The proposal addresses the differences between IFN1 and IFN3 in terms of their potency in antiviral activity and anti-proliferative effects relevant to cancer therapy. The study hypothesizes that one of the differences is in the affinity between the cytokine receptor and the intracellular JAK kinases. By engineering the IFN3 receptor to bind the JAK family kinase TYK2 more tightly, the preliminary data show that IFN1 signaling is reduced while simultaneously IFN3 amplitude is potentiated. This suggests the receptors compete for JAK kinases. The proposal will use high throughput screening in Aim 1 to identify small molecules that can modulate cytokine signaling in a receptor-dependent manner. This is not a goal that is not currently possible with broad JAK inhibitors. The strategy will employ A549-Dual cells with a readout for IFN signaling by Luciferase followed by secondary screening for NFkB signaling in response to TNFalpha by colormetric alkaline phosphatase. Functional screening of hits that inhibit or potential IFN1 and IFN3 signaling will be cytotoxicity and antiviral (VSV) activity. The preliminary data also evaluated the role of receptor geometry in IFN signaling. This is done by inserting alanines to cause a rotation of the receptor with doubling of the signaling occurring with a 327 degree rotation. This optimized IFN3 signaling can equal IFN1 antiviral activity against VSV and enhance anti-proliferative activities. The grant hypothesized that 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. the approach can be used to screen dozens of cytokine receptors for orientation-mediated signal modulation and that many cytokine pathways will be amenable to tuning signaling amplitudes. A strategy is proposed in which the chimeric receptors formed by fusion of the IFN1 extracellular domains with the transmembrane and intracellular domains of the targeted cytokine receptors.

The impact of the work is high as there is the potential for basic science understanding of how receptors and JAK kinases interact with the added potential for discovery of inhibition strategies that can impact cancer.

The team conducting this work is very strong.

Northwestern Professor Curt Horvath has expertise across more than 3 decades in JAK/STAT signaling, particularly in response to viruses. He also heads the NU-HTAL lab and thus is an expert in performance of cell based HTP assays in Aim 1.

A major positive for this award in the context of the CBC Catalyst award is the pairing with a newer assistant professor from the University of Chicago. Dr. Mendoza joined UC in 2018. He is cross trained in computational, functional and structural biology expertise and is well suited to the proposed work in Aim 2.