ribosomal proteins (RPs) or rRNA increases cytoplasmic levels of RPs, in particular RPL5/uL18 and RPL11/uL5, culminating in the stabilization of p53, consequently triggering cell cycle arrest and apoptosis via activation of the nucleolar stress response.

In this study, we focus on various mutations in the RPL5 gene to investigate their impact on ribosome synthesis and function using as a model lymphoblastoid cell lines with DBA-related RPL5 mutations and wild-type (wt). This choice is driven by the pivotal involvement of RPL5 in orchestrating the nucleolar stress response. Since it is hypothesized that DBA results, at least in part, from altered ribosomal biogenesis leading to reduced ribosomal availability, we measured the amount of available cytoplasmic ribosomes in cells with different genotypic backgrounds. In addition, we purified ribosomes in highly stringent conditions and analyzed their composition by mass spectrometry. To define the impact of the mutations on intrinsic ribosomal activity, we exploited a unique cell-free assay, established in our lab, based on a ribosome-free rabbit reticulocyte lysate (RRL) which is reassembled with ribosomes purified from DBA lymphoblastoid cell lines and *in vitro*-transcribed reporter mRNAs. The preliminary results from this study indicate that in DBA RPL5 mutations have varying impacts on ribosomal synthesis and function, including the possibility of a mutation-specific mechanism.

Once defined the impact of RPL5 mutations on ribosome biogenesis and function, we will shift our focus on the effects of the mutations on the cellular environment at different levels, including transcription and translation, to build up a broader picture of the molecular pathogenesis of DBA, potentially paving the way for novel targeted therapeutic strategies.

P2

IPSC DISEASE MODELS TO STUDY AND TREAT DIAMOND-BLACKFAN ANEMIA SYNDROME (DBAS)

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Introduction: Disease mechanisms driving anemia in DBAS are not completely elucidated, and research is hampered by the scarce availability of patient samples and disease models. Recently, we have developed an induced pluripotent stem cell (iPSC) culture to RBC differentiation platform, providing a powerful tool to study erythroid development. The TRACER-consortium aims to generate novel DBAS disease models, to genetically correct DBAS-iPSCs to produce cultured RBCs for transfusion, or corrected HSCs for autologous stem cell transplantation (Fig.1A). Currently, DBAS treatment is limited to glucocorticoids, blood transfusions, or allogeneic SCT, associated with toxicity and risk.

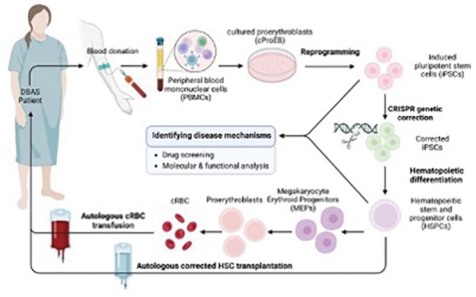
Methods: Patients were selected from the Dutch Registry (DBAN; Fig.1B) and PBMC-derived culturedproerythroblasts (proEB) were reprogrammed using the non-integrative Cytotune-Sendai-IPS2.0-kit. The DBAS-iPSC pluripotency marker expression and differentiation potential is demonstrated. DBAS-iPSC derived embryoid bodies (EBs) give rise to ‘hematopoietic organoids’ (HO) that produce hematopoietic production cells(HPCs) which are characterized by flow cytometry, and further expanded to produce proEBs and enucleated cRBCs.

Results: ProEBs of three DBAS patients were reprogrammed and these DBAS-iPSCs form EBs, HO, and HPCs,similar to healthy control iPSCs. However, the DBAS-HPCs display a slight bias to the myeloid lineage, and a reduced proEB expansion potential. Nonetheless, this platform allows the sustainable production of HPCs for further mechanistic studies.

Conclusion: In DBAS there is a clinical need for novel therapeutic strategies to treat severe anemia and reduce organ toxicity. DBAS-iPSC lines provide a sustainable source of DBAS models, in which erythropoiesis, novel therapeutics, including gene therapy, can be studied for clinical applications.

Figure 1.

A.



B.

Patient	Molecular defect	Current treatment	glucorticoid response	*In vitro* proerythroblast culture	Input reprogramming
#P1	Unknown*	Transfusions	Partial	Possible	Cultured Proerythroblasts
#P2	Unknown*	Transfusions	Partial	Possible	Cultured Proerythroblasts
#P4	Unknown*	Transfusions	Unresponsive	Unable, poor	PBMC
#P5	*RPS26*	None	Good	Good	Cultured Proerythroblasts
#P6	*RPS24*	GC low dose	Good	T.B.D.	PBMC
#P8	*RPS26*	None	Good	Very Good	NA
#control1 (parent of P1 & P2)	Unknown	NA		Possible / Good	NA
#control 2 (parent of P1&P2)	Unknown	NA		Possible / Good	NA

* diagnosis confirmed based on preRNA analysis (Northern Blot, Gleizes Lab Toulouse)

P3

TOWARDS AN IMPROVED ERYTHROID PRODUCTION FROM IPSCS

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Introduction:

Rare but severe anemia's such as Sickle cell anemia and Diamond Blackfan anemia syndrome are debilitating diseases that of yet have for many patients no cure available. Thus, new treatments are very much needed. Induced pluripotent stem cells (iPSC) are a promising source of new potential treatments as they can be differentiated into many different cell types, including erythrocytes. We have formulated a protocol that shows differentiation of human iPSC into hematopoietic stem and progenitor cells (HSPCs) and terminally differentiated effector cells such as erythrocytes. Unfortunately, so far *in vitro* differentiation has proven a significant challenge. This is mainly due to developmentally immature/non-adult hematopoiesis, mimicking aspects of spatio-temporal, independent hematopoietic waves during fetal development. Definitive waves of hematopoiesis originate from hemogenic endothelium (HE) in a process termed endothelial to hematopoietic transition (EHT). This occurs both in the yolk sac, producing erythro-myeloid progenitors (EMPs), and in the AGM-region, where the first hematopoietic stem cells (HSCs) are generated. Improved understanding of iPSC differentiation will provide ways to resemble definitive hematopoietic waves. This project aims to unravel the differentiation process and identify factors involved in the erythroid specification.

Methods:

Directed hematopoietic specification from iPSC was induced by differentiation of iPSC colonies using specific growth factors and cytokines that support the formation of mesoderm, endothelial and hematopoietic cells in a temporal manner. Single cell RNA sequencing of disrupted iPSC derived hematopoietic organoids revealed the presence of hemogenic endothelium, endothelial (CD73+) and hematopoietic cells (CD43). To track endothelial (CD73+) or hematopoietic (CD43+) differentiation from HE within the differentiating hematopoietic organoid, specific reporter constructs have been generated

Results:

These constructs have been nucleofected in K562 cells which resulted in a knock-in efficiency of 3,98% and 4,90% for CD43 and CD73 constructs, respectively. Subsequently, iPS cell lines have been successfully generated with these constructs. In combination with a Cas9 expressing iPSC line, we will perform a CRISPR library screen to identify genes/TFs that control the erythroid differentiation during iPSC-derived hematopoiesis.

Conclusion / Discussion:

There is a need for new therapies and increased insight for rare anemia's. iPSC derived hematopoietic red blood cells but in future also hematopoietic stem cells could provide both a new treatment as well as new model systems to study those diseases. We will study iPSC derived hematopoiesis and erythropoiesis through the newly developed reporter cell lines.

P4

ELUCIDATING GLUCOCORTICOID-RESPONSIVENESS IN DIAMOND-BLACKFAN ANEMIA SYNDROME USING IPSC-DERIVED ERYTHROBLASTS

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Introduction

Diamond-Blackfan anemia syndrome (DBAS) is a rare congenital ribosomopathy that usually presents in infancy and is characterized by hypoplastic anemia, congenital malformations and a predisposition to cancer. Glucocorticoids (GCs) are, next to red blood cell (RBC)-transfusions and allogeneic stem cell transplantation, the only available treatment option for anemia in DBAS. Although initially, the majority (~80%) of patients respond to GC treatment, long-term GC treatment is unsuccessful in many patients as a result of reduced efficacy, unacceptably high doses and unacceptable side effects. The aim of our studies is to understand the exact mechanism by which GCs can alleviate anemia. We want to identify the GC-Receptor (GCR) binding sites in target genes to investigate GC responsiveness and to identify pathways crucial for the therapeutic effect of GC. Ultimately, we hope to activate these pathways independent of GC.

Methods

As patient material is difficult to obtain, patient-derived induced pluripotent stem cell (iPSC)-lines are used to generate hematopoietic organoids (HeOs) from which RBC precursors can be obtained.

GC stimulation experiments with proerythroblasts allow us to identify GC-responsive genes necessary for setting up CUT&Tag experiments. CUT&Tag experiments give us a high-resolution mapping of the DNA binding sites of the glucocorticoid-receptor (GCR) and the genes involved in the GC-response. Comparing the mappings of healthy GC responsive and DBAS-derived non-responsive RBC precursors should hint at the specific pathways that are involved in their response.

Results

Here, we show the successful generation of RBC precursors from HeOs that were generated with DBAS patient-derived iPSC-lines. We also identify GC-responsive genes in proerythroblasts (GILZ, TXNIP) and an IP-functional GCR-antibody that is likely to be suitable for CUT&Tag experiments.

Conclusion

DBAS patient-derived HeOs can serve as a platform to provide patient-specific RBC precursors and the GC-responsive genes GILZ and TXNIP together with the suitable GCR-antibody will be used to set up CUT&Tag experiments.

P5

THE BIOLOGY OF RPS19-R62W: INTERACTING PROTEINS AND THEIR ROLES IN RED BLOOD CELL DIFFERENTIATION

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The genetics of Diamond-Blackfan anemia syndrome (DBAS) entails single point mutations and full deletions, mostly in genes encoding for ribosomal proteins. We are particularly interested in the role of ribosomal proteins harboring missense mutations and leading to DBAS. For this purpose, we focus on a family that harbors the most common mutation, R62W, in the most commonly mutated ribosomal protein, RPS19. In this family, the father is a silent carrier of the mutation and two children rely on blood transfusions.

We aim to address the following questions: is RPS19-R62W present in biologically relevant levels in patient cells? Is it excluded from ribosomes as previously described in cell line models? If it is expressed and extraribosomal, is it stable and interacting with other proteins with a potentially detrimental effect in red blood cell differentiation?

We generated stable HEK293 cell lines ectopically expressing N- and C-terminal FLAG-tagged versions of wild-type (WT) and mutant (R62W) RPS19, and C-terminal FLAG-tagged versions of five additional RPS19 missense mutations. Polysome gradient analyses revealed that only RPS19-WT is incorporated into ribosomes, but none of the mutant RPS19 proteins. We immunoprecipitated FLAG-RPS19 WT and missense mutant proteins and identified interactors exclusively binding to RPS19-R62W, but not to RPS19-WT, by mass spectrometry. Binding of the top two hits, LRP1 and PPM1G, to RPS19-R62W has already been validated by western blot analysis. Both proteins have potential roles in red blood cell differentiation. We are currently evaluating the interactors in fibroblasts derived from the family.

What is the physiological meaning of these interactions? Are RPS19-R62W binding proteins important for differentiation of red blood cells? To address this critical question, we are silencing the expression of the identified interactors in PBMCs and in HUDEP-2 cells, scoring for a potential defect in differentiation through a robust assay already established in our Lab. We have also obtained induced pluripotent stem cells (iPSCs) from the family and are currently generating bone marrow organoids thereof, enabling the depletion of candidate genes in iPSCs to monitor red blood cell differentiation in organoids. A positive outcome may point towards a gain-of-function behavior, rather than haploinsufficiency, for RPS19-R62W.

This work promises to unveil novel genes of interest for DBAS biology which may provide future targets for drug development.

P6

DE NOVO HETEROZYGOUS RPL8 MISSENSE VARIANT IN A PATIENT FROM YAKUTIA WITH DIAMOND-BLACKFAN ANEMIA

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Background

Diamond-Blackfan anemia (DBA) is a rare congenital disorder characterized by erythroid hypoplasia in bone marrow and various phenotypic abnormalities, growth retardation as well as increased susceptibility to malignancy. Clinical signs may range from non-specific changes in complete blood count (CBC) to severe developmental anomalies. Currently, approximately 23 genes have been associated with DBA phenotype. The *RPL8* gene belongs to the L2P family of ribosomal proteins and encodes one of the components of 60S large ribosomal subunit.

Case report

A 2-year-old boy with isolated anemia (Hb 44 g/L and RBC 1.72×1012/L), first identified in CBC at the age of 1 year, was referred to our center for further examination. The patient had no previous history of reduced hemoglobin or erythrocyte counts. His bone marrow biopsy showed a reduction oferythroid germ cells and maturation arrest of granulocytes at the myelocyte stage. Otherwise, the patient shows normal development without any phenotypic abnormalities. The family was suggested to undergo whole genome sequencing (WGS). Results of the trio WGS revealed a novel missense variant in *RPL8* (NM_001317782.2) c.332C>G, p.(Thr111Arg) that was absent in the samples of bothparents.

Conclusion

So far, there is no certain phenotype in Online Mendelian Inheritance in Man database that is related to *RPL8* gene. To date, there are 2 genetic variants in the *RPL8* gene that have been previously described in patients with DBA phenotype: c.413C>T in a girl who is anemic but does not exhibit any phenotypic abnormalities, and c.113A>G in a patient with DBA-like phenotype that included failure to thrive, thumb anomalies and slight macrocytosis along with normal hemoglobin level. This is the thirdclinical case of a patient with a clinical diagnosis of DBA and an identified mutation in the *RPL8*gene. Although the variant has not yet been functionally evaluated, our data may provide additional evidence that the candidate *RPL8* gene should be included in the list of genes associated with DBA.

Acknowledgments

We would like to thank Biotechcampus (Moscow, Russia) for carrying out WGS.

P7

LEARNING THROUGH REGISTRIES, A DESIRED AND DEMANDED NEED BY PATIENT ASSOCIATIONS, CLINICIANS AND INVESTIGATORS. PRELIMINARY DATA FROM THE SPANISH REGISTRY OF BLACKFAN DIAMOND ANEMIA SYNDROME (DBA).

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Material and Methods

February 2023 started the inclusion of DBA patients inside the pre-existing Spanish Registry of Hemoglobinopathies and Rare Anemias (REHem-AR). Informed consent was obtained from patients or their legal guardians. Data recording and analysis were approved by local ethics committees, Prosecutor's Office for Minors, Spanish Data Protection Agency and reported to the Spanish Agency of Medicines and Medical Devices. Variables were entered pseudonymized into the REDCap web application by the treating physicians or a common data manager and include personal data, diagnostic items, treatment and follow-up. We present the results corresponding to the patients registered up to April 2024. Data collection is done retrospectively at registration and prospectively thereafter, with a follow-up on an annual basis. Pediatricians and hematologists monitoring DBA patients were invited to participate through the Spanish Bone Marrow Failure and Red Cell Disorder Groups.

Results:

57 patients from 21 Spanish hospitals have been registered, age range from 1-52 years (75.4% < 18 years, 25.6% > 18 years old), 56% males: 44% females, 90% of Spanish origin, 57% diagnosed in their first year of life, 37% before the age of 10 and 3% at older ages. Anemia was the most common reason of consultation (90%), followed by familial history. The range of hemoglobin at diagnosis was 2.2-11.4 g/dl (median 6.6). Genetic studies were performed in most of them with mutations in RPS19 30%, RPL 5 12.5%, RPL 11 and RPS 26 10.7%, RPS17 and RPS10 5.4%, RPS7 3.6%, RPL35A 1.8%. 19.9% had no mutation or genetic study was not done. Malformations were described in 39%. At the moment of analysis 41% was on steroids, 34.5 % on transfusions, 21% with no treatment and 3.5% have been transplanted. 3 patients developed a malignancy (colorectal cancer, breast cancer, testicular seminoma). 1 patient died due to an aortic thrombosis. 3 have lost follow up for other reasons.

Conclusions:

As learned over the years from existing registries in DBA and other rare diseases, registries are crucial to improve the understanding and management of patients. We hope to contribute in this sense in the future and help to move forward.

References:

- American, German, French, Italian, etc. DBA registries
- Spanish Registry of Hemoglobinopathies and Rare Anemias (REHem-AR)

P8

IMPLEMENTING HUMAN PHENOTYPE ONTOLOGY INTO THE GERMAN DBA REGISTRY – WHAT'S THE BENEFIT?

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Background: Human phenotype ontology (HPO) provides standardized vocabulary to describe phenotypes of human diseases. Each HPO term describes one phenotypic characteristic, e.g. absent thumb. HPO is under continuous development. Associations between genotypes and phenotypes can be displayed. HPO tools can be used for differential diagnostics in (rare) diseases and translational research.[1,2] Associations of HPO-terms could lead to the discovery of previously unknown gene alterations. Diamond-Blackfan-Anemia presents with impaired erythropoiesis and is often associated with short stature or congenital malformations. It is caused by mutations in ribosomal genes, however, no mutation is found in around 20-30% of patients.[3] Irrespective of the mutation noted, there is no close relationship between the severity of symptoms or clinical course of the disease and the underlying genetics.

Methods: Description of a common set of HPO terms necessary to comprise a DBA phenotype. Comparison of terms available in HPO and applied in the German DBA registry. Detection of sets of HPO terms necessary to identify a DBA patient. Calculation of sensitivity and specifity of the most suitable HPO sets to 100 real DBA patients of the German registry.

Results: We will summarize the current HPO vocabulary (~190 terms) describing DBA. Many terms of the current registry do not match with HPO vocabulary, some result in redundancy or wrong comprehension when searched systematically. Pathognomonic HPO sets are available and reach relevant sensitivity and specifity in the German registry.

Conclusion: The implementation of HPO into the German DBA registry is possible and its use can be applied to all patients. HPO is a powerful tool that can be utilize for differential diagnoses in (rare) genetic diseases like DBA as well as for structured analyses in research. HPO should be implemented in all databases in order to standardize vocabulary. Comprehensive analyses using HPO could lead to a better understanding of genotype-phenotype correlations, therapeutic stratification and expansion of the diagnostic spectrum.

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Atendee Index

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