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**APPLICATION NUMBER: 021087**

**MICROBIOLOGY REVIEW(S)**

**Microbiology Review**  
**Division of Antiviral Drug Products (HFD-530)**

NDA: 21-087      Serial No. ~~000~~      Reviewer:      N. Battula  
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Sponsor:      Hoffmann-La Roche Inc.  
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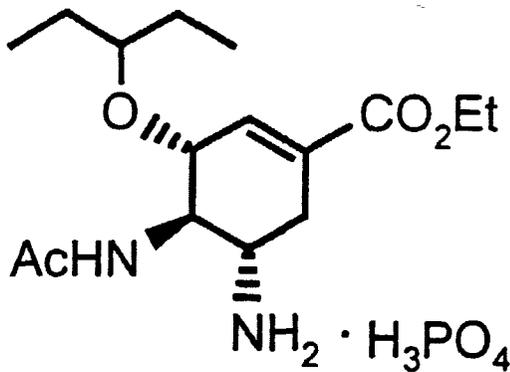
Product names:      Proprietary:      Tamiflu  
                                 Nonproprietary:      Oseltamivir phosphate  
                                 Code:      Ro 64-0796

Chemical name:      (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid ethyl ester, phosphate

Empirical formula:      C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub> (free base)

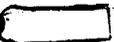


Structural formula:



Dosage form:      Oral capsules, strength 75mg 

Indication:      Treatment of influenza

Related documents:      IND 

## BACKGROUND

In this NDA Hoffmann-La Roche Inc. is requesting approval of Tamiflu™ (capsules for oral use) for the treatment of uncomplicated acute illness due to influenza infection in adults who have been symptomatic for no more than two days. The proposed indication is based on studies in which the predominant influenza infections were with influenza virus A, and in a small number of patients with influenza virus B. In support of this indication, Hoffmann-La Roche Inc. submitted data from two-phase 3 placebo-controlled and double blind clinical trials, one conducted in the USA, and one conducted in Europe. Treatment effects of Tamiflu™ were based on the assessment of the symptomatic course of influenza illness. The primary endpoint in these studies was the median time to alleviation of clinically significant signs and symptoms of influenza, and secondary endpoints involved multiple criteria including quantitative evaluation of influenza virus titers.

The applicant presented the microbiology portion of the NDA in several sub-sections. They include: the mechanism of action, anti-neuraminidase activity in vitro, antiviral activity in vitro, antiviral activity in animal models, and the development of resistance to the drug. Studies on the development of resistance were sub-divided into 3-sections: resistance to oseltamivir in vitro, resistance to Tamiflu in human challenge studies and resistance to Tamiflu in natural influenza virus infection. The microbiology review that follows addresses the data described, and includes a background devoted to the virology of influenza virus (1).

**Influenza virology:** Three influenza virus types A, B and C are members of a family of related enveloped viruses with a diameter in the range of 80 to 120 nM and of indistinguishable morphology. The division of influenza viruses into types A, B and C is based on the antigenic differences between their nucleoproteins and their matrix protein antigens.

Influenza viruses replicate in the nucleus of the infected cells. Their genome is segmented, single-stranded linear RNA. The viral RNA is 'negative-stranded' (i.e., the virion RNA is complementary to the messenger RNA) and thus the RNA itself cannot serve as messenger RNA for the synthesis of viral proteins. However, in infected cells, the negative-stranded influenza virus RNA is directly transcribed to generate positive-stranded RNA by the combined action of viral and host cellular enzymes. The positive-

stranded RNA then serves both as a template for the synthesis of the viral genomic RNA segments as well as viral proteins.

Influenza viruses A and B contain 8 segments of RNA and code for 10 proteins, which include two envelope proteins. The RNA segments 4 and 6 code for the viral envelope glycoproteins; the former codes for the glycoprotein hemagglutinin (HA), and the latter codes for the glycoprotein neuraminidase (NA). In contrast to influenza virus types A and B, the influenza virus C contains 7 segments and codes for 9 proteins with a single envelope glycoprotein. A single envelope glycoprotein is coded for by the RNA segment 4 of influenza virus C and is referred to as HEF, because it contains the hemagglutinin, esterase and fusion activities. Therefore, NA inhibitors, such as the approved drug Relenza, and the drug of this NDA, Tamiflu, as well as other experimental drugs undergoing FDA evaluation are expected to inhibit the replication of influenza viruses A and B but not influenza virus C.

The envelope glycoproteins, HA and NA, of influenza virus types A and B play an important role in the initiation of viral infection and pathogenicity. Several studies indicate that the envelope proteins, HA and NA function in a complementary manner in that they mutually compensate the receptor binding function. Influenza virus HA recognizes and binds to the cell receptors i.e., sialic acids (neuraminic acid) and the NA activity releases the virus from receptor sialic acid, thereby promoting the virus release. The molecular target of oseltamivir is the viral NA and more specifically the NA enzyme activity portion of the protein without direct effect on the antigenic properties of the NA. Considering the molecular interactions between HA and NA proteins and their complementary role in the viral infection, replication, spread, and pathogenicity, some additional molecular aspects of these two proteins are summarized briefly.

**Influenza virus hemagglutinin:** HA derives its name from its role in the agglutination of erythrocytes by attachment to specific sialic glycoprotein receptors. The HA is encoded by the 4th largest RNA segment and accounts for 25-27% of the virion protein that is distributed evenly on the surface of the virions. The HA is responsible for the attachment of the virus particle to the cell surface via a sialic acid receptor and also for subsequent fusion of viral and cell membranes which allows the entry of the virus genome into the cell cytoplasm. HA is the major antigen of the virus against which neutralizing antibodies are produced. The variability of HA has been the main factor in the continuing evolution of influenza epidemics and controlling those epidemics by immunization.

HA is a trimeric envelope glycoprotein. It is initially synthesized as a single 567-amino acid polypeptide that undergoes glycosylation. During infection, the 567 amino acid polypeptide (HA<sub>0</sub>) undergoes post-translational cleavage by cellular proteases. The cleavage results in the formation of two subunits, HA<sub>1</sub> (328 amino acids) and HA<sub>2</sub> (221 amino acids) that covalently attach to each other by disulfide bonds. The active HA is a trimer of this disulfide-linked HA<sub>1</sub>/HA<sub>2</sub> subunit. Cleavage of HA<sub>0</sub> to form HA<sub>1</sub> and HA<sub>2</sub> polypeptides is required for the viral infectivity because it is the HA<sub>2</sub> fragment that mediates fusion between the viral envelope and the host cell membrane.

**Influenza virus neuraminidase:** The target of oseltamivir is the viral NA. The function of NA is to cleave the sialic acids from the viral HA, viral NA itself, and host cell substrates. This cleavage is believed to prevent virus aggregation, elutes virus from infected cells, and facilitates trafficking of the virions to reach new cells for infection.

Influenza virus NA is a tetrameric envelope glycoprotein of identical subunits. The NA is coded by the 6th largest RNA segment of influenza virus and accounts for about 6% of the virion protein. NA, also known as sialidase or acylneuraminyl hydrolase, catalyzes the cleavage of the  $\alpha$ -ketosidic linkage between the terminal sialic acid and an adjacent sugar residue of glycoproteins, glycolipids, and oligosaccharides. Removal of the sialic acids by the NA assists both the entry and release of influenza virus from cells. The NA of human influenza viruses cleaves the NeuAc $\alpha$ 2,6 linkage. The HA of human influenza viruses preferentially recognize NeuAc $\alpha$ 2,6 linkage for binding and initiation of the infection process.

NA is a bifunctional protein in that it acts both as an enzyme, by removing sialic acids from glycoproteins, and as a major antigenic determinant, in that it can undergo antigenic variation. The enzyme plays a role in preventing aggregation of newly synthesized virus particles by the sialidase-catalyzed hydrolysis of acetylneuraminic acid, which is the receptor binding site for the HA of influenza virus. NA may also allow virus to move through the mucous present in the respiratory tract. NA has been crystallized and its three-dimensional structure determined (2). Oseltamivir is designed as a substrate analogue of the NA active site, therefore it should bind and inhibit its activity.

**Nomenclature of influenza virus subtypes:** As stated earlier, influenza viruses are divided into types A, B and C on the basis of antigenic differences of their nucleocapsid and matrix proteins. Influenza A viruses are further subdivided into subtypes on the basis of antigenic differences in the HA and NA. Fourteen HA, and 9 NA subtypes are recognized from all animals. However, to date only 3 HA (H1, H2 and

H3) and 2 NA (N1 and N2) subtypes have been recognized in epidemics of human influenza A infection. The most common subtypes of HA and NA combination that have been found to circulate in humans are H1N1, H2N2 and H3N2. The nomenclature system recommended by the World Health Organization include the virus type, geographic origin, isolate number and the year of isolation. The antigenic description of the HA and NA is given in parenthesis, e.g., A/Shanghai/11/87 (H3N2).

**Drug nomenclature:** In 1997 [redacted] submitted the original IND [redacted] for the evaluation of oseltamivir as a treatment of influenza virus infection. [redacted]

[redacted] Subsequently, Hoffmann-La Roche pharmaceuticals obtained the non-proprietary name, oseltamivir phosphate, for the drug substance and the trade mark, Tamiflu, for the drug product.

In the microbiology review, the name of the drug substance, oseltamivir, is used for antiviral activity studies in vitro and in animal models, and the name of the drug product, Tamiflu, is used in clinical studies, unless stated otherwise. Oseltamivir phosphate is the prodrug and its deacetylated product is the active form of the drug. In all of the in vitro studies and in animal studies the active form of oseltamivir or the oseltamivir prodrug were used, respectively. The drug component in the formulated form, Tamiflu, is oseltamivir phosphate.

## SUMMARY

**Mechanism of neuraminidase inhibition by oseltamivir:** Influenza virus NA is a glycohydrolase that cleaves the terminal  $\alpha$ -ketosidic linkage between the terminal sialic acid residue and an adjoining sugar residue found on a array of glycoproteins, glycolipids and oligosaccharides. Molecular studies on the x-ray crystal structure of NA defined the active site of the enzyme in 3-dimensions. Mechanistic studies of NA-directed catalysis revealed that the hydrolytic cleavage of the sialic acid proceeds through an oxonium cation transition-state. The dual knowledge of the enzyme active site and the catalytic mechanism through the transition state structure provided opportunities for rational design of NA inhibitors using computational methods. Oseltamivir is one of several inhibitors of NA rationally designed by logical substitutions on the sialic acid substrate-based template.

The function of NA in the influenza virus life cycle is not completely understood. The enzyme does not seem to play a direct role in viral entry, replication, assembly, or budding (3). Instead the enzyme binds to the sialic acid containing glycoconjugates and cleaves the terminal sialic acids. Therefore, NA is believed to facilitate the prevention of virus aggregation and promote virus trafficking through the mucus of the respiratory track.

The overall amino acid homology of the ten NAs (nine NA subtypes of influenza virus A and one of B) is low (~30%). However, the 21 amino acids that make up the catalytic site of the enzyme in all of the ten NAs are completely conserved. This highly conserved nature of the enzyme active site predict that rationally designed drugs like zanamivir, oseltamivir and other experimental inhibitors block the activity of the NA of different subtypes. Consistent with this expectation, oseltamivir was found to inhibit the activity of all of the NAs and the inhibitory concentrations were within a narrow range (see below).

The applicant selected oseltamivir as their choice for the inhibition of NA because of the inhibitor's higher binding affinity ( $K_i = \sim 0.3-1.7 \times 10^{-9}$  M) to the different NAs than the NAs affinity to the substrate ( $K_m = \sim 15-40 \times 10^{-6}$  M). This high differential binding property of oseltamivir by isosteric conformation permits competitive inhibition of the enzyme activity. Designers of sialic acid-based inhibitors (sialomimetics) also pointed out that mutants of the highly adaptable influenza virus could emerge by reversal in the differential binding properties of the substrate and inhibitor, i.e., preferential binding of the substrate over the inhibitor. Indeed NA mutants of influenza virus have been isolated from both in vitro and in clinical settings after exposure of the virus to oseltamivir (see below).

**Inhibition of neuraminidase activity by oseltamivir:** In the initial exploratory studies, the potential of oseltamivir to inhibit influenza virus NA activity was evaluated by determining the inhibitory constants ( $K_i$ ) for the enzymes derived from both influenza virus types A and B. In the NA enzymatic assays, a low molecular weight, fluorogenic synthetic compound, 2'-(4-methylumbelliferyl)- $\alpha$ -D-N-acetylneuraminic acid (MUNANA) was used as a substrate. The NA enzyme cleaves the  $\alpha$ -glycosidic linkage of the substrate, thereby releasing the fluorescent compound, 4-methylumbelliferone, which can be measured fluorimetrically.

Table 1. Kinetic constants for the inhibition of influenza viral A and B neuraminidase subtypes by oseltamivir and zanamivir

Virus	$K_i$ (nM) <sup>a</sup> Oseltamivir	$K_i$ (nM) Zanamivir
A/PR/8/34 (H1N1)	0.5	0.3
A/Victoria/3/75 (H3N2)	0.2	0.9
4/Tokyo/3/77 (H2N2)	0.2	Nd <sup>b</sup>
B/Lee/40	1.2	1.1
A/Mississippi/9/93	3.6	Nd

a =  $K_i$ : values were determined using MUNANA as substrate

b = Nd: not determined

Results in Table 1, show that oseltamivir inhibits the NA activity of laboratory strains of influenza A/N1, A/N2 and B with a  $K_i$  of less than 4 nM. Zanamivir (an approved drug for the treatment of influenza), an inhibitor of NA activity, showed similar inhibitory activity against the NAs of laboratory isolates.

In additional studies, the sponsor determined the ability of oseltamivir to inhibit the NA activity of multiple laboratory strains and clinical isolates of human influenza viruses. As shown in Table 2, oseltamivir inhibited the activity of all NAs tested with an  $IC_{50}$  (concentration of the inhibitor required to inhibit 50% of the enzyme activity) of less than 3 nM. Comparison of NA inhibitory concentrations of oseltamivir and zanamivir show that both drugs inhibit the activity of NA at similar concentrations.

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Table 2. Inhibition of the neuraminidase activity of influenza A and B viruses by oseltamivir

Virus	Oseltamivir <sup>a</sup>		Zanamivir <sup>a</sup>
	IC <sub>50</sub> (µg/ml)	IC <sub>50</sub> (nM)	IC <sub>50</sub> (nM)
<b>Clinical Isolates</b>			
A/Texas/36/91 (H1N1)-like	0.00014	0.5	0.3
A/Taiwan/1/86/(H1N1)-like	0.00037	1.3	0.5
A/Victoria/2/87 (H3N2)-like	0.00020	0.7	2.6
A/Virginia/305/95 (H3N2)	0.00003	0.1	0.6
A/Shangdong/9/93 (H3N2)-like	0.00006	0.2	0.7
B/Beijing/184/93-like	0.00074	2.6	1.2
B/Victoria/2/87-like	0.00074	2.6	1.4
<b>Laboratory Strains</b>			
A/WS/33/ (H1N1)	0.00028	1.0	0.7
A/Texas/36/91 (H1N1)	0.00011	0.4	0.5
A/Victoria/3/75 (H3N2)	0.00014	0.5	1.7
A/Port Chalmers/1/83 (H3N2)	0.00009	0.3	1.1
A/Johannesburg/33/94 (H3N2)	0.00023	0.8	4.6
A/NWS/G70C	0.00009	0.3	0.9
A/NWS/G70C	0.00011	0.4	1.0
B/Mass/3/66	0.00023	0.8	1.7
B/Hong kong/5/72	0.00048	1.7	1.0
B/Harbin/07/94	0.00057	2.0	2.1

a = IC<sub>50</sub> values determined using 200 µM MUNANA as substrate.

To evaluate the capability of oseltamivir to inhibit the NAs of human and animal influenza viruses the applicant also determined the effect of oseltamivir on all 9 subtypes influenza virus A neuraminidases as well as influenza virus B neuraminidase. The results in Table 3 show that oseltamivir inhibited all of the ten NA subtypes of influenza viruses. The IC<sub>50</sub> in all cases was in the range of 0.3 to 1.7 nM. This relatively uniform inhibitory concentration of all NAs was anticipated because of the conserved nature of the NA active site and the rational design of the drug to bind to the active site and inhibit activity of the enzyme.

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Table 3. Inhibition of the ten influenza neuraminidase subtypes by oseltamivir

Virus	Subtype	IC <sub>50</sub> (nM)
A/WS/33	A/H1N1	1.0
A/Japan /305/57	A/H2N2	0.3
A/Duck/Germany/1215/73b	A/H2N3	0.3
A/Turkey/Ontario/6118/68b	A/H8N4	0.4
A/Duck/Alberta/60/76b	A/H12N5	1.5
A/Duck/Czechoslovakia/56b	A/H4N6	0.4
A/Chick/Germany/N/49b	A/H10N7	0.8
A/Duck/Ukraine/1/63b	A/H3N8	0.8
NWS/G70C	A/H1N9	0.3
B/Hong Kong/5/72	B	1.7

**Specificity of neuraminidase inhibition:** Neuraminidases are ubiquitous enzymes that participate in multiple essential metabolic activities. In order to evaluate the specificity of oseltamivir, the applicant determined the inhibitory activity of oseltamivir against one purified human NA (from liver), two NAs from other viruses, two NAs from 2 strains of bacteria and two NAs from two different tissues of the rat (Table, 4). In these enzyme specificity studies, the low molecular weight synthetic substrate, MUNANA, was used. Results of these studies suggest that oseltamivir is a weak inhibitor of the human lysosomal NA. In comparison to the influenza virus NA the concentration of oseltamivir required for the inhibition of human NA was greater by at least five orders of magnitude than the concentration required for inhibition of influenza virus NA. The inhibitory activity of oseltamivir against other viral, bacterial, and mammalian NAs showed substantially weaker inhibitory activity compared to that of influenza virus NA. Based on this limited data the sponsor concluded that oseltamivir is a specific inhibitor of influenza virus NA.

Humans have many endogenous NAs that participate in a range of metabolic activities. The limited data (lack of activity against a single human NA) are insufficient to draw the conclusion of the selective inhibition of oseltamivir to influenza virus NA. Furthermore, in these studies the purity of the enzymes, the metal ion requirements for optimal activity of the enzymes, the substrates and a variety of other factors were not normalized or optimized. In brief, the studies reported on the selectivity of oseltamivir for inhibition of influenza NA are inadequate to draw conclusions on its selectivity.

Table 4. Inhibition of neuraminidase activity from different sources by oseltamivir

Neuraminidase Source	K <sub>i</sub> (nM)
<b>Infuenza Virus</b>	
A/PR/8/34 (H1N1)	0.2
A/Victoria/3/75 (H3N2)	0.8
B/Lee/40	1.2
<b>Other viral</b>	
Parainfluenz virus	>500,000
Newcastle disease virus	>500,000
<b>Bacterial</b>	
<i>C.perfringens</i>	370,000
<i>V.cholerae</i>	>500,000
<b>Mammalian</b>	
Human liver	>500,000
Rat liver	>500,000
Rat uterus	>500,000

K<sub>i</sub> values determined using MUNANA substrate.

The low molecular weight synthetic substrate (MUNANA) used in the anti-NA activity studies represents the minimal molecular requirements for cleavage by the viral NA. The natural substrates for NA are numerous and of multiple molecular forms (glycoproteins, glycolipids and oligosaccharides) and conformations. The sialic acid in the natural substrates may be hidden with limited accessibility to the enzyme compared to that of the simplistic substrate. The IC<sub>50</sub> values determined using natural substrates is predicted to be higher than that with the simple substrate used in all of the studies reported in the NDA.

**In Vitro anti-influenza virus activity:** The applicant carried out several studies to determine the anti-influenza virus activity of oseltamivir. In these studies, both laboratory adapted and clinical isolates of influenza A and B viruses were used. In parallel experiments, the antiviral activity of oseltamivir was compared to another influenza virus NA inhibitor, zanamivir, a drug recently approved for the treatment of influenza. The in vitro antiviral activity studies have evaluated the effect of oseltamivir on: (a) influenza virus induced plaque formation on indicator MDCK cells, (b) virus replication as measured by the yield of virus in the culture medium of influenza virus

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infected MDCK cells, and (c) the reduction in cytopathic effect of influenza virus due to the treatment of cells with oseltamivir.

Table 5. Inhibition of the replication of laboratory strains of influenza viruses by oseltamivir.

Virus	IC <sub>50</sub> (nM)	
	Oseltamivir	Zanamivir
<b>Plaque reduction assay</b>		
A/WS/33 (H1N1)	21	21
A/Fort Meyers/1/47 (H1N1)	24	20
A/Victoria/3/75 (H3N2)	0.56	2.8
A/Port Chalmers/1/73 (H3N2)	0.77	3.1
B/Mass/3/66	124	140
B/Hong Kong/5/72	155	200
A/nws/g70c (H1N9)	1.1	9.0
<b>Virus yield assay</b>		
A/nws/G70c (H1N9)	11	90
<b>CPE assay</b>		
A/Texas/36/91 (H1N1)	99	79
A/WS/33 (H1N1)	26,000	60,000
A/Johannesburt/32/94 (H3N2)	52	230
A/Victoria/3/75 (H3N2)	200	400
A/Shangdong/09/93 (H3N2)	1,800	10,000
B/Harbin/07/94	93	29
B/Hong Kong/5/72	2,300	1,200

Results presented in Table 5, show that oseltamivir was active against different laboratory isolates influenza virus with a broad range of sensitivity. In this set of experiments the IC<sub>50</sub> values for oseltamivir ranged from 0.56 nM to 26.0 μM. In parallel experiments, the inhibitory activity of oseltamivir was also compared with zanamivir. The IC<sub>50</sub> for zanamivir was comparable to that of oseltamivir and ranged from 2.8 nM to 60 μM. In several other studies the applicant determined the inhibitory concentrations of a broad range of viruses including laboratory and clinical isolates (results not shown). In

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these studies the IC<sub>50</sub> and IC<sub>90</sub> ranged from 0.0008 μM to >35 μM and 0.004 μM to >100 μM, respectively.

Comparisons of the results of anti-NA enzyme activity with anti-influenza viral activity by oseltamivir show that they are in marked contrast to each other. The viral NA is highly sensitive with inhibitory effects in a narrow range (6-fold) for all of the subtypes of NAs (Table 3). On the other hand the range of antiviral activity was extremely broad (>40,000-fold) indicating wide variation in the IC<sub>50</sub> for viral replication (Table 5). This result indicates that different subtypes of influenza virus have a widely varying need of NA activity for viral replication.

**Selectivity of oseltamivir:** To distinguish cytotoxic effect from antiviral effect of oseltamivir, the applicant investigated the in vitro cytotoxicity of the drug on MDCK cells. In parallel experiments, the antiviral activity of oseltamivir against 4 influenza viruses and cytotoxicity on MDCK cells were determined. In these studies, the test cells were exposed to different concentrations of the drug for 4 days with appropriate controls in parallel and the cell viability determined. Results in table 6 shows the therapeutic index of oseltamivir is in the range of >39 to > 5000.

Table 6: Antiviral activity and cytotoxicity of oseltamivir

Virus	IC <sub>50</sub> (μM)	CC <sub>50</sub> (μM)	TI
A/NWS/33 (H1N1)	26.0	>1000	>39
A/Victoria/3/75 (H3N2)	0.2	>1000	>5000
A/Shangdong/09/93 (H3N2)	1.8	>1000	>555
B/HongKong/5/72	2.3	>1000	>435

IC<sub>50</sub> = 50% virus inhibitory concentration

CC<sub>50</sub> = 50% cell inhibitory concentration

TI = Therapeutic index (IC<sub>50</sub> ÷ CC<sub>50</sub>)

The applicant stated that in other studies (data not shown) the cytotoxic effect of oseltamivir to five established cell lines of epithelial or fibroblastoid origin (MDCK, MRC-5, VERO, MK and 293 cells) which supported influenza virus replication were tested for cytotoxicity of oseltamivir. These cells were exposed to different concentrations of the drug for 5 days with appropriate controls in parallel and the cell viability determined. There was no detectable cytotoxic effect on these cells when exposed to oseltamivir concentrations of up to 100 μM.

**Efficacy of oseltamivir in animal models of influenza virus infection:** Three animal models of influenza virus infection, mouse, ferret and chicken, are conventionally used to evaluate the anti-influenza virus activity of test compounds. In these animal models, the influenza infection is predominantly confined to the respiratory tract, as is the case with the majority of human influenza virus infections. The ferret model more closely matches the clinical course of human infection in that the infected ferrets develop a self-limited disease with signs similar to those observed clinically in humans: fever, increased nasal signs and lethargy. In the mouse model, infected animals develop pneumonia and show high mortality rate. Chickens infected with highly pathogenic avian influenza virus strains develop disseminated infection with very high mortality rate.

The applicant investigated the prophylactic and therapeutic anti-influenza virus efficacy of oseltamivir in the mouse and ferret models. The proposed NDA is for a treatment indication of influenza, therefore the review of the data mainly deals with the therapeutic effect of oseltamivir in the mouse and ferret models.

Efficacy in the mouse model: Female pathogen-free BALB/c mice were anaesthetized by intra-peritoneal injection of phenobarbital and infected intranasally with a 90% lethal dose of mouse-adapted influenza A/NWS/33 (H1N1). The volume of the inoculum or the infectious titer of the influenza virus was not stated. Each of the treatment arms contained 10 infected animals, and the placebo arm contained 26 infected animals. Treatment with oseltamivir was initiated at 24, 36, 48 or 60 hours post-infection with the treatment (10 mg/kg/day) continuing twice daily for 5 days. Deaths were noted daily for 21 days, and the arterial oxygen saturation (SaO<sub>2</sub>) determinations were made on days 3 to 10. The applicant stated that toxicity controls were not included because the concentration of the drugs used in this experiment was well tolerated (data not shown).

Results presented in Table 7 show that treatment with oseltamivir increased the mean survival time and survival benefit compared to the infected untreated control. Treatment with oseltamivir provided complete protection against lethal effects of infection even when therapy was initiated at 60 hours post infection. SaO<sub>2</sub>, used as an indicator of the extent of pulmonary disease in the surviving animal, showed no significant change between treated and untreated infected animals. The applicant has not reported the effect of the drugs on virus titer and further if the effect is dose related in the mouse model. The therapeutic effect of oseltamivir on influenza virus B infection in this model was not presented.

Table 7. Effect of oseltamivir on influenza virus infections in mice

Compound	Treatment initiation post-infection (h)	Surv/ Total	MST <sup>a</sup> (days)	Mean SaO <sub>2</sub> <sup>b</sup> (%)
Oseltamivir	24	10/10	>21	87.0
	36	10/10	>21	86.4
	48	10/10	>21	86.3
	60	10/10	>21	87.0
Saline	24	4/26	11.3	85.2

a = Mean survival time of mice dying on or before day 21.

b = Mean of days 3 to 10 readings.

Efficacy in the ferret model: Adult ferrets (average weight 1.4 kg) under anaesthesia were inoculated intranasally with 10<sup>6</sup> 50% egg infective dose of influenza virus A/England/939/69 (H3N2). Oseltamivir was dosed orally b.i.d. 2-hours post-infection for 3 days at 5 mg/kg or at 25 mg/kg. Control ferrets received vehicle alone. The infected ferrets were assessed for infection in the upper respiratory tract (URT) by virus titration of nasal lavage, pyrexia, and inflammatory response to infection (the influx of leukocytes) by counting cells in the nasal lavage, and for infection in the lower respiratory tract by virus titration in the lung homogenates.

The applicant stated that there was no difference in the influenza virus titers between the untreated group and those animals dosed at 5 mg/kg dose, whereas, an effect was seen at the higher dose of 25 mg/kg at 30 and 36 hours post-infection. At both doses oseltamivir substantially reduced the febrile response and reduced nasal cell influx. The applicant stated that, "there was no indication that the lower respiratory virus was reduced in the lung homogenates at 96 hours post-infection in any of the groups of ferrets. Control and treated animals had similar titer of virus," (data not shown).

The over all results of influenza virus infection in the ferret model were mixed. Based on the data, the applicant stated that oseltamivir, "appears to be a potent inhibitor of influenza virus induced disease. However, as gauged by the compound's ability to inhibit viral replication in the URT, the compound does not appear to be an effective inhibitor." The applicant repeated the experiment in the ferret model and again concluded that; "while the virus titers seemed to be little affected by the antiviral, cell counts were considerably suppressed."

**Variation in influenza virus and mechanisms of drug resistance development:** As an RNA virus, the influenza viruses inherently exhibit high mutation rates that are primarily attributed to the lack of proofreading during RNA replication. The estimated mutation rate of  $1.5 \times 10^{-5}$  mutations per nucleotide per infectious cycle of influenza viruses is similar to other RNA viruses (1). This high rate of mutation itself provides an ample source of selection of naturally occurring variants. In addition, the rate of mutation for some of the component genes of influenza virus varies. The mutation rate for HA (a neutralizing antigen) is estimated at  $6.7 \times 10^{-3}$ , suggesting that the accelerated rate of mutation in this gene may be selected by immune pressure (1). The combined effects of accumulation of point mutations due to replication errors and immune pressure accelerate the genetic drift.

In addition to genetic drift other events such as RNA recombination, genetic reassortment among and between human and animal strains due to the segmented nature of the viral genome and other genetic effects may further enhance the rate of variation contributing to genetic shifts, drug resistance, and potential pathological consequences.

Available evidence on the inhibition of anti-influenza virus activity by the use of sialomimetic drugs designed to block NA activity appear to induce yet another type of variation in influenza virus. Previous studies with NA inhibitors indicate those genetic changes in HA alone without changes in the activity or sensitivity of NA, appear to be another mechanism of escape from NA inhibition. Thus, multiple mechanisms (inherent, induced and those due to molecular interactions) come into play to overcome drug pressure and allow the virus to rapidly adapt to new drug challenges.

**Resistance to oseltamivir in vitro:** The applicant investigated the potential of oseltamivir to induce changes in susceptibility of influenza viruses as a consequence of exposure to the drug. In these studies recipient MDCK cells infected with different laboratory strains of human influenza viruses [A/Victoria/3/75 (H3N2), A/Port Chalmers/1/73 (H3N2), A/WS/33 (H1N1) and B/Hong kong/5/72] were passaged in vitro in increasing concentrations of the drug. The resulting virus isolates were then sequentially analyzed for changes in their phenotype and genotype.

It is important to note that in all of the susceptibility studies, the experiments were designed to decrease the likelihood of selecting for HA mutants with reduced susceptibility to oseltamivir (4) but were designed to identify mutants in NA with reduced sensitivity to oseltamivir. This selection was achieved by modifying the virus

infection procedure to eliminate that virus population which binds weakly to the sialic acid receptor because of mutations in the HA. In the modified infection procedure, virus was only allowed to adsorb for 15 min at 37°C instead of the conventional adsorption of 60 min. Any weakly bound virus which had not completed the adsorption and initiated the penetration step was washed off with pre-warmed phosphate buffered saline.

The applicant defined phenotypic resistance as decrease in the sensitivity of NA activity as determined by an in vitro enzyme assay with a synthetic low molecular substrate, MUNANA. The genotypic basis of resistance, defined as mutations in the NA or HA or both, was determined by sequencing most of NA gene and, in selected cases part of the HA gene by reverse transcriptase mediated polymerase chain reaction (RT-PCR).

Table 8 shows the genotypic and phenotypic results for seven virus selections using 5 different laboratory strains of human influenza virus. The results indicate that 6 of the 7 selections had mutations in the NA gene that correlated with a decrease in the sensitivity (4 to 30,000-fold) of NA to oseltamivir, compared to the NA of parent. In three independent selections (using 2 different H3N2 viruses), a conservative substitution of arginine at amino acid position 292 to lysine (represented as Arg292Lys) in the NA active site was observed. This mutation correlated with a 30,000-fold decrease in sensitivity to oseltamivir when tested (Table 8).

The effect of Arg292Lys mutation on the NA was characterized by use of the in vitro enzyme assay. Data presented in table 9 show that the mutation, Arg292Lys, caused a reduction in both the affinity of the enzyme for the substrate ( $K_m$ ) and its relative activity ( $V_{max}$ ). Most importantly, the single mutation appeared to be responsible for a 30,000-fold reduction in sensitivity to oseltamivir, effectively negating the inhibitory effect of the drug on this mutant enzyme. These results demonstrate that the mutation compromised both the enzyme activity and sensitivity to oseltamivir.

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Table 8. In vitro selection of human influenza virus neuraminidase mutants with decreased sensitivity to oseltamivir.

Selection #	Virus	Passage # <sup>a</sup>	Neuraminidase Mutation	Oseltamivir Sensitivity (fold change)
1	A/Victoria/3/75 (H3N2)	12	R292K	30,000
2	A/Victoria/3/75 (H3N2)	12 17	I222T R292K	4 30,000
3	A/Port Chalmers/1/83 (H3N2)		R292K	nd <sup>b</sup>
4	A/Texas/36/91 (H1N1)	7 12	I222V I222V, H274Y	2 1000
5	A/WS/33 (H1N1)	14	H274Y	200
6	A/WS/33 (H1N1)	11	H274Y	300
7	B/Lee/40		None	

a = Passage number at which mutation was detected.

b = nd: not determined.

Results in Table 8 also show that in addition to the Arg292Lys mutation a threonine substitution for the isoleucine residue at residue 222 (Ile222Thr) in the NA active site in A/Victoria/3/75 (H3N2) was detected. This mutation produced a 4-fold decrease in NA sensitivity to oseltamivir. The applicant stated that this mutant did not survive subsequent passage in the presence of increasing concentrations of the inhibitor and therefore could not be followed further.

Table 9. Characterization of neuraminidase of influenza A/H3N2 mutant selected in vitro in the presence of oseltamivir

Virus	Amino acid at residue 292	MUANA Substrate		Oseltamivir K <sub>i</sub> (nM)
		K <sub>m</sub> (μM)	Relative V <sub>max</sub>	
A/Victoria/3/75	Arg (wt)	84	17	0.13
Mutant (292K)	Lys	337	1	3.600

Passage of the H1N1 strains (A/Texas/36/91 and A/WS/33) in increasing concentrations of oseltamivir resulted in the emergence of mutations at Ile222Val and His274Tyr in the active site of the NA. The Ile222Val single mutation showed a minor change in sensitivity to oseltamivir. The single mutation His274Tyr appeared to cause a 200 to 300-fold decrease in NA sensitivity to oseltamivir. In contrast, NA containing this mutation in addition to the Ile222Val mutation was more than 1000-fold less sensitive to inhibition by oseltamivir than the wild type enzyme. However, in contrast to what was observed with the Arg292Lys mutation, the His274Tyr mutation did not change the affinity of the enzyme for the substrate (Table 10).

Table 10. Characterization of neuraminidase of influenza A/H1N1 mutant selected in vitro in the presence of oseltamivir

Virus	Amino acid at residue 274	MUANA Substrate		Oseltamivir IC <sub>50</sub> (nM)
		K <sub>m</sub> (μM)	Relative V <sub>max</sub>	
A/WS/33	His (wt)	41	1	4
A/WS/33-mut	Tyr	46	1	800

The applicant stated that although the mutations in NA caused significant reductions in sensitivity to oseltamivir in vitro, these mutations markedly reduced viral infectivity in mice and suggested that the NA mutations with reduced sensitivity to oseltamivir are likely to be of limited clinical significance (data not shown).

**Oseltamivir resistance due to mutations in hemagglutinin:** It was previously demonstrated that decreased susceptibility to NA inhibitors during in vitro selection can occur due to changes in the targeted NA or in the non targeted viral HA or in both. It was also observed that HA mutants have been easier to generate than the NA mutants and that resistance mutations in HA precede the mutations in the NA (5).

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Table 11. Inhibition of wild-type and variant viruses in a plaque reduction assay

	Encoded Amino acid <sup>a</sup>			Fold change in IC <sub>50</sub> <sup>b</sup>	
	292 in	28 in	124 in		
	NA	HA1	HA2	Oseltamivir	Zanamivir
A Victoria/ 3/75	Arg(wt)	Arg(wt)	Arg(wt)	1(0.008μm)	1(0.033μM)
Passage 8 <sup>c</sup>	Arg	Thr	Met	8.6	8.5
12-B1	Arg	Thr	Met	6.9	6.4
12-S3	Lys	Thr	Met	3.230	6.0

a = Genotypic results are indicated for amino acid 292 of the neuraminidase (NA), amino acid 28 of sub-unit 1 of the hemagglutinin (HA1), and amino acid 124 of sub-unit 2 of the hemagglutinin (HA2).

b = Data are shown as fold change in IC<sub>50</sub> relative to values obtained for the wild-type virus passaged in MDCK cells in the absence of inhibitor. Numbers shown in parentheses indicate IC<sub>50</sub> values for wild-type virus passaged in MDCK cells. IC<sub>50</sub> values are from a single experiment in which all samples were tested in parallel.

c = IC<sub>50</sub> values are given for a plaque purified variant isolated from virus harvested after eight passages in the presence of oseltamivir.

To investigate the potential emergence of HA resistance to oseltamivir, influenza A/Victoria /3/75 (H3N2) virus was passaged in increasing concentrations of the drug in MDCK cells. Results of these studies are summarized in table 11. After the eighth passage, the virus showed a decrease in susceptibility to oseltamivir. Genotypic analysis of the NA and HA genes showed no mutations in the NA but showed two mutations in the HA gene resulting in an Ala28Thr substitution of HA1 and an Arg124Met substitution of HA2. These mutations were not located in the sialic acid binding (receptor) site of the HA but instead were located in the stalk region of the HA. This HA variant virus compared to the parent virus was approximately 9-fold less susceptible to both zanamivir and oseltamivir as determined by plaque reduction assay.

Further passage of the HA mutant resulted in a selection of new variants with similar decreases in susceptibility to oseltamivir (Table 11). Passage 12 virus showed two populations, 12-B1, and 12-S3. The 12-B1 variant exhibited the same mutations as the passage 8 variants with HA mutations, but without NA mutations. The 12-S3 variant, in addition to the HA mutations, exhibited a NA mutation at Arg292Lys. The 12-S3 variant containing mutations in both HA and NA showed a similar decrease in susceptibility to both oseltamivir and zanamivir. These mutations in the variants have been shown to be

stable since after 10 passages in the absence of oseltamivir the variant genotype and phenotype were maintained.

In summary, resistance to oseltamivir in influenza virus can occur. The resistance could be due to mutations in the viral HA, in the viral NA, or both in the HA and NA. This pattern of emergence of resistance to oseltamivir is similar to that reported for zanamivir (an influenza virus NA inhibitor approved for the treatment of influenza).

**Resistance to oseltamivir in challenge studies:** The applicant conducted three influenza virus challenge studies in sero-susceptible (hemagglutinin-inhibition activity titers of  $\leq 1:8$ ) human volunteers. In one study challenge strain A/Texas/36/91 (H1N1) was used and in the other two studies challenge strain B/yamagata/16/88 was used. Influenza virus isolates derived from the nasal washings of pre-treatment and post-treatment matched virus were evaluated for potential emergence of resistance to oseltamivir.

To obtain sufficient amounts of influenza virus for the NA assay, nasal wash samples from influenza virus infected and treated subjects were propagated in MDCK cells. NA inhibition assays were performed using detergent-solubilized virus from the MDCK cell culture supernatant as the source of the enzyme, and MUNANA as the substrate. The  $IC_{50}$  (concentration of the inhibitor necessary to reduce NA activity by 50% relative to the activity containing no inhibitor) of NA for the placebo and Tamiflu treated isolates was determined.

Phenotypic resistance of NA to the inhibitor was defined as a measurable decrease in the in vitro sensitivity. Resistance to the inhibitor is said to occur when the  $IC_{50}$  of the post-treatment virus NA was greater than the mean + 2 SD of the pre-treatment virus NA of the corresponding influenza virus subtype. Results from matched isolates of a total of 102 subjects (53 from influenza A challenge study and 49 from influenza B challenge studies) showed that three of the isolates (all from Influenza virus A challenge study) had phenotypic resistance to oseltamivir anti-NA activity in vitro.

To identify the genotype responsible for the reduced sensitivity of NA to oseltamivir, the nucleotide sequence of NA for the three resistant isolates was determined. Viral RNA of the control virus inoculum and the post-treatment resistant virus isolates was converted to DNA by RT-PCR and the nucleotide sequence of the product DNA encompassing the NA active site (amino acids 100-400) was determined. Comparison of the NA amino acid sequences of the 3 virus isolates and the control inoculum showed a tyrosine

substitution for the conserved amino acid histidine at amino acid position 274 (His274Tyr) in the NA active site.

Characterization of the mutant NA containing the His274Tyr substitution showed a 400-fold reduced sensitivity to oseltamivir but without significant effect on the affinity of the enzyme to the substrate ( $K_m$ ) or the activity of the enzyme ( $V_{max}$ ). In antiviral activity assays, the His274Tyr mutation in the NA of A/texas/36/91 (H1N1) reduced the whole virus sensitivity to inhibitor by 3000-fold and in anti-NA enzyme assays the mutation caused a 400-fold reduction in sensitivity of the enzyme to the inhibitor.

In the challenge studies, 3% (3/102) of the treated isolates developed resistance to the drug as determined by changes in susceptibility of the NA enzyme to the drug. In these studies, the contribution of HA toward resistance has not been evaluated. The mutation, His274Tyr, that was selected in vivo in the challenge studies has also been selected in vitro when the same challenge strain [A/texas/36/91 (H1N1)] was passaged in presence of oseltamivir (Table 8). The phenotypic resistance profiles of the in vivo-selected and in vitro-selected resistant mutants were similar. These results suggest that the development and nature of in vitro resistance to oseltamivir is predictive of in vivo resistance.

**Resistance to oseltamivir in naturally acquired infection:** In clinical studies of naturally acquired infection with influenza virus the applicant evaluated pre-treatment and post-treatment matched virus isolates from 301 influenza virus infected and treated subjects. The manner of influenza virus sample collection in clinical studies involving natural infection was different from the challenge studies in that in the former nasal swabs were used as a source of virus, and in the latter, nasal washings were used as the source of the virus. Specimen collection for virus recovery by nasal wash is considered more efficient than by nasal swabs, and therefore, results derived from nasal washings are likely to be more accurate than those of swabs.

Phenotypic analysis of the clinical isolates of influenza virus, determined by change in the sensitivity of NA to oseltamivir, showed that four out of the 301 post-treatment isolates had a large decrease in NA sensitivity, indicating the emergence of oseltamivir resistant influenza variants. In addition, there were also several other post-treatment isolates that showed marginal decreases of NA inhibition by oseltamivir but the applicant considered them non-resistant because of the marginal change in the sensitivity of the NA. All of the four resistant isolates were of the H3N2 subtype. The overall incidence of decreased drug susceptibility to NA was 1.3%.

To define the genotypic basis for the decrease in NA sensitivity to the inhibitor, the nucleotide sequence of the NA gene from the 4 resistant isolates was determined. Comparison of the NA amino acid sequences of the 4 resistant isolates and the pre-treatment control virus showed single mutations in the NA of each of the 4 resistant phenotypes. Three of the four resistant isolates had an Arg292Lys mutation and the fourth resistant isolate had a Glu119Val mutation.

Characterization of the influenza virus carrying the NA mutation, Arg292Lys in an A/victoria/3/75 (H3N2) background, showed a 30,000-fold decrease in sensitivity to NA by the inhibitor. The catalytic activity of NA was decreased to about 2% of that of the wild type enzyme with effects on both the affinity of the enzyme to the substrate ( $K_m$ ) and the activity of the enzyme ( $V_{max}$ ). The Glu119Val mutation in the NA of influenza virus increased the enzyme's affinity to the substrate slightly and decreased the sensitivity of enzyme to the inhibitor by 20 to 40 folds.

In clinical studies of natural infection with influenza virus 1.3% (4/301) of the treated isolates developed resistance to the drug as evaluated by changes in susceptibility to NA enzyme only. In these studies, the contribution of HA toward resistance is not known. The mutation, Arg292Lys, that was selected in vivo in the clinical studies has also been selected in vitro when influenza A virus (H3N2) was passaged in presence of oseltamivir (Table 8). The resistance profiles of the in vivo-selected and in vitro-selected resistant mutants were similar. The resistance evaluations in clinical studies indicate that in vitro resistance to oseltamivir is predictive of in vivo resistance. The properties of resistant clinical isolates are similar to that of the in vitro generated mutants.

**Cross-resistance:** Resistance to sialomimetics conferred by the viral NA was primarily due to mutations in the active site of the enzyme where the drugs were designed to tightly bind and interfere with the natural substrate, the sialic acid. In resistance studies with oseltamivir both the in vitro and clinical isolates showed a decrease in sensitivity to the drug that correlated with mutations at amino acid positions: 119, 222, 274 and 292. Each one of these mutations imparted a decrease in the sensitivity of the enzyme to oseltamivir, but the magnitude of resistance was variable. Each of these mutations has also shown varying degrees of cross-resistance to zanamivir.

Genotypic analysis of oseltamivir resistant in vitro generated and clinical isolates showed that two of the oseltamivir generated mutations were the same as those found in zanamivir resistant in vitro generated mutants (at amino acid positions Arg292Lys and Glu119Val). There was also unique, drug-specific NA mutations that were not shared

between oseltamivir and zanamivir. The sponsor investigated the cross-resistance of oseltamivir resistant mutants with zanamivir. The Arg292Lys mutation reduced the sensitivity of NA to zanamivir by 20 to 30-fold but the reduction in sensitivity to oseltamivir was several thousand fold. On the other hand the Glu119Val mutation reduced the activity of zanamivir by 100-fold but the reduction in NA sensitivity to oseltamivir was 20-fold. Varying degrees of cross-resistance has also been reported among other rationally designed experimental inhibitors of NA (6).

Resistance to sialomimetic drugs occur by more than one mechanism; due to mutations in the viral NA, or viral HA or both. Mutations in the HA that reduce the affinity for receptor binding appear to circumvent the need for significant NA activity for virus release. Such HA mutants that alter the host cell-virus interactions predict cross-resistance, among all neuraminidase inhibitors (6). Thus, zanamivir (recently approved for treatment of influenza) resistance due to mutations in HA is expected to be cross-resistant to oseltamivir and to all other sialomimetics and vice versa. In vitro resistance to oseltamivir due to mutations in the HA was observed. The effect of oseltamivir-associated mutations for cross-resistance to other NA inhibitors was not been tested directly but is predicted to impart cross-resistant to other NA inhibitors.

**Immune response:** The applicant determined the effect of Tamiflu on humoral immune response, by comparing the pre-treatment and the post-treatment viral antibody titers in the respective sera. Results of these studies showed that the antibody titers were similar in the placebo and Tamiflu treated sera of subjects with natural influenza virus infection as well as in the sera of influenza virus challenge subjects. In addition, the magnitude of increase in antibody titer was similar in the placebo and Tamiflu treated sera. In approximately 90% of the subjects in each group, there was an increase in antibody titer of at least 4-fold. These results indicate that in both naturally acquired infection and experimental infection with influenza virus, treatment with Tamiflu did not appreciably impair humoral antibody response to influenza virus infection. However, the applicant has not conducted an influenza virus vaccine interaction study.

## CONCLUSIONS

In vitro anti-NA activity studies demonstrate that oseltamivir is a strong, competitive inhibitor of the NA of influenza virus A and B. In vitro antiviral activity studies show that oseltamivir inhibits the replication of laboratory strains and clinical isolates of influenza virus. The in vitro anti-NA activity and antiviral activity data are consistent

with the observed antiviral efficacy in animal models and in experimental infection of human volunteers.

The applicant stated that oseltamivir is specific to the influenza virus NA by comparing the drug's effect on a single human enzyme. The extent to which oseltamivir compromises the endogenous activities of multiple human NAs was not adequately evaluated, and therefore, the conclusion that oseltamivir is specific for viral NA could not be supported. However, long term exposure of animals to oseltamivir appear to show no significant toxicity (see Pharmacology review).

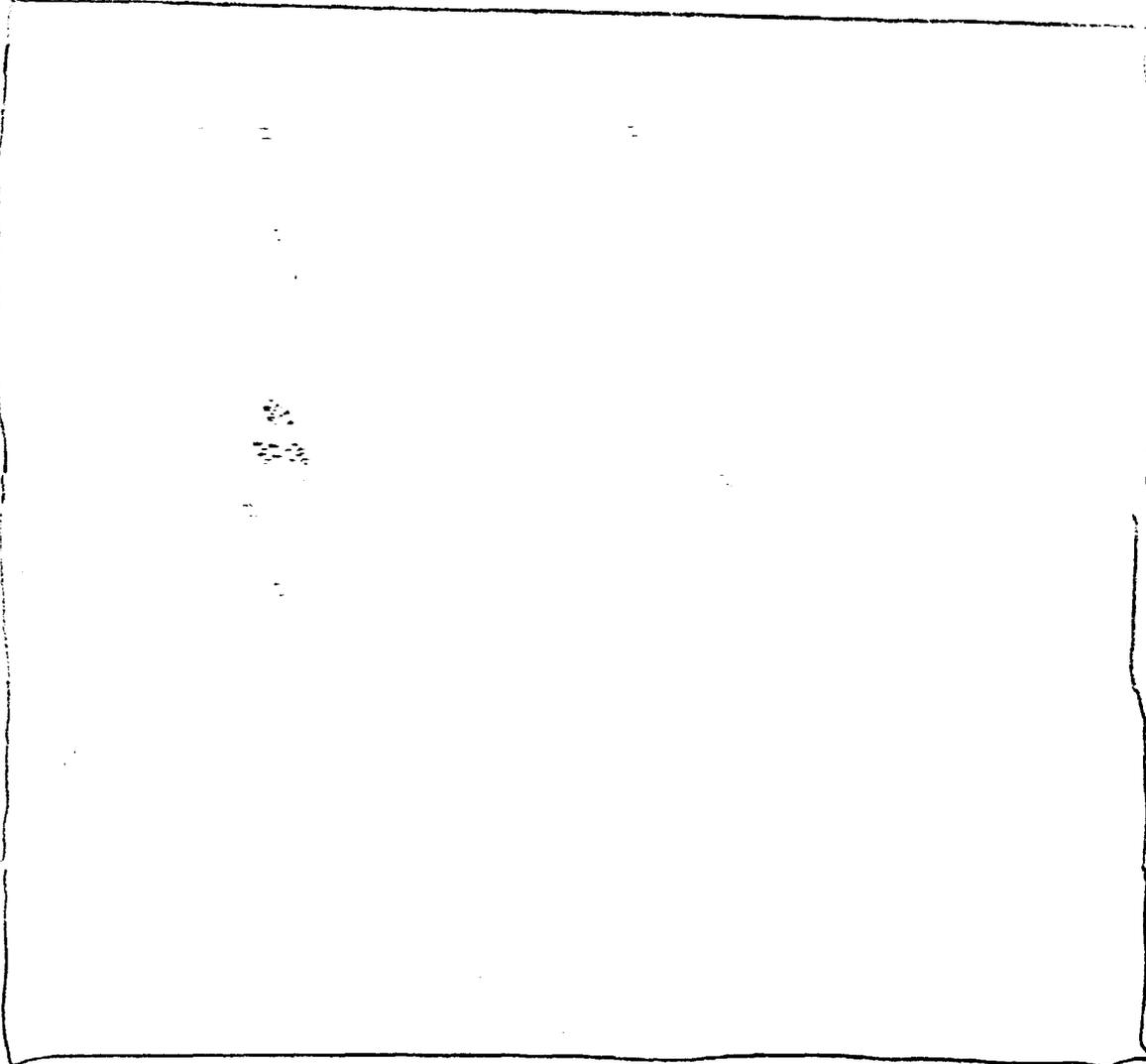
The  $IC_{50}$  for natural substrates is likely to be higher than that with the low molecular weight, minimal synthetic substrate used in the determination of anti-NA activity of oseltamivir. The natural substrates for NA are numerous and are high molecular weight glycoconjugates with multiple conformations. Furthermore, in vivo desialation of mucopolysaccharides is the net result of two competing enzyme systems (equilibrium); the multiple cellular sialyl transferases which add sialic acids to a multitude of cellular substrates and a single influenza NA that can remove the sialic acid residues. Extrapolation of this information suggests the need for higher concentrations of the drug for antiviral activity in vivo than that projected from the anti-NA activity studies.

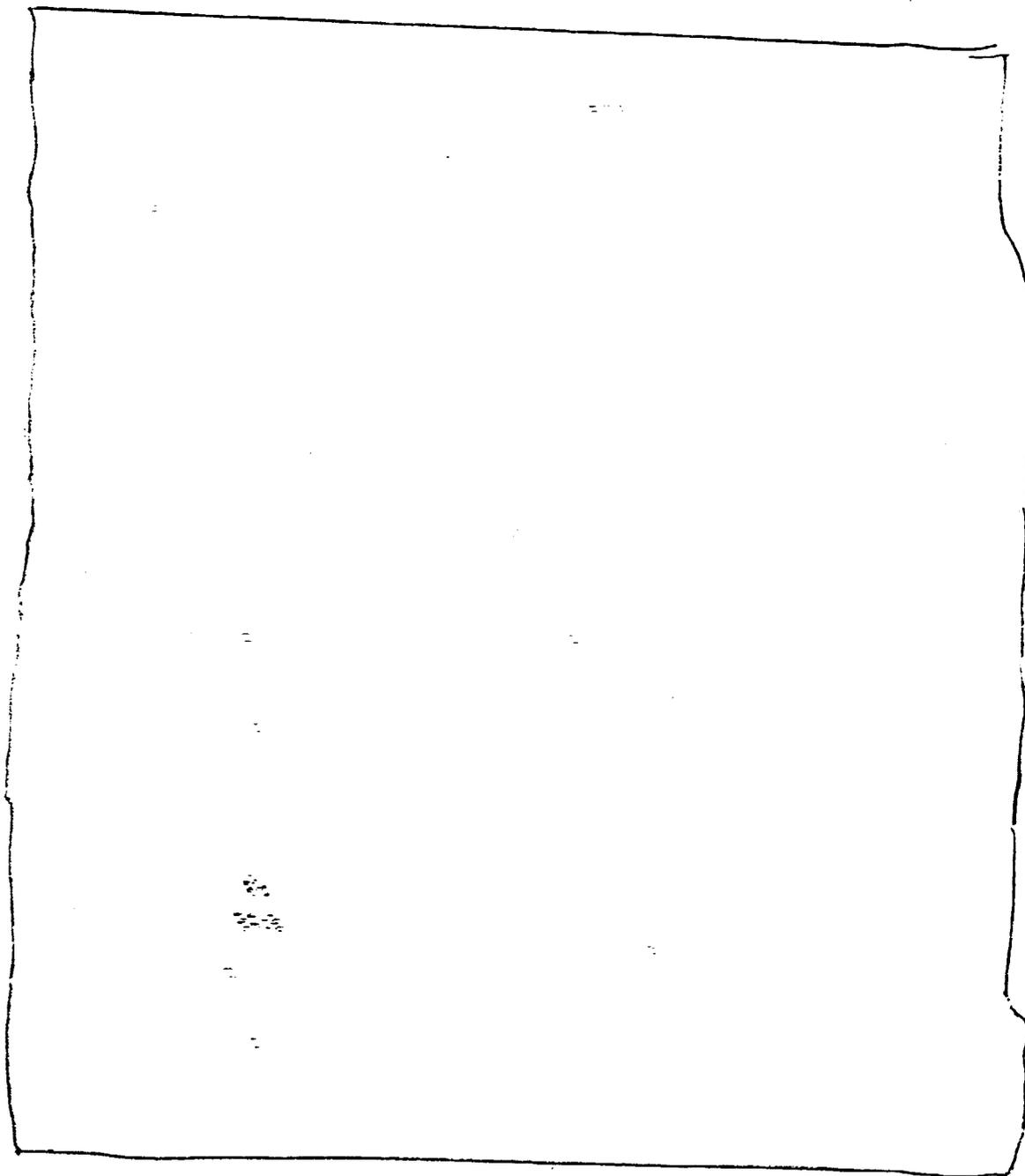
The role of NA in influenza virus replication is not well understood. NA may be a suboptimal target for efficient inhibition of influenza virus replication because it does not appear to play a role in viral entry, replication, assembly or budding (7), mutants (in vitro or clinical isolates) with NA activity of <5% of the parent enzyme replicate well in cell culture and in ferrets (8). However, if the viral titer is directly related to influenza pathology, NA enzyme inhibitors could potentially lessen the severity of infection.

The remarkable adaptability of influenza virus, including the error-prone nature of its replication, endows the virus with the possible pre-existence of any conceivable mutant, providing opportunities for selection and selective amplification of the mutants under drug pressure. Oseltamivir susceptibility studies showed the emergence of drug resistance within 10 passages of the virus in vitro and within 20 replication cycles in vivo (1). Additionally, the drug has a low genetic barrier for resistance, i.e., a single mutation is sufficient to overcome the drug pressure. However, the emergence of resistance to oseltamivir appears to be slower, with lower frequency, than the licensed anti-influenza drugs, amantadine and rimantadine.

The molecular basis for the emergence of resistance and cross-resistance to sialomimetic drugs is due to mutations in the viral NA and HA. These viral envelope glycoproteins are the major antigenic determinants and the drug-selected mutations in these genes can result in antigenic variation beyond the inherent variation. The HA in particular is immunologically very important in that it is this antigen against which protective antibodies develop and its variability has been the main factor in the continuing evolution of influenza epidemics. Therefore, the potential of the sialomimetic drugs like oseltamivir or zanamivir to select for variants in these antigenic molecules, makes it crucial to characterize them for changes in transmissibility and virulence.

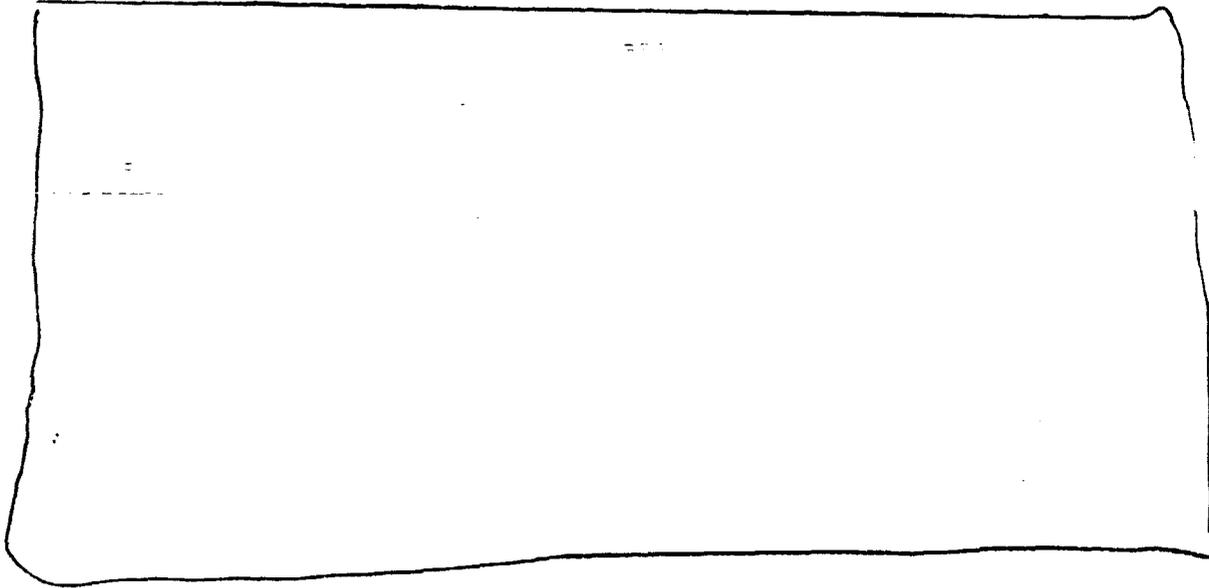
**Draft microbiology label:** Attached is the microbiology label as of 10-27-99. There may be revisions to portions of the label.





**Phase 4 considerations:** The following is a list FDA requested microbiology issues for consideration as Phase 4 commitments by the sponsor.





## RECOMMENDATION

With respect to microbiology, this application is recommended for approval.

## REFERENCES

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/S/

Narayana Battula

**CENTER FOR DRUG EVALUATION AND RESEARCH**

**APPLICATION NUMBER for: 021087**

**ADMINISTRATIVE DOCUMENTS and  
CORRESPONDENCE**



Pharmaceuticals

March 8, 1999

Mellon Bank  
Three Mellon Bank Center  
27th Floor (FDA 360909)  
Pittsburgh, Pennsylvania 15259-0001

Ladies and Gentlemen:

RE: NDA 21-087 - OSELTAMIVIR PHOSPHATE  
HUMAN DRUG APPLICATION FEE - I.D. No: [redacted]

Enclosed please find a check in the amount of \$ [redacted] made payable to the U.S. Food and Drug Administration. This payment represents the user fee required for our original New Drug Application for oseltamivir phosphate.

If you have any questions, please do not hesitate to contact the undersigned.

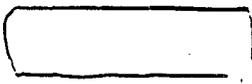
Sincerely,

HOFFMANN-LA ROCHE INC.

Elisa Scordato Mandra  
Associate, Labeling  
Drug Regulatory Affairs  
(973) 562-3683 (telephone)  
(973) 562-3700/3554 (fax)

ESM:eh

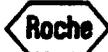
Enclosure: Check No. [redacted]



Hoffmann-La Roche Inc.

340 Kingsland Street  
Nutley, New Jersey 07110-1199

Trade Name (oseltamivir phosphate)  
Capsules 75 mg



NDA 21-087  
Debarment Certification

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**DEBARMENT CERTIFICATION**

Hoffmann-La Roche Inc. hereby certifies that it did not and will not use in any capacity the services of any person debarred under Section 306 of the Federal Food, Drug, and Cosmetic Act in connection with this application.

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**Re: Pending NDA 21-087**

**TAMIFLU™ (oseltamivir phosphate), Capsule**

**Patent Information/Market Exclusivity Request**

Pursuant to the provisions of Section 505(c)(3)(D)(ii) and Section 505(j)(4)(D)(ii) of the Federal Food, Drug and Cosmetic Act ("Act") as amended, and the provisions of 21 CFR 314.50(j)(3), we hereby claim a five (5) year market exclusivity period based upon the fact that no active ingredient (including an ester or salt of the active ingredient) of the drug covered by this NDA has been approved in any other application submitted under Section 505(b)(1). During this market exclusivity period, no other application may be accepted for submission by FDA which refers to oseltamivir before the expiration of five (5) years from the date of the approval of the application for oseltamivir except that such an application could be submitted after the expiration of four (4) years if the application contains a certification of patent invalidity or non-infringement as further described in the Act.

In accordance with the further amendments to the Act, when the approval is made by the Food and Drug Administration, it is our understanding that this market exclusivity information will be included at the same time in the Approved Prescription Drug Product List ("Orange Book").

**Choose one:**

- An updated Patent Information form is herewith attached.
- A copy of the Patent Information originally submitted on (date) is herewith attached.

Rev. 12/97

89065

**EXHIBIT A**

**UPDATED PATENT INFORMATION FOR NDA NO. 21-087**

1)	Active Ingredient(s)	oseltamivir phosphate
2)	Strength(s)	75 mg
3)	Trade Name	TAMIFLU™
4)	Dosage Form and Route of Administration	capsule for oral administration
5)	Applicant (Firm) Name	Hoffmann-La Roche Inc.
6)	NDA Number	21-087
7)	First Approval Date	Not yet approved*
8)	Exclusivity: Date first ANDA could be submitted	ANDA can not be submitted for at least five (5) years from the date pending NDA is approved
9)	Patent Information	See Attachment

**CONFIDENTIAL INFORMATION**

\*Since the New Drug Application has not yet been approved, this submission is considered as constituting trade secrets or commercial or financial information which is privileged or confidential within the meaning of the Freedom of Information Act (5 USC 552). It is requested that this submission not be published until the New Drug Application has been approved.

Rev. 12/97

**ATTACHMENT TO EXHIBIT A**

**First US Patent Number: 5,763,483**

**Expiration Date: December 27, 2016 subject to patent term extension.**

**Type of Patent-Indicate all that apply (check applicable boxes):**

- |    |  |                                     |   |                          |   |
|----|--|-------------------------------------|---|--------------------------|---|
| 1. | Drug Substance (Active Ingredient)     | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |
| 2. | Drug Product (Composition/Formulation) | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |
| 3. | Method of Use                          | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |

If patent claims method(s) of use, please specify approved uses or uses for which approval is being sought that is covered by patent:

Therapy of Influenza

**Name of Patent Owner: Gilead Sciences, Inc.**

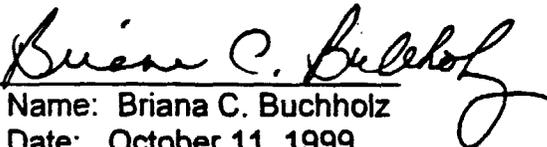
**The following declaration statement is required if the above listed patent has Composition/Formulation or Method of Use claims.**

The undersigned declares that the above stated United States Patent Number **5,763,483** covers the composition, formulation and/or method of use of oseltamivir phosphate. This product is:

currently approved under the Federal Food, Drug, and Cosmetic Act.

OR

the subject of this application for which approval is being sought.

By:   
Name: Briana C. Buchholz  
Date: October 11, 1999  
Title: Senior Counsel  
Telephone Number: (973) 235-6208

**Third US Patent Number: 5,952,375**

**Expiration Date: February 2, 2016 subject to patent term extension.**

**Type of Patent-Indicate all that apply (check applicable boxes):**

- |    |  |                                     |   |                          |   |
|----|--|-------------------------------------|---|--------------------------|---|
| 1. | Drug Substance (Active Ingredient)     | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |
| 2. | Drug Product (Composition/Formulation) | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |
| 3. | Method of Use                          | <input type="checkbox"/>            | Y | <input type="checkbox"/> | N |

If patent claims method(s) of use, please specify approved uses or uses for which approval is being sought that is covered by patent:

\_\_\_\_\_

**Name of Patent Owner: Gilead Sciences, Inc.**

**The following declaration statement is required if the above listed patent has Composition/Formulation or Method of Use claims.**

The undersigned declares that the above stated United States Patent Number **5,952,375** covers the composition, formulation and/or method of use of oseltamivir phosphate. This product is:

currently approved under the Federal Food, Drug, and Cosmetic Act.

OR

the subject of this application for which approval is being sought.

By: 

Name: Briana C. Buchholz

Date: October 11, 1999

Title: Senior Counsel

Telephone Number: (973) 235-6208

**EXHIBIT A1**

**PATENT INFORMATION FOR NDA NO. 21-087**

1)	Active Ingredient(s)	oseltamivir phosphate
2)	Strength(s)	75 mg
3)	Trade Name	
4)	Dosage Form and Route of Administration	capsule for oral administration
5)	Applicant (Firm) Name	Hoffmann-La Roche Inc.
6)	NDA Number	21-087
7)	First Approval Date	Not yet approved*
8)	Exclusivity: Date first ANDA could be submitted	ANDA can not be submitted for at least five (5) years from the date pending NDA is approved
9)	Patent Information	See Attachment

**CONFIDENTIAL INFORMATION**

\*Since the New Drug Application has not yet been approved, this submission is considered as constituting trade secrets or commercial or financial information which is privileged or confidential within the meaning of the Freedom of Information Act (5 USC 552). It is requested that this submission not be published until the New Drug Application has been approved.

Rev. 12/97

ATTACHMENT 1 TO EXHIBIT A1

This format repeats to allow up to three patents. If there are additional patents, please copy and attach.

First US Patent Number: 5,763,483

Expiration Date: December 27, 2016 subject to patent term extension.

Type of Patent-Indicate all that apply (check applicable boxes):

- |   |                                     |   |                          |   |
|---|-------------------------------------|---|--------------------------|---|
| 1. Drug Substance (Active Ingredient)     | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |
| 2. Drug Product (Composition/Formulation) | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |
| 3. Method of Use                          | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |

If patent claims method(s) of use, please specify approved uses or uses for which approval is being sought that is covered by patent:

Therapy of Influenza

Name of Patent Owner: Gilead Sciences, Inc.

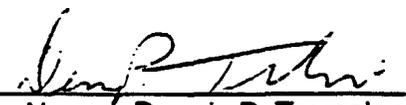
The following declaration statement is required if the above listed patent has Composition/Formulation or Method of Use claims.

The undersigned declares that the above stated United States Patent Number 5,763,483 covers the composition, formulation and/or method of use of oseltamivir phosphate. This product is:

currently approved under the Federal Food, Drug, and Cosmetic Act.

OR

the subject of this application for which approval is being sought.

By: 

Name: Dennis P. Tramaloni

Date: March 10, 1999

Title: Senior Counsel & Managing Attorney

Telephone Number: (973) 235-4475

**Second US Patent Number: 5,866,601**

**Expiration Date: February 2, 2016 subject to patent term extension.**

**Type of Patent-Indicate all that apply:**

- |   |                                     |   |                          |   |
|---|-------------------------------------|---|--------------------------|---|
| 1. Drug Substance (Active Ingredient)     | <input checked="" type="checkbox"/> | Y | <input type="checkbox"/> | N |
| 2. Drug Product (Composition/Formulation) | <input type="checkbox"/>            | Y | <input type="checkbox"/> | N |
| 3. Method of Use                          | <input type="checkbox"/>            | Y | <input type="checkbox"/> | N |

**Name of Patent Owner: Gilead Sciences, Inc.**

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A copy of the above information should be submitted with the NDA. For patents issued after the NDA is filed or approved, the applicant is required to submit that information within 30 days of the date of issuance of the patent.

To expedite publication in *The Orange Book*,\* a deskcopy should be submitted to:

Mailing address: (US Mail)

US Food and Drug Administration  
Center for Drug Evaluation and Research  
Division of Data Management and Services  
Drug Information Services Team  
HFD-93  
5600 Fishers Lane  
Rockville, MD 20857

OR

Location address: (for Federal Express deliveries)

US Food and Drug Administration  
Center for Drug Evaluation and Research  
Division of Data Management and Services  
Drug Information Services Team  
HFD-93 Room #235  
Nicholson Lane Research Center  
5516 Nicholson Lane  
Building A  
Kensington, MD 20895  
Phone (301) 827-5470  
OR faxed to: (301) 594-6463

EXCLUSIVITY SUMMARY FOR NDA # 21-087 SUPPL # \_\_\_\_\_

Trade Name Tamiflu → Generic Name oseltamivir phosphate

Applicant Name Hoffmann-La Roche HFD # 530

Approval Date If Known 10/27/99

**PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?**

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

a) Is it an original NDA?  
YES  NO

b) Is it an effectiveness supplement? N/A  
YES  NO

If yes, what type? (SE1, SE2, etc.) \_\_\_\_\_

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")  
YES  NO

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

\_\_\_\_\_  
\_\_\_\_\_

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

This is an original NDA not a supplement.

d) Did the applicant request exclusivity?

YES // NO //

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?  
5 years

e) Has pediatric exclusivity been granted for this Active Moiety?

No, studies are ongoing.

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule, previously been approved by FDA for the same use? (Rx to OTC switches should be answered NO-please indicate as such)

YES /\_\_\_/ NO //

If yes, NDA # \_\_\_\_\_ Drug Name \_\_\_\_\_

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /\_\_\_/ NO //

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

## PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

### 1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /\_\_\_/ NO //

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# \_\_\_\_\_

NDA# \_\_\_\_\_

NDA# \_\_\_\_\_

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /  / NO /  /

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# \_\_\_\_\_

NDA# \_\_\_\_\_

NDA# \_\_\_\_\_

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES" GO TO PART III.

**PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS**

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2 was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /\_\_\_/ NO /\_\_\_/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /\_\_\_/ NO /\_\_\_/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

---

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /\_\_\_/ NO /\_\_\_/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO.

YES /\_\_\_/ NO /\_\_\_/

If yes, explain:

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /\_\_\_/ NO /\_\_\_/

If yes, explain:

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.



4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1 !

IND # \_\_\_\_\_ YES / \_\_\_ / ! NO / \_\_\_ / Explain: \_\_\_\_\_

!  
!

Investigation #2 !

IND # \_\_\_\_\_ YES / \_\_\_ / ! NO / \_\_\_ / Explain: \_\_\_\_\_

(b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1 !

YES / \_\_\_ / Explain \_\_\_\_\_ ! NO / \_\_\_ / Explain \_\_\_\_\_

!  
!

\_\_\_\_\_ ! \_\_\_\_\_

!

Investigation #2 !

YES / \_\_\_ / Explain \_\_\_\_\_ ! NO / \_\_\_ / Explain \_\_\_\_\_

!  
!

\_\_\_\_\_ ! \_\_\_\_\_

\_\_\_\_\_ ! \_\_\_\_\_



**PEDIATRIC PAGE**

(Complete for all original applications and all efficacy supplements)

NDA/PLA/PMA # 21-087 Supplement # \_\_\_\_\_ Circle one: SE1 SE2 SE3 SE4 SE5 SE6

HFD-530 Trade and generic names/dosage form: Tamiflu™ (oseltamivir phosphate) Action:  AP AE NA

Applicant Hoffmann-La Roche Therapeutic Class 7030120 Antiviral - Anti-Influenza - Systemic.

Indication(s) previously approved: none.

Pediatric information in labeling of approved indication(s) is adequate \_\_\_ inadequate  X

Indication in this application Treatment of influenza.

1. **PEDIATRIC LABELING IS ADEQUATE FOR ALL PEDIATRIC AGE GROUPS.** Appropriate information has been submitted in this or previous applications and has been adequately summarized in the labeling to permit satisfactory labeling for all pediatric age groups. Further information is not required.

2. **PEDIATRIC LABELING IS ADEQUATE FOR CERTAIN AGE GROUPS.** Appropriate information has been submitted in this or previous applications and has been adequately summarized in the labeling to permit satisfactory labeling for certain pediatric age groups (e.g., infants, children, and adolescents but not neonates). Further information is not required.

3. **PEDIATRIC STUDIES ARE NEEDED.** There is potential for use in children, and further information is required to permit adequate labeling for this use.

a. A new dosing formulation is needed, and applicant has agreed to provide the appropriate formulation.

b. A new dosing formulation is needed, however the sponsor is either not willing to provide it or is in negotiations with FDA.

c. The applicant has committed to doing such studies as will be required.

(1) Studies are ongoing,

(2) Protocols were submitted and approved.

(3) Protocols were submitted and are under review.

(4) If no protocol has been submitted, attach memo describing status of discussions.

d. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

4. **PEDIATRIC STUDIES ARE NOT NEEDED.** The drug/biologic product has little potential for use in pediatric patients. Attach memo explaining why pediatric studies are not needed.

5. If none of the above apply, attach an explanation, as necessary.

**ATTACH AN EXPLANATION FOR ANY OF THE FOREGOING ITEMS, AS NECESSARY.**

/S/  
Signature of Preparer and Title

Regulatory Management Officer

10/19/99  
Date

cc: Orig NDA/PLA/PMA # 21-087  
Div File  
NDA/PLA Action Package  
HFD-006/ SOImstead (plus, for CDER/CBER APs and AEs, copy of action letter and labeling)

CDER LABELING AND NOMENCLATURE COMMITTEE

CONSULT # 1186 HFD# 530 PROPOSED PROPRIETARY NAME: PROPOSED ESTABLISHED NAME:  
ATTENTION: Grace N. Carmouze Tamiflu oseltamivir phosphate  
RE: NDA/IND # 21-087

A. Look-alike/Sound-alike

Potential for confusion:

THERAFLU (OTC)	XXX	Low	Medium	High
TAMBOCOR	XXX	Low	Medium	High
TANAFED		Low	XXX Medium	High
		Low	Medium	High
		Low	Medium	High

B. Misleading Aspects:

C. Other Concerns:

	Capturing the indication in the name (flu), may violate reminder ad provisions. "Tami" implies "Tame"?
--	---

D. Established Name

Satisfactory  
 Unsatisfactory/Reason

[Empty box for Unsatisfactory/Reason]

Recommended Established Name

[Empty box for Recommended Established Name]

E. Proprietary Name Recommendations:

ACCEPTABLE  UNACCEPTABLE

F. Signature of Chair/Date

ISI 8/9/99

MRD-500-1111111111

JUN 2 1999

Hoffmann-La Roche Inc.  
Attention: Linda Robertson, Ph.D.  
Program Manager, Drug Regulatory Affairs  
340 Kingsland Street  
Nutley, New Jersey 07110

Dear Dr. Robertson:

We have received your new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for the following:

Name of Drug Product: Tradename (oseltamivir phosphate) capsules

Therapeutic Classification: Priority (P)

Date of Application: April 29, 1999

Date of Receipt: April 29, 1999

Our Reference Number: 21-087

Unless we notify you within 60 days of our receipt date that the application is not sufficiently complete to permit a substantive review, this application will be filed under section 505(b) of the Act on June 2, 1999 in accordance with 21 CFR 314.101(a). If the application is filed, the user fee goal date will be October 29, 1999.

Be advised that, as of April 1, 1999, all applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred (63 FR 66632). If you have not already fulfilled the requirements of 21 CFR 314.55 (or 601.27), please submit your plans for pediatric drug development within 120 days from the date of this letter unless you believe a waiver is appropriate. Within 120 days of receipt of your pediatric drug development plan, we will notify you of the pediatric studies that are required under section 21 CFR 314.55.

If you believe that this drug qualifies for a waiver of the study of the pediatric study requirement, you should submit a request for a waiver with supporting information and documentation in accordance with the provisions of 21 CFR 314.55 within 60 days from the date of this letter. We will notify you within 120 days of receipt of your response whether a waiver is granted. If a waiver is not granted, we will ask you to submit your pediatric drug development plans within 120 days from the date of denial of the waiver.

Pediatric studies conducted under the terms of section 505A of the Federal Food, Drug, and Cosmetic Act may result in additional marketing exclusivity for certain products (pediatric exclusivity). You should refer to the *Guidance for Industry on Qualifying for Pediatric Exclusivity* (available on our web site at [www.fda.gov/cder/pediatric](http://www.fda.gov/cder/pediatric)) for details. If you wish to qualify for pediatric exclusivity you should submit a "Proposed Pediatric Study Request" in addition to your plans for pediatric drug development described above. If you do not submit a Proposed Pediatric Study Request within 120 days from the date of this letter, we will presume that you are not interested in obtaining pediatric exclusivity and will notify you of the pediatric studies that are required under section 21 CFR 314.55. Please note that satisfaction of the requirements in 21 CFR 314.55 alone may not qualify you for pediatric exclusivity.

Under 21 CFR 314.102(c) of the new drug regulations, you may request an informal conference with this Division (to be held approximately 90 days from the above receipt date) for a brief report on the status of the review but not on the application's ultimate approvability. Alternatively, you may choose to receive such a report by telephone.

Please cite the NDA number listed above at the top of the first page of any communications concerning this application. All communications concerning this NDA should be addressed as follows:

U.S. Postal Service:

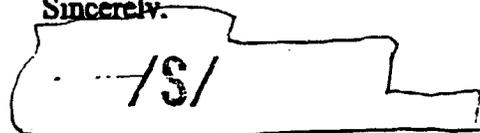
Food and Drug Administration  
Center for Drug Evaluation and Research  
Division of Antiviral Drug Products, HFD-530  
Attention: Division Document Room  
5600 Fishers Lane  
Rockville, Maryland 20857

Courier/Overnight Mail:

Food and Drug Administration  
Center for Drug Evaluation and Research  
Division of Antiviral Drug Products, HFD-530  
Attention: Division Document Room  
9201 Corporate Blvd.  
Rockville, Maryland 20850-3202

If you have any questions, contact Ms. Grace N. Carmouze, Regulatory Project Manager, at 301/827-2335.

Sincerely,



Anthony W. DeCicco, R.Ph.  
Chief, Project Management Staff  
Division of Antiviral Drug Products  
Office of Drug Evaluation IV  
Center for Drug Evaluation and Research