Specific Aims.

The long term goal of this research project is to identify the optimal dose and schedule of administration of drugs active against influenza viruses that will prevent and/or cure people with influenza without causing the emergence of resistant viruses. The adamantanes and neuraminidase inhibitors have been used for the prevention and/or treatment of influenza. However, they often fail because treatment with these drugs leads to the emergence of resistant viruses in the treated population.

Adamantanes have historically been used in the treatment and prevention of influenza A virus infections (1). Recently, viruses that are resistant to these inexpensive drugs have emerged, rendering them less useful for the therapy of influenza (2, 3). Neuraminidase inhibitors represent a new class of agents for use against type A and type B influenza virus infections (1). While shown to be effective, there have been instances of emergence of resistance or reduced sensitivity during therapy with neuraminidase inhibitors (4-6). This has been seen especially in children where high clearances for these agents in general and oseltamivir in specific are the norm (5).

The hollow fiber infection model (HFIM) system has been used to determine the optimal dose and schedule of administration of antibacterial, antifungal and antiviral compounds for use in the treatment of individuals infected with bacteria, fungi, and viruses (7-16). We propose to use the HFIM system to study the effects of amantadine and the neuraminidase inhibitor, oseltamivir carboxylate, on the replication of influenza viruses and to identify the pharmacodynamically-linked variables for these antiviral drugs, alone and in combination, with respect to inhibition of virus replication. We also propose to identify whether a different pharmacodynamically-linked variable is present for suppression of emergence of resistance. **We hypothesize that the HFIM system can be used to provide information on resistance selection in humans and that the HFIM system can be used to determine the dose and administration schedule of antiviral compounds and combinations of antiviral compounds that will inhibit the replication of influenza viruses while preventing the emergence of resistance.**

Specific Aim #1. Validate the HFIM system as a model for antiviral drug-induced resistance in humans by demonstrating that: 1) influenza viruses that are resistant to amantadine and oseltamivir carboxylate can be generated in the HFIM system when these antiviral compounds are delivered as monotherapy using the recommended human doses and PKs; and 2) that the resistant strains generated in the HFIM system have similar characteristics as those isolated from clinical settings. Several influenza virus clinical isolates and laboratory strains including the recombinant H5N1 influenza virus, rgA/Vietnam/1203/2004xA/PR/8/34 (a surrogate for H5N1 influenza virus), will be used in these studies.

Specific Aim #2. Use these viruses in the HFIM system to optimize the dosing strategy of amantadine and oseltamivir carboxylate to minimize the emergence of drug resistance by performing dose ranging and dose fractionation studies of these drugs in influenza virus-infected cells under monotherapy conditions.

Specific Aim #3. Use the HFIM system to determine the pharmacodynamically-linked variables of combinations of amantadine and oseltamivir carboxylate with the aim of suppressing or preventing the emergence of resistance to these drugs in cells infected with these influenza viruses.

The results of these studies will help establish protocols for the use of these antiviral compounds for the treatment of patients during influenza epidemics and pandemics.

Our research strategy involves a multifaceted, translational collaboration designed to optimize the move from research discovery to clinical application. The collaborators in this activity include a non-profit research institute (Ordway Research Institute. Albany, NY), a non-profit genomics research institute (Translational Genomics Research Institute, Flagstaff, AZ), and a private biotech company (Adamas Pharmaceuticals, Inc, Emeryville, CA). This strategy has proven successful in other activities including a current and ongoing research project involving the above partners.