CBC CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Modulating the potency of cytokine signaling by optimizing receptor-kinase engagement

Name, degree, title, institution, and contact information including the email address of each PI

JUAN L. MENDOZA, PH.D.
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Northwestern University
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Do you have any current or pending grant applications that potentially overlap with this application?
Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

(ENTER THE OVERLAP HERE)

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

LAY SUMMARY: Cytokines, the signaling proteins of the immune system, are essential for controlling infections, mediating inflammation and establishing long-term immunity. Cytokine families use receptors outside of the cell to access shared signaling components inside of the cell, called the JAK-STAT pathway(1) , to carry out their unique functions. The ability to control the intensity of JAK-STAT pathways would greatly enhance therapies for cytokine-mediated diseases. We recently studied two families (IFN1 and IFN3) and discovered that (i) altering the affinity between the JAK and the receptor and (ii) changing the orientation between JAKs at the receptor are properties that can be manipulated to potentiate signaling. We propose to find small molecules that can control cytokine activity by altering receptor-JAK interactions, and screen dozens of additional cytokine pathways to find new targets for future therapeutic and drug development.

HIGH RISK/HIGH REWARD: A comprehensive analysis of IFN1 and IFN3 receptors revealed molecular determinants that control the extent and quality of antiviral and anticancer signaling. Through distinct transmembrane receptors, both IFNs can activate JAK kinases and STAT transcription factors(2). We hypothesized that JAK associations with receptors contribute to the intensity of the response. Utilizing yeast surface display, we found that affinity between cytokine receptors and the JAKs change the intensity of a response. Here, we plan to find small molecules that can either increase or decrease IFN signaling by changing the affinity between a receptor and JAK. Small molecule modulators would define a new class of receptor-specific drugs as opposed to the currently available broad-acting JAK inhibitors(3). Second, we found that the IFN3 receptor-JAK complex exists in a sub-optimal geometry. Changes that re-orient the JAKs dramatically improved the amplitude of the response, increasing IFN3 to the level of IFN1(4). *Based on these findings and the potential for drug discovery, our recent AIM proposal for Abbvie Ventures was a semi-finalist*. Identifying additional cytokine signaling pathways would strengthen future prospects and/or help obtain funding from the NIH to drive further discovery in cytokine signaling and function relevant to a large number of human diseases.

PROPOSAL STATEMENT: DESCRIPTION OF RESEARCH

AIM 1. Identify small molecules that specifically modulate cytokine receptor-JAK interactions. IFN1 induces cell signaling at an amplitude three times stronger than IFN3, resulting in 100x potency in antiviral activity(5) and increased anti-proliferative effects relevant to cancer therapy(2). **(Fig. 1).** Since the two IFN families use identical signaling components(6), **we hypothesized that one of the differences was in the affinity between the cytokine receptor and the intracellular JAK kinases.** Indeed, we measured the affinities for TYK2 to IFN1 receptors to be ~10 μM but ~100 μM to IFN3 receptors. By engineering the IFN3 receptor to bind TYK2 more tightly (100 nM affinity) we show in cells expressing the engineered IFN3 receptor that IFN1 signaling is reduced but 10% while simultaneously IFN3 amplitude is potentiated by 25% **(Fig 1)**. <u>This suggests a competition for JAK usage between different receptors and supports our hypothesis that we can identify small molecules that will modulate cytokine signaling in a receptor-dependent manner, a goal not possible with current broad JAK inhibitors.</u> *While cytokine receptors have motifs that bind the JAKs(7), only 10 of 30 residues are invariant, providing sufficient variability to support specific receptor-JAK small molecule modulators.*

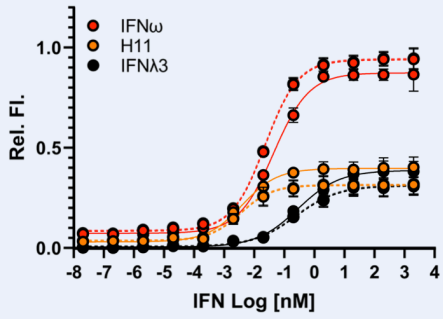

Figure 1- Affinity of cytokine-receptor-JAK interactions tune signaling amplitude. IFN3 signals at 33% amplitude relative to IFN1 (red dash). A high affinity IFN3 ligand does not change signaling amplitude only sensitivity along the x-axis (orange dash). When a higher affinity IFN3 receptor is used, IFN1 signaling is reduced by 10% (solid red) and IFN3 signaling is potentiated by 25% when treated with either the high-affinity ligand or wild-type (orange and black solid lines, respectively).

A live cell HTS will be carried out with A549-Dual cells, a line that allows simultaneous analysis and real-time monitoring of IFN signaling with secreted Lucia luciferase and NFκB signaling with secreted alkaline phosphatase. Cells will be treated with compounds from NU-HTAL libraries and effects on IFN1 and IFN3 signaling assayed directly following IFN1 or IFN3 stimulation and counter-screened in parallel against NFκB stimulation by TNFα. Specific IFN modulators identified will be ranked for follow-up and tested for cytotoxicity and titrated to estimate efficacy. The additional functional screening will be carried out with the biological endpoint of IFN-stimulated antiviral activity. The ability of compounds to alter virus replication will be tested using GFP-labelled Vesicular Stomatitis Virus (VSV-GFP), and the ability of compounds to control IFN1 and IFN3 -dependent antiviral states will be quantified. Top candidates will be followed up with specific assays for JAK kinase phosphorylation, using moderate-throughput assay as in Fig. 1. Independent assessment of candidates will determine if their actions are directly and indirectly associated with the receptor and kinase, using a diverse number of comprehensive biological assays and biophysical measurements.

AIM 2. Identify cytokine pathways that can be functionally controlled through reorientation of the intracellular JAKs. To evaluate the role of receptor geometry in IFN signaling, we engineered cell lines that express wild-type and mutant IFN3 cognate receptors (IFNλR1) with rotated intracellular registers by inserting alanines in the transmembrane (TM) helix that incrementally rotates the TM and intracellularly bound JAK by 109° **(Fig. 2, left panel)**. Our approach enables direct assaying of downstream signaling and biological activities of IFN3 as a function of varied JAK-JAK geometry within a complex. We found that the optimal rotation of the IFNλR1-JAK1 by 327° (insertion of 3 alanines) achieved a doubling of the signaling Emax **(Fig. 2, left panel)**. For many viruses, IFN1s are 2-logs more potent when comparing the half-maximal inhibitory concentration (IC_{50}) in antiviral assays whereas for the anti-proliferative effect in our assay, IFN1s are nearly 4-logs more potent(4). When IFN3 signaling is optimized, IFN3 treatment equals IFN1 antiviral activity against VSV and the anti-proliferative activities are enhanced by 3-logs(4) **(Fig 2)**. These studies suggest a high-affinity ligand that is able to reorient the receptor complex would potentiate the IFN3 system and would be a more ideal drug compared to IFN1s. Moreover, the limited tissue expression of IFN3 receptors is expected to result in vastly lower toxicity.

Here, we will use this approach to screen dozens of cytokine receptors for orientation-mediated signal modulation. Placement of JAKs in different orientations will reveal a previously hidden cytokine modulation code. In addition to our IFN3 work, the signaling amplitude of other cytokines can be modulated with fixed JAK orientations(8-10), and we have engineered IFN2 agonists that differential mediate therapeutic gene expression(9). It is likely many if not all cytokine pathways will be amenable to tuning signaling amplitudes.

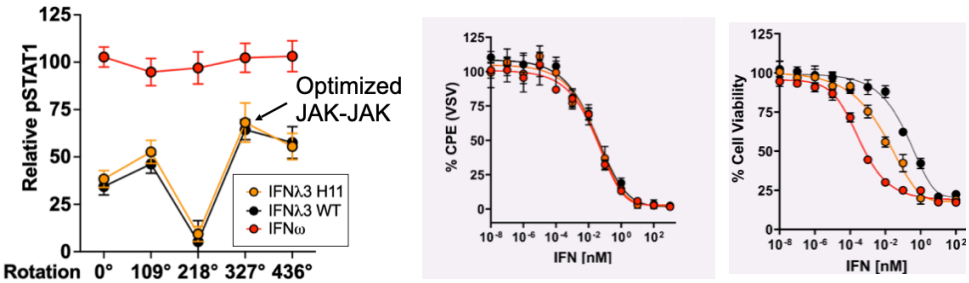

Fig 2. Re-orientation of JAKs in a signaling complex can tune cytokine signaling and function. (Left panel) Insertion of alanines into a IFN3 receptor to re-orient intracellular JAKs tunes signaling (relative to IFN3) from 0% (218° rotation) to 200% (327° rotation). When IFN3 complex is optimized to ~75% of IFN1, (middle panel) antiviral potency matches IFN1 (black) and (right panel) anticancer activity is improved from a ~4-log difference (data not shown) to a 30-fold difference when a high-affinity ligand (orange) is used.

We will construct and screen dozens of chimeric cytokine receptor pairs sampling different JAK geometries and determine signaling amplitudes as in Fig. 2. We will fuse the extracellular domains of IFN1 with the transmembrane and intracellular domains of the target cytokine receptors. Chimeric receptors will be expressed in cell lines lacking IFN1 receptors, enabling use of a single ligand for screening a

multitude of cytokine responses. For the chimeric receptor pairs that exhibit different signaling amplitudes, we will then validate in the context of the full-length receptors (not chimeric) and measure signaling amplitude, a subset of representative genes, and relevant functional assays. Potential applications and targets include inflammatory cytokines such as IL1 that contribute to neurodegeneration(11); anti-inflammatory cytokines including IL4, IL10, and TGF-beta(12, 13). Further cytokines pathways active in immune cell function such as prolonged signaling of IL2 and IFN1-2 may contribute to T-cell exhaustion(14). Thus, by modulating signaling amplitude on T-cells, we may balance between maintenance of T-cell activity to prevent the overstimulation that could be of great interest to CAR T-cell technologies. Again, this aim is to identify cytokine pathways but not engineer ligands at this stage as those approaches are still being developed.

NATURE OF THE INTER-INSTITUTIONAL COLLABORATION: Dr. Mendoza is an Assistant Professor at UC, following a highly successful and fruitful postdoc at Stanford focused on IFN-Receptor interactions and their therapeutic manipulation. Dr. Horvath is a Professor at NU with a long history in the IFN field with diverse significant contributions to the understanding of IFN1 responses, JAK-STAT signaling, and antiviral gene regulatory networks. We met in 2019, when JM was the recipient of the international Milstein Young Investigators award. Since then, CH has served as a mentor guiding his development as a future leader in the Cytokine and Interferon fields. The current project was born out of JM's interests in cytokine receptor structure and function, and CH's interest in understanding the similarities and differences of IFN1 vs. IFN3. We began a close collaboration, the basis of an earlier unfunded Catalyst award application. However, we continued that work and have major findings to pursue this application, a manuscript in revision at Immunity(4), and gained interest from two separate VCs. This is the perfect moment to spark this project into action with the Catalyst award and make significant translatable advances. We communicate often to discuss science relative to our collaboration, new ideas, topics regarding careers, and grants.

CRITERIA FOR MEASURING SUCCESS OF THE PROJECT: Success in this project will be measured by our ability to carry out these innovative experiments and publish one or two high-profile manuscripts with the results incorporating both the screening/mutagenesis and functional analysis. Longer-term metrics include the development of selective drugs for tuning the responses to IFN1 or IFN3, to intensify or interfere with the control of downstream effects.

LONG-TERM FUNDING PLAN FOR CONTINUATION BEYOND THE CATALYST AWARD:
One of the ultimate goals for this project and definition of success is to use the catalyst to generate sufficient preliminary data for NIH funding, through NIAID or NIGMS as either co-PIs on R01 or P01 mechanisms. An additional marker of success will be the identification of new cytokine receptor pathways and "tuned" properties that are of interest. Lastly, identification of small molecules that can either potentiate or inhibit IFN signaling in a receptor-JAK-specific manner. Meeting these objectives will provide proof of concept needed for both obtaining NIH funding and attracting VC interest.

TRANSLATIONAL POTENTIAL: As the primary response essential for controlling infections, and a major immune activator essential for cancer therapies as well as heritable diseases, the IFN system is inherently translational. Defects in IFN responses have been linked to the severity of acute viral infections including SARS-CoV-2, and heritable gain-of-function results in chronic auto-inflammatory diseases such as SLE or Aicardi- Goutieres syndrome. The results from our proposal will directly inform therapeutic approaches to modulate both chronic and acute IFN responses, raising or lowering the level of immune activation as a thermostat. Many viruses have developed strategies that interfere with IFN1 or IFN3, and we imagine a future where we could selectively boost alternate IFN pathways to provide antiviral responses to highly evolved viral pathogens. Targets from the genetic screen will further expand the cytokine pathways and types of indications that would benefit from our studies.

Relevant Cited Scientific References

1. Philips RL, Wang Y, Cheon H, Kanno Y, Gadina M, Sartorelli V, Horvath CM, Darnell JE, Jr., Stark GR, O'Shea JJ. The JAK-STAT pathway at 30: Much learned, much more to do. Cell. 2022;185(21):3857-76. Epub 2022/10/15. doi: 10.1016/j.cell.2022.09.023. PubMed PMID: 36240739.
2. Mendoza JL, Schneider WM, Hoffmann H-H, Vercauteren K, Jude KM, Xiong A, Moraga I, Horton TM, Glenn JS, de Jong YP, Rice CM, Garcia KC. The IFN-λ-IFN-λR1-IL-10Rβ Complex Reveals Structural Features Underlying Type III IFN Functional Plasticity. Immunity. 2017;46(3):379-92. doi: 10.1016/j.immuni.2017.02.017. PubMed PMID: 28329704.
3. McLornan DP, Pope JE, Gotlib J, Harrison CN. Current and future status of JAK inhibitors. Lancet. 2021;398(10302):803-16. Epub 2021/08/30. doi: 10.1016/S0140-6736(21)00438-4. PubMed PMID: 34454676.
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5. Pervolaraki K, Rastgou Talemi S, Albrecht D, Bormann F, Bamford C, Mendoza JL, Garcia KC, McLauchlan J, Hofer T, Stanifer ML, Boulant S. Differential induction of interferon stimulated genes between type I and type III interferons is independent of interferon receptor abundance. PLoS Pathog. 2018;14(11):e1007420. Epub 2018/11/30. doi: 10.1371/journal.ppat.1007420. PubMed PMID: 30485383; PMCID: PMC6287881.
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14. Huang Y, Jia A, Wang Y, Liu G. CD8(+) T cell exhaustion in anti-tumour immunity: The new insights for cancer immunotherapy. Immunology. 2022. Epub 2022/10/04. doi: 10.1111/imm.13588. PubMed PMID: 36190809.

Program Director/Principal Investigator (Last, First, Middle): Horvath, Curt

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 06/01/2023 THROUGH 05/31/2025

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Curt Horvath	PD/PI		.18		152,775	3,182	865	4,047
Patrick Parisien	Technical	6			87,800	43,900	11,414	55,314
SUBTOTALS →						47,082	12,279	59,361

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* TC reagents	1139
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)* HTA user fees, screening reagents	2000

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 62500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 62500

Program Director/Principal Investigator (Last, First, Middle): Horvath, Curt

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	59,361	59,361			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	1139	1139			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	2000	2000			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	62500	63,361			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	62500	63,361			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD **$** 125000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Cell culture reagents (media, plasticware)- $1139
HTA reagents and services -$2000

Program Director/Principal Investigator (Last, First, Middle): Mendoza, Juan Luis

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 06/01/2023 THROUGH 05/31/2025

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Juan Mendoza	PD/PI	.24			129,836	2597	392	3254
Shima Shabani	Grad	12			42,396	42,396		42,396
Isabella Castillo	Technician	2			38,000	6333	1,608	7494
SUBTOTALS →						51326	2000	53326

CONSULTANT COSTS

EQUIPMENT *(Itemize)*

SUPPLIES *(Itemize by category)*
Media for cell cultures and protein expression: bacteria, insect and mammalian. Antibodies for flow cytometry and westerns. Gene synthesis. Reagents for molecular biology and sequence verification. 8424

TRAVEL 500

INPATIENT CARE COSTS

OUTPATIENT CARE COSTS

ALTERATIONS AND RENOVATIONS *(Itemize by category)*

OTHER EXPENSES *(Itemize by category)*
Publications 250

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 62,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 62,500

Program Director/Principal Investigator (Last, First, Middle):

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	53326	53326			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	8424	8424			
TRAVEL	500	500			
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	250	250			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*	62,500	62,500			
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	62,500	62,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 125,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Personnel
Juan L. Mendoza, PhD, Principal Investigator (0.24 academic months), will share scientific leadership of the project with Dr. Horvath and directly oversee the work described in Aims 1 and 2.

Shima Shabani, graduate student (12.0 months per each calendar year effort), will execute experiments in Aim 2. She is experienced working with lentivirus for cell transduction, producing proteins, and a number of functional assays. She will be a major force to perform the experiments in a timely manner.

Isabella Castillo, technician (2 months per calendar year effort), will offer support the project with efforts in molecular biology, preparing reagents, execution of the assays, and protein production efforts for Aim 2.

Materials and Supplies:
We have budgeted ~$8400 per for materials and supplies and including reagents, antibodies, enzymes, chemicals, culture media, kits, glassware, plasticware, other disposables for protein production, gene synthesis, cloning, and assays.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Horvath, Curt M.

eRA COMMONS USER NAME (credential, e.g., agency login): curthorvath

POSITION TITLE: Professor of Molecular Biosciences

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Illinois State University, Normal, IL, USA	BS	5/1988	Biological Sciences
Northwestern University, Evanston, IL USA	PhD	12/1992	Biochem/Mol Biol/ Virology
Cornell University Medical School, NY, NY, USA	Postdoc	01/1993	Signal Transduction
The Rockefeller University, NY, NY, USA	Postdoc	05/1998	Molecular Cell Biology

A. Personal Statement

With >25 years devoted to virology, innate immunity, and cytokine signal transduction, **I am uniquely suite for this project**. I am a recognized leader in RLR-MAVS-IFN-JAK-STAT signaling, and both host restriction of virus replication and virus evasion of host. Our lab studies RNA virus-host interactions including those mediated by influenza A and B viruses as well as diverse Paramyxoviruses. One of the first described viral mechanisms of IFN evasion, our discovery of Paramyxovirus V protein-mediated STAT degradation and targeting by the VDC Ub ligase stands as a mechanistic paradigm of viral antagonism and host cell hijack. Expertise in gene regulation and genomics demonstrates the ability to conduct successful large scale, data driven projects. This combined expertise has driven the development of this proposal to study Ub ligase functions in Zika virus infections. Research in the Horvath laboratory at Northwestern University focuses on the molecular events controlling inducible gene regulation, with emphasis on the production of and response to cytokines and especially interferons. This includes biochemistry, molecular cell biology, mammalian signal transduction, pattern recognition, innate immunity, virology, genomics, and regulated gene expression in both normal cellular function and during acute infections and in cancerous transformation. Contemporary projects take a variety of molecular and genomic approaches to analyze virus-induced RLR-MAVS signal transduction and activation of host antiviral transcription, including analysis of genome-wide chromatin, transcription factor and RNA polymerase activation by viruses and IFNs, inducing mRNAs, microRNAs, and novel noncoding RNAs. Expertise in RNA virus biology, IFN signaling, and IFN evasion mechanisms, notably the structural and functional analysis of Paramyxovirus and Flavivirus -mediated STAT protein targeting to K48 Ubiquitination and proteasomal degradation is particularly relevant to this proposal, as is the characterization of K63-Ubiqutin regulation by the RLR immune sensor, LGP2. As Director of the NU High Throughput Analysis Laboratory, Dr. Horvath oversees a team of dedicated scientists and technical staff working in a premier open resource laboratory committed to providing Chicago's life science community with affordable means of high-throughput assay design, execution and data analysis from massively parallel experiments using state-of-the-art liquid handling and analytical instruments and high content microscopes. The CBC Catalyst application represents a new direction for the Horvath lab and an innovative new collaboration with Dr. Juan Mendoza from U. Chicago. The project will provide synergy between structural

engineering at the receptor cytoplasmic interface and genomic and functional consequences of IFN1 and IFN3 families that are key regulators of antiviral and anti-inflammatory immunity.

Ongoing and recently completed projects that I would like to highlight include:

1R21AI148949-01A1
Horvath (PI)
05/20/20 – 04/30/22
Mechanisms and Modifiers of Zika Virus Innate Immune Evasion

5P30CA060553-20
Platanias (PI), Role: Program Leader, Metabolism, Organelles, and Membranes Basic Science Program
09/16/13 - 07/31/23
The Robert H. Lurie Comprehensive Cancer Center

5P01HL071643-15
Sznajder (PI), Chandel (Proj. 3 Lead), Role: Co-investigator
09/01/15 - 06/30/21
PPG: Pathophysiology of Acute Lung Injury - "Project 3: Metabolic regulation of acute lung injury"

5R01 GM111652-04
Horvath (PI)
08/01/15 - 07/31/20
Analysis of Novel Virus-Induced RNAs

Citations:

1. Parisien J.-P., Lenoir J.J., Alvarado G., **Horvath C.M**. The Human STAT2 Coiled-Coil Domain Contains a Degron for Zika Virus Interferon Evasion, Journal of Virology; 2022 Jan 12;96(1):e0130121. PMID: 34643427
2. Lenoir JJ, Parisien JP, **Horvath CM.** Immune regulator LGP2 targets Ubc13/UBE2N to mediate widespread interference with K63 polyubiquitination and NF-κB activation. Cell Rep. 2021 Dec 28;37(13):110175. doi: 10.1016/j.celrep.2021.110175. PMID: 34965427
3. Grant R.A., Morales-Nebreda L., Markov N.S., Swaminathan S., Querrey M., Guzman E.R., Abbott D.A., Donnelly H.K., Donayre A., Goldberg I.A., Klug Z.M., Borkowski N., Lu Z., Kihshen H., Politanska Y., Sichizya L., Kang M., Shilatifard A., Qi C., Lomasney J.W., Argento A.C., Kruser J.M., Malsin E.S., Pickens C.O., Smith S.B., Walter J.M., Pawlowski A.E., Schneider D., Nannapaneni P., Abdala-Valencia H., Bharat A., Gottardi C.J., Budinger G.R.S., Misharin A.V., Singer B.D., Wunderink R.G., *NU Script Study Investigators (**Curt Horvath**) Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia. Nature. 2021 Feb;590(7847):635-641. doi: 10.1038/s41586-020-03148-w. Epub 2021 Jan 11. PMID: 33429418 **NU/NIAID Successful Clinical Response in Pneumonia Treatment (SCRIPT) Center*.
4. **Horvath C.M.** Weapons of STAT destruction. Interferon evasion by paramyxovirus V protein, Eur J Biochem. 271:4621-4628, 2004. PMID: 15606749.
5. Parisien JP, Lenoir JJ, Mandhana R, Rodriguez KR, Qian K, Bruns AM, **Horvath CM**. RNA sensor LGP2 inhibits TRAF ubiquitin ligase to negatively regulate innate immune signaling. EMBO Rep. 2018 Jun;19(6):e45176. PMID: 29661858

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2018-present Director, Northwestern University Center for Cell, Development, and Systems Biology
2017-present Director, Northwestern University High Throughput Analysis Laboratory (HTAL)
2007-present Professor, Department Microbiology and Immunology, Northwestern University, IL
2007-present Professor, Department Medicine, Northwestern University, IL
2007-present Professor, Department Molecular Biosciences, Northwestern University, IL
2004-2009 Senior Scientist, Evanston Northwestern Healthcare, IL
2004-2006 Associate Professor, Department of Biochemistry, Molecular, Cell Biology, Northwestern, IL
2004-2006 Associate Professor, Department of Medicine, Northwestern University, IL
2001-2004 Associate Professor, Immunobiology Center, Mt. Sinai School of Medicine, NY

1998-2001 Assistant Professor, Immunobiology Center, Mt. Sinai School of Medicine, NY
1998-2004 Adjunct Faculty, Rockefeller University, NY
1993-1998 Postdoctoral Fellow (NIH/LLS), The Rockefeller University, NY; Dr. James Darnell
1992-1993 Postdoctoral Fellow (NIH), Weill-Cornell Medical College, NY; Dr. Moses Chao

Other Experience and Professional Memberships

Editorial Board member, J. Virology, Virology, J. of JAK-STAT Pathways, Cytokine and Growth Factor Reviews, ad hoc reviewer for Nature, Cell, Molecular Cell, PNAS, Cell Host and Microbe, PLoS Pathogens, JBC, MCB, et. al.

2019 Executive Council, International Cytokine and Interferon Society (ICIS)
2017 Liaison to Federation of Clinical Immunology Societies (FOCIS)
2014- Executive Council, International Cytokine and Interferon Society (ICIS)
2013- Member, ICIS Meetings Committee
2011- Member, Steering Committee, Signal Transduction in Cancer Training Program
2011- Member, Steering Committee, Cellular and Molecular Basis of Disease Training Program
2009 Member and Co-Director of Signal Transduction in Cancer Program, Lurie Cancer Center
2005- Member, Virus Replication Training Program, Northwestern University, IL
2004-2009 Member and Co-Director of Viral Oncogenesis Program, Lurie Cancer Center
2002- Member, American Society for Virology (Lifetime Member)
2001- Member, American Society for Microbiology
1999- Member, International Cytokine and Interferon Society (**ICIS**; Lifetime Member)

Honors and awards

2022 Distinguished Service Award, ICIS
2011 American Society for Microbiology Distinguished Service Citation
2009 Soretta and Henry Shapiro Professor in Molecular Biology
2008 Jean Ruggles Romoser Chair in Cancer Research
2004-2009 American Cancer Society Research Scholar Award
2000-2003 NY City Council Speakers Fund for Biomedical Research
1998 American Liver Foundation Ira Jacobsen Liver Scholar Award
1996-1999 Leukemia Society of America Special Fellowship
1993-1995 NIH NRSA Postdoctoral Fellowship

C. Contributions to Science

1. Cytokine and IFN JAK-STAT Signaling

The primary means by which cytokines mediate biological responses is through transcriptional activation via Receptor-JAK-STAT pathways. As cytokines are the essential for all aspects of immune system regulation and also feature as causative agents of human diseases, these pathways are of high biomedical and therapeutic relevance. From the pioneering days of the JAK-STAT pathway to present, study of the molecular mechanisms underlying Signal Transduction and Activation of Transcription have been the focus of my group. We have made fundamental contributions to the understanding of interferon and cytokine biology, and generated novel insights in the areas of STAT signal transduction and gene regulation. We apply innovative biochemistry and molecular cell biology approaches to reveal the fundamental mechanisms underlying these biomedically important systems. Particular expertise in the IFN response system includes receptor JAK-STAT signaling, STAT protein DNA and chromatin interactions, epigenetic signaling, and resulting consequences for innate antiviral immune responses.

a. **Horvath CM**, Stark GR, Kerr IM, Darnell JE. Interactions between STAT and non-STAT proteins in the Interferon stimulated gene factor 3 transcription complex. Mol Cell Biol. 1996;16(12):6957-64.
b. Aaronson DS, **Horvath CM**. A road map for those who don't know JAK-STAT. Science. 2002 May 31;296(5573):1653-5. Review. PMID: 12040185.
c. Philips RL, Wang Y, Cheon H, Kanno Y, Gadina M, Sartorelli V, **Horvath CM**, Darnell JE Jr, Stark GR, O'Shea JJ. The JAK-STAT pathway at 30: Much learned, much more to do. Cell. 2022 Oct 13;185(21):3857-3876. doi: 10.1016/j.cell.2022.09.023.

d. PMID: 36240739 Review.Au-Yeung N, **Horvath CM**. Histone H2A.Z Suppression of Interferon-Stimulated Transcription and Antiviral Immunity Is Modulated by GCN5 and BRD2. iScience. 2018;6:68-82. doi: 10.1016/j.isci.2018.07.013. PMID: 30240626; PMCID: PMC6137307.

2. Mechanisms of Virus and Cytokine -Inducible Gene Transcription

Lab investigations focused on STAT proteins in IFN signaling, nuclear trafficking, and transcriptional mechanisms, connecting STAT proteins to fundamental transcription regulators, including the RNA polymerase Mediator complexes and histone-modifying acetylation and deacetylation machinery. The discovery of a requirement for HDACs in the positive regulation of IFN transcriptional responses provided a particularly unique insight into the fundamental mechanisms of inducible gene regulation. Contemporary work on IFN-stimulated gene targets identified nucleosome dynamics activate by IFN, including prominent associations with histone H2A.Z that is remodeled by acetyl transferase, GCN5, and the bromodomain protein, BRD2.

a. Lau JF, Nusinzon I, Burakov D, Freedman LP, **Horvath CM**. Role of Metazoan Mediator Proteins in Interferon-Responsive Transcription. Mol Cell Biol. 2003;23(2):620-8.
b. Nusinzon I, **Horvath CM**. Interferon-stimulated transcription and innate antiviral immunity require deacetylase activity and histone deacetylase 1. Proc Natl Acad Sci U S A. 2003;100(25):14742-7. PMID: 14645718.
c. Nusinzon I, **Horvath CM**. Histone deacetylases as transcriptional activators? Role reversal in inducible gene regulation. Sci STKE. 2005 Aug 9;2005(296):re11. doi: 10.1126/stke.2962005re11. PMID: 16091625.
d. Au-Yeung N, **Horvath CM.** Transcriptional and chromatin regulation in interferon and innate antiviral gene expression. Cytokine Growth Factor Rev. 2018;44:11-7. Epub 2018/12/05. doi: 10.1016/j.cytogfr.2018.10.003. PMID: 30509403; PMCID: PMC6281172.

3. Mechanisms of Virus Innate Immune Evasion

Our group was the first to characterize virus-encoded interferon evasion mechanisms that target the JAK-STAT pathway. Our demonstrations of paramyxovirus hijacking of cellular ubiquitin ligases to target STAT proteins demonstrated the diversity of viral evolution to target host immunity and established experimental procedures for a generation of virologists to study “IFN antagonism”. Specific contributions include definitive characterization of the molecular mechanisms for virus-induced immunosuppression by infectious agents including parainfluenza virus 5, human parainfluenza virus 2, measles virus, influenza A virus, and the emerging Nipah and Hendra virus. This work led to the discovery of RLR targeting by paramyxoviruses and continues to inform therapeutic and vaccine oriented basic research. Our discovery of STAT2 as host range restriction factor is the first example of of what is now known to underlie restriction of many human pathogens from murine models.

a. Parisien JP, Lau JF, **Horvath CM**. STAT2 acts as a host range determinant for species-specific paramyxovirus interferon antagonism and simian virus 5 replication. J Virol. 2002 Jul;76(13):6435-41. PMID: 12050355; PMCID: PMC136248.
b. Parisien JP, Bamming D, Komuro A, Ramachandran A, Rodriguez JJ, Barber G, Wojahn RD, **Horvath CM**. A shared interface mediates paramyxovirus interference with antiviral RNA helicases MDA5 and LGP2. J Virol. 2009 Jul;83(14):7252-60. doi: 10.1128/JVI.00153-09. Epub 2009 Apr 29. PMID: 19403670; PMCID: PMC2704796.
c. Ramachandran A, Parisien JP, **Horvath CM**. STAT2 is a primary target for measles virus V protein-mediated alpha/beta interferon signaling inhibition. J Virol. 2008 Sep;82(17):8330-8. doi: 10.1128/JVI.00831-08. Epub 2008 Jun 25. PMID: 18579593; PMCID: PMC2519631.
d. Parisien J.-P., Lenoir J.J., Alvarado G., **Horvath C.M**. The Human STAT2 Coiled-Coil Domain Contains a Degron for Zika Virus Interferon Evasion, Journal of Virology; 2022 Jan 12;96(1):e0130121. PMID: 34643427

4. Mechanisms of Pathogenic RNA Recognition and Interferon Production

In addition to the STAT protein targeting, we recognized that viruses also blocked innate RNA pattern recognition by a selection of RLR proteins. We found that Paramyxovirus V proteins antagonize MDA5 but not RIG-I, and while mapping the sites of V protein contact with MDA5, we found a second RLR target to be LGP2. In addition to demonstrating the mechanisms of RLR antagonism, investigations of RLR pathways were expanded to incorporate an integrative interdisciplinary approach that combined virology and innate immunology with biophysics, computational biology, biochemistry, molecular cell biology, and proteomics to study mechanisms that regulate virus-host interactions. We revealed that LGP2 acts as a biphasic regulator that enhances MDA5

RNA binding, antiviral responses, and signal transduction at low concentrations, yet antagonizes both RIG-I and MDA5 at high concentrations. My group provided a mechanistic context for LGP2 in RLR signaling and also helped to frame MDA5 RNP filament formation into a biological context by solving mechanistic questions LGP2 enhancement of antiviral signaling by MDA5. This work has recently completed the circle by demonstrating the effective use of Paramyxovirus evasion proteins to combat the hyperactive IFN signatures activated in patients suffering from inherited MDA5 mutations that cause familial autoimmune syndromes. Ongoing projects examine the alternative role of LGP2 as an attenuator of antiviral signaling, and we have described a concentration-dependent mechanism that results in biphasic LGP2 signal transduction. One target for LGP2-based negative regulation is the TRAF ubiquitin ligase, an essential NFκB signaling protein family. Recent work has expanded LGP2 negative regulation to included diverse TRAF-mediated stimuli including TNF and IL-1 among others.

a. Bruns AM, Pollpeter D, Hadizadeh N, Myong S, Marko JF, **Horvath CM**. ATP hydrolysis enhances RNA recognition and antiviral signal transduction by the innate immune sensor, laboratory of genetics and physiology2 (LGP2). J Biol Chem. 2013;288(2):938-46. PMCID: PMC3543043.
b. Bruns AM, Leser GP, Lamb RA, **Horvath CM**. The innate immune sensor LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament assembly. Mol Cell. 2014;55(5):771-81. PMCID: PMC4156907.
c. Parisien JP, Lenoir JJ, Mandhana R, Rodriguez KR, Qian K, Bruns AM, **Horvath CM.** RNA sensor LGP2 inhibits TRAF ubiquitin ligase to negatively regulate innate immune signaling. EMBO Rep. 2018 Jun;19(6). pii: e45176. doi:10.15252/embr.201745176. Epub 2018 Apr 16. PMID: 29661858; PMCID: PMC5989757.
d. Lenoir JJ, Parisien JP, **Horvath CM.** Immune regulator LGP2 targets Ubc13/UBE2N to mediate widespread interference with K63 polyubiquitination and NF-κB activation. Cell Rep. 2021 Dec 28;37(13):110175. doi: 10.1016/j.celrep.2021.110175. PMID: 34965427

5. Genome-Scale Analysis of Virus and IFN-induced Gene Regulation

Transcription regulation in the antiviral system has been a long-term interest in the laboratory. Next-generation sequencing approaches to study antiviral immune responses, and genome-scale research of mRNA, microRNA, and ncRNA transcription in the lab is providing a new understanding of the fundamental process of activated gene expression in humans. Our group has characterized small and large RNA transcriptomes activated by infection with RNA viruses including Sendai virus and influenza A virus and others. We have also analyzed chromatin packaging and nucleosome dynamics of the human type I IFN gene cluster, and described genome-wide NFkB and IRF3 binding sites that correspond to RNA polymerase activity. This work defined a widespread genome activation in response to virus infection and interferon signaling that was previously unappreciated, thereby defining a vast cohort of novel innate immune mediators that includes both coding and non-coding RNAs. Recent followup to these studies has identified hundreds of previously unrecognized transcripts activated by diverse virus infections and IFN. These finding complement the lab's microRNA profiling, which identified inducible miRNAs and elucidated their targets among cellular mRNAs. Similar analysis of IFN-stimulated gene targets identified nucleosome dynamics activate by IFN, including prominent associations with histone H2A.Z that is remodeled by GCN5 and BRD2 co-activators. This fundamental work on ISG regulation creates essential understanding of both the IFN response and the mechanical workings of mammalian transcription.

a. Buggele WA, **Horvath CM**. MicroRNA profiling of Sendai virus-infected A549 cells identifies miR-203 as an interferon-inducible regulator of IFIT1/ISG56. J Virol. 2013 Aug;87(16):9260-70. doi: 10.1128/JVI.01064-13. Epub 2013 Jun 19. PMID: 23785202; PMCID: PMC3754065.
b. Freaney JE, Kim R, Mandhana R, **Horvath CM**. Extensive cooperation of immune master regulators IRF3 and NFκB in RNA Pol II recruitment and pause release in human innate antiviral transcription. Cell Rep. 2013 Sep 12;4(5):959-73. doi:10.1016/j.celrep.2013.07.043. Epub 2013 Aug 29. PMID: 23994473; Central: PMC3792498.
c. Au-Yeung N, **Horvath CM**. Histone H2A.Z Suppression of Interferon-Stimulated Transcription and Antiviral Immunity Is Modulated by GCN5 and BRD2. iScience. 2018;6:68-82. doi: 10.1016/j.isci.2018.07.013. PMID: 30240626; PMCID: PMC6137307.
d. Mandhana R, **Horvath CM**. Sendai Virus Infection Induces Expression of Novel RNAs in Human Cells. Sci Rep. 2018;8(1):16815. doi: 10.1038/s41598-018-35231-8. PMID: 30429577; PMCID: PMC6235974.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Mendoza, Juan

eRA COMMONS USER NAME (credential, e.g., agency login): jlmendoza

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
San Francisco State University, San Francisco, CA	BS	2003	Biochemistry
University of Texas Southwestern Medical Center, Dallas, TX	PHD	12/2011	Molecular Biophysics
Stanford University, Stanford, CA	Postdoctoral Fellow	11/2018	Structural Immunology

A. Personal Statement

I have a history of using computational biology and structure-function studies to understand the limitations of current-day therapeutics and developing new strategies to overcome such limitations. In my laboratory at the University of Chicago, I am continuing to build upon the powerful set of scientific tools to further our understanding of the cytokine structure-function relationship and to shed light on important aspects of human health, disease, and improve the impact of therapeutics. My recent breakthroughs in protein engineering uniquely positions my lab to exploit engineered ligands or receptors to solve structures of challenging complexes and to study cytokine signaling to a new level of detail. By leveraging my knowledge of macromolecular structures and applying my computational and engineering expertise, we will interrogate the contributions of every protein-protein interaction involved in cytokine signaling. My lab's expertise in protein engineering, structural biology, IFN biology, and functional analyses places us in a unique position to achieve the goals outlined in the CBC Catalyst Award proposal.

During my graduate studies at UTSW, I analyzed the natural evolutionary record of the ABC Transporter family to identify positions in the cystic fibrosis transmembrane conductance regulator (CFTR) to alter either protein stability, the folding pathway, and/or function. Engineering CFTR variants with a broad range of CFTR at the plasma membrane, allowed me to correlate protein folding with CFTR function, demonstrate that the most common CF-causing mutation was defective in two steps of protein folding, and establish new tools for future drug discovery efforts. In 2011, I joined the lab of Dr. K. Christopher Garcia (Stanford University) for a postdoctoral fellowship to gain expertise in structural and combinatorial biology. During my fellowship at Stanford, I made contributions to both computation and cytokine biology. I developed an algorithm to predict peptides found in nature that can elicit a TCR functional response; the accuracy of these predictions was>94%. I have engineered cytokines from all members of the Interferon (IFN) superfamily. The engineered type I IFNs demonstrate how ligands can have diverse anti-cancer potencies but that antiviral activity could not be improved upon. By solving the structure of the type II IFN (IFN-γ) signaling complex, I show that IFN-γ partial agonists can decouple MHC I antigen from PD-L1 expression and could be used as drugs to accentuate IFN-γs anti-cancer activity. For type III IFNs (IFN-λs), I found the antiviral and anti-cancer activities can be improved upon through protein engineering, but to reach type I IFN levels of activities requires more advanced approaches for ligand design that are made possible through the team assembled in this proposal.

In addition to my dedication to basic science, I'm also interested in developing tools and molecules that can be taken out of the lab to the clinic, or that inform clinical studies. For example, my studies on CFTR had a direct impact on how small molecule correctors should be screened and created a change in the industry.

Because of this change, there is now a very effective treatment for the most common CF-causing mutation. During my time as a postdoctoral scholar at Stanford University, I filed five patents. These technologies are the core intellectual properties in the founding of two startups, Synthekine and 3T Biosciences. As an independent investigator at the University of Chicago, I have filed two patents that include my collaborator, Dr. Jeffrey Hubbell. The University and the School of Molecular Engineering are fully supportive of intellectual property. As evidence of this commitment, the Polsky Center for Entrepreneurship and Innovation was established. The Center assists in patent filing, licensing technologies, and the formation of startups. We envision the results from the Catalyst Award will contribute to new intellectual properties and will strengthen the already existing interest from investors for a startup company and or serve as the basis for NIH funding with our collaborator, Dr. Horvath.

1. Mendoza JL, Fischer S, Gee MH, Lam LH, Brackenridge S, Powrie FM, Birnbaum M, McMichael AJ, Garcia KC, Gillespie GM. Interrogating the recognition landscape of a conserved HIV-specific TCR reveals distinct bacterial peptide cross-reactivity. Elife. 2020 Jul 27;9 PubMed Central PMCID: PMC7384859.
2. Mendoza JL, Escalante NK, Jude KM, Sotolongo Bellon J, Su L, Horton TM, Tsutsumi N, Berardinelli SJ, Haltiwanger RS, Piehler J, Engleman EG, Garcia KC. The structure of the IFNγ receptor complex guides the design of biased agonists. Nature. 2019 Mar;567(7746):56-60. PubMed Central PMCID: PMC6561087.
3. Mendoza JL, Schneider WM, Hoffmann HH, Vercauteren K, Jude KM, Xiong A, Moraga I, Horton TM, Glenn JS, de Jong YP, Rice CM, Garcia KC. The IFN-λ-IFN-λR1-IL-10Rβ Complex Reveals Structural Features Underlying Type III IFN Functional Plasticity. Immunity. 2017 Mar 21;46(3):379-392. PubMed Central PMCID: PMC5510750.
4. Birnbaum ME, Mendoza JL, Sethi DK, Dong S, Glanville J, Dobbins J, Ozkan E, Davis MM, Wucherpfennig KW, Garcia KC. Deconstructing the peptide-MHC specificity of T cell recognition. Cell. 2014 May 22;157(5):1073-87. PubMed Central PMCID: PMC4071348.

Ongoing projects that I would like to highlight include:

1R35GM147179-01
Mendoza (PI)
07/01/22 – 06/31/27
Shedding new light on cytokine signaling through molecular engineering

For this NIH MIRA award, we proposed to measure JAK and STAT binding affinity for a broad range of receptors to better understand JAK-STAT signaling. It does not propose any small molecule or genetic screens and does not overlap with the Catalyst Award proposal.

B. Positions and Honors

Positions and Employment

1998 - 2001	Manager System Administration, Excite@Home Inc, Redwood City, CA
2011 - 2017	Postdoctoral Scholar, Stanford University, Stanford, CA
2017 - 2018	Basic Research Scientist, Stanford University, Stanford, CA
2018 -	Assistant Professor, University of Chicago, Chicago, IL

Other Experience and Professional Memberships

2017 -	Member, International Cytokine & Interferon Society

Honors

2003 - 2004	Post-Baccalaureate Research Education Program, NIH
2004 - 2005	Louis Stokes Alliance for Minority Participation, NSF
2004	Edwin Motel Award– Most Promising Student, SFSU

2011 Dean's Discretionary Award– Academic and Research Excellence, UTSW
2011 Best Graduate Student Poster, UTSW
2012 Damon Runyon Cancer Research Foundation Fellow, Damon Runyon Cancer Research Foundation
2013 Helen Hay Whitney Foundation Fellow, Helen Hay Whitney Foundation
2019 Milstein Young Investigator Award, International Cytokine & Interferon Society

C. Contribution to Science

1. Cytokine Engineering. Over the last several years, I became immensely interested in understanding protein-protein interactions of cytokines bound to their receptors and their role in modulating the human immune system. By using existing structural information, we can apply protein engineering methods to specifically alter the strength of the interactions between cytokines and receptors, the potency of the JAK/STAT signaling, and cytokine function. In engineering the type I IFNs, I was able to use the existing receptor complex to specifically target interactions between the ligand and each of the IFNAR receptors. These studies provided insights into the IFN function by demonstrating the tunable IFN function to be the anti-cancer activity since the antiviral activity of the wild-type IFNs was already at a maximum. In cases where a structure is not known, such as the IFN-λ and IFN-γ receptor complexes, I applied a novel non-biased engineering approach to gain insights into both structure and function. The engineering workflow I developed combines error-prone PCR with DNA family shuffling for creating combinatorial libraries in a two-step process. Using this approach, I evolved a higher affinity IFN-λ ligand and IFN-γR1 receptor enabling the solution of each complete type II and III IFN signaling complexes, respectively. In contrast to the type I IFNs, a higher affinity IFN-λ demonstrated that both the anti-cancer and antiviral activities could be improved upon. Excitingly, an in vivo study suggests the more potent IFN-λ is no more toxic than the wild-type, which combination of properties makes it a promising therapeutic. The IFN-λ complex also helped explain how IL-10Rβ recognizes the different cytokines of the IL-10 superfamily. Using the same engineering approach, I recently engineered a higher affinity IFN-γR1 receptor to stabilize the IFN-γ signaling complex and solve the structure. By gaining an atomic resolution map of all the ligand-receptor interactions, I was able to engineer IFN-γ partial agonists by modulating the number of receptors recruited by each of the ligands. Surprisingly, these new IFN-γ ligands had lowered potencies in JAK/STAT signaling which decoupled a source of IFN-γ pleiotropy. The partial agonists fully induce MHC I antigen expression, an anti-cancer activity, but result in limited PD-L1 expression, a pro-tumor activity. In addition to understanding the structure and activity of natural cytokines, I was a part of a team of scientists creating new molecules termed synthekines. Synthekines are molecules that can dimerize cytokine receptors from different families to generate a new cell signal. These molecules have great potential for generating tissue-specific cytokines or new cytokine activities.

   a. Mendoza JL, Escalante NK, Jude KM, Sotolongo Bellon J, Su L, Horton TM, Tsutsumi N, Berardinelli SJ, Haltiwanger RS, Piehler J, Engleman EG, Garcia KC. The structure of the IFNγ receptor complex guides the design of biased agonists. Nature. 2019 Mar;567(7746):56-60. PubMed Central PMCID: PMC6561087.
   b. Moraga I, Spangler JB, Mendoza JL, Gakovic M, Wehrman TS, Krutzik P, Garcia KC. Synthekines are surrogate cytokine and growth factor agonists compel signaling through non-natural receptor dimers. Elife. 2017 May 12;6 PubMed Central PMCID: PMC5429090.
   c. Mendoza JL, Schneider WM, Hoffmann HH, Vercauteren K, Jude KM, Xiong A, Moraga I, Horton TM, Glenn JS, de Jong YP, Rice CM, Garcia KC. The IFN-λ-IFN-λR1-IL-10Rβ Complex Reveals Structural Features Underlying Type III IFN Functional Plasticity. Immunity. 2017 Mar 21;46(3):379-392. PubMed Central PMCID: PMC5510750.
   d. Spangler JB, Moraga I, Mendoza JL, Garcia KC. Insights into cytokine-receptor interactions from cytokine engineering. Annu Rev Immunol. 2015;33:139-67. PubMed Central PMCID: PMC4445396.

2. Predicting Protein-Protein Interactions. I have an ongoing interest in understanding proteins in terms of their linear and three-dimensional structures. By leveraging the amassed sequence information either from

databases or “in-house” synthetically generated protein libraries, I developed algorithms to predict protein-protein interactions. I have used co-evolutionary analysis of the ABC transporter family to predict mutations throughout the five domains of CFTR that alter the protein stability, folding efficiency, and inter-domain interactions. Astoundingly, all sixteen mutations I identified as evolutionarily of interest altered folding; fourteen mutations resulted in lower levels of CFTR at the plasma membrane (PM) and two improved folding of CFTR. The success rate of the predictions using this computational approach was surprising since several studies had demonstrated proteins to be robust and tolerant of mutations. I have also developed an algorithm to use sequence information from libraries of synthetic peptide sequences to describe the cross-reactivity of T-Cell Receptor (TCR) and peptide-Major Histocompatibility Complex (pMHC) interactions. Libraries of peptide-MHC were displayed on yeast and those peptides that bound to a TCR of interest were isolated and subjected to next-generation sequencing (NGS). I developed the software pipeline to process, analyze, and predict peptides that bind specific MHC/TCRs with >94% prediction accuracy. This algorithm provides a powerful tool to identify cancer antigens recognized by orphan TCRs in patients whose peptides may be useful for developing peptide-based vaccines.

a. Mendoza JL, Fischer S, Gee MH, Lam LH, Brackenridge S, Powrie FM, Birnbaum M, McMichael AJ, Garcia KC, Gillespie GM. Interrogating the recognition landscape of a conserved HIV-specific TCR reveals distinct bacterial peptide cross-reactivity. Elife. 2020 Jul 27;9 PubMed Central PMCID: PMC7384859.

b. Riley TP, Hellman LM, Gee MH, Mendoza JL, Alonso JA, Foley KC, Nishimura MI, Vander Kooi CW, Garcia KC, Baker BM. T cell receptor cross-reactivity expanded by dramatic peptide-MHC adaptability. Nat Chem Biol. 2018 Oct;14(10):934-942. PubMed Central PMCID: PMC6371774.

c. Birnbaum ME, Mendoza JL, Sethi DK, Dong S, Glanville J, Dobbins J, Ozkan E, Davis MM, Wucherpfennig KW, Garcia KC. Deconstructing the peptide-MHC specificity of T cell recognition. Cell. 2014 May 22;157(5):1073-87. PubMed Central PMCID: PMC4071348.

d. Mendoza JL, Schmidt A, Li Q, Nuvaga E, Barrett T, Bridges RJ, Feranchak AP, Brautigam CA, Thomas PJ. Requirements for efficient correction of ΔF508 CFTR revealed by analyses of evolved sequences. Cell. 2012 Jan 20;148(1-2):164-74. PubMed Central PMCID: PMC3266553.