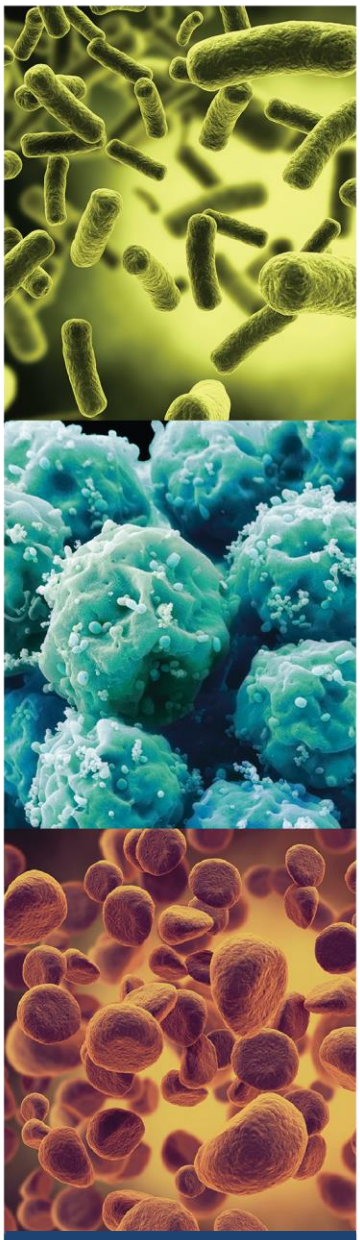
ImQuest BioSciences



Influenza Antiviral Drug Development

Efficacy, Toxicity, Range of Action, Mechanism of Action

Introduction

New influenza virus inhibitors effective against all virus subtypes that are well tolerated and that have a higher genetic barrier for resistance are urgently needed. At present, only four drugs - amantadine (AMT), oseltamivir carboxylate (OSC), ribavirin (RBV), and zanamivir (ZNV) – have been approved for the prevention and treatment of seasonal influenza virus infections.

The clinical usefulness of two of the four drugs is limited due to their specificity for influenza A virus, the increasing incidence of resistant viruses in the population, and the associated neurological side effects of the drugs. Many seasonal influenza strains are resistant to AMT, OSC or both. Therefore, the development of novel and improved anti-influenza drugs remains an urgent international priority.

ImQuest BioSciences provides cost-effective preclinical services for the expeditious evaluation of efficacy, toxicity, range and mechanism of action of potential anti-influenza drugs in accordance with the FDA's requirements for an IND filing.

The antiviral evaluation of RBV, AMT, and OSC described herein was performed using our panel of wild-type and drug-resistant influenza A and B viruses. Similar studies can be conducted for new and novel antiviral agents under development.

Methodology

Influenza Panel: ImQuest possesses a large and continuously growing collection of clinical virus strains from the CDC and other commercial or repository sources. These low-passage clinical viruses have been obtained from patient samples and amplified for long-term use without multiple passages through established cell lines.

Anti-Influenza Virus Cytoprotection Assay: MDCK cells (1 x 10^4 cells per well) were seeded in 96-well flat-bottom tissue culture plates and allowed to adhere overnight at 37° C, 5% CO_2. Following incubation, the medium was removed from the cell monolayers and the cells were washed with DPBS.

Six serial dilutions of RBV, AMT and OSC (purchased from Sigma Aldrich) were prepared.

The efficacy and toxicity of test agents is compared to known control compounds evaluated in parallel. The control agents typically possess mechanisms of action similar to the test agent.

The endpoints of the assay are the effective (EC) and toxic (TC) concentrations of the test and control agents. These concentrations can be calculated at the 25%, 50%, 95% or 99% level.

The therapeutic, or selectivity, index (TI or SI) is determined by dividing the observed TC concentration by the EC concentration.

ImQuest BioSciences is a preclinical contract research and development company that evaluates the potential of new and novel pharmaceutical products. We specialize in the development of drugs, vaccines and biologic products for the treatment and prevention of infectious disease, cancer and inflammatory disease.

Robert W. Buckheit, Jr., Ph.D.
Chief Scientific Officer
rbuckheit@imquestbio.com

Influenza virus strains were diluted to a pre-determined titer to yield 85% to 95% cell killing at 4 days post-infection and were added to the plate.

Following incubation at 37° C, 5% CO_2 for four days, cell viability was measured by XTT tetrazolium dye staining. The optical density was determined spectrophotometrically at 450 and 650 nm using Softmax Pro 4.6 software.

Percent reduction of the cytopathic effects (CPE) of the virus-infected wells and the percent cell viability of uninfected drug control wells were calculated by four parameter curve fit analysis.

Time-of-Drug Addition Assay: RBV, AMT, OSC, and the polymerase inhibitor T705 were added to MDCK cells at 0, 1, 4, 9 and 24 hours post-infection with wild-type influenza virus A/PR/8/34 or OSC–resistant influenza virus A/NJ/15/07 containing the H274Y neuraminidase mutation. Time-of-drug-addition assays allow determination of the mechanism of antiviral action by defining when a compound loses effectiveness relative to the temporal and sequential progression of influenza virus replication.

Results

The range of antiviral action of RBV, AMT, and OSC against representative wild-type and resistant influenza virus strains is summarized in the table below.

Influenza Strain	Ribavirin (µg/mL)			Amantadine (µM)			Oseltamivir (µg/mL)		
	EC_{50}	TC_{50}	TI	EC_{50}	TC_{50}	TI	EC_{50}	TC_{50}	TI
A/Denver/1/57 (H1N1)	1.58	>100	>63.3	0.17	>10	>58.8	>1	>1	---
A/NWS/33 (H1N1)	1.68	>100	>59.5	>10	>10	---	0.2	>1	>5
A/WS/33 (H1N1)	2.05	>100	>48.8	>10	>10	---	0.02	>1	>50
A/HK/8/68 (H3N2)	0.56	>100	>179	1.78	>10	>5.62	0.008	>1	>125
A/PR/8/34 (H1N1)	1.87	>100	>53.5	7.33	>10	>1.36	0.37	>1	>2.7
A/Fort Monmouth/1/47 (H1N1)	2.1	>100	>47.6	0.09	>10	>111	>1	>1	---
A/Aichi/2/68 (H3N2)	0.81	>100	>123	0.51	>10	>19.6	0.006	>1	>167
A/CA/27/07 (H1N1)	1.41	>100	>70.9	0.99	>10	>10.1	0.23	>1	>4.35
A/NY/18/09 (H1N1)	2.11	>100	>47.4	>10	>10	---	0.02	>1	>50
A/CA/04/09 (H1N1)	1.48	>100	>67.6	0.71	>10	>14.1	0.02	>1	>50
A/CA/05/09 (H1N1)	1.65	>100	>60.6	>10	>10	---	0.007	>1	>143
A/New Caledonia/20/99 (H1N1)	3.20	>100	>31.3	0.43	>10		0.17	>1	>5.88
A/Wisconsin/06/1994 (H3N2) A30V	16.7	>100	>5.99	>10	>10	---	0.00001	>0.1	>10,000
A/Venezuela/6971/2005 (H3N2) V27A	21.3	>100	>4.69	>10	>10	---	0.02	>1	>50
A/Virginia/01/2006 (H1N1) S31N	4.45	>100	>22.5	>10	>10	---	0.07	>1	>14.3
A/Wisconsin/25/2007 (H1N1) L26F	7.68	>100	>13	>10	>10	---	2.45	>10	>4.08
A/HK/2652/2006 (H1N1) S31N	8.92	>100	>11.2	>10	>10	---	0.09	>1	>11.1
A/Brisbane/10/2007 (H3N2) S31N	0.85	>100	>118	>10	>10	---	>1	>1	---
A/Taiwan/760/2007 (H3N2) S31N	23.1	>100	>4.33	>10	>10	---	0.05	>0.1	>2
A/New Jersey/15/2007 (H1N1) H274Y	2.45	>100	>40.8	0.77	>10	>13	>1	>1	---
A/Wuhan/395/95-like (H3N2)	1.23	>100	>81.3	0.94	>10	>10.6	0.67	>1	>1.49
A/HK/2369/2009 (H1N1) H274Y	1.66	>100	>60.2	>10	>10	---	>1	>1	---
B/Taiwan/2/62	0.99	>100	>101	>10	>10	---	0.02	>1	>50
B/Mass/3/66	1.81	>100	>55.2	>10	>10	---	0.001	>0.1	>100
B/MD/1/59	0.86	>100	>116	>10	>10	---	0.52	>1	>1.92
B/Allen/45	0.87	>100	>115	>10	>10	---	0.27	>1	>3.7
B/Bridgit	2.73	>100	>36.6	>10	>10	---	>1	>1	---
B/Great Lakes/1739/1954	0.63	>100	>159	>10	>10	---	0.07	>1	>14.3
B/Lee/40	0.78	>100	>128	>10	>10	---	0.02	>1	>50
B/Memphis/20/96	0.91	>100	>110	>10	>10	---	>1	>1	---
B/Memphis/20/96 R152K	0.57	>100	>175	>10	>10	---	0.11	>1	>9.09

The figures below depict the dose response curves for both wild-type and drug-resistant viruses as would typically be performed in a range of action evaluation for a new compound.

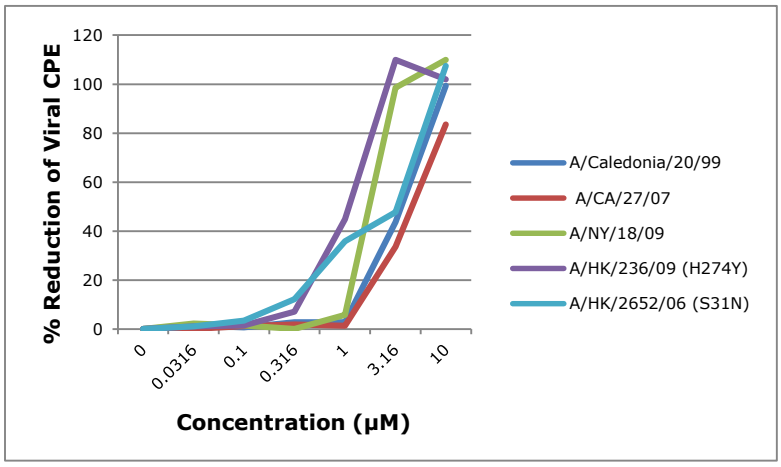


Figure 1. Anti-Influenza Activity of Ribavirin

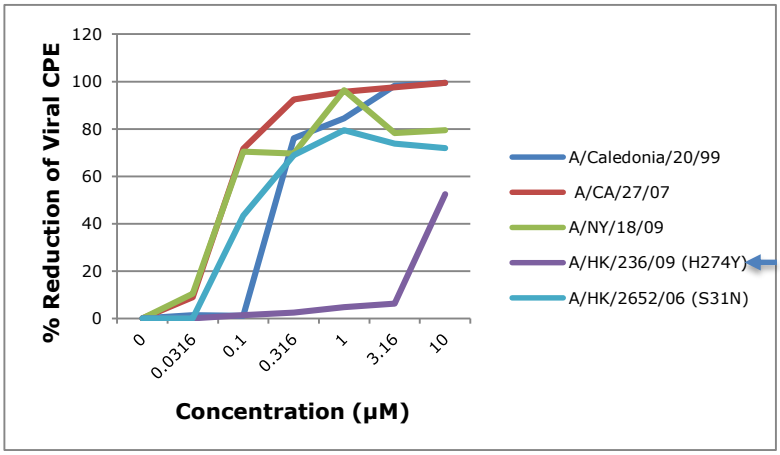


Figure 2. Anti-Influenza Activity of the Neuraminidase Inhibitor, Oseltamivir Carboxylate

The influenza virus A/HK/236/09 clinical isolate contains the H274Y amino acid substitution in the neuraminidase gene conferring resistance to OSC.

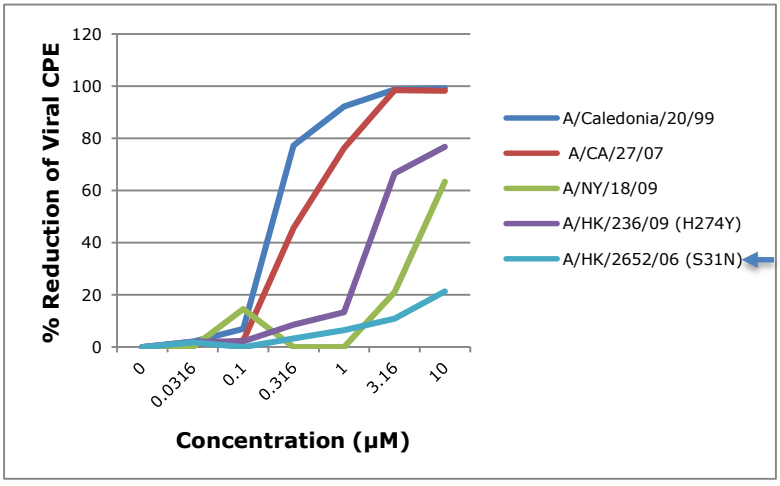


Figure 3. Anti-Influenza Activity of the M2 Ion Channel Inhibitor Amantadine

The influenza A/HK/2652/06 isolate contains the S31N mutation conferring resistance to the M2 ion channel inhibitor, AMT.

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Time-of-drug-addition assays are used to determine when a test agent loses effectiveness relative to the timing of steps in virus replication thereby providing information on antiviral mechanism of action. Presented in the figures below are the results of assays in which RBV, AMT, OSC, and the polymerase inhibitor, T705, were added at various times post infection.

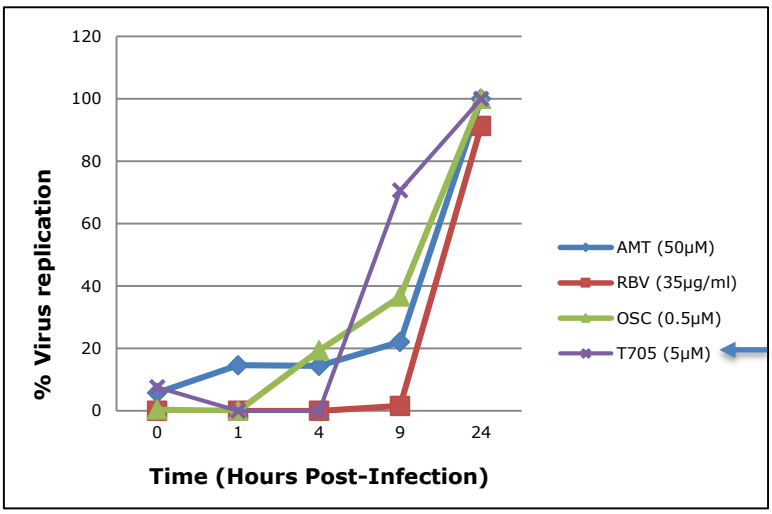

Figure 4. Anti-Influenza Virus Time-of-Drug-Addition Assay with Wild Type Virus A/PR/8/34

RBV, M2 ion channel inhibitor AMT, and neuraminidase inhibitor OSC remained active against wild type influenza virus when added up to 9 hours post-infection.

The nucleoside polymerase inhibitor T705 remained active when added to influenza virus infected MDCK cells up to 4 hours post-infection.

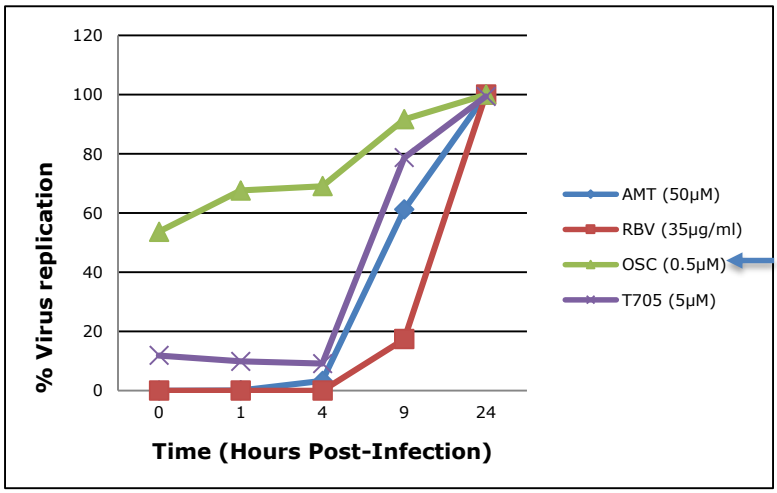

Figure 5. Anti-Influenza Virus Time-of-Drug-Addition Assay with A/NJ/15/07 (H275Y)

Influenza virus A/NJ/15/07 is resistant to OSC as a result of the H274Y mutation in the neuraminidase gene. Therefore, in this assay, the ability of OSC to protect cells from infection is compromised.

Summary

ImQuest BioSciences provides a variety of cell-based antiviral assays to define the efficacy and toxicity of new and novel test compounds to inhibit influenza viruses as compared to FDA-approved drugs. In addition, drug-resistant virus strains and cell-based assays such as time-of-drug-addition assays can be used to determine range and mechanism of antiviral action.

ImQuestSUCCESS

Select drug candidates with the highest probability of clinical success

The ImQuest*SUCCESS* preclinical services platform is used to critically evaluate the potential of a test compound and to assure that its efficacy, toxicity, and pharmaceutical properties are evaluated in a comprehensive and interactive way. Successful completion of platform objectives provides significant confidence in the potential of a test compound to transition to human clinical trials, enhances the robustness of drug development efforts and reduces the risk of expensive clinical development failures by the exclusion of candidates which are likely to fail during advanced preclinical and clinical development at early (and less expensive) time points.

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