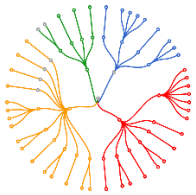
Letters of Support – INDEX



No.	SubTopic	Topic	Contact
1	Large Cohorts	REDS III - Recipient Epidemiology and Donor Evaluation Study	Mike Busch (REDS-III)
2	Large Cohorts	REDS-III - RBC storage	Mike Busch
3	Large Cohorts	Sickle Cell Disease	Brian Custer
4	Large Cohorts	ZIKV DENV Chikungunya	Mars Stone
5	Large Cohorts	RELEVANCE	Anna Bogdanova
6	Model animals	Mouse models of band 3	Jim Zimring
7	Model animals	Hypoxia	Yang Xia
8	Aging	IUGR RBCs	Theresa Powell
9	Aging	Premature, Newborn, ECMO	John Kim
10	Aging	Cardiopulmonary Bypass (adults, senile)	Nathan Clendenen
11	Aging	Aging	James DeGregori
12	Aging	Parabiosys	Laura Santambrogio
13	Aging	Down syndrome & Alzheimer's Dis.	Joaquin Espinosa
14	Aging	Multiple Sclerosis	Pluchino
15	Aging	Parkinson's Disease	Leehey
16	Environment	Fat diet	Tiffany Thomas
17	Environment	Sports	San Millan
18	Environment	Inflammation	Pietras
19	Environment	Iron depletion	Eldad Hod
20	Environment	Iron overload	Britta Will
21	Environment	Sepsis	Allan Doctor
22	Environment	Pulmonary Hypertension	Stenmark
23	Environment	COPD	Fini
24	Environment	Emergency Medicine	Monte
25	Environment	Ischemia	Faubel
26	Environment	High altitude	Rob Roach
27	Genetics - Gender	Testosterone	Tamir Kanias
28	Genetics - Gender	Transgender/PCOS	Cree Green
29	Genetics	G6PD deficiency	Richard Francis
30	Genetics	PK deficiency	Holmes Morton
31	Genetics	G1Pase	Dan Ambruso
32	Genetics	Beta Thalassemia	Antonelou
33	Genetics	Homocysteinuria	MacLean
34	Genetics	RBC metabolism in AML patients	Jordan
35	Genetics	Zoomics - Denver Zoo	Anneke Moresco
36	Storage	RBC storage	Spitalnik
37	Storage	Non Human Primates	Buehler
38	Storage	Sickle cell disease - rejuvenated resuscitation	Weslby
39	Storage	Storage	Dumont
40	Storage	Age of stored RBCs and sickle patients	Karafin
41	Genetics	From Omics to Structure/Function	Eisenmesser
42	Storage	Rat RBCs and HS	Cabrales
43	Bioinformatics	Systems Biology	Palsson
44	Statistics	Statistics	Kechris

September 4, 2018

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Assistant Professor
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Dear Dr. D'Alessandro,

The NHLBI-funded Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) central laboratory which serves as Biorepository for biospecimens collected for the REDS-III research studies has identified the following biospecimens requested for your proposal:

- 13,800 frozen RBC samples derived from leukocyte-reduced, end-of-storage red blood cell concentrates from the REDS-III RBC omics study
- 300 confirmed Zika virus positive RBC samples from US blood donors from the REDS-III US Natural History Cohort of Zika Virus (ZIKV) RNA Positive Blood Donors
- 3,000 RBC aliquots from the REDS-III Brazil Sickle Cell Disease cohort

Upon funding and justification, the samples will be made available. Consent will be verified for intended research use, and samples will be either anonymized or de-identified per the IRB-approved consent. Note that the final biospecimens are subject to visual inspection when retrieved from storage. Problem vials will be substituted with equivalent specimens wherever feasible in consultation with the study investigator.

All REDS-III data associated with the biospecimens being requested will be available through the NHLBI Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC at https://biolincc.nhlbi.nih.gov/home/) or the database of Genotypes and Phenotypes (dbGaP at https://www.ncbi.nlm.nih.gov/gap).

Further details about the REDS-III program can be obtained from the REDS-III website https://reds-iii.rti.org/Home.aspx

Sincerely,

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LOS - no. 1

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September 4, 2018

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SUBJECT: REDS-III RBC-Omics Samples Evaluation for your MIRA Proposal

Dear Angelo,

I am excited to provide my unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". The letter from the REDS-III Biorepository confirms the availability of samples.

Unique RBC samples derived from leukocyte-reduced (LR), end-of-storage red blood cell (RBC) concentrates were collected from consenting volunteer whole blood donors at four different U.S. blood centers (Blood Systems Research Institute in Northern California, Blood Center of Wisconsin, American Red Cross in Connecticut, and Institute for Transfusion Medicine in Pittsburgh) within the framework of the Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) RBC-Omics Study, which I coordinated over the past five years.

Within the framework of the RBC-Omics project, we obtained DNA from WBC derived from LR filters from these RBC components and performed a GWAS which enabled analysis of Single Nucleotide Polymorphisms (SNPs) and Copy Number Polymorphisms (CNPs) for ~300,000 common traits of relevance to red cell physiology, erythropoiesis, iron and redox metabolism, energy metabolism and hypoxia. RBC samples from the end of storage (~42 days post-donation) were tested in real time for spontaneous storage hemolysis (one of the FDA gold standards to determine the quality of stored RBCs) and for the propensity of washed RBCs to hemolyze when challenged with pro-oxidant (AAPH) or osmotic stressors (pink test). This genetic and phenotypic information complements detailed records on donor sex, age, ethnicity, donation frequency, ferritin status, as well as records from the REDS-III clinical linked donor-recipient database reporting transfusion outcomes in thousands of recipients or LR-RBC transfusions from these donors.

Your state of the art metabolomics and proteomics methods, which you have already successfully applied to a subset of 599 samples from 250 donors (including time points of storage day 10, 23 and 42), have generated data that has immediately resulted in two successful publications, with an additional 6-7 pending over the next 8-12 months. For example, we have recently noted that some donors' who's stored RBCs had extremes in oxidative

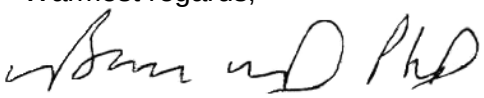
hemolysis, were characterized by increased methionine consumption. Following up on this observation with proteomics analyses and metabolic flux analysis with ^{13}C ^{15}N-methionine, your team has revealed a role for oxidative stress in methylating deamidated asparagine or aspartate residues in critical proteins involved in RBC oxygen-dependent metabolic modulation – including the N-terminal cytosolic domain of band 3, glyceraldehyde 3-phosphate dehydrogenase and many other enzymes. Though previous studies had described the phenomenon of protein damage repair in RBCs as a response to oxidative stress, none of these studies had investigated the relevance of such a phenomenon for RBC storage, or its amenability to iatrogenic intervention (e.g. via hypoxic storage or storage with antioxidant). Moreover the state of the art proteomics methods your lab has mastered allowed you to map, for the first time, the actual proteins and residues that are methylated through this process. This is but a paradigmatic example of how likely your proposal is to generate relevant translational information (especially when coupled to transfusion outcomes in thousands of recipients) which I believe will impact the fields of transfusion medicine and hematology in general.

We recognize and value your extraordinary knowledge, talent and enthusiasm, which is prototypical of an extremely promising early stage investigator. In your relatively short academic career you have already become a highly regarded, leading world expert in RBC metabolism. You have established and are driving a state-of-the-art laboratory equipped with top instrumentation both in high-throughput metabolomic and proteomic analyses, and with computational capacity and pipelines required for analyses of these complex data. For this and many other reasons, above all your unconditional love for the science of red blood cells, we were happy to welcome you as ***Affiliate Investigator to our Blood Systems Research Institute*** based in Denver and San Francisco.

Logistics for the successful progress of this project have already been worked out within the framework of the successful pilot study, including Material Transfer Agreements between the REDS III group and the University of Colorado Denver generated for that purpose.

I wish you the best of luck with your proposal and wholeheartedly hope that this grant gets funded so we can continue and expand this amazing collaboration that has the potential to revolutionize the field of transfusion medicine and our understanding of red blood cell biology at large.

Warmest regards,



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4 September 2018

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Dear Angelo,

I am excited to provide an enthusiastic letter of support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". This letter represents our complete commitment for a proposed collaboration with your lab to analyze samples from ~3,000 patients in the REDS-III Brazil Sickle Cell Disease cohort. The letter from the REDS-III Biorepository confirms the availability of samples.

Enrollment samples, including red blood cell (RBC) aliquots were collected between 2013 and 2015 from 2795 participants in six different sites in Brazil. The cohort included a slight predominance of children <18 years (55.9%) and females (53.0%). Hemoglobin (Hb) SS was the most common SCD genotype (70.7%), followed by HbSC (23%), Sβ0 (3.0%) and Sβ+ (2.9%). In addition, we have follow-up samples collected in 2017 and 2018 for nearly all members of the cohort. Targeted studies focused on transfusion outcomes have been conducted and are currently undergoing data analysis, including identification of clinical and genetic predictors of red blood cell (RBC) alloimmunization and vaso-occlusive pain episodes, determinants of the impact of chronic transfusion therapy on clinical and laboratory outcomes, and characterization of blood utilization and transfusion adverse events in the cohort.

The entire SCD cohort has been extensively phenotyped and genotyped using the transfusion medicine array developed as part of REDS-III program, and the cohort also has whole genome sequencing completed as part of the NHLBI Trans-Omics for Precision Medicine (TOPMed) program. In addition, we have transcriptomic data for a subset of the cohort (~100 individuals) at multiple time points. It is highly relevant for us to participate in in-depth trans-omics investigations of the overall cohort. Studies that seek to understand the metabolome of red cells are of exceptional interest to the SCD study team.

As you know, we have been collaborating for more than a year within the framework of the REDS-III study and our interactions have been positive and constructive. Your demonstrated capabilities and expertise in the area of RBC metabolism are evident to the community and your undertaking with this project is very likely to generate data relevant to many research endeavors.

In the light of these considerations, I am very pleased to be able to provide my unconditional support to your proposal and look forward to the critical scientific insights we will gain.

Warmest regards and best of luck,

Brian Custer, PhD, MPH
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September 4, 2018

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Dear Angelo,

I am writing to provide my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". The letter from the REDS-III Biorepository confirms the availability of samples.

The NHLBI funded **US Natural History Cohort of Zika Virus (ZIKV) RNA Positive Blood Donors** enrolled consenting volunteer whole blood donors from Puerto Rico and the continental US identified confirmed as Zika infected through NAT blood screening IND studies into longitudinal follow-up studies. As part of follow-up study design, red blood cell samples collected at each study visit are accompanied by demographic data and assessment of clinical outcomes through detailed symptom questionnaires, extensive characterization of RNA persistence in various blood compartments and body fluids as well as serology profiles over time. Of particular relevance are the donors who were identified in the early pre-seroconversion phase of infection and followed through acute infection and most informative to understanding of Zika pathogenesis and development of host response to infection. Similar studies of Dengue infected donors, identified before the emergence of Zika in the Americas, have banked comparable red blood cell samples.

Further characterization of these samples, especially with your state of the art high-throughput metabolomics platforms, are likely to generate data necessary to formulate specific hypotheses, to be tested in future mechanistic studies. It will be interesting for example to understand whether RBCs from infected, asymptomatic subjects display quantifiable metabolic markers that can be used for future, cost-affordable, prospective testing; of particular importance to transfusion medicine, and blood screening and diagnostic testing. Markers of inflammation or oxidative stress, as well as indications of metabolic reprogramming could inform on as of yet unappreciated impact of viral infection on red cell physiology and function, including oxygen carrying and off-loading capacity.

Additionally, these studies can inform our understanding of virus-host interactions at the individual and population level, for example by identifying potential RBC-generated mosquito chemoattractants that could ease viral spread across the population, as previously observed for malaria (Emami et al. Science. 2017 Mar 10;355(6329):1076-1080).

In the light of the considerations above, I am happy to wholeheartedly offer support to this project.

Warm regards,

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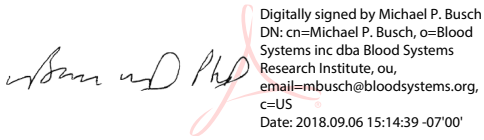

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To whom it may concern

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https://www.researchgate.net/profile/Anna_Bogdanova

CoMMiTMenT, FP7 project: http://rare-anaemia.eu

RELEVANCE, HORIZON2020: http://relevance.arivis.com

Zurich, September 3rd, 2018

RELEVANCE group support for your MIRA Proposal

On behalf of the RELEVANCE group (Regulation of red cell life-span, erythropoiesis, survival, senescence and clearance), I am excited to provide our support to your Early Stage R35 proposal entitled “MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage”.

As you know, RELEVANCE is an international consortium of thirteen partners representing academic research centres, diagnostic labs, blood supply centres, and small industries that combines basic and translational research to improve prognostic, diagnostic and therapeutic approaches on red blood cells production, function, and clearance in healthy humans and patients. Though located across Europe, the team of investigators involved in RELEVANCE shares with you the passion for RBC biology and the role of erythrocytes in systems physiology. Your omics platforms would complement the expertise of our current portfolio and samples sets we may have banked within the framework of RELEVANCE could well fit within the broader scope of your proposal, i.e. to assess the impact of aging, genetics, environment and storage on RBC metabolism as gleaned through state-of-the-art proteomics and metabolomics approaches.

LOS - no. 5

We have recently interacted through the editorial initiative I launched which focuses on RBC biology as part of the Frontiers in Physiology journal, within the framework of which you have joined our editorial board of experts in the field.

In the light of the considerations above, all the members of the RELEVANCE group are all glad to renew our support to your proposal and wish you best of luck with your submission.

With best regards

Anna Bogdanova

Anna Bogdanova, Prof. Dr. rer. nat. habil.

Representing all members of the RELEVANCE consortium:
Lars Kaestner, University of Saarland, Germany
Giampaolo Minetti, University of Pavia, Italy
Richard van Wijk, University Medical Center of Utrecht, the Netherlands
Marieke von Linders and Robin van Bruggen, Sanquin Blood Supply, the Netherlands
Stéphane Egée, Biological Station Roscoff, France
Ashley Toye University of Bristol, UK
Wassim el Nemer INSERM France
Joan Lluís Vives Corrons and María del Mar Mañú Pereira Josep Carreras Leukemia Research Institute, Spain
Tim Ryan Epigem Ltd, UK
Andrea Brueggemann and Markus Rapedius, Nanion, Germany
Christian Götze Arivis AG, Germany
Andrew Rafael Bañas and Jesper Glückstad, OptoRobotix ApS, Denmark

LOS - no. 5

Bloodworks Northwest

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9/9/2018

Dear Angelo,

I am excited to provide my enthusiastic and unequivocal support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

I have developed an acute appreciation for your rigorous and highly productive approach and methods as a result of our collaboration over the past two years, a collaboration that has resulted in the publication of a series of important papers for the field of transfusion medicine, including Nemkov et al. Haematologica 2018; Reisz et al. Transfusion 2018. Our almost daily conversations have given me a detailed insight into your approach to science, and your keen talent of using the power of your analytic platforms to run the gamut from hypothesis generation to in depth mechanistic studies and rigorous mechanistic elucidations of complex biological systems.

Within the framework of this project and in the context of my acute interest and expertise in hematology, with a special emphasis on immunology and red cell biology, I will be happy to provide you red blood cells from the animal models we have and will be generating for our projects focused on red cell storage and redox biology. Given our frequent dialog, including visiting each other's labs in the last year, I am familiar with the hypotheses you are investigating and the questions you are asking. I find your approach to be innovative, intriguing, and potentially transformative for the field of RBC biology. Along these lines, and in support of your efforts, I am happy to provide you with red cells from my mouse strains and KOs (including mice expressing human N-term of band 3, KO for the high-affinity binding site for hemoglobin or for the ~10 amino acid residues that represent the anchor site for glycolytic enzymes). These tools will provide you the ability to perform mechanistic elucidations of the biology in question and to assess your central hypothesis that red cell oxygen-dependent metabolic regulation plays a key role on systems physiology as a function of age, genetics (including gender), environment and storage.

In addition to the strains listed above, my lab has all the tools necessary to (i) sort RBC populations depending on fine biotin/GFP-dependent labeling strategies, (ii) store red blood cells under conditions that mimic blood bank storage and test transfusion efficacy through state of the art flow cytometry-based sorting strategies; (iii) create any additional mice that may be useful to your project and pave the way for future collaborative work through, for example, the submission of joint proposals to further the observations you will report within the framework of this study. Importantly, I am happy to use our mice and perform manipulations with resources outside your R35 budget, and in response to your needs and

requirements, as a collaborator dedicated to supporting your work through fine manipulation of whole animal systems.

With 15 years of experience in this field, developing methods and tools to assess new hypotheses, I feel highly qualified to support you in your innovative efforts. I would like to commit to you personally, and state for the reviewers, that I am eager to bring approaches and resources to bear on your studies. I am eager to work with you as a source of experimental intervention so as to assure that you are able to generate the biological samples and conditions you require, in order to test your current hypotheses and new hypotheses that come forth from them. Given your innovation, energy, and immense productivity, I have no doubts that (if supported) this project will break through barriers and challenge paradigms of RBC biology. I am eager to support this in any way I can; and commit that you will have full access to myself, my lab, and our methods and tools – as well as active collaboration in generating new tools and methods as required.

Good luck on your highly exciting proposal,

With warmest regards,

James C Zimring M.D.,Ph.D.
Chief Scientific Officer and Director of BloodworksNW Research Institute
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LOS - no. 6

UTHealth
The University of Texas
Health Science Center at Houston

Medical School
Department of Biochemistry and Molecular Biology

Denver, August 28th, 2018

Angelo D'Alessandro
Department of Biochemistry and Molecular Genetics
University of Colorado Denver – Anschutz Medical Campus
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Dear Angelo,

I am writing in enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". I will be happy to provide my expertise on RBC biology, biochemistry, hypoxic responses in vivo and in vitro, and mouse models to investigate RBC biology as necessary.

As you know, my team is pursuing mechanistic studies on aging and hypoxia, using the CD73/ADORA2B/AMPK or PKA/SPHK1 signaling axis as a mechanistic key to investigate systemic responses to hypoxia.

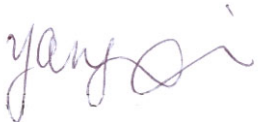
Our collaborations over the past three years have been incredibly productive. Just to highlight some of our research products, I would like to mention Anren's Nature Communications 2017 paper, Kaiqi's Nature Communications (2016 – with you as co-first author) and Scientific Reports (2017), Liu Hong's Circulation paper (2016), and your recent Haematologica paper (Reisz et al. 2018) out of a total of over >10 papers. Our collaborations have resulted in your early career awards (National Blood Foundation 2016 and Boettcher Webb-Waring 2017) and paved the way for follow up studies on RBC metabolic responses to hypoxia in health and disease we are currently working on together.

Your support and expertise in the field of omics technologies and their applications to the study of RBC biology has been instrumental for the success of some of my recent R01 proposals and I hope we will continue working together for many years to come.

As we discussed several times, we share an unconditional love for RBCs and I do believe that the proposal you assembled here will be instrumental to a long series of innovative studies in the field of hematology, not necessarily exclusively related to erythrocyte biology.

For the above-mentioned reasons I am excited to provide my enthusiastic support to your proposal and I hope it will be favorably received by the review panel.

Sincerely,



LOS - no. 7

Yang Xia, M.D., PhD,
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September 1, 2018

SUBJECT: RBCs from IUGR infants for your MIRA proposal

Dear Angelo,

We are excited to provide our unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As you know, our laboratory is investigating intrauterine growth restriction (IUGR) in infants, a phenomenon that gravely impacts fetal growth and threatens mothers and fetuses alike, often resulting in a premature delivery. While maternal infection or preeclampsia-related complications may underlie the etiology of IUGR, in our current ongoing cohort we are investigating the impact of placenta metabolism and transport capacity on nutrient delivery to the infant. Even though we would have routinely thrown away red blood cell samples from the mother and the baby, we will start banking these samples for your omics analyses as described in your proposal. Clearly, this will represent an extreme case scenario of red cells from the youngest possible subjects, as well as a subset with clinically relevant hypoxia in that oxygen can be one of the poorly delivered nutrients in IUGR fetuses.

We wish you good luck with your proposal.

Sincerely,

Theresa Powell, PhD
Professor
Department of Pediatrics, Division of Neonatology
Department of Obstetrics and Gynecology,
Division of Reproductive Sciences

Stephanie Chassen, MD
Assistant Professor
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LOS - no. 8

Children's Hospital Colorado

www.childrenscolorado.org

Heart Institute

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August 29, 2018

SUBJECT: Newborn, children, and teen RBC samples (with and without ECMO) for your MIRA proposal

Dear Angelo,

As we recently discussed, I am confirming my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As you know, over the next 12-24 months I will be collecting RBC samples from newborn infants, children, and teenagers suffering from systemic hypoxemia at baseline and following therapy through extracorporeal membrane oxygenation (ECMO). This is an IRB-approved prospective study. Children's Hospital Colorado is a tertiary regional referral center for critical cardiovascular and pulmonary disease; and is an Extracorporeal Life Support Organization (ELSO) center of excellence supporting 30-40 children with ECMO each year. As a member of the ECMO Advanced Clinical Team and faculty in the Heart Institute, not only do I have access to patients requiring ECMO but the potential for additional collaboration and recruitment of children before and after repair of cyanotic heart disease.
As we discussed, it will be interesting to investigate the metabolic and proteomics (especially redox PTMs) of RBCs when circulated through the extremely hyperoxic (~100% oxygen saturation) environment of ECMO. We anticipate that RBCs from newborn patients, characterized by fetal hemoglobin with higher affinity for oxygen than adult hemoglobin, will suffer from such pro-oxidant conditions more than adult RBCs – which our colleague Dr. Clendenen will provide independently under currently approved IRB protocols, as discussed.

The approach your team has exploited in Reisz et al. Blood 2016 is perfectly suited to address the questions above. Anticipated results could inform the clinical community on potential caveats associated with ECMO and how to potentially address them in a critical, at risk population. Incidentally, these samples will also open a window on the metabolic mechanisms of hypoxia in RBCs from (very) young individuals, which perfectly fits within the main scope of your proposal.

I am looking forward to our collaborations in the near future.

Kindest regards,

John S. Kim, MD MS
Assistant Professor of Pediatrics – Cardiology
University of Colorado School of Medicine

LOS - no. 9

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August 29, 2018

SUBJECT: Adult RBC samples (pre/post-ECMO) and cardiopulmonary bypass for your MIRA proposal

Dear Angelo,

Following up on our extensive and productive collaborations over the past two years, I am writing to renew my unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As extensively discussed, I will be able to provide RBC samples from cardiopulmonary bypass patients prior to anesthesia, during cardiopulmonary bypass (a condition of potential systemic hypoxemia), both at baseline and following therapy through extracorporeal membrane oxygenation (ECMO). The University of Colorado is a tertiary referral center for cardiovascular disease and cardiac surgery that performs over 600 procedures with cardiopulmonary bypass each year in addition to approximately 100 patients per year requiring ECMO. As a founding member of the Human Biologics Cooperative, I direct sample collection under a currently approved and actively recruiting biorepository with capacity for enrolling 20,000 patients with multiple sample collections at different time points before, during, and after surgery.

As we discussed, it will be interesting to investigate the metabolic and proteomics (especially redox PTMs) of RBCs when circulated through the extremely hyperoxic (~100% oxygen saturation) environment of ECMO. Currently approved IRB protocols are already in place and we have already started collecting samples for this project. Our samples will complement paediatric samples (+/- ECMO) which will be provided by Dr. Kim and his team.

The approach your team has exploited in Reisz et al. Blood 2016 is perfectly suited to address the questions above. Anticipated results could inform the clinical community on potential caveats associated with ECMO and how to potentially address them in a critical, at risk populations, such as patients with end stage cardiovascular disease.

SCHOOL OF MEDICINE
Department of Anesthesiology
UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

On a side note, I am happy to highlight the success of our collaborative work to date, which contributed to my promotion to Assistant Professor in the Department of Anesthesiology last year, as well as our continuous direct collaboration – as noted by the daily presence of one of my team members, Rachel Henderson, in your lab and our recent joint publications.

To conclude, I wish you the best of luck with this interesting proposal and hope to expand our collaborations in the near future.

Sincerely,

Nathan Clendenen, MD, MS
Assistant Professor of Anesthesiology
Department of Anesthesiology
University of Colorado Denver

University of Colorado
Cancer Center
An NCI Designated Comprehensive Cancer Center

James DeGregori, Ph.D.
Deputy Director, UCCC
Professor, Department of Biochemistry and Molecular Genetics

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Aurora, Colorado 80045
Phone 303 724-3230
Fax 303 724-3215
e-mail : james.degregori@ucdenver.edu

September 3, 2018

SUBJECT: RBC from young/old mice for your MIRA proposal

Dear Angelo,

Following up on our extensive and productive collaborations over the past several years, I am writing to emphasize my unconditional support for your Early Stage R35 proposal entitled “MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage”.

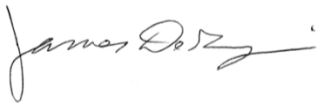
As an ***expert in the field of aging*** *(Marusyk et al. 2008; Le et al. 2010; Henry et al. PNAS 2010; Aging 2011; JCI 2015; Rohzok et al. Aging 2014; PNAS 2016; Trends Cancer 2016)* and evolutionary biology *(DeGregori – Adaptive Oncogenesis - Harvard University Press 2018)*, **I will be happy to provide advice and to collaborate on this interesting project** investigating the role of RBC metabolic reprogramming as a function of aging and inflammation. My lab is highly invested in understanding how metabolism is reprogrammed by aging and inflammation, focusing on early hematopoietic progenitors and other tissues. As discussed, we have been banking RBC samples for you to support this project.

As your **academic mentor and close collaborator** (*Gregory et al. PNAS 2017; Exp Haematology 2018; Henry et al. Haematologica 2017; Jones et al. Cancer Cell, in press*), I believe that you and your team have all the expertise necessary to successfully complete the proposed study. Indeed, despite your Early Career Stage, you have demonstrated a strong expertise in the fields of RBC biology and the application of omics technologies to investigate the role of RBCs in health and disease. Incidentally, your metabolomics expertise has been instrumental to providing state of the art metabolomics support to our School of Medicine and Cancer Center, as well as the Linda Crnic Institute for Down Syndrome (of which we are fellow grant recipients). Owing to the increasingly appreciated link between inflamm-aging, oncogenesis and neurocognitive impairment (e.g. in aging and/or Down syndrome), most of your models will provide valuable insights and/or biological material to test specific hypotheses complementary to our ongoing studies with you, Drs. Eisenmesser, Hansen and Dinarello on the role of anti-inflammatory pathways (e.g. IL-37, A1AT) in aging and cancer.

LOS - no. 11

In the light of the considerations above, I am glad to confirm my unconditional support to your proposal and I wish you good luck with the submission.

Sincerely,



James DeGregori
Professor

LOS - no. 11

Science at the heart of medicine

Laura Santambrogio
Professor
Department Pathology, Microbiology & Immunology

Jack and Pearl Resnick Campus
1300 Morris Park Ave., room 140
Bronx, NY 10461
tel 718.430.3458 fax 718.430.8541
laura.santambrogio@einstein.yu.edu

New York September 1st, 2018

SUBJECT: Red blood cells from parabiotic mice for your MIRA proposal
Angelo D'Alessandro, PhD
Dept. Biochemistry and Molecular Genetics
University of Colorado Denver
Anschutz Medical Campus
12801 East 17th Ave
room L18-9118 - RC1 South
Aurora, CO, USA 80045

Dear Angelo,

I am happy to provide my support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As you know, research in my lab focuses around mechanisms of "inflammaging", i.e. the chronic-inflammatory-like state that is observed progressively with aging. To investigate this facet of aging, we have established a mouse model of parabiosys where the circulatory system of young and old mice are surgically connected. While we had originally set to investigate the plasma metabolome of these mice in collaboration with your team, we will be happy to share with you the otherwise discarded red blood cells from these mice (young-young, old-old, young-old – with blood collected either from the young or the old mouse).

Best of luck with your proposal!

Warm regards,

Laura Santambrogio, MD. PhD.
Professor of Pathology, Immunology and Microbiology
Albert Einstein College of Medicine

LOS - no. 12

Joaquín M. Espinosa, PhD
Executive Director, Linda Crnic Institute for Down Syndrome
Professor, Department of Pharmacology, University of Colorado School of Medicine
Co-Leader, Molecular Oncology Program, UC Cancer Center
Director, The Functional Genomics Facility

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12700 E. 19th Avenue, Room P15-4008
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Office: 303 724-9907 / Fax: 303 724-5741
Joaquin.Espinosa@ucdenver.edu

SCHOOL OF MEDICINE
Department of Pharmacology
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

LINDA CRNIC INSTITUTE FOR DOWN SYNDROME™

Denver, August 28th, 2018

Subject: *Letter of support –investigation of Down syndrome RBCs by Dr. Angelo D'Alessandro*

Dear Angelo,

We are glad to provide our enthusiastic support to your Early Stage R35 proposal entitled "MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage".

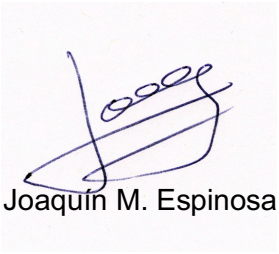
We are glad that the support originally provided to you by the ***Linda Crnic institute for Down Syndrome***, of which you are an ***affiliate investigator***, has resulted in the generation of the preliminary data necessary to warrant further research in the field of red blood cell metabolic reprogramming in Down syndrome.

Our joint collaborations have already resulted in an exploratory paper on red blood cell metabolism in Down syndrome (Culp-Hill et al. Blood Advances 2017), while additional research products are currently being finalized describing alterations of tryptophan metabolism in the plasma metabolome of individuals with Down syndrome (Powers et al. *under review*). We confirm that the Crnic Institute can provide plasma and red blood cells from well-characterized mouse models of Down syndrome, including Dp10, Dp16, and Dp17 mice.

We are familiar with your outstanding approaches and expertise, and we are supportive of your passion for RBCs. Indeed, we are happy to count on you for the metabolomics arm of our ongoing cohort study of the population with Down syndrome, known as the ***Human Trisome Project*** (www.trisome.org). Within the framework of that study, additional plasma and red blood cell samples will be made available to you for further research activity, as planned. Your proposal represents the perfect continuation of your Crnic-funded research activity and promises to provide novel therapeutic strategies to prevent and/or mitigate some of the hypoxia-related comorbidities observed in the Down syndrome population.

In the light of the considerations above, we are happy to provide our enthusiastic support to your proposal and wish you the best of luck with this submission.

Warm regards,



Joaquín M. Espinosa

LOS - no. 13

UNIVERSITY OF CAMBRIDGE
Department of Clinical Neurosciences

Stefano Pluchino, MD, PhD
University Reader in Regenerative Neuroimmunology
and Honorary Consultant in Neurology
Our Ref: **LoS MIRA proposal- D'Alessandro**

31th August 2018
Cambridge, UK

Re: RBCs from Multiple Sclerosis patients/animal models for your MIRA proposal

Dear Angelo,

I am happy to provide my unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment***

As you well know, our research focuses on multiple sclerosis (MS) and etiological contributors to MS-associated neurocognitive complications.

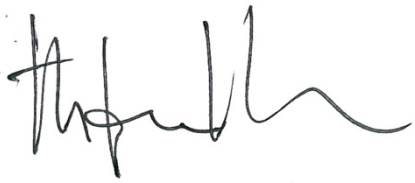
In a series of recent studies, we have demonstrated that some pro-inflammatory metabolites like succinate accumulate in the cerebrospinal fluid of rodents with experimental autoimmune encephalomyelitis (EAE), and patients with MS (Peruzzotti-Jametti, D'Angelo, et al. *unpublished*), which in turn promotes mononuclear phagocyte infiltration and secondary damage to the central nervous system during chronic inflammation (Peruzzotti-Jametti et al. *Cell Stem Cell* 2018).

Though our studies have not been hitherto focused on red blood cell biology, within the framework of follow studies my team will be happy to collect and provide erythrocytes from laboratory animals with EAE and patients with MS to support your omics analyses. Evidence is already available that red cell abnormalities (fatty acid and kynurenine metabolism) are observed in people with MS (Hartai et al. Acta Neurol Scand. 2005 Aug;112(2):93-6.; Hon et al. J Membr Biol. 2009 Dec;232(1-3):25-34.), though these early studies have not leveraged the power of the state-of-the-art omics tools you have available in your lab.

In the light of these considerations and given the impressive span of oxidative stress-related conditions you will be testing in your proposal, I have a good confidence that this additional set of samples may represent a perfect complement to your proposal.

Sincerely,

Stefano Pluchino, MD, PhD



LOS - no. 14

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University of Colorado | Anschutz Medical Campus
Department of Neurology School of Medicine
Movement Disorders Center

Maureen A. Leehey, MD, FAAN
Professor of Neurology
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Fax: (303) 724-2212

Clinic address:
University of Colorado Hospital
Anschutz Outpatient Pavilion
Neurology Department, 4th floor
1635 Aurora Ct.
Aurora, CO 80045
Telephone: (720) 848-2080

SUBJECT: RBCs from Parkinson's Disease patients for your MIRA proposal

Dear Angelo,

I am writing in support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment***

During the past few months, we have been interacting within the framework of a project related to metabolomics analyses of plasma from patients suffering from Parkinson's Disease as part of a clinical trial I am running here in Denver to investigate the potential therapeutic effects of cannabidiol in these patients. While not originally part of my study, I appreciate the relevance of existing literature on the impact of PD on RBC redox homeostasis and amino acid metabolism (Gomes-Trolin et al. Exp Neurol 2002). In this view, your omics analyses of the samples I could provide would be much more informative than existing literature based on previous generation analytical methods. Your hypothesis of a central role of red blood cell metabolism in modulating systemic responses to hypoxia and oxidative stress is intriguing and I hope that my patient population will benefits from the mechanistic understanding of the phenomena that you wish to investigate in your proposal.

With warm regards,

Maureen A. Leehey, MD, FAAN
Professor of Neurology
Chief, Movement Disorders Division

LOS - no. 15

COLUMBIA UNIVERSITY
College of Physicians and Surgeons

Tiffany Thomas, Ph.D.
Assistant Professor of Pathology & Cell Biology
Biochemical Genetics Laboratory Director

Department of Pathology & Cell Biology
630 West 168th Street
HP3-325
New York, NY 10032
646.317.5360 Tel
212.305.7553 Fax
tt2254@cumc.columbia.edu

SUBJECT: RBC populations for your MIRA proposal

Dear Angelo,

In the light of our recent email exchanges and given our shared love for red blood cells, I am writing to confirm my support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

Over the past 2 years, we have been productively (Nemkov et al. Haematologica 2018) collaborating to investigate the impact of diets on RBC storage. As I plan on using dietary interventions in mice by feeding high fat diets based on different fatty acid compositions as well as other dietary supplements that can potentially affect RBC lifespan and storage, it would be interesting to determine the impact of such interventions on RBC metabolism as a function of mouse age, gender, etc. In this view, I am delighted to collaborate with you on this and parallel projects from my lab that pertain to complementary aspects of your proposal.

Sincerely,

Tiffany Thomas

LOS - no. 16

Aurora, Colorado, Sept, 6th, 2018

SUBJECT: RBC populations for your MIRA proposal

Dear Angelo,

I am writing to confirm my most enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As we have discussed extensively over the past two years, my laboratory has worked hard to secure IRB approval to collect blood samples from sedentary subjects, individuals suffering from type 2 diabetes or other metabolic syndrome, as well as from recreational athletes, semi-professional and professional athletes from different disciplines. Though I cannot disclose as of now additional information about the latter group owing to non-disclosure agreement limitations that are currently being worked out prior to the formalization of work contracts, I will have access to blood from world class professional cyclists as well as elite and world-class athletes from different sports. We are planning studies on systemic metabolic responses at baseline, during training and competition and during the recovery phase at the end of training or competitive stage. In this view, it is worth noting that, while I had originally planned on just collecting plasma and whole blood, your arguments on the relevance of RBC metabolism in oxygen binding/off-loading has convinced me to pursue this additional analysis in collaboration with your group, whereas your proposal will be successful in securing funding.

In the light of the considerations above, I do really hope that your proposal will be successful and, in any case, I hope we will be able to foster and expand our collaborations.

Warm regards,

Iñigo San Millán, PhD
Assistant Professor, Department of Physical Medicine and Rehabilitation
Director, Sports Performance
University of Colorado School of Medicine
12631 E 17th Ave, Aurora, CO 80045

School of Medicine
UNIVERSITY OF COLORADO
ANSCHUTZ MEDICAL CAMPUS

LOS - no. 17

SCHOOL OF MEDICINE
Division of Hematology
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Eric M. Pietras, PhD
Assistant Professor
Boettcher Investigator
University of Colorado
Anschutz Medical Campus
12700 East 19th Ave
Aurora, CO 80045
eric.pietras@ucdenver.edu
www.medschool.ucdenver.edu/hematology

August 29, 2018

RE: Letter of collaboration for MIRA proposal

Dear Angelo,

I am pleased to provide my enthusiastic support to your Early Stage R35 proposal entitled, "*MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage*".

As you know, my research program focuses on understanding the impact of chronic inflammation on the production and function of multiple hematopoietic lineages, including erythroid cells, also known as red blood cells (RBC; *see Pietras, Blood 2017*). As collaborators and fellow Boettcher Investigators, we have collaborated extensively over the last two years to investigate stress erythropoiesis in a background of chronic inflammation by combining state of the art metabolomics approaches developed in your lab with the unique chronic and acute IL-1 injection models my lab developed in mice. This synergy has led to the development of novel hypotheses that extends from my original interest around hematopoiesis and erythroid precursors to mature RBC, where our preliminary data suggest that the mature RBC metabolome and proteome may represent a subject worth investigating. Indeed, our preliminary findings are likely to recapitulate mature erythrocyte metabolism in response to pathological inflammatory conditions in human patients, such as in the case of rheumatoid arthritis (another project we are collaborating on), Down syndrome (your work with the Espinosa's group is relevant in this context and we have also recently started investigations this model) or physiological aging (from an "inflammaging" perspective).

To help facilitate your work, we are happy to provide red blood cells (RBC) from IL-1β-treated mice for your MIRA proposal, as well as RBC from our other chronic inflammatory disease models. We are also happy to provide our expertise in hematopoiesis and inflammation. Our ongoing collaborations with the DeGregori and Dinarello labs at the University of Colorado, who will provide additional letters of support, further warrants the generation of samples that will fit well within the broad scope of your interesting proposal.

I am glad to confirm my support to your early stage investigator award. Please don't hesitate to

Warm regards,

Sincerely,

LOS - no. 18

Eric M. Pietras, PhD

COLUMBIA UNIVERSITY
College of Physicians and Surgeons

Eldad A. Hod, M.D.
Associate Professor of Pathology & Cell Biology
Director, Center for Advanced Laboratory Medicine

Department of Pathology & Cell Biology
630 West 168th Street
P&S 14-434
New York, NY 10032
212.342.5648 Tel
eh2217@cumc.columbia.edu

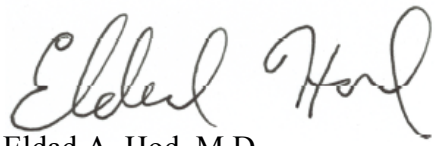
Re: Red blood cells from mice and donors for your MIRA proposal

Dear Angelo,

I am writing in support of your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". Over the past few years, we have had the pleasure of working together on different aspects of the red cell storage lesion and how this impacts erythrocyte metabolism and transfusion outcomes, as determined by measurements of post-transfusion recovery with ^{51}Cr radiolabeled red cells and in vivo markers of hemolysis. These studies, currently being finalized and almost ready for publication, paved the way for appreciating the impact of iron availability on erythropoiesis and the role this plays on factors relevant to blood donor health and red blood cell quality. In light of these results, we have successfully secured R01 funding to investigate the role of donor iron deficiency and dietary iron supplementation on red blood cell quality. Within the framework of this study, we are banking red blood cells from human blood donors, who are undergoing post-transfusion recovery studies while iron deficient and following randomization to total body iron repletion. Furthermore, we also have banked samples from our mouse model of nutritional iron deficiency. Within the framework of your proposal, we will happily share aliquots of all of our banked samples for your proteomics and metabolomics analysis.

In light of the considerations above, I am glad to provide my unconditional support for your initiative and I hope we will have the chance to collaborate on this and many other projects in the years to come.

Respectfully yours,



Eldad A. Hod, M.D.
Department of Pathology and Cell Biology
Division of Transfusion Medicine and Stem Cell Therapy
Columbia University Medical Center

EINSTEIN
Albert Einstein College of Medicine

Science at the Heart of Medicine

Albert Einstein Cancer Center

Gottesman Institute for
Stem Cell and Regenerative Medicine Research

Britta Will, Ph.D.

Assistant Professor
Department of Medicine (Oncology)
Department of Cell Biology

Director, Cancer Stem Cell
Pharmacodynamics Unit
Albert Einstein Cancer Center

Jack & Pearl Resnick Campus
1300 Morris Park Ave., Bronx, NY 10461
Chanin Building, Room 401

Tel 718.430.3786 Fax 718.430.8574

britta.will@einstein.yu.edu

Bronx, 9/05/2018

RE: Red blood cells from iron chelation experiments for your MIRA proposal

Dear Angelo,
I am very happy and excited to provide my unconditional support for your Early Stage R35 proposal entitled *MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage*.

As you know, our laboratory has been investigating the role of eltrombopag in hematopoiesis over the last years. Eltromopag is a small-molecule thrombopoietin receptor mimetic, FDA-approved for the alleviation of thrombocytopenia in patients with idiopathic thrombocytopenia or severe aplastic anemia. Our past work demonstrated efficacy and safety of this drug for counteracting life-threatening thrombocytopenia in patients with myelodysplastic syndrome (*Will et al., Blood 2009*). Importantly, our new study shows that eltrombopag also stimulates multilineage hematopoiesis - an effect that is largely driven by the compound's intracellular iron chelation capacity (*Kao et al., Sci Transl Med 2018*).

Although our main research focus is on healthy hematopoietic and leukemic stem cells, we will be able to share with you mature red blood cells (RBC) or any other erythroid progenitor cell type (if required) from our various murine and human primary cell assay systems. Our interactions over the past few months elucidating the molecular mechanism of action of eltrombopag in purified immature hematopoietic cells have been immensely constructive and highly successful; in light of your lab's expertise in RBC omics analyses, I feel very confident about the success of the proposed omics analyses.

With my best wishes,

Britta Will, PhD
Assistant Professor in Medicine

Washington University in St. Louis
SCHOOL OF MEDICINE

Department of Pediatrics

Research Units
Pathobiology Unit
Patient Oriented Research Unit
Developmental Biology and Genetics Unit

August 30, 2018

Angelo D'Alessandro, PhD
Assistant Professor
Metabolomics Core Director
Boettcher Investigator
Department of Biochemistry and Molecular Genetics
University of Colorado, Denver

SUBJECT: RBC populations for your MIRA proposal

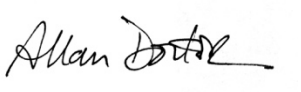
Dear Angelo,

I am writing in support to your Early Stage R35 proposal entitled "MIRAGES – Metabolic Investigation of Red Blood Cells as a function of Aging, Genetics, Environment and Storage". Over the past two years, we have been extensively and successfully (*Nemkov et al. Haematologica 2018; Rogers et al. under review*) collaborating on investigations related to RBC capacity to cope with oxidative stress, under physiological conditions, in response to cryopreservation. As you know well, my R01 focused on Sepsis Induced RBC Dysfunction (SiRD) has a component of NMR-based approaches to investigate RBC metabolic shift from glycolysis to the pentose phosphate pathway through the use of stable isotope-labeled tracers. While initially we did not involve you in the original proposal, following our meeting at FDA in DC and subsequent interactions stemmed from you *Reisz et al. (Blood 2016) publication*, we believe that your team holds the expertise necessary to analyze RBC metabolic responses to sepsis. As preliminary data seems promising and sample collection is ongoing, we are glad to provide these samples for you to analyze as part of this MIRA application, if and when you will be forced to drop any other support from NIGMS-funded investigations.

In your proposal you will be also investigating RBC metabolic responses to viral, other than bacterial infection (e.g. Zika, Dengue, Chikungunya), which would make a direct comparison against sepsis even more telling and appealing from a translational and basic science perspective.

In the light of the considerations above, I wish you the best with your proposal and hope to expand our collaborations in the future.

Warm regards,



Allan Doctor, MD
Professor of Pediatrics and Biochemistry

LOS - no. 21

University of Colorado **Kurt R. Stenmark, MD**

Anschutz Medical Campus
School of Medicine
Developmental Lung Biology and CVP Research Labs

Section Head
Pediatric Critical Care Medicine

August 30, 2018

SUBJECT: RBC Pulmonary Hypertensive subjects/animal models for your MIRA proposal

Dear Angelo,

I am writing to provide my unconditional support to your Early Stage R35 proposal entitled "*MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage*". Our collaborations over the past two years have been extremely productive and have contributed to the generation of a significant body of data in the field of pulmonary hypertension (PH). In particular, our joint effort identified a role for metabolic reprogramming of fibroblast, smooth muscle cells and macrophages in the development of PH, an observation that was supported by mechanistic evidence about the involvement of the miR-124/PTBP1/PKM2 axis in the etiology of metabolic reprogramming in PH. As you have tried to convince people, over the past two years, the role of red blood cell metabolism in a disease associated with systemic hypoxemia may be non-marginal. In this view, we will be providing you with RBCs from control or PH subjects and/or animal models of PH for your omics analysis.

Some of our models, e.g. the bovine model, are also important for USDA relevant studies, in that the development of PH, when these bovines are reared at high altitude, may result in premature death and significant economic loss for the farmer.

In the light of the considerations above and given the success of our ongoing collaborative work, I am happy to confirm my support to your proposal and wish you the best of luck.

Sincerely,

Kurt R. Stenmark, MD
Professor of Pediatrics and Medicine

LOS - no. 22

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Webpage: http://www.ucdenver.edu/CriticalCare

University of Colorado Denver

Anschutz Medical Campus
School of Medicine

Pulmonary & Critical Care Medicine
CVP Research Laboratories

August 31, 2018

Angelo D'Alessandro
Assistant Professor
Director, Metabolomics Core
Department of Biochemistry and Molecular Genetics
Aurora, CO 80045

Re: RBC from COPD subjects for your MIRA proposal

Dear Angelo,

I am writing to provide my support to your Early Stage R35 proposal entitled "MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage". As you know, I have access to a patient population suffering from Chronic Obstructive Pulmonary Disease (COPD), a disease associated with systemic hypoxemia. While the focus of our research is on plasma, red blood cells may be available for you to perform omics analyses, in keeping with the broader scope of your proposal to investigate RBC metabolism in health and disease.

Warm regards,

Mehdi A. Fini, MD
Assistant Professor of Medicine
Department of Medicine, Division of Pulmonary
Cardiovascular Pulmonary Research Lab &
VA Eastern Colorado Health Care System
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SCHOOL OF MEDICINE
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emergency.medicine@ucdenver.edu
www.medschool.ucdenver.edu/emergencymedicine

September 12, 2018

SUBJECT: RBCs from Emergency Department patients for your MIRA proposal

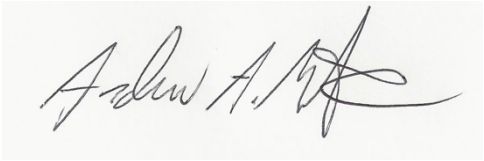
Dear Angelo,

I am glad to provide my unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment.*** As an Associate Professor of Emergency Medicine and Medical Toxicology at the University of Colorado I am perfectly positioned to provide the samples that are critical to your work. As you know, I direct the Emergency Medicine Specimen Bank through my own National Institute General Medical Science grant and I have a robust infrastructure as part of my personalized medicine research program.

Our work together to investigate the impact of metabolic changes in plasma from patients enrolled in our emergency department at UC Health at the Anschutz Medical Campus has been is exciting. As you know, we have operationalized our Emergency Medicine Specimen Bank and have enrolled over 5,000 patients and have already collected more than 1,500 blood samples that we will examine with your metabolomic pipeline. Within the framework of this project – that has already generated publishable data (Son et al. *in preparation*) you are serving as an external support for 'omics analyses. While the main focus of our prior work is on plasma, we will be able to bank erythrocytes (which we usually discard) for some categories of patients (e.g. critically ill, hemorrhage patients). In fact, we have formalized our relationship with the Trauma Program at the University of Colorado Level 1 Trauma Center to enroll trauma patients and collect samples throughout their hospitalization. This category of patients represents an extreme case scenario of clinically-relevant hypoxia and represent the perfect continuation of your studies in collaboration with the Trauma Research Center here in Denver.

In the light of the considerations above, I am happy to renew my support to your proposal and wish you and your team the best of luck.

Sincerely,



Andrew A. Monte, MD, PhD
Associate Professor
University of Colorado
Leprino Building, 7th Floor, Campus Box B-215
12401 E. 17th Ave
Aurora, CO 80045
&
Rocky Mountain Poison & Drug Center
Denver Health and Hospital Authority
Denver, CO
Email: Andrew.monte@ucdenver.edu

LOS - no. 24

Department of Medicine
SCHOOL OF MEDICINE
UNIVERSITY OF COLORADO
ANSCHUTZ MEDICAL CAMPUS

Division of Renal Diseases and Hypertension
School of Medicine, Department of Medicine

C281
12700 E 19th Ave | Aurora, CO 80045
o 303 724 4852 | **f** 303 724 4868
ucdenver.edu/renal

September 1, 2018

SUBJECT: RBC from animal models of kidney ischemia/reperfusion for your MIRA proposal

Dear Angelo,
I am writing in support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". Over the past two years, we have been collaborating on projects related to kidney ischemia (*Fox et al. under review*) and how the ischemia/reperfusion injury impacts distant organs, like heart, lungs and liver. In this view, it may be worthwhile to investigate the impact of ischemia and reperfusion on red blood cell metabolism, for which we will be collecting samples from the murine models currently up and running in the lab. I believe your proposal is innovative and will provide comprehensive new information on RBC biology (in health and diseases) and storage.

Warm regards,

Sarah Faubel, MD
Professor of Medicine
Associate Division Chief, Renal Division
University of Colorado Denver and Denver VA Medical Center Internal Medicine, Renal, and Pediatric Nephrology, Children's Hospital Colorado

University of Colorado
Anschutz Medical Campus

ALTITUDE RESEARCH CENTER
Division Pulmonary Sciences and Critical Care
Medicine, Department of Medicine
School of Medicine

Campus Box F-524
12469 E. 17th Place
Aurora, CO 80045

303 724 1670 (office)
303 724 1660 (fax)

www.altituderesearch.org

Wednesday, September 5, 2018

SUBJECT: Stored blood from humans for your MIRA proposal

Dear Angelo:

I am glad to provide my enthusiastic support to your Early Stage R35 proposal entitled **"MIRAGES – Metabolic investigation of red blood cells as a function of Aging, Genetics, Environment and Storage".**

As director of the Altitude Research Center in Denver and expert in human systemic responses to high-altitude hypoxia. Over the past few years, you and I have collaborated extensively on defining molecular mechanisms underlying RBC metabolic responses to high-altitude hypoxia. Despite the exciting progress and numerous publications in top scientific journals (e.g. Circulation, Nature Communications, commentaries in Science, etc.), our understanding of how to leverage RBC metabolism to improve acclimatization to high-altitude hypoxia or training at high altitude is still partial and incomplete.

In this view, I believe that the proposal you are putting together will be relevant to the RBC community at large and at the same time, fits well within the scope of my current research project and warrants the opportunity to provide you with new samples sets to investigate further relevant questions such as "how does RBC metabolism respond to high-altitude hypoxia in subjects who do not acclimatize well?

Overall, you have my unconditional support for your proposal.

Sincerely,

Robert Roach, PhD
Director, Altitude Research Center

Blood Systems Research Institute

717 Yosemite Street / Denver, CO 80230
(303) 363-2400

August 30, 2018

SUBJECT: RBC from testosterone-treated subjects and mice undergoing orchiectomy for your MIRA proposal

Dear Angelo,

I am pleased to write this letter in support of your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". As a Principal Investigator at Blood Systems Research Institute in Denver, I have a long-standing interest in advancing the knowledge of genetic, biologic, and metabolomic modifiers of red blood cell function and hemolysis. I am particularly interested in understanding the mechanisms, by which testosterone modulates erythroid biology leading to enhanced susceptibility of male red blood cells to hemolysis in cold storage and in hemolytic disease. As a member of NHLBI's REDS-III RBC-Omics study, my team has identified a role for male sex and donor age as predictors of RBC predisposition to oxidative hemolysis. These findings have been confirmed and further elucidated in your pilot metabolic study, which clearly demonstrated sex- and age-specific differences in the levels of reduced glutathione, the key hydrophilic antioxidant in RBCs. I must note that my collaborators on RBC-Omics and I are highly impressed by the quality and outstanding performance of your work, which has significantly advanced the impact of our findings. In this view, the project you are proposing aligns well with REDS-III research goals, and to my own R01 project.

Given the challenges in red cell research due to the lack of DNA, an Omic approach that covers in-depth metabolomics and proteomics characterization of red blood cells from testosterone-treated subjects is essential to our collaborative studies. In this view, we will provide you access (under the umbrella of MTAs already being processed by our institutions) to red blood cells from blood donors with polycythemia secondary to testosterone replacement therapy, from male and transgender male patients before and after testosterone exposure, and from orchiectomy or intact male mice. As a Blood Systems Research Institute investigator and successful collaborator in a series of recent studies (both by D'Alessandro et al. and appearing in the upcoming special issue of Transfusion devoted to REDS-III), I am confident about the success of this initiative and wish you the best of luck with your proposal.

Warm regards,

Tamir Kanias

Tamir Kanias, PhD
Associate Investigator
Blood Systems Research Institute
717 Yosemite St, Denver, CO 80230
Direct: (303) 361-3107

Children's Hospital Colorado

www.childrenscolorado.org

September 3, 2018

SUBJECT: RBC from transgender youth starting gender-affirming hormone therapy for your MIRA proposal

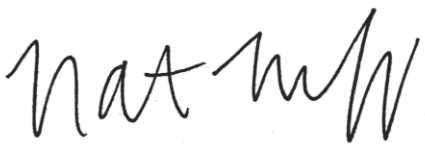
Dear Angelo,

As you know, Natalie and I have been working to understand the role of sex steroids in influencing metabolic changes in youth, in particular girls who have polycystic ovarian syndrome (PCOS) or youth who are receiving estradiol or testosterone as part of gender reassignment. As you know, we have received 2 pilot grants for cross-sectional and 1-month longitudinal analyses, both of which revolve around discovery metabolomics work with you and your lab. During our recent conversations, we agreed on our mutual interest on understanding the longer-term impact on plasma (our group) and red blood cell (your team) metabolism of hormone therapy among transgender adolescents before and after initiation of gender-affirming hormones. In this view, we are writing to renew our unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***," within the framework of which we will be providing samples at baseline, and then 1 and 12 months after initiation of gender-affirming hormones (testosterone or estradiol) in adolescents.

Clearly, these samples provide an interesting piece of the broad puzzle on the role of genetics on red blood cell metabolism, genetics here meant in the sense of gender as a result of chromosome XX/XY-dependent dimorphism.

As a fellow Boettcher Investigator (Melanie), our collaborations are welcomed and fostered by the local scientific community and appreciated by both teams, especially in the light of our interactions over the past year. In confirming our support to your proposal, we wish you and your team best of luck with this initiative.

Warm regards,



Natalie Nokoff, MD
Assistant Professor,
Research Director, TRUE Center for Gender Diversity
University of Colorado Anschutz Medical Campus
Children's Hospital Colorado

Melanie Cree Green, MD, PhD
Assistant Professor,
Director, Multi-disciplinary PCOS clinic
University of Colorado Anschutz Medical Campus
Children's Hospital Colorado

LOS - no. 28

COLUMBIA UNIVERSITY
College of Physicians and Surgeons

Department of Pathology & Cell Biology
Laboratory of Transfusion Biology
630 West 168th Street
P&S 14-434
New York, NY 10032
212.342.4569 Tel
rof3@cumc.columbia.edu email

August 30, 2018

Re: RBC from G6PD deficient donors for your MIRA proposal

Dear Angelo,

As we have been extensively collaborating over the past year and a half, you are well aware that my research activity focuses on the impact of glucose-6-phospahte dehydrogenase (G6PD) deficiency on red blood cell storage quality and transfusion outcomes. Since G6PD is the rate limiting enzyme of one of the main antioxidant pathways in RBCs, the pentose phosphate pathway, we sent you samples in the recent past to document metabolic changes in stored RBCs donated by G6PD-deficient and G6PD-normal donors. However, in those studies we did not address the impact of G6PD deficiency (variants with different levels of residual activity exist) on metabolic phenotypes, not just during storage, but also at steady state and/or following exposure to pro-oxidant compounds or drugs. Therefore, I am writing to provide my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". As part of this project, we will provide you access to red blood cells from G6PD deficient individuals that can be tested for [1,2,3]-^{13}C-glucose tracing at baseline and during routine storage in the blood bank, redox proteomics (especially irreversible oxidation of Cys152 and 156 of GAPDH) and protein methylation as a function of oxidative damage repair for proteins targeted by the storage lesion as a function of storage duration.

We have enjoyed previous successful collaborations, resulting in the publication of a series of papers (e.g. Karafin et al. Curr Opin Hematol 2018; Nemkov et al. Haematologica 2018), with many others in preparation. In light of these considerations I am confident about the success of your initiative and wish you the best of luck with your proposal. You have my full support.

Sincerely,

Richard O. Francis

Richard O. Francis, M.D., Ph.D.
Assistant Professor, Department of Pathology & Cell Biology,
Columbia University P&S

Director, Special Hematology and Coagulation Laboratory,
New York Presbyterian Hospital-Columbia University Medical Center

LOS - no. 29

Central Pennsylvania Clinic

Becoming

A Medical Home for Special Children and Adults

PO Box 5806
4527 East Main Street, Suite E
Belleville, PA 17004
Telephone: 717-935-2065
Fax: 717-935-5560

D Holmes Morton MD

September 11, 2018

Angelo D'Alessandro, PhD
University of Colorado
Anschutz Medical Campus, 80045
Aurora, CO, USA.
Tel.: +1 (303) 724-5798; angelo.dalessandro@ucdenver.edu,
RE: RBC from PK deficient donors for your MIRA proposal

Dear Angelo,
I will provide enthusiastic support to your Early Stage R35 Research Proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

Our clinic's paper **Erythrocyte Pyruvate Kinase Deficiency: Longitudinal Risk and Disease Management. (2011) American Journal of Hematology E-published August 2011** remains an important study about natural history and patients care. Currently my practice at the **Central Pennsylvania Clinic** in Belleville provides care for more than 75 children and adults with this elsewhere rare disorder, which represents one of the largest patient cohorts in the world. I have also recently been involved in a world-wide study of the PK deficiency in collaboration with Boston Children's, Stanford University and many other centers and have enjoyed a 40-year working-friendship with Dr. David G Nathan, who introduced me to this interesting disorder thriugh a case presentation in my first week at Harvard Medical School in 1979.

I frequently sample pyruvate kinase (PK) deficient patients, before and after splenectomy, whose reticulocytes and red blood cells are unstable as a result of impaired ATP-generation and increased oxidative stress, both problems apparently made worse by low production of pyruvate, iron overload, and glutathione depletion. Reticulocytes counts increase after splenectomy and with increasing iron loading to 40-60%. Your red-cell & plasma metabolomic profiles promise to provide new insights into the biochemical basis of the unstable PK-deficient reticulocytes and may help us develop innovative therapeutic options to induce more effective transitions from reticulocytes to mature red cells, limit hemolysis, and there-by increase Hgb & Hct in my patients. Our studies may ultimately allow us to avoid excessive red-cell transfusions, therapeutic splenectomies, and iron overload.

I will be happy to share with you red-cell & plasma samples for your metabolomics analyses and otherwise collaborate with you and your lab staff.
I wish you the best of luck with this interesting and clinically important work.

Sincerely,

LOS - no. 30

D. Holmes Morton MD

University of Colorado
Anschutz Medical Campus
School of Medicine

Department of Pediatrics
Hematology/Oncology/BMT
Children's Hospital Colorado

Mail Stop B115
13123 East 16th Avenue
Aurora, CO 80045
Office: 720-777-6458
Fax: 720-777-7289

September 13, 2018

Angelo D'alessandro, PhD

Department of Biochemistry and Genetics

University of Colorado Denver, Anschutz Medical Campus

12801 East 17th Ave.

Room L18-9118-RC1 South

Aurora, CO 80045

Dear Angelo,

I am happy to provide my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As you know, I am a world leading expert in pediatric hematology with a solid expertise in blood biology and Transfusion Medicine. Over the past two years, I have involved you in a case-study of a poor child suffering from a rare enzymopathy, impacting the activity of the enzyme glucose 1-phosphatase (G6PC3 deficiency). This enzyme is fundamental for proper neutrophil activity, though we do not exclude additional impacts on other blood cell metabolism. In this view, during the next visit to this subject, instead of discarding the erythrocyte component when enriching for neutrophils, we will collect red blood cell samples for you to analyze with your omics tools.

We are happy to include this as part of our broader collaborative projects, recently resulted in a paper just about to be submitted for consideration of publication (McKinneyey et al).

In the light of the considerations above, I believe you hold the expertise necessary to perform such analysis and obtain meaningful results and wish you the best of luck with your proposal.

Warm regards,

Daniel R. Ambruso MD

Daniel R. Ambruso, MD
Professor of Pediatrics and Pathology
University of Colorado Denver, Anshcutz Medical Campus
Pediatric Hematologist
Center for Cancer and Blood Disorders
Children's Hospital Colorado
Aurora, CO 80045

HELLENIC REPUBLIC
National and Kapodistrian
University of Athens
--------- EST. 1837----------

SCHOOL OF SCIENCE
DEPARTMENT OF BIOLOGY
SECTION OF CELL BIOLOGY & BIOPHYSICS

Marianna H. Antonelou, Assist. Professor

Panepistimiopolis,
157 84 Athens,
GREECE

Fax: ++ 30 210-727 4742
manton@biol.uoa.gr

Athens, 30 August 2018

SUBJECT: RBCs from carriers of beta thalassemia mutations for your MIRA proposal

Dear Angelo,

As we have been extensively collaborating over the past four years (see D'Alessandro et al. Transfusion 2015; Tzounakas et al. Free Rad Biol Med 2016; Reisz et al. Frontiers in Medicine 2018, or Nemkov et al. Haematologica 2018), you are well aware that my research activity also focuses on the impact of beta thalassemia on red blood cell physiology and transfusion outcomes. Since thalassemia results from mutations causing -among other- excess oxidative stress to RBCs, we are currently banking biological samples to send to you to investigate metabolic and redox proteomics changes in these subjects. In this view, I am writing to provide my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". Your analyses will be complemented by electron microscopy, physiological evaluation (redox homeostasis, proteostasis, death signaling) and immunoblot-based assays in RBCs and extracellular vesicles that our laboratory has pioneered in the field over the past 15 years.

As a friend and colleague, we cherish our interactions and hope to collaborate for many more years to come.

Warm regards,

Marianna

University of Colorado Health Sciences Center at Fitzsimons

Mental Retardation and Developmental Disabilities Research Center
Department of Pediatrics
School of Medicine
Mail Stop 8313, P.O. Box 6511
Aurora, CO 80045-0511
Fax: 303-724-3838

SUBJECT: RBC from homocystinuria subjects for your MIRA proposal

9th September, 2018

Dear Angelo,

Further to our previous conversations, I am writing to express my enthusiasm and willingness to support your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As you know, I am a world leading expert in the field of homocystinuria and inherited diseases of thiol and folate metabolism. My lab has used a range of omics platforms including metabolomics to elucidate the pathogenic mechanisms by which mutation induced dysregulation of transsulfuration and the folate, methionine and gamma glutamyl cycles results in sequelae as diverse as cognitive impairment, connective tissue disorders and thrombosis. It is worth noting that this approach resulted in a subsequent FDA-funded multi-site clinical trial of the use of taurine as a new treatment for homocystinuria that we completed this year.

This trial successfully demonstrated that treatment with the amino sulfinic acid taurine completely ablated endothelial dysfunction in patients with homocystinuria. In addition to constituting the first advance in treatment for this condition in over 45 years, this finding also serves as a powerful example of how a better understanding of fundamental metabolic changes in disease can result in the rational design of novel therapeutic strategies.

During the course of our collaborations during the past two years, I have come to realize that your approach of examining changes in the metabolome of RBC in both health and disease is a highly original strategy with very strong potential to both improve our understanding of fundamental biochemical processes and how they are perturbed in multiple disease states. My experience to date indicates that this approach can be successfully mined and translated into exciting potential new therapies. Consequently, I am happy to provide both my expertise and share banked samples from subjects suffering from homocystinuria to support your proposal and foster additional collaborations between our teams.

I am confident that your study will significantly impact our understanding of RBC metabolism and further shed light on this currently neglected area of biochemical physiology.

For these reasons I am happy to confirm my unconditional support to your proposal.

Warm regards,

Ken Maclean PhD
Professor of Pediatrics
Ehst-Hummel-Kaufmann Family Endowed Chair
in Inherited Metabolic Disease

SCHOOL OF MEDICINE
Division of Hematology
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Craig T. Jordan, PhD
Nancy Carroll Allen Professor
Chief, Division of Hematology
University of Colorado
Anschutz Medical Campus
12700 East 19th Ave, Room 9122
Research Complex 2, Campus Box B170
Aurora, CO 80045
craig.jordan@ucdenver.edu
www.medschool.ucdenver.edu/hematology

August 30, 2018

Angelo D'Alessandro
Assistant Professor
Dept of Biochemistry and Molecular Biology
University of Colorado Medical Center

Re: Red blood cells from AML patients undergoing chemotherapy/radiotherapy for your MIRA proposal

Dear Angelo,

I am glad to confirm my support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". As you know, my expertise is in hematological malignancies and, in particular, acute myeloid leukemia and cancer stem cells. We have extensively collaborated on studies focusing on these topics over the past three years, i.e. since I moved to Colorado where I lead the Division of Hematology in the Department of Medicine. In light of our successful collaborative work (Pei et al. JBC 2016; Stevens et al. Nature Communications 2018; Pollyea et al. Nature Medicine *in press*) I have been happy to support your secondary appointment to the Hematology Division.

Within the framework of our studies, we are routinely collecting samples from AML patients undergoing chemotherapy and/or radiotherapy. While we usually discard erythrocytes during our primary leukemia cell collection protocols, I would be happy to bank red blood cells from these subjects for your omics investigations. Indeed, your preliminary high-throughput screening data suggest that some of the compounds involved in mainstay treatments for AML and other cancers can impact RBC metabolism in ways that may be telling of more systemic untoward effects. Understanding the molecular mechanisms behind these off-target effects may help relieving unnecessary health burdens in these categories of patients associated with current standard of practice in anti-cancer therapies or inform the formulation/testing of next-generation drugs with similar efficacy but reduced off-target effects.

Sincerely,

Craig T. Jordan, Ph.D.
Chief of Division of Hematology
Vice Chair of Basic Research, Department of Medicine
University of Colorado Anschutz Medical Campus

Denver Zoological Foundation
2300 Steele Street Denver, CO 80205 | P: 720.337.1400 | F: 720.337.1401 | denverzoo.org

August 31, 2018

Angelo D'Alessandro, PhD
Dept. Biochemistry and Molecular Genetics
University of Colorado Denver
Anschutz Medical Campus
12801 East 17th Ave
room L18-9118 - RC1 South
Aurora, CO, USA 80045

SUBJECT: Banked blood from Denver Zoo animals for your MIRA proposal

Dear Angelo,
The collaboration we have pursued of the past 12 months to investigate metabolic changes in blood of equids undergoing anesthesia, has been very rewarding. The Denver Zoo routinely banks serum from animals that undergo anesthesia for routine procedures, thus we have a large archive with a variety of species. There are many new and exciting potential projects that can be explored with these biomaterials, additional samples can be obtained prospectively. Samples can be made available to you and your project following the appropriate requests and documentation, as we have done for ongoing projects. These samples, no doubt would be of potential interest to your proposal, and at the same time would contribute to fulfill our research goals while contributing the Denver Zoo's mission to improve animal health and welfare.
In this view, I am happy to provide my unconditional support to your Early Stage R35 proposal entitled "MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage".

Best of luck with the submission!
Kindest regards

Anneke Moresco, DVM, PhD
Research Manager

Denver Zoological Foundation
2300 Steele Street | Denver, CO 80205

COLUMBIA UNIVERSITY
College of Physicians and Surgeons

Steven Spitalnik, M.D.
Executive Vice Chairman, Professor of Pathology & Cell Biology

Department of Pathology & Cell Biology
Division of Laboratory Medicine
Laboratory of Transfusion Biology
630 West 168th Street
P&S 14-434
New York, NY 10032
212.305.2204 Tel
212.305.3693 Fax
ss2479@columbia.edu

August 30, 2018

Angelo D'Alessandro, Ph.D.
Department of Biochemistry and Molecular Genetics
University of Colorado: Anschutz Medical Campus
Aurora, 80045 CO

Dear Angelo,

It is a distinct pleasure for me to provide this letter in support of your Early Stage R35 proposal entitled "**MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage.**"

I believe that your work is extremely innovative, well performed, and highly insightful. In my opinion, you are the best person in the world pursuing the metabolism of red blood cells, using an array of cutting-edge "omics" methods. Not only do you have the relevant technical and technological expertise, but you also are applying these to fundamental biological problems with an excellent feel for the underlying biology. In addition, the problems you are studying (e.g., sickle cell disease and trauma) are medically important and your findings are highly likely to have significant medical relevance.

Over the last three years, we have had a chance to collaborate extensively on many projects and I would be happy to contribute, even if only by providing my expertise on red blood cell storage and red cell biology, to the success of your study. I am happy to help in any way.

In summary, I believe that you are the ideal candidate to pursue these important issues, and that you have the appropriate resources in an ideal academic location for these studies. Please do not hesitate to reach out to me for any help you may need. I wish you the best of luck with this new proposal!

Yours truly,

Steven Spitalnik

Digitally signed by Steven Spitalnik
DN: cn=Steven Spitalnik, o, ou, email=ss2479@cumc.columbia.edu, c=US
Date: 2018.08.30 07:11:23 -04'00'

Steven L. Spitalnik, M.D.
Professor and Executive Vice Chairman

LOS - no. 36

SUBJECT: Stored blood from Non-human Primates for your MIRA proposal

Dear Angelo,

I am glad to provide my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

To the best of my knowledge, to date genetics factors such as interspecies genetic variability with respect to red cell metabolism and proteome have been scarcely or at all investigated.

During the past few months we have initiated a collaboration that prompted us to start collecting and storing blood from non-human primates, including rhesus, cynomolgus and baboons. We will be happy to share with your team such samples for omics characterization and hope that such analyses will foster future mechanistic or pre-clinical work relevant to the field of transfusion medicine. To the best of my knowledge, despite the incredible potential relevance of such analyses – if only for comparative biology purposes – nobody has ever performed a detailed metabolomics and proteomics characterization of stored RBCs from these animals. Determining the differences across non-human primate species is a key factor in our laboratory's efforts at FDA that are focused on systematic approaches to optimize translational animal model selection and development in transfusion research. We consider your work in RBC omics as critical toward advancing these efforts.

In the light of the considerations above, I renew my interest and support to your proposal and wish you and your team the best of luck.

Kindest regards,

Paul W. Buehler, Pharm.D., Ph.D.
Senior Scientist, Pharmacology/Toxicology
Laboratory of Biochemistry and Vascular Biology
Division of Blood Components and Devices
10903 New Hampshire Avenue
Silver Spring, MD 20993

DukeHealth

Department of Anesthesiology

Ian J Welsby, BSc, MBBS, FRCA
Associate Professor of Anesthesiology

August 30, 2018

Angelo D'Alessandro, PhD
Assistant Professor
Boettcher Investigator
Linda Crnic Investigator
Affiliate Investigator - Blood Systems Research Institute
Associate Editor - Blood Transfusion
Director, Metabolomics Core - School of Medicine Department of Biochemistry and Molecular Genetics University of Colorado Denver - Anschutz Medical Campus

SUBJECT: RBC from sickle cell disease subjects before and after transfusion with rejuvenated RBCs for your MIRA proposal

Dear Angelo,

As we have been extensively collaborating over the past two years (see Gehrke et al. and Culp-Hill et al. Transfusion 2018), I can confirm the availability of red blood cells from sickle cell patients receiving transfusion therapy with standard red cell units or bedside-rejuvenated units. In this view, I am writing to provide my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

I am confident about the success of your ambitious initiative and wish you the best of luck with your proposal.

Warm regards,

Ian Welsby, BSc, MBBS, FRCA
Associate Professor of Anesthesiology
Department of Anesthesiology and Critical Care
2301 Erwin Road, 5691-H
Durham, NC 27710
Office Ph.: 919-684-0862
Fax: 919-287-2720
Email: ian.welsby@dm.duke.edu

LOS - no. 38

Blood Systems Research Institute

717 Yosemite Street / Denver, CO 80230
(303) 363-2400

September 4, 2018

Angelo D'Alessandro, PhD
Department of Biochemistry and Molecular Genetics
University of Colorado Denver – Anschutz Medical Campus
Aurora, CO

SUBJECT: Support with blood units for research purposes and in-depth omics analyses for your MIRA proposal

Dear Angelo,

I am glad to confirm my support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

Over the past few years, we have been collaborating extensively on investigations pertaining to red blood cell storage under standard or alternative conditions, including but not limited to alkaline additives (D'Alessandro et al. Transfusion 2016), anaerobic storage (Dumont et al. Transfusion 2016) or oxidative modifications to stored red blood cells involving methylation of deamidated asparagines or aspartate residues of key structural and functional proteins in RBCs (Reisz et al. Transfusion 2018). Since I joined Blood Systems Research Institute as Director in 2016, we have welcomed you as an Affiliated Investigator. Within the framework of this affiliation, we have also finalized a material transfer agreement to provide you with leukocyte-reduced, red blood cell concentrates for research purposes, like the one you used for your high-throughput screening of 1366 FDA-approved drugs (Burgoyne et al. *in preparation*). Given our longstanding collaboration on a number of projects and our physical proximity in Denver, I am happy to confirm my support to your research initiative, and wish you the best of luck with your project.

Sincerely,

Larry J. Dumont, MBA, PhD
Director, Senior Investigator

LOS - no. 39

BLOODCENTER
of WISCONSIN™

Blood Research Institute

Doing more good than you know

SUBJECT: Support with blood units for research purposes and in-depth omics analyses for your MIRA proposal

Dear Angelo,

I happy to provide my unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

We have started our collaboration a few months ago, when we both joined a collaborative group on RBC redox biology also involving Drs. Spitalnik, Zimring, Hod, Francis and Thomas, who also provided letters of support to your proposal.

My contribution relates to my expertise in sickle cell biology and transfusion medicine. Under approved protocols and funded studies I am testing the impact of the age of stored blood on transfusion outcomes in sickle cell recipients. Though initially not planned for this study, I will be happy to provide packed red blood cell aliquots from the unit and recipients (before and after treatment) to test the impact of stored RBC metabolism on recipient systemic hypoxemia and blood metabolism. Indeed, our institutions are currently working on the finalization of material transfer agreements to ease the finalization of such study which I hope will be finalized, provided funding will be secured through this MIRA initiative you are putting together.

Overall, our successful collaborations so far (*Karafin et al. Curr Opin Haematol 2018*) hold the promise to expand into programmatic initiatives over the next few years and I hope that this proposal you are assembling will represent one of the early steps towards a long a prolific collaborative experience between our labs.

Warm regards,

Matthew S. Karafin, MD MS
Medical Director, Medical Sciences Institute, BloodCenter of Wisconsin/Versiti
Associate Investigator, Blood Research Institute
Associate Professor, Department of Pathology and CTSI, Medical College of Wisconsin
Office Phone: 414-937-6809
Email: matthew.karafin@bcw.edu

LOS - no. 40

University of Colorado Denver, School of Medicine

Biochemistry & Molecular Genetics
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August 30th, 2018

Angelo D'Alessandro
Department of Biochemistry and Molecular Genetics
University of Colorado Denver – Anschutz Medical Campus
12801 East 17th Ave – L18-9118
Aurora, CO, USA - 80045

Dear Angelo,
I am excited to provide my unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As an expert in protein dynamics and structural biochemistry, I extremely enjoyed our collaboration aimed at the acquisition of some of the preliminary data you included in your submission, a collaboration that also involved Drs. Zimring and Hansen, co-investigators in your proposal. Our recent successful collaborative efforts have resulted in a series of publications (Reisz et al. Transfusion 2018; Paukovic et al. JMB 2018*;* Kendrick et al. Oncotarget 2017; Cavalli et al. PNAS 2017) and fostered continuous interactions between our two laboratories, both located on the 9th floor of the Research Complex 1 south at the University of Colorado Denver – Anschutz Medical Campus.

The model you are proposing focuses around the N-terminal cytosolic domain of band 3 and its oxygen-dependent competitive interaction with deoxyhemoglobin and glycolytic enzymes. Of note, this very model was **part of my PhD thesis**, as we have discussed extensively in the past few years. In this view, I was happy to model the impact of methylation of deamidated asparagines and aspartate residues of GAPDH in our recent joint publication (Reisz et al. Transfusion 2018). I will be happy to provide similar expertise for the analyses of the data you will generate within the framework of this proposal.

In the light of these considerations, I am happy to offer my enthusiastic support to your initiative and am looking forward to further collaborations with your group.

Sincerely,

Elan Eisenmesser, Ph.D.
Associate Professor
School of Medicine
Dept. Biochemistry/Mol.Genetics
University of Colorado Denver
Aurora, CO 80045

LOS - no. 41

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PEDRO CABRALES Ph.D.
PROFESSOR

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pcabrales@ucsd.edu

September 4, 2018

RE: RBC from subjects suffering from beta thalassemia for your MIRA proposal

Dear Angelo,

During the last two years we have been successfully collaborating on investigations focused on plasma metabolic reprogramming following trauma/hemorrhagic shock and resuscitation, leveraging well-established rat models (see Williams et al. under review). Trauma and hemorrhagic shock – and resuscitation – represent a clinically relevant model of metabolic responses to hypoxia. Understanding molecular mechanisms underlying RBC metabolism and metabolism-dependent oxygen biding and off-loading capacity could inform the next generation of therapies in the field of emergency medicine. In this view, I am happy to provide my unconditional support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***". The body of observations that will be generated within the framework of this study will be instrumental to the finalization and submission of follow up joint research projects by our groups.

Best of luck with the submission!

Sincerely yours,

Pedro Cabrales

Pedro Cabrales

LOS - no. 42

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August 31, 2018

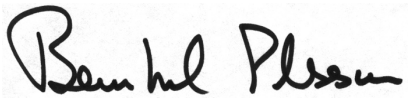
SUBJECT: Support Systems Biology analyses for omics data from your MIRA proposal

Dear Angelo,

I happy to provide my enthusiastic support to your Early Stage R35 proposal entitled "***MIRAGES – Metabolic Investigation of Red blood cells as a function of Aging, Genetics, Environment and Storage***".

As you know, we share an unconditional love for red blood cells. Despite their apparent simplicity, owing to the lack of nuclei and organelles, red cells and red cell metabolism are still incompletely understood. As a world leader in the field of Systems Biology, I spent the best part of the past forty years engineering computational models of red cell metabolism in silico, models that have been optimized over the last decade owing to significant advances in omics technologies. Still, recent joint papers from our teams showing active remnants of cytosolic carboxylate metabolism in mature erythrocytes (D'Alessandro et al. Transfusion 2017; Paglia et al. Blood 2016) clearly show that our models of red blood cell metabolism are incomplete. Your proposal and the body of knowledge that will be made available to scientific community as a result of your – very likely – success will be transformative as it will foster a generational leap in our understanding of red blood cell metabolism in health and disease, as a function of aging, genetics (including gendere and enzymopathies) and environment. In the light of the above, I am excited to provide my strong support to your initiative and wish you the best of luck with your proposal.

Warm regards,



Bernhard Palsson

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September 5, 2018

Angelo D'Alessandro
Department of Biochemistry and Molecular Genetics
University of Colorado Denver – Anschutz Medical Campus
12801 East 17th Ave – L18-9118
Aurora, CO, USA - 80045

Dear Angelo,

It is a pleasure to write in support to your NIH R01 grant application entitled, "**PIMT1 in Red Blood Cell Senescence and Aging**".

As an **expert in the field of statistics** and the application of biostatistics to big data analysis and, in particular, omics data analysis (Russell et al. BMC Res Notes 2018; Keene et al. Am J Resp Crit Care Med 2017; Miller et al. Plos One 2017; Reinhold et al. Plos One 2017), **I will be happy to advise and collaborate on data analysis and statistical methods** to identify the role of protein methylation at aspartate and deamidated asparagine residues in red blood cell senescence, aging and in response to oxidative stress.

In the past two years, our labs have initiated a series of collaborations to facilitate the application of proper computational and biostatistical methods to the data generated by your lab and core. For example, we are both serving on the thesis committee of Ms. Rani Powers, a Computational Bioscience PhD student. We also both contributed a chapter on computational methods in metabolomics for your upcoming Springer Nature book on *High-throughput metabolomics*. Apart from the considerations above, we have been extensively collaborating on the finalization of joint projects and I hope that this one will be part of our broader successful collaborative initiatives.

In confirming my support to this initiative, I wish you good luck with your submission and I hope your interesting proposal will be positively received by the review panel.

Sincerely,

LOS - no. 44

Katerina Kechris, PhD
Professor
Department of Biostatistics and Informatics, Colorado School of Public Health
Division of Biomedical Informatics and Personalized Medicine, School of Medicine
University of Colorado Anschutz Medical Campus