Fiscal Year:	FY 2021	**Task Last Updated:**	FY 07/22/2021
PI Name:	McDonald, Karen Ph.D.		
Project Title:	A Plant-Based Platform for "Just in Time" Medications		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	TRISH--TRISH		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	95616-5270	**Congressional District:**	3
Comments:			
Project Type:	GROUND	**Solicitation / Funding Source:**	2020 TRISH BRASH1901: Translational Research Institute for Space Health (TRISH) Biomedical Research Advances for Space Health
Start Date:	04/01/2020	**End Date:**	03/31/2022
No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	2	**No. of Master' Degrees:**	0
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	TRISH
Contact Monitor:		**Contact Phone:**	
Contact Email:			
Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Lane, Nancy M.D. (University of California, Davis) Sudarshana, Mysore Ph.D. (United States Department of Agriculture) Nandi, Somen Ph.D. (University of California, Davis) Paul, Debashis Ph.D. (University of California, Davis)		
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Task Description:

The objectives of this proposal are to design, develop, and evaluate a plant-based bioproduction platform for rapid production of three NASA-relevant human therapeutic biologics, recombinant parathyroid hormone residues 1-34 (PTH) for osteoporosis, granulocyte colony stimulating factor (G-CSF) for acute radiation treatment, and Trypsin (TRP) in treatment of burns, skin abrasion, or skin laceration in Lactuca sativa (lettuce). Plants offer many advantages as a biological host for production of medicines since they are safe, will already be available during deep space missions, require minimal external resources, can utilize in situ resources (light and carbon dioxide) for growth, and do not propagate mammalian viruses. Plants can be used for production of the therapeutics as well as purification reagents, and even offer a potential for oral delivery of the therapeutics in the future. To achieve production, purification, and delivery of just-in-time biologics made in lettuce within 24 hours we will develop novel plant viral expression systems, production and purification protocols, and viral immunosorbent nanoparticles.
Our Specific Aims are:

Specific Aim #1: Development and evaluation of transgene constructs and plant viral expression vectors for transient production of three therapeutic biologics, in Lactuca sativa (lettuce), for NASA-medically relevant conditions.

Specific Aim #2: Development and testing of methods for delivery and utilization of plant viral expression cassettes in lettuce plants/tissues and evaluation of the production kinetics and levels (mg/kg fresh weight) of these biologics.

Specific Aim #3: Development and testing of methods for rapid purification of the three biologics using plant-made plant viral immunosorbent nanoparticles (VINs) for affinity separation and evaluation of additional purification strategies to meet Topic 6 ("Just in time" medications") constraints.

Specific Aim #4: Characterization of the purity, efficacy, and potency of the purified plant-made biologics. This proposal is innovative due to the development of novel recombinant protein expression technologies in plants as well as purification strategies that are fast and simple. The proposed approach minimizes mass, volume, power, and cold chain requirements.

Rationale for HRP Directed Research:

Research Impact/Earth Benefits:

This research project addresses the following NASA risks and gaps:
Risks Addressed: Primary: Risk of Adverse Health Outcomes & Decrements in Performance due to Inflight Medical Conditions. Secondary: Risk of Ineffective or Toxic Medications During Long-Duration Exploration Spaceflight.

Gaps Addressed:

Medical-701: We need to increase inflight medical capabilities and identify new capabilities that (a) maximize benefit and/or (b) reduce costs on human system/mission/vehicle resources.

Medical-601: We need an quantitative method for assessing whether the value of new exploration medical capability research is of sufficient benefit vs. cost to perform.

Medical-201: We need to characterize the resource costs associated with each relevant exploration medical capability.

Medical-301: We need to prioritize the relevant exploration medical capabilities in order to perform medical system trade space analysis.

Medical-401: We need to characterize the predicted resource or risk constraints associated with exploration missions in order to determine which capabilities (and associated resources) should be included in an exploration medical system.

Med02: We do not have the capability to provide a safe and effective pharmacy for exploration missions.

ExMC 4.22: Limited capability to diagnose and treat radiation sickness (Closed)

The paper by McNulty et al. (2021) describes how the technologies that we are developing address these NASA risks and gaps. In addition, the technologies that we are developing have impact here on Earth in terms of being able to rapidly produce countermeasures to respond to infectious disease outbreaks. An example is the analysis presented in the paper by McDonald and Holtz (2020) in which we show the potential for using field-grown lettuce to produce SARS-CoV-2 antigens for COVID-19 diagnostic tests. The paper by Bernardi et al. (2020) describes a novel COVID-19 therapeutic, ACE2-Fc, which we have made in plants. [Ed. Note: Referenced publications are listed in the Bibliography section below]

1) Original Project Aims/Objectives
The objectives of this proposal are to design, develop, and evaluate a plant-based bioproduction platform for rapid production of three NASA-relevant human therapeutic biologics, recombinant parathyroid hormone residues 1-34 (PTH) for osteoporosis, granulocyte colony stimulating factor (G-CSF) for acute radiation treatment, and Trypsin (TRP) in treatment of burns, skin abrasion, or skin laceration in Lactuca sativa (lettuce). Plants offer many advantages as a biological host for production of medicines since they are safe, will already be available during deep space missions, require minimal external resources, can utilize in situ resources (light and carbon dioxide) for growth, and don't propagate mammalian viruses. Plants can be used for production of the therapeutics as well as purification reagents, and even offer a potential for oral delivery of the therapeutics in the future. To achieve production, purification, and delivery of just-in-time biologics made in lettuce within 24 hours we will develop novel plant viral expression systems, production and purification protocols, and viral immunosorbent nanoparticles.

Our Specific Aims are:

Specific Aim #1: Development and evaluation of transgene constructs and plant viral expression vectors for transient production of three therapeutic biologics, in Lactuca sativa (lettuce), for NASA-medically relevant conditions.

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Specific Aim #3: Development and testing of methods for rapid purification of the three biologics using plant-made plant viral immunosorbent nanoparticles (VINs) for affinity separation and evaluation of additional purification strategies to

Task Progress:	meet Topic 6 constraints. Specific Aim #4: Characterization of the purity, efficacy, and potency of the purified plant-made biologics. This proposal is innovative due to the development of novel recombinant protein expression technologies in plants as well as purification strategies that are fast and simple. The proposed approach minimizes mass, volume, power, and cold chain requirements. 2) Key Findings We have made progress in the construction of a Lettuce mosaic virus (LMV)-based expression system for transient expression of target biologics in plants. For the LMV system we are starting with a California isolate which we have fully sequenced. For the Bean yellow dwarf virus (BeYDV)-based expression system, we have designed two BeYDV fragments to enable building a disarmed virus vector for expression, codon optimized these fragments for expression in Lactuca sativa, and had them commercially synthesized. We have developed preliminary protocols for gene delivery in lettuce using the BioRad Helios gene gun. We are initially testing delivery of a plasmid containing a reporter protein, green fluorescent protein GFP, in different types of lettuce and N. benthamiana as a control. We have successfully loaded the plasmid DNA onto gold microparticles, prepared cartridges (bullets), and bombarded lettuce with these constructs. We have developed a statistical framework that utilizes a resampling based statistical interference procedure that we can use for analysis of production kinetics. It allows us to compare time-to-harvest (and other functionals) for recombinant protein production, under different experimental setups, based on a limited number of measurements, while ensuring false discovery rate control. 3) Impact of Key Findings The development of the plant-based expression vectors will help address the risk associated with being able to make a biologic quickly enough to meet unanticipated medical needs of crew. The development of the particle bombardment gene delivery approach will help address the risk associated with rapid production of a biologic therapeutic and will reduce mass, volume, power, and cold chain requirements, since a plasmid library or preloaded cartridges can be brought. The development of the statistical framework will help us to efficiently design the time course experiments for transient production of our target biologics in plant tissue. The method will help us quickly optimize production strategies with minimal experiments. It may also be a useful tool for process optimization on planet that by reducing resource requirements, including crew time. 4) Proposed Research Plan for the Coming Year Due to COVID-19 and restricted lab access we have reduced the scope of our project to eliminate work on trypsin and will instead focus on PTH (1-34) and G-CSF. In addition, for Specific Aim #4 we will not perform any cell-based assays but instead will focus on biophysical characterization and binding assays. Our plan for the coming year is to complete the design and construction of the plant-based expression vectors, test these vectors for transient expression of green fluorescent protein (GFP) in lettuce and N. benthamiana using different gene delivery strategies to optimize both the speed and expression level, use optimized methods for transient production of PTH (1-34) and G-CSF in lettuce and N. benthamiana, develop VIN systems for purification of these products, and apply the Equivalent System Mass analysis to additional steps in the plant-based bioproduction process to help inform and guide process development.
Bibliography Type:	Description: (Last Updated: 07/12/2023)
Articles in Peer-reviewed Journals	Bernardi A, Huang Y, Harris B, Xiong Y, Nandi S, McDonald KA, Faller R. "Development and simulation of fully glycosylated molecular models of ACE2-Fc fusion proteins and their interaction with the SARS-CoV-2 spike protein binding domain." PLoS One. 2020 Aug;15(8):e0237295. https://doi.org/10.1371/journal.pone.0237295 ; PMID: 32756606; PMCID: PMC7406073 , Aug-2020
Articles in Peer-reviewed Journals	McNulty MJ, Xiong YM, Yates K, Karuppanan K, Hilzinger JM, Berliner AJ, Delzio J, Arkin AP, Lane NE, Nandi S, McDonald KA. "Molecular pharming to support human life on the moon, Mars, and beyond." Crit Rev Biotechnol. 2021 Sep;41(6):849-64. Epub 2021 Mar 9. https://doi.org/10.1080/07388551.2021.1888070 ; PMID: 33715563 , Sep-2020
Articles in Peer-reviewed Journals	McDonald KA, Holtz RB. "From farm to finger prick - a perspective on how plants can help in the fight against COVID-19." Front Bioeng Biotechnol. 2020 Jul;8:782. https://doi.org/10.3389/fbioe.2020.00782 ; PMID: 32714921; PMCID: PMC7351482 , Jul-2020
Awards	Haddad K. "Deans Distinguished Graduate Fellowship, April 2020." Apr-2020