CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-246

MICROBIOLOGY REVIEW

Background and Summary: On October 27, 1999, Hoffmann-La Roche Inc. received approval for Tamiflu™capsules (NDA # 21-087) for the treatment of uncomplicated acute illness due to influenza infections in adults who have been symptomatic for no more than two days. The indication was based on studies of naturally occurring influenza in which the predominant infection was influenza A, and influenza challenge studies in which antiviral activity of Tamiflu™ was supported for influenza virus types A and B.

On May 22, 2000, Hoffmann-La Roche Inc. submitted a supplemental NDA #21-087 SE1-002, requesting approval of Tamiflu™ capsules for the prophylaxis of influenza in adults and adolescents. On November 17, 2000, Hoffmann-La Roche Inc. received approval for Tamiflu™ (NDA # 21-087 SE1-002) capsules for the prophylaxis of influenza in adults and adolescents of 13 years and older. The indication was based on prophylaxis studies of naturally acquired influenza involving subjects in community settings, in nursing home settings, in settings of family transmission and in influenza challenge studies in which therapeutic and prophylactic effect of Tamiflu™ was supported for influenza virus types A or B.

On June 15, 2000, Hoffmann-La Roche Inc. submitted an original NDA #21-246, for use of Tamiflu™ in the treatment of influenza in children. In this NDA Hoffmann-La Roche Inc. is requesting approval for oral suspension of Tamiflu™ for use in the treatment of uncomplicated acute illness due to influenza virus infection in patients older than one year of age who have been symptomatic for no more than two days. In support of the pediatric indication for Tamiflu™, the applicant submitted several clinical studies. The studies include a pivotal study (WV15758) in the treatment of children aged 1-12 years and a supportive study (WV15759/WV15871) in asthmatic children aged 6-12 years.

In the initial Tamiflu™ application for the treatment of adult influenza (NDA 21-087), Hoffman La Roche Inc. submitted detailed virology study reports. The submitted virology studies included: the mechanism of action of oseltamivir; anti-neuraminidase activity in vitro; anti-viral activity in vitro; efficacy of oseltamivir in influenza virus infected mice and ferrets; phenotypic resistance due to decrease in the sensitivity of the neuraminidase activity to oseltamivir carboxylate; genotypic resistance due to mutations in the neuraminidase gene; resistance in human influenza virus challenge studies; resistance in naturally acquired infection; cross-resistance to other neuraminidase inhibitors; and the effect of Tamiflu™ on humoral immune responses. The microbiology submission of the adult treatment indication was reviewed in detail (please refer to the microbiology review of NDA21-087) and those studies are not repeated in this review.

In three clinical studies of this original submission (NDA#21-246) for the pediatric indication, the sponsor evaluated patient influenza virus samples for the emergence of resistance induced by Tamiflu™ treatment. In these studies, different drug forms were used and the antiviral resistance was defined differently from the conventional traditional manner. Therefore, for clarity the drug nomenclature and resistance measures used are described below.

Drug forms used: In the nonclinical and clinical studies, different forms of the drug and the drug products were used. Therefore, to facilitate in the reading of this review, a brief description of the different drug forms used in these studies is provided. Tamiflu™ is the formulated drug product for the treatment and prophylaxis of influenza. Oseltamivir phosphate is the ethyl ester prodrug that is in the formulated drug product. Oseltamivir carboxylate is the active pharmaceutical ingredient that is formed by ester hydrolysis of the prodrug. In nonclinical virology studies, the prodrug, oseltamivir phosphate, was used in the determination of antiviral activity, efficacy in animal models and in the support studies of treatment and prophylaxis in experimental infection of human volunteers with attenuated influenza virus type A or B. In the neuraminidase enzyme sensitivity assays for the determination of resistance, the active pharmaceutical ingredient, oseltamivir carboxylate was used. In clinical studies for the treatment and prophylaxis indication in adults and adolescents, Tamiflu™ capsules were used. In the current NDA for the treatment of children older than one year, an oral aqueous suspension of Tamiflu™ was used.

Definition of resistance: The applicant evaluated clinical samples of influenza virus for loss of sensitivity of neuraminidase to the inhibitor, oseltamivir carboxylate. In these studies, the pre- and post-treatment samples obtained from the placebo arm and the Tamiflu™ treated arm were evaluated. The loss of neuraminidase sensitivity to inhibition by oseltamivir carboxylate (expressed as fold increase in the IC_{50}) has been referred to as "Resistance to Tamiflu™." It is to be noted that the resistance determination in these studies is an anti-enzyme assay, which measures the loss of sensitivity of the viral neuraminidase to the inhibitor. Thus, the assay is strictly an anti-enzyme assay. Conventionally, antiviral assays measure the ability of a candidate antiviral agent to inhibit virus replication. In this case, the antiviral assay should measure the inhibitory effect of oseltamivir phosphate or oseltamivir carboxylate on the replication of influenza virus. The applicant stated that they were unable to do the antiviral assay for lack of an appropriate cell culture system in which influenza virus replication can be determined. In all of the resistance studies reported here, unless stated otherwise, resistance in influenza virus implies loss of sensitivity of neuraminidase to the inhibitor, oseltamivir carboxylate, the active pharmaceutical ingredient in Tamiflu™.

Resistance evaluation in clinical studies: In three of the clinical studies conducted for the pediatric indication, the sponsor evaluated influenza virus samples from the patients for the loss of sensitivity of neuraminidase to the drug. Influenza virus was sampled from most patients by nasal and throat swabs at baseline, 2, 4, 6, and 10 days post Tamiflu™ treatment. The clinical studies in which the influenza virus samples were collected for assessment of changes in neuraminidase sensitivity are briefly described below and are summarized in Table 1.

(1) Study WV15758 was a pivotal trial conducted in children aged 1 to 12 with naturally acquired influenza virus infection. This was a double blind, randomized, placebo-controlled study comparing 2mg/kg of oseltamivir with placebo. A total of 698 patients were recruited into the study. Of these, 452 (65%) were confirmed to be influenza infected either by viral culture or hemagglutinin inhibition antibody testing and 422 (60%) were culture positive for influenza virus (219 in the placebo arm and 203 in the treatment arm). Assays for the determination of sensitivity of neuraminidase activity was blinded for all of the pre- and post-treatment virus samples in both placebo and Tamiflu™ treated groups. By this analysis, matched pre- and post-treatment IC_{50} was obtained for 97 children in the treatment arm and for 129 children in the placebo arm.

(2) Study WV15759/WV 15871 (rollover study) was conducted in asthmatic children older than 6 years of age. A total of 335 patients entered the combined study (includes 185 patients rolled over from study WV15759). Of these, 179 (53%) were confirmed to be influenza infected either by viral culture or hemagglutinin inhibition antibody testing and 129 subjects (39%) were culture positive for influenza virus. Nose and throat swab samples for culture and neuraminidase sensitivity testing were taken pre-treatment and on study day 6 only. In this study, for the determination of IC_{50} there were 3 matched pairs in the treatment arm and 11 matched pairs in the placebo arm.

(3) Study WV15731 was a small (n=10) dose ranging (1 mg/kg, 2 mg/kg and 3 mg/kg) pharmacokinetic study in children aged 1 to 12 years. Five out of the ten were confirmed to have influenza infection by culture. Pre- and post-treatment virus was recovered from the five culture patients and all of the 5 matched pairs were in the treatment group.

Table 1 shows the clinical studies in which resistance analysis was conducted, the total number of patients recruited into the studies, influenza virus culture positive patients in the placebo and Tamiflu™ treatment arm and the number of patients with matched pre- and post treatment virus isolates. The data presented in the table indicate that the average success rate of obtaining matched isolates from culture positive patients was 49% for the placebo arm, and 39% for the Tamiflu™ treated arm. In these controlled studies, it should have been possible to improve the percentage of patients from which virus samples could be collected for a better evaluation of viral response to Tamiflu™ treatment.

Table 1. Summary of influenza virus culture positive subjects in the pediatric studies

Study	Number Of patients	Culture positive (%)	Placebo		Tamiflu™	
			Culture positive	Matched Pairs (%)	Culture positive	Matched pairs (%)
WV15758	698	422 (60)	219	129 (59)	203	97 (48)@
WV15759/15871	355	129 (36)	67	11 (16)	62	3 (5)#
WV15731	10	5 (50)	*	*	5	5 (100)
Total	**556**	**245 (44)**	**286**	**140 (49)**	**270**	**105 (39)**

@ = Last day (2, 4 or 6) culture positive sample evaluated for neuraminidase activity

A single collection of virus sample on post-treatment day 6.

* No placebo group

Assay for neuraminidase activity: Influenza virus neuraminidase is a glycohydrolase. The enzyme cleaves the terminal sialic acid residues found on the cell surface of an array of glycoproteins, glycolipids, and oligosaccharides. (The cell surface sialic acids are also the receptors to which the influenza virus hemagglutinin attaches and penetrates into the cell). In the determination of the neuraminidase enzyme assay, the applicant used a synthetic substrate [instead of natural substrate(s)] that meets the minimal molecular requirements for cleavage by neuraminidase. The synthetic substrate used is a low molecular weight, fluorogenic compound, 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid. Neuraminidase cleaves the α-glycosidic linkage of the substrate, thereby releasing the fluorescent compound, 4-methuylumbilliferone, which can be measured fluorimetrically. Loss of sensitivity to neuraminidase is expressed as an increase in the IC_{50} of post-treatment isolate compared to the pre-treatment isolate from the same patient.

Phenotypic resistance: Phenotypic resistance to oseltamivir was defined as a measurable decrease in the in vitro sensitivity of the neuraminidase activity to the inhibitor.

Resistance to the inhibitor is said to occur when the IC_{50} of the post treatment virus neuraminidase was greater than the mean + 2SD of the pre-treatment influenza virus neuraminidase. The incidence of resistance was calculated based on the number of phenotypically resistant virus cultures in the numerator and a denominator reflecting the total number of matched pre and post-treatment influenza virus cultures.

Genotypic resistance: To identify the genotype responsible for the reduced neuraminidase sensitivity to oseltamivir carboxylate, the nucleotide sequence of the neuraminidase gene of the matched isolates was determined. Viral RNA of the pre-treatment neuraminidase-sensitive virus isolates and the post-treatment neuraminidase-resistant virus isolates was converted into DNA by RT-PCR and the nucleotide sequence of the DNA encompassing the neuraminidase active site (amino acids 100-400) was determined. Change(s) in the nucleotide sequence that result in amino acid substitution of the post-treatment resistant virus isolate as compared to the pre-treatment neuraminidase-sensitive control virus nucleotide sequence indicates the genotypic changes and the amino acid(s) that contribute to the genotypic resistance in the neuraminidase.

Table 2 shows the number of matched pairs in the placebo and treatment arms of the three clinical trials in which resistance was evaluated and the incidence of resistance calculated. The overall incidence rate of resistance in the Tamiflu™ treatment group was 8.6% (9/105) with no detected resistance in the placebo group. The incidence of resistance of 8.6% in the children is much higher than that of the 1.3% (4/301) in the adult treatment studies. Pediatric patients generally carry higher virus titer than adults and they shed virus longer than adults. The chance of emergence of resistance increases with increased viral titer and with the length of ongoing replication. Thus, in the pediatric patients, both the higher viral titer and longer duration of shedding may account for the higher incidence (8.6%) of resistance than in the adults (1.3%).

Table 2. Neuraminidase phenotype in matched clinical samples

Study	Placebo		Tamiflu™		Incidence of resistance
	Matched pairs	Resistant	Matched pairs	Resistant	
WV15758	129	None	97	9	9.3%
WV15759/15871	11	None	3	None	None
WV15731	*	*	5	None	None
Total	**140**	**0**	**105**	**9**	**8.6%**

* No placebo group

Resistance data presented in Table 3 shows the patient number, the last day of culture positivity, age of the children, change in the neuraminidase sensitivity, indicating phenotypic resistance (fold change in the IC_{50} of oseltamivir carboxylate) and the genetic mutation that contributed to the phenotypic resistance. The results indicate that the emergence of resistance was rapid (in 4-6 days of treatment initiation with Tamiflu™) with a large decrease in the sensitivity of neuraminidase (73-86,850-fold) to oseltamivir carboxylate.

Table 3. Resistance (phenotypic and genotypic) in matched pairs

Patient number	Sample day @	Age (yr.) #	Fold change in IC_{50} *	Mutation in neuraminidase
2757	6	6	15,300	
6012	6	1	390	
6796	6	3	1,440	
6801	6	1	46,800	
6853	6	5	33,850	
7047	4	11	73	
7695	6	5	1,060	
8932	6	6	86,850	
8938	6	2	33,700	

@ = Last culture positive sample

* = Ratio of neuraminidase activity of the last collected post-treatment isolates to the pre-treatment isolate

= Pre-treatment isolate was not available

Genotyping of the neuraminidase gene of the influenza virus from these 9 resistance cases showed that each one of them carry a mutation in the neuraminidase gene. The predominant mutation (7 cases) was a

(1 case) or a

(1 case).

Studies of clinically derived resistant viruses in ferrets: The applicant evaluated the relative infectivities and pathogenicities of the clinically derived resistant mutant viruses with the corresponding sensitive non-mutant wild type virus in ferrets. Groups of animals (n=3/4) were infected with a range of challenge doses of the wild type or mutant

viruses with log_{10} dilutions (10^{-1} to 10^{-6}) of the stock virus. The relative infectivities of wild type and mutant viruses were compared by titration of infectious virus recoverable from nasal wash samples taken daily from days 1 through 6, following infection. The relative pathogenicities of wild type and mutant virus were compared by the measurements of inflammatory cell counts in nasal washes, body temperature, and whole body weights.

Based on the results of these studies, the applicant concluded that the infectivity/ replication capability of the viruses carrying point mutations at amino acid positions 292, 119, or 274 in the neuraminidase gene was severely compromised. Titers of the resistant mutant virus were 2 to 3 logs less than that of the corresponding wild type virus. The pathogenic responses induced by infection with viruses carrying the point mutations was significantly reduced in comparison to the response induced by infection with the wild type virus.

Animal data on the relative infectivity and pathogenicity of the wild type and mutant virus presented by the applicant are of questionable validity. The primary concern in these experiments was that influenza virus lacking sialidase activity or containing < 5% of the wild type virus activity can undergo replication in cell culture, eggs and ferrets [Refs: Hughes, MT et al., J. Virol. (2000) 74: 5206; Colacino, JM. In Brown, LE et al., (ed) Elsevier Sciences (1996) 741; Gubareva, LV et al., J infect Dis. (1998) 178: 1257]. The amount of neuraminidase protein or the activity of the enzyme in the resistant viruses is unknown and it was not tested prior to infection of ferrets (see conclusions). Furthermore, in the preparation of stock virus using recipient MDCK cells for animal experiments, virus particle numbers were normalized on the basis of the hemagglutinin content without consideration of the neuraminidase. The sponsor claimed that the patient virus sample expanded in MDCK cells for animal infection may not be representative of the primary patient virus (because of the receptor differences in MDCK cells and human respiratory cells). In spite of these pitfalls, evaluation of the submitted animal data indicated that the mutant virus displayed complex growth kinetics. Under certain infection conditions, the resistant virus grew equally well, better or worse when compared with the non-resistant wild type virus. Based on the manner in which the animal experiments were conducted and the data presented, no conclusions could be drawn.

Resistant virus in pediatric patients: The titers of influenza virus in 'resistant children' was significantly higher (by several logs) compared to titers of all influenza virus positive treatment population, or titers of virus positive Tamiflu™ treatment group or titers of virus positive patients on post treatment day 6. The applicant concluded that, "on day 6, titers of patients carrying resistant virus were high (significantly above the 75th

percentile)." This result suggests that the resistant virus that emerged in Tamiflu™ treated patients acquired growth advantage over the non-resistant virus of Tamiflu™ treated patients.

The applicant stated that all of the patients, however, were culture negative on the 10th day. It was also stated that the patients carrying the resistant virus cleared normally and showed no clinical deterioration. The latter statement by the sponsor, however, was not supported by the data presented on the symptoms of illness or freedom from illness.

With regard to the clinical consequences of resistant virus in children, the symptoms (days to freedom from illness) lasted longer and the intensity of the symptoms appear to have increased in the 'resistant children' compared to the non-resistant virus in children treated with Tamiflu™ or placebo (see clinical and statistical review for details). Tamiflu™ resistant virus emerged rapidly in treated children. The resistant virus acquired enhanced replication efficiency. The "resistant virus containing subjects" showed a tendency toward higher and prolonged symptoms of illness. The enhanced replication of the resistant virus coupled with increases in illness symptom prompts for recommendations of better surveillance on the emergence of Tamiflu™ resistance, transmission of resistance, enrichment of resistance in the population and the clinical consequences of resistant influenza virus.

Draft microbiology label:

MICROBIOLOGY: ***Mechanism of Action:*** Oseltamivir is an ethyl ester prodrug requiring ester hydrolysis for conversion to the active form, oseltamivir carboxylate. The proposed mechanism of action of oseltamivir is via inhibition of influenza virus neuraminidase with the possibility of alteration of virus particle aggregation and release.

Antiviral Activity In Vitro: The antiviral activity of oseltamivir carboxylate against laboratory strains and clinical isolates of influenza virus was determined in cell culture assays. The concentrations of oseltamivir carboxylate required for inhibition of influenza virus were highly variable depending on the assay method used and the virus tested. The 50% and 90% inhibitory concentrations (IC50 and IC90) were in the range of 0.0008 μM to >35 μM and 0.004 μM to >100 μM, respectively (1 μM=0.284 μg/mL). The relationship between the in vitro antiviral activity in cell culture and the inhibition of influenza virus replication in humans has not been established.

Drug Resistance: Influenza A virus isolates with reduced susceptibility to oseltamivir carboxylate have been recovered in vitro by passage of virus in the presence of increasing concentrations of oseltamivir carboxylate. Genetic analysis of these isolates showed that

reduced susceptibility to oseltamivir carboxylate is associated with mutations that result in amino acid changes in the viral neuraminidase or viral hemagglutinin or both.

In clinical studies of post-exposure and seasonal prophylaxis, determination of resistance was limited by the low overall incidence rate of influenza infection and prophylactic effect of TAMIFLU.

In clinical studies in the treatment of naturally acquired infection with influenza virus, 1.3% (4/301) of post-treatment isolates in adult patients and adolescents, and 8.6% (9/105) in pediatric patients aged 1 to 12 years showed emergence of influenza variants with decreased neuraminidase susceptibility to oseltamivir carboxylate.

Genotypic analysis of these variants showed a specific mutation in the active site of neuraminidase compared to pretreatment isolates. The contribution of resistance due to alterations in the viral hemagglutinin has not been fully evaluated.

Cross-resistance: Cross-resistance between zanamivir-resistant influenza mutants and oseltamivir-resistant influenza mutants has been observed in vitro.

Due to limitations in the assays available to detect drug-induced shifts in virus susceptibility, an estimate of the incidence of oseltamivir resistance and possible cross-resistance to zanamivir in clinical isolates cannot be made. However, one of the three oseltamivir-induced mutations in the viral neuraminidase from clinical isolates is the same as one of the three mutations observed in zanamivir-resistant virus.

Insufficient information is available to fully characterize the risk of emergence of TAMIFLU resistance in clinical use.

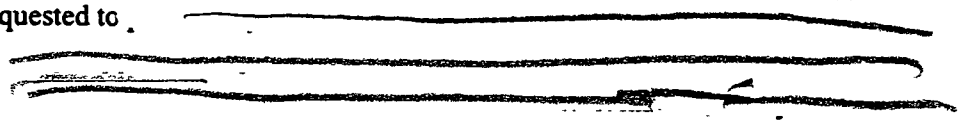
Immune Response: No influenza vaccine interaction study has been conducted. In studies of naturally acquired and experimental influenza, treatment with TAMIFLU did not impair normal humoral antibody response to infection.

CONCLUSIONS: To determine the efficacy of Tamiflu™ in the treatment of naturally occurring influenza illness in patients older than one year of age, the applicant conducted several clinical studies. In three of the clinical studies (Table1), the applicant collected matched pre and post-treatment influenza virus samples for the determination of the emergence of resistance. The definition of resistance in these studies was the loss of sensitivity of neuraminidase to the drug and thus it is anti-enzyme assay and not an antiviral assay.

Resistance analysis of the pre and post-treatment matched pairs showed large increases (73 to 86,850- fold) in the IC_{50} in 9 patient isolates indicating emergence of resistance to Tamiflu™ in these patients (Table 3). The resistance incidence rate of 8.6% (Table 2) in the pediatric patients is remarkably different from the 1.3% found in the adults and adolescent studies. By genotypic analysis, it was found that resistance to neuraminidase in each case was conferred by a single mutation in the neuraminidase gene. The predominant mutation was at the amino acid position 292 (7 cases) with one case of each at positions 119 and 274. The genetic threshhold for the emergence of resistance in influenza virus neuraminidase was low (i.e., a single mutation in the neuraminidase confers high level of resistance), rapid (within 4-6 days of initiation of therapy) and substantial (8.6%).

The incidence rate of resistance in children is remarkably higher (8.6%) than that in the adults (1.3%). Part of the explanation for the higher rate of resistance in children may be due to the higher viral titers and longer length of ongoing replication, both of which provide opportunities for the virus to evolve rapidly in response to the drug pressure. An additional contributing factor for higher rate of resistance may be the lower immunocompetancy of small children compared to adults. From the higher resistance rate observed in children, it can be surmised that immunocompromised patients may be an important population in which the virus replicates at higher rates for prolonged periods. In this setting, anti-neuraminidase therapy for influenza could potentially lead to increased rates of emergence of resistance and nosocomial spread of the resistant viruses.

The primary method of scoring for the emergence of resistance to Tamiflu™ was on the basis of a decrease in the in vitro susceptibility of influenza virus neuraminidase enzyme activity in the post-treatment virus isolates compared to the pre-treatment isolates of the same patient, i.e., enzyme resistance. It is well recognized that influenza virus escapes inhibition by neuraminidase inhibitors not only by mutations in the target neuraminidase but also by mutations in the viral hemagglutinin. Therefore, it is important to evaluate for the antiviral resistance by directly assaying for changes in the antiviral sensitivity of the whole virus to the drug in cell culture i.e., antiviral resistance. The applicant has been requested to



The initiation of influenza virus infection and the viral spread is mediated by the dynamic interactions between the receptor (sialic acid) binding activity of viral hemagglutinin and the receptor destroying activity of the viral neuraminidase. Studies on the emergence of resistance to neuraminidase inhibitors showed that resistance occurs both in vitro and in vivo, and that the resistance was mediated by mutations in the viral neuraminidase or

hemagglutinin or both. Measures for the phenotypic and the genotypic changes should include both the neuraminidase and hemagglutinin, to reflect the true incidence of resistance. Therefore, studies on the emergence of resistance and surveillance of resistance should include the genotyping of the neuraminidase and the hemagglutinin genes (both HA1 and HA2 portions of the hemagglutinin molecule.)

In vitro studies on the neuraminidase-resistant influenza virus variants for the assessment of potential class cross-resistance among neuraminidase inhibitors showed cross-resistance among them. The cross-resistance could be due to mutations either in the targeted neuraminidase gene or the non-targeted hemagglutinin gene. Mutations that decrease the affinity of hemagglutinin to its receptor sialic acid make the virus less dependent on neuraminidase activity and thus less sensitive to all neuraminidase inhibitors as a class. Resistance mutations of this type have been reported in influenza B virus that was derived from neuraminidase inhibitor treated patients. Therefore, it is important to carry out additional studies to assess class cross-resistance among the neuraminidase inhibitors, due to mutations in the targeted neuraminidase and the non-targeted hemagglutinin genes.

The cumulative observations of the rapid emergence of resistance (4-6 days following the initiation of treatment), higher incidence rate (8.6%) of resistance, enhanced infection/ replication efficiency of the resistant variants and prolongation of illness symptoms that appear to be of a greater intensity prompts us to be vigilant and recommend to the sponsor to do additional follow up studies related to the emergence of resistance and its consequences. The recommendations should include: (

Drug-resistant influenza virus could be either drug-dependent or drug-nondependent. The drug-nondependent viruses can grow with about the same efficiency in the presence or in the absence of the drug. Drug-dependent viruses, on the other hand, primarily show their effects in the presence of the drug (i.e., enhanced replication and the effects of replication) and not in the absence of the drug. In vitro studies with neuraminidase inhibitors of influenza virus have demonstrated that inhibitor-dependent influenza variants emerge in vitro [Ref: McKimm-Breschkin, JL et al., (1996) Antimicrob. Agents Chemother. 40, 40-46]. These in vitro drug-dependent influenza virus variants in addition to being drug-dependent have acquired altered growth properties (i.e., increases in the rate of virus replication and virus yield), and altered cytotoxic properties (i.e., increase in both plaque number and plaque size).

In view of the observed emergence of drug-dependent variants that show altered biological properties and in consideration of the prolonged exposure in prophylaxis and treatment, it is important, to investigate for the potential emergence of drug-dependent variants in Tamiflu™ exposed subjects. In addition, characterization of the molecular and biological properties of the drug-dependent variants should be determined. In this NDA, Tamiflu™ resistant viruses were found on post-treatment day 6 that was also the last day of treatment. The titers of the resistant viruses detected on that day was high compared to their counter part non resistant viruses that were cleared. The applicant stated that on day 10 post-treatment i.e., 4-days after the treatment stoppage, no virus could be cultured in any of the resistant patient samples. This abrupt drop in viral titer suggests the possibility that the resistant virus could be drug-dependent. Therefore, the applicant should be requested to test the available resistant isolates for potential drug-dependency

The applicant stated that the results of their animal data indicate that the mutant viruses resistant to Tamiflu™ were severely compromised in their infectivity/replicative ability in vivo in mouse and ferret. Based on this statement they requested an addition to the microbiology section of the label that,

The animal experiments reported were fraught with pitfalls to draw any conclusions. The primary concern in these experiments was that influenza virus lacking sialidase activity or containing < 5% of the parent virus activity can undergo replication in cell culture, eggs and ferrets [Refs: Hughes, MT et al., J. Virol. (2000) 74: 5206; Colacino, JM. In Brown, LE et al., (ed) Elsevier Sciences (1996) 741; Gubareva, LV et al., J infect Dis. (1998) 178: 1257]. The amount of neuraminidase protein or its enzymatic activity in the resistant viruses used to infect the animals was unknown. The wild type and mutant virus stocks for animal experiments were normalized by equivalent hemagglutinin content without consideration of neuraminidase protein or its activity. In addition, the sponsor claimed that the patient virus sample expanded in MDCK cells for animal infection may not be representative of the primary patient virus (because of the receptor differences in MDCK cells and human respiratory cells). In spite of these short falls, the resistant viruses showed complex growth patterns. Depending on the infection conditions, the growth of the mutant virus was similar, better or worse than the non-resistant virus. Thus, the animal experiments were deficient in multiple respects, difficult to interpret and draw conclusions. Thus, the requested statement for the label was deleted.

RECOMMENDATIONS: With respect to microbiology, the application for the pediatric indication is supported. However, in consideration of the genetic variation (mutations in resistant virus) induced by Tamiflu™ and the potential for the emergence of antigenic variation (mutations in both neuraminidase and hemagglutinin), the applicant is

reminded to address any outstanding Phase 4 commitments agreed to on October 25,1999 for the adult and adolescent treatment indication and on November 17, 2000 for the prophylaxis indication. In addition, the sponsor is requested to address the following Phase 4 commitments.

Phase 4 considerations:

1. Please utilize the resistant clinical isolates that you have to evaluate for potential cross-resistance to other neuraminidase inhibitors.

2. Please evaluate the resistant clinical isolates that you have for the emergence of drug-dependent variants.

3. [redacted]

4. [redacted]

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Concurrence:
HFD 530/Assoc. Dir.
HFD 530/TLMicro

Distribution:
Original IND
HFD-530/Division File
HFD-530/RMO: Carmouze, G.
HFD-530/MO
HFD-530/TLMicro
HFD-530/Reviewer Micro

/s/

Narayana Battula
12/14/00 11:08:38 AM
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Tamiflu for pediatric indication

Walla Dempsey
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