Fiscal Year:	FY 2022	**Task Last Updated:**	FY 01/19/2023
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		
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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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Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2020 TRISH BRASH1901: Translational Research Institute for Space Health (TRISH) Biomedical Research Advances for Space Health
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No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	3
No. of Bachelor's Candidates:	3	**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:	We have developed protocol and construct a Lettuce mosaic virus (LMV)-based expression system for transient expression of target biologics in plants. For the LMV system, we have started with a California isolate which we have fully sequenced and submitted to Genbank with accession number MZ318158. The LMV isolate from California were tested and confirmed its ability to infect N benthamiana by sap inoculation. This technology can be used in any future research project to produce recombinant molecule in various plant species. 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 lettuce and had them commercially synthesized. The expression vector TAG was tested in combination with the DNA-B component of bean dwarf mosaic virus (BDMV); which facilitates the systemic expression of TAG). We also studied RNA silencing suppressor P19 in N benthamiana and lettuce to enhance the TAG vector expression efficiency. Fluorescence microscopic observations and western blotting confirmed that TAG can express the GFP in both N benthamiana and lettuce by 2 hours and 4 hours respectively. We have developed preliminary protocols for gene delivery in lettuce using the BioRad Helios gene gun. We have initially tested delivery of plasmids containing RBD and G-CSF in different types of lettuce and N. benthamiana as a control. RBD and G-CSG could be produced in both N. benthamiana and romaine in as little as 18 and 4 hours respectively. All the above technologies can be used independently or in combination for various research and development projects that primarily aim towards produce recombinant protein in very short period of time or "just in time." 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 experimental data, while ensuring false discovery rate control.
	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 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. 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 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 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. 2) Key Findings We have developed the construct of a Lettuce mosaic virus (LMV)-based expression system for transient expression of

Task Progress:	target biologics in plants. For the LMV system, we have started with a California isolate which we have fully sequenced. The LMV isolate from California was tested and we confirmed its ability to infect N benthamiana by sap inoculation. 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 lettuce, and had them commercially synthesized. We attempted to make the LMV infectious clone by Gibson assembly but due to toxicity induction or internal recombination events, the LMV plasmid was quite unstable inside the E coli. We have developed preliminary protocols for gene delivery in lettuce using the BioRad Helios gene gun. We have initially tested delivery of plasmids containing receptor-binding domain (RBD) and G-CSF in different types of lettuce and N. benthamiana as a control. RBD and G-CSG could be produced in both N. benthamiana and romaine in as little as 18 and 4 hours respectively. The expression vector TAG was tested in combination with the DNA-B component of bean dwarf mosaic virus (BDMV), which facilitates the systemic expression of TAG. We also studied RNA silencing suppressor P19 in N benthamiana and lettuce to enhance the TAG vector expression efficiency. Fluorescence microscopic observations and western blotting confirmed that TAG can express the green fluorescent protein (GFP) in both N benthamiana and lettuce by 2 hours and 4 hours respectively. 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. Due to the lack of sequence information, the complete genome of LMV isolate was sequenced by RNA-seq followed by Sanger sequencing and submitted to GenBank with accession number MZ318158. 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 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), G-CSF, and RBD. In addition, for Specific Aim #4 we did not perform any cell-based assays, but instead will focus on biophysical characterization and binding assays. Our plan for future is to complete the testing of all these vectors for transient expression of GFP in lettuce and N. benthamiana using different gene delivery strategies to optimize both the speed and expression level.

Bibliography Type:	Description: (Last Updated: 07/12/2023)
Articles in Peer-reviewed Journals	McNulty MJ, Schwartz A, Delzio J, Karuppanan K, Jacobson A, Hart O, Dandekar A, Giritch A, Nandi S, Gleba Y, McDonald KA. "Affinity sedimentation and magnetic separation with plant-made immunosorbent nanoparticles for therapeutic protein purification." Front Bioeng Biotechnol. 2022 Apr 27;10:865481. https://doi.org/10.3389/fbioe.2022.865481 ; PMID: 35573255; PMCID: PMC9092175 , Apr-2022
Articles in Peer-reviewed Journals	Lobato Gómez M, Huang X, Alvarez D, He W, Baysal C, Zhu C, Armario-Najera V, Blanco Perera A, Cerda Bennasser P, Saba-Mayoral A, Sobrino-Mengual G, Vargheese A, Abranches R, Abreu IA, Balamurugan S, Bock R, Buyel JF, da Cunha NB, Daniell H, Faller R, Folgado A, Gowtham I, Häkkinen ST, Kumar S, Ramalingam SK, Lacorte C, Lomonossoff GP, Luís IM, Ma JK, McDonald KA, Murad A, Nandi S, O'Keefe B, Oksman-Caldentey KM, Parthiban S, Paul MJ, Ponndorf D, Rech E, Rodrigues JCM, Ruf S, Schillberg S, Schwestka J, Shah PS, Singh R, Stoger E, Twyman RM, Varghese IP, Vianna GR, Webster G, Wilbers RHP, Capell T, Christou P. "Contributions of the international plant science community to the fight against human infectious diseases - part 1: epidemic and pandemic diseases." Plant Biotechnol J. 2021 Oct;19(10):1901-20. https://doi.org/10.1111/pbi.13657 . Epub 2021 Jul 19. PMID: 34182608; PMCID: PMC8486245 , Oct-2021
Articles in Peer-reviewed Journals	He W, Baysal C, Lobato Gómez M, Huang X, Alvarez D, Zhu C, Armario-Najera V, Blanco Perera A, Cerda Bennaser P, Saba-Mayoral A, Sobrino-Mengual G, Vargheese A, Abranches R, Alexandra Abreu I, Balamurugan S, Bock R, Buyel JF, da Cunha NB, Daniell H, Faller R, Folgado A, Gowtham I, Häkkinen ST, Kumar S, Sathish Kumar R, Lacorte C, Lomonossoff GP, Luís IM, K-C Ma J, McDonald KA, Murad A, Nandi S, O'Keef B, Parthiban S, Paul MJ, Ponndorf D, Rech E, Rodrigues JCM, Ruf S, Schillberg S, Schwestka J, Shah PS, Singh R, Stoger E, Twyman RM, Varghese IP, Vianna GR, Webster G, Wilbers RHP, Christou P, Oksman-Caldentey KM, Capell T. "Contributions of the international plant science community to the fight against infectious diseases in humans - part 2: Affordable drugs in edible plants for endemic and re-emerging diseases." Plant Biotechnol J. 2021 Oct;19(10):1921-36. https://doi.org/10.1111/pbi .13658 . Epub 2021 Jul 19. PMID: 34181810; PMCID: PMC8486237 , Oct-2021
Articles in Peer-reviewed Journals	McNulty MJ, Berliner AJ, Negulescu PG, McKee L, Hart O, Yates K, Arkin AP, Nandi S, McDonald KA. "Evaluating the cost of pharmaceutical purification for a long-duration space exploration medical foundry." Front Microbiol. 2021 Oct 11;12:700863. https://doi.org/10.3389/fmicb.2021.700863 . PMID: 34707576; PMCID: PMC8542935. , Oct-2021
Articles in Peer-reviewed Journals	McNulty MJ, Hamada N, Delzio J, McKee L, Nandi S, Longo ML, McDonald KA. "Functionalizing silica sol-gel with entrapped plant virus-based immunosorbent nanoparticles." J Nanobiotechnology. 2022 Mar 4;20(1):105. https://doi.org/10.1186/s12951-022-01303-1 . PMID: 35246160; PMCID: PMC8895542 , Mar-2022

Articles in Peer-reviewed Journals	Bhattacharjee S, Liao S, Paul D, Chaudhuri S. "Inference on the dynamics of COVID-19 in the United States. " Sci Rep. 2022 Feb 10;12(1):2253. https://doi.org/10.1038/s41598-021-04494-z . PMID: 35145115; PMCID: PMC8831615 , Feb-2022
Articles in Peer-reviewed Journals	Huang Y, Harris BS, Minami SA, Jung S, Shah PS, Nandi S, McDonald KA, Faller R. "SARS-CoV-2 spike binding to ACE2 is stronger and longer ranged due to glycan interaction." Biophys J. 2022 Jan 4;121(1):79-90. https://doi.org/10.1016/j.bpj.2021.12.002 . Epub 2021 Dec 7. PMID: 34883069; PMCID: PMC8648368 , Jan-2022
Articles in Peer-reviewed Journals	Liao S, Macharoen K, McDonald KA, Nandi S, Paul D. "Analysis of variability of functionals of recombinant protein production trajectories based on limited data." Int. J. Mol. Sci. 2022 July 10;23(14):7628. https://doi.org/10.3390/ijms23147628 ; PMID: 35886973; PMCID: PMC9317391 , Jul-2022
Articles in Peer-reviewed Journals	Xiong Y, Hirano H, Lane NE, Nandi S, McDonald KA. "Plant-based production and characterization of a promising Fc-fusion protein against microgravity-induced bone density loss." Front bioeng biotechnol. 2022 Sep 12;10:962292. https://doi.org/10.3389/fbioe.2022.962292 ; PMID: 36172011; PMCID: PMC9511166 , Sep-2022
Awards	McDonald KA. "D.I.C. Wang Award for Excellence in Biochemical Engineering, May 2022." May-2022
Awards	McDonald KA. "Fellow of the American Institute of Chemical Engineering, May 2021" May-2021