Sequence studies of Respiratory Syncytial Virus (RSV) isolates from a 2012-2013 community outbreak in USA to identify signature sequences associated with disease severity and transmission.

As the single most important cause of serious lower respiratory tract disease in infants and young children in the United States and globally, RSV is of considerable public health importance and a high priority for vaccine development. There are two major antigenic groups of RSV, A and B, and multiple genotypes within the two groups. RSV causes lower respiratory tract illness and potentiates childhood wheezing and asthma. It has been found that RSV pathogenicity is strain-dependent in the mouse model. To establish that specific RSV genotypes of virulent RSV strains are associated with early childhood wheezing, severe acute bronchiolitis and subsequent asthma development and asthma pathogenesis, plan to utilize RSV isolates obtained from a retrospective bronchiolitis-to-asthma infant cohort (INSPIRE) with defined parameters of illness such as bronchiolitis severity score (BSS) and prolonged wheezing. The current study design is to perform sequencing whole RSV genotypes. We will also measure cytokine/chemokine levels in nasal washes to define how RSV strains impact bronchiolitis, inflammation, and wheezing. RSV genotypes and/or mutations associated with clinical disease will be studied functionally. Using RSV reverse genetics system to produce recombinant RSV, we will introduce virulence-associated mutations into wild-type RSV. The effects of these mutations will be studied in cells and mice to determine virulence mechanisms where host genetics and pre-existing immunity are controlled. This "**bedside to bench**" approach combines epidemiological and clinical data with mechanistic viral genetics and mouse models. The mouse model of RSV pathogenesis that we have developed utilizes quantitative, digital pathology, pulmonary pathophysiology, and recapitulates RSV-induced mucus expression.

The complete genome sequence data from viruses collected during this season not only will expand our knowledge of the virulence factors, but provide detailed information on evolution and transmission of RSV strains during this season. It will also provide the detailed sequence data needed to identify strains with common lineage and understand change during the course of the outbreak and identify changes that may have selective advantage to the virus. Whole genome sequencing will also help us identify possible epistatic interactions between RSV genes. JCVI is already involved in similar large scale sequencing projects of parainfluenza viruses from other parts of the world. Whole genome sequencing of 100 RSV sample covering the entire 2012-2013 season will provide a key piece to similar studies of RSV.

Infants at risk for RSV infection and subsequent asthma have been frequently found to have bacterial super-infections and there are high incidences of pulmonary bacterial co-infection in children with severe respiratory syncytial virus (RSV) bronchiolitis. Whether this reflects a defective innate immune response very early in life among those predisposed to develop severe RSV infection and/or asthma, or whether this altered colonization or co-infection results or causes more severe disease is not known. This project will also take advantage of the same nasopharyngeal samples to study the effects of RSV infection on the lungs and upper respiratory track microbiome. Sequencing of the microbiome will address if there are changes in microbial dynamics in patients with high disease severity compared to those with low disease severity. This is a critically important question, as we do not yet understand the respiratory

microbiome of infants during health and disease, the association with respiratory morbidity, and whether early life modification could ultimately be predictive of, or be modified to prevent subsequent respiratory morbidity.