Fiscal Year:	FY 2024	**Task Last Updated:**	FY 09/20/2023
PI Name:	Schwertz, Hansjorg M.D., Ph.D.		
Project Title:	Megakaryocytes Orbiting in Outer Space and Near Earth: The MOON Study		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology		
Space Biology Cross-Element Discipline:	(1) Immunology		
Space Biology Special Category:	None		
PI Email:	hansjorg.schwertz@u2m2.utah.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	801-946-0924
Organization Name:	University of Utah, Salt Lake City		
PI Address 1:	Department of Family and Preventive Medicine		
PI Address 2:	391 S Chipeta Way, Suite C		
PI Web Page:			
City:	Salt Lake City	**State:**	UT
Zip Code:	84108-1263	**Congressional District:**	2
Comments:			
Project Type:	FLIGHT	**Solicitation / Funding Source:**	2020 Space Biology NNH20ZDA001N-SB E.12. Flight/Ground Research
Start Date:	12/01/2021	**End Date:**	11/30/2024
No. of Post Docs:	1	**No. of PhD Degrees:**	
No. of PhD Candidates:		**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA ARC
Contact Monitor:	Griko, Yuri	**Contact Phone:**	650-604-0519
Contact Email:	Yuri.V.Griko@nasa.gov		
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	none		
COI Name (Institution):	Rondina, Matthew M.D. (University of Utah, Salt Lake City) Rowley, Jesse Ph.D. (University of Utah, Salt Lake City)		
Grant/Contract No.:	80NSSC22K0255		
Performance Goal No.:			
Performance Goal Text:			
Task Description:	Megakaryocytes (MKs) and their progeny, platelets (PLTs), are dynamic effector cells with recently discovered novel functions, which bridge the inflammatory, immune, and hemostatic continuum. Changes in bone marrow MKs, resulting in low PLT numbers, (thrombocytopenia, which occurs in astronauts during spaceflight) are associated with dysregulated host inflammatory/immune responses. MKs and PLTs sense and respond to environmental cues. MKs also differentially invest developing PLTs with RNAs and proteins that alter functions of newly-released cells, influencing cellular and host responses. Surprisingly, there is a paucity of data regarding in-flight, long-term dynamics of MK development and function, as well as PLT function and production. Given previously identified and published space-travel associated risks on dysregulated inflammation, immune responses, thrombus formation, and hemostatic systems, filling this critical knowledge gap is important for the health of spaceflight crewmembers during and after missions. Moreover, as other blood cells (e.g., red blood cells, leukocytes, etc.) may be altered by microgravity, data generated are likely to contribute to our understanding of how spaceflight affects other hematopoietic processes. This proposal is based on our robust preliminary data demonstrating that conditions mimicking microgravity (rotating wall vessel culture, RWVC) markedly alter human MK morphology and gene expression. We hypothesize that microgravity will re-program MKs and newly-released PLTs, resulting in critical changes in their transcriptome, proteome, and alterations in PLT number and function. We will determine how microgravity and space radiation conditions on board the International Space Station (ISS) alter human MK and PLT maturation/production, gene expression (DNA, RNA, and protein), and cellular function. We will study in vitro human hematopoietic progenitor cell (HPC)-derived MKs in Earth-based experiments under standard or microgravity conditions. In parallel, human MKs will be studied on the ISS. Integrated, cutting-edge OMICS toolsets (e.g., RNA-sequencing and ribosomal footprinting [Ribo-seq]), comprehensive morphologic		

	studies, and cell production kinetic studies will be used. They will provide unprecedented insight into adaptation processes needed for MK and PLT function under conditions experienced by humans performing spaceflights. These studies will directly address crew health concerns that currently limit human space exploration and will assist in developing targeted countermeasures. This proposal concurs with the major National Research Council (NRC) Decadal Survey Recommendations for cellular and molecular biology studies using state- of-the-art tools coupled with systems biology, and for studies evaluating the physiological interplay of cardiopulmonary and immune functions during application of spaceflight. Furthermore, we will address goals of the NASA Space Biology Science Plan 2016-2025, including: (1) determine the effects of the space environment on DNA function, (2) develop a systems biology-based understanding of the cellular and molecular changes to explain how gravitational changes in spaceflight effects organisms and causes phenotypic changes, and (3) identify how spaceflight affects the ability of cells to generate and maintain their complex internal cyto-architecture, processes critical for MKs and PLTs.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Results from the proposal o have immediate mechanistic implications for the development of countermeasures for unexpected clot formation, and dysregulated systemic inflammation in ISS crew members. o may be broadly applicable to other cells, such as leukocytes, monocytes, and red blood cells, that are altered in spaceflight settings. o allowing us to translate ex vivo findings into a relevant in vivo situation via comparing longitudinal RNA expression studies in human PLTs and durable reprogramming of PLTs in astronauts (pre- vs. post-flight) mitigating cell-autonomous changes only observed in culture systems. o Identified target genes across ground and flight, cell culture and astronaut projects will be verified for functional and systems biology relevance by using cell culture CRISPR-Cas9 approaches. • Exploration Benefits o Advance knowledge will help the development of countermeasures for unexpected clot formation, and dysregulated systemic inflammation in spaceflight crew members. o Obtained data will be translatable to address known “ground-based” dysfunctions of human MKs and PLTs, may lead to the discovery of new drug targets and therapeutic interventions, and initiate new research avenues. • Earth Benefits o Since results generated may be broadly translatable to other cell types, such as leukocytes, monocytes, and red blood cells, the analysis data sets will be applicable to numerous inflammatory and pro-thrombotic conditions observed in the medical arena. Furthermore, the direct comparison of ground-simulations and ISS-flight experiments, and the integration of ex vivo findings will help in validating commonly used spaceflight simulation cell culture conditions.
Task Progress:	The progress and accomplishments will be divided into administrative/ organizational, and experimental tasks. • Administrative/Organizational accomplishments: o The flight hardware solicitation was posted, flight hardware was selected and kick-off meeting happened took place on 07/13/2023. o The PI and Project Scientist prepared and finished the Science Requirement Document. o After the PI team was granted IRB exempt status, travel and experimental procedures to and at NSRL were administratively organized, and NSRL irradiation was performed. A rerun was granted by ASBCB, and is scheduled for Fall 2024. o NASA IRB approved our human subject research application needed for Specific Aim 3. In addition, the University of Utah IRB accepted the NASA IRB as single IRB of record, while NASA IRB accepted the University of Utah as a study site for aforementioned IRB. o NASA IRB was extended for an additional year. o Mini-ED and crew briefing materials were submitted and approved. o Two flight crews were briefed. o The PI, the Project Scientists, and the implementation partner are preparing the pre-flight testing review package. o The science verification test plan has been worked on, is continuously updated and anticipated to be finalized in within this funding period. o Flight Mission Patch for MeF1 payload was designed, and is in production. • Experimental accomplishments: Since the flight hardware selection process was delayed for various reasons, several hardware-dependent science verification experiments could not be performed during the last 10 month. However, since a flight hardware was assigned, such SVT related experiments were initialized. These include, but are not limited to, biocompatibility and toxicity tests of the proposed flight hardware using CD34+ hematopoietic stem cells. In addition, cell seed density and cell survival rate verification tests will be implemented in the next months. Furthermore, experimental testing of microscopy capabilities, including clarity and bubble forming tests will be performed. Finally, the impact of media changes and the implemented media change techniques on cell culture performance will be tested using the flight hardware culture device. The following experiments were accomplished and generated valuable insights and data: • Experiments related to Specific Aim 1: o Experiments using n=6 mobilized adult CD34+ hematopoietic stem cell cultures were performed comparing standard suspension versus rotating wall vessel culture. We were able to demonstrate an appropriate increase in cell numbers, and stable cell viability. Flowcytometric data were analyzed, as well as cell morphology and ability to form proplatelet extension by means of live-microscopy and confocal immuno-cytochemistry. Cells were harvested and submitted to the University of Utah sequencing core facility for further sequencing analysis. o Data gathered from the total RNA sequencing runs are analyzed and verification experiments targeting transcripts of interest will be initiated in the next months. o The experiment simulating galactic cosmic rays (simGCR) was performed using the NSRL facilities at Brookhaven National Laboratory. A rerun of the simGCR experiment (due to anomaly experienced during the initial run) was applied for and will take place in Fall of 2024. • Experiments related to Specific Aim 2: Several standard ground cell culture conditions need to be adjusted due to in-flight conditions on-board the ISS. o During preliminary experiments, we detected that preserving CD34+ hematopoietic stem cells with RNAlater would result in a dissociation of ribosomes from the respective target RNA sequence, and therefore, making ribosomal protected fragment sequencing impossible. Extensive testing demonstrated that different strategies resulted in preservation of cells providing integrity of ribosome-RNA complexes (using polysome analysis), and furthermore, resulting in RNA integrity numbers (RIN) well within the mission success criteria defined in the SRD. Furthermore, cell cultures used for experiments described in Specific Aim 1 were preserved using such method, and post -sequencing quality control variables demonstrated total read counts and read coverage being within the mission success criteria for excellent scientific return. o On-orbit cell culture experiments will include morphologic studies conducted using live cell imaging approaches. Since the tubulin cytoskeleton is a major contributor to regulated proplatelet formation, we selected different tubulin probes suitable for live microscopy for further testing. All tested live cell imaging dyes can be used without additional washing steps per manufacturer’s protocol, which will significantly reduce astronaut hands-on time. The experimental results demonstrated sufficient staining characteristics of the selected live imaging probes when incubating the cells for different time points. In addition, using a 20x objective, we were able to readily identify detailed tubulin cytoskeletal structures within proplatelet extensions. This test-series was completed, and an appropriate on-orbit live-staining protocol can be implemented. o On-orbit cell culture experiments will require extended fixation of cells using paraformaldehyde (PFA) or alternative approaches. A series of experiments demonstrated that CD34-derived megakaryocytes can be stored for 5 weeks in the appropriate PFA solution at 4?C while preserving morphology and antigenicity. o After identification of the flight hardware, the PI team conducted cell growth / differentiation experiments using the flight hardware materials (as provided by IP). CD34+ hematopoietic stem cells were in direct contact to such materials during the entire culture period, and results were compared to standard lab cell culture conditions. CD34+ hematopoietic stem cells demonstrated tolerance towards being exposed to materials which will be used for the experimental setting on board the ISS. Cells expanded as expected and demonstrated viability percentages comparable to regular culture conditions, even when media changes were performed using syringe and cannula. Furthermore, proplatelet formation was preserved, which was also verified by an unaltered performance of the tubulin live stain. • Experiments related to Specific Aim 3: The amount of whole blood which can be obtained from the human subjects in Specific Aim 3 is limited, we performed preliminary experiments mimicking the experimental set-up planned to be implemented for Specific Aim 3. o Platelets were isolated from healthy volunteers using whole blood volumes proposed in Specific Aim 3. Harvested numbers of platelets were suitable to perform proposed experiments.
Bibliography Type:	Description: (Last Updated: 10/04/2023)

Articles in Other Journals or Periodicals	Seylani A, Galsinh AS, Tasoula A, RI A, Camera A, Calleja-Agius J, Borg J, Goel C, Kim J, Clark KB, Das S, Arif S, Boerrigter M, Coffey C, Szewczyk N, Mason CE, Karouia F, Schwertz H, Beheshti A, Tulodziecki D. "Space flight for all: One giant leap for ethics?" Nature Communications, submitted September 2023. , Sep-2023
Articles in Peer-reviewed Journals	Campbell RA, Manne BK, Banerjee M, Middleton EA, Ajanel A, Schwertz H, Denorme F, Stubben C, Montenont E, Saperstein S, Page L, Tolley ND, Lim DL, Brown SM, Grissom CK, Sborov DW, Krishnan A, Rondina MT. "IFITM3 regulates fibrinogen endocytosis and platelet reactivity in nonviral sepsis. " J Clin Invest. 2022 Dec 1;132(23):e153014. https://doi.org/10.1172/JCI153014 ; PMID: 36194487; PMCID: PMC9711880 , Dec-2022
Articles in Peer-reviewed Journals	Freitag M, Schwertz H. "A new role of NAP1L1 in megakaryocytes and human platelets." Int J Mol Sci. 2022 Nov 24;23(23):14694. https://doi.org/10.3390/ijms232314694 ; PMID: 36499021; PMCID: PMC9737020 , Nov-2022
Significant Media Coverage	Schwertz H. "Cooperative Centers of Excellence in Hematology funded by National Institute of Diabetes and Digestive and Kidney Diseases – U24." Social media announcement, June 1, 2022. https://twitter.com/CCEH_U24/status/1532042770226876416?cxt=HHwWgMCgwYmy9MIqAAAA , Jun-2022
Significant Media Coverage	Schwertz H. "Cooperative Centers of Excellence in Hematology funded by National Institute of Diabetes and Digestive and Kidney Diseases – U24." Web announcement, Yale Cooperative Center of Excellence in Hematology general news, June 6, 2022. https://cceh.io/articles/members-of-our-niddk-sponsored-centers-of-excellence-in-hematology-are-studying-hematopoietic-development-in-space , Jun-2022
Significant Media Coverage	Dollemore D. (Schwertz H and Rondina M interview). U of U Health experiment prepared for launch to International Space Station. "Drs. Schwertz and Rondina interviewed for University of Utah faculty/staff profile." University of Utah website, March 29, 2022. https://attheu.utah.edu/facultystaff/u-of-u-health-experiment-prepared-for-launch-to-international-space-station/ , Mar-2022
Significant Media Coverage	Schwertz H. "American Board of Preventive Medicine (ABPM) announcement." Social media announcement on LinkedIn. https://www.linkedin.com/feed/update/urn:li:activity:7013837484853796864/ , Jan-2023