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Utilisation des antiviraux dans le traitement de la grippe

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L'UNIVERSITE PIERRE ET MARIE CURIE (PARIS VI)**

Spécialité Epidémiologie

Ecole doctorale Pierre Louis de Santé Publique (ED 393)

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par Monsieur Thierry Blanchon

Pour obtenir le grade de
Docteur de l'Université Pierre et Marie Curie

**« Utilisation des antiviraux dans le traitement de
la grippe »**

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A ma famille

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1 Introduction

La grippe saisonnière est une maladie infectieuse respiratoire fréquente, qui se manifeste par un syndrome grippal associant de la fièvre, des myalgies et des signes respiratoires, d'évolution aigüe (2). Elle peut entraîner des complications graves et potentiellement mortelles, en particulier chez certaines populations à risque comme les personnes âgées ou celles atteintes de pathologies chroniques. Chaque année en France, entre novembre et mars, on recense 700 000 à 4 800 000 consultations pour syndromes grippaux, soit 1 à 8% de la population générale (3, 4). A travers le monde, on compte, selon les épidémies saisonnières, 3 à 5 millions de cas graves et 250 000 à 500 000 décès par an (2), et sensiblement plus lors de la survenue de pandémies. Le retentissement socio-économique de la grippe est estimé en France à 2,9 +/- 2,5 journées de travail perdues par personne et par épisode grippal, chez les patients qui consultent pour un syndrome grippal (5).

Les épidémies de grippe saisonnières actuelles sont dues au virus *Influenzae* de types A et B (6, 7). Le virus de la grippe de type A comporte de nombreux sous-types en fonction de deux protéines de surface qui le composent, l'hémagglutinine (de H1 à H17) et la neuraminidase (de N1 à N9), et de leurs différentes associations.

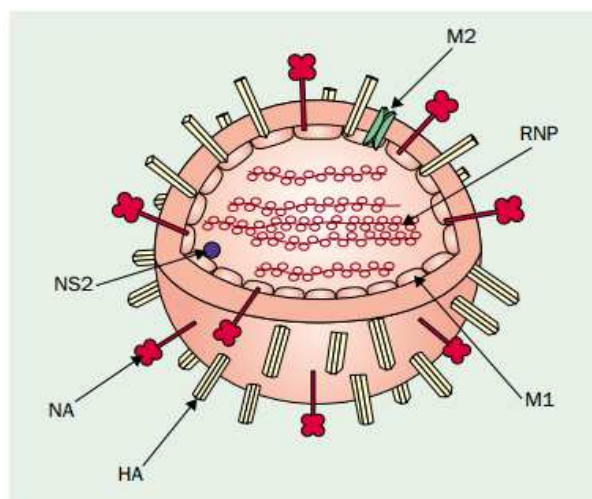


Figure 1: Schematic representation of influenza A virion
Eight ribonucleoprotein segments (RNP) are surrounded by layer of matrix (M1) protein and lipid bilayer taken from host cell at budding. NS2 (NEP) protein is associated with M1. Three viral proteins are incorporated into the lipid bilayer: HA, NA, and M2 protein. HA trimers and NA tetramers form spikes on the surface of the virion. RNP segments contain viral RNA surrounded by nucleoprotein and associated with the polymerase complex.

d'après Gubavera LV et al, Lancet 2000 (1)

Deux sous-types sont actuellement rencontrés chez l'homme lors des épidémies saisonnières, A(H1N1) et A(H3N2). Le virus de la grippe de type B ne possède pas différents sous-types, mais deux lignages antigéniquement distincts co-circulent depuis 1983 selon les différentes régions du monde, les lignages Victoria et Yamagata.

Exceptionnellement, un nouveau sous-type de virus influenza de type A peut être introduit chez l'homme. Ce phénomène a été observé trois fois au cours du 20^e siècle. Cette introduction d'un nouveau virus peut résulter d'un réassortiment du génome entre un virus grippal humain et un virus animal (virus des pandémies de 1957, 1968 et 2009), ou d'une adaptation d'un virus animal à l'homme par accumulation de mutations d'adaptation (pandémie de 1918) (8-12), graphe ci-dessous. Ces introductions de nouveaux sous-types (aussi appelées cassures antigéniques) sont responsables de pandémies dont l'impact peut être considérable, tant en terme de mortalité que de morbidité (13). En effet, ces nouveaux sous-types émergent le plus souvent dans des populations immunologiquement naïves ou peu protégées, ce qui occasionne un taux d'attaque très élevé, des formes cliniques plus graves et un taux de létalité plus important que lors de la grippe saisonnière.

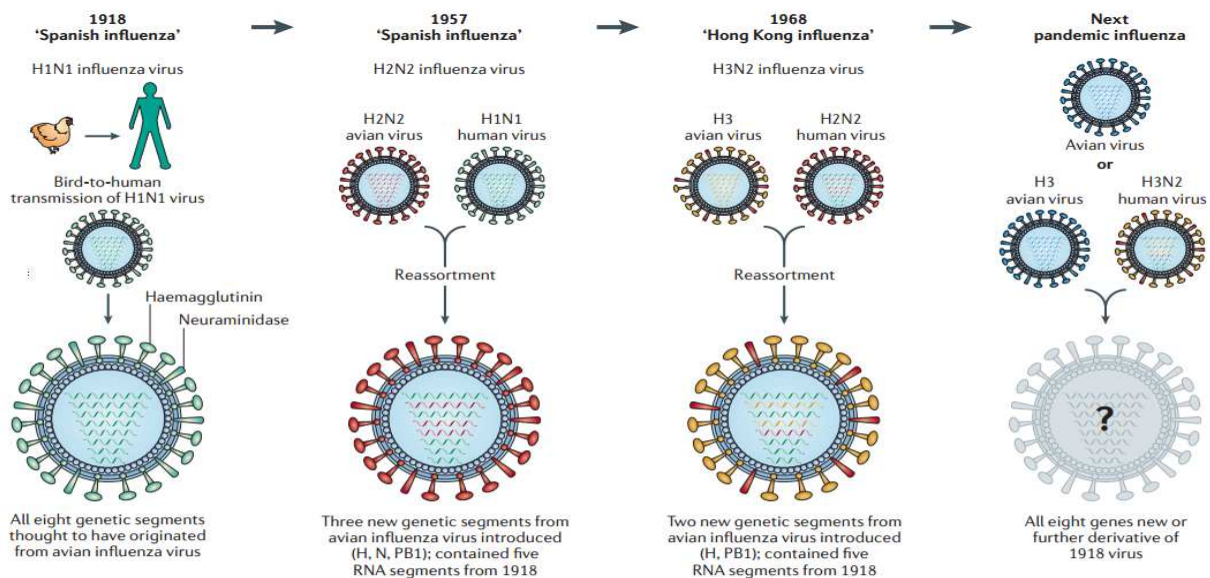


Figure 1 | **The two mechanisms by which pandemic influenza originates.** In 1918, the 'Spanish influenza' H1N1 virus, closely related to an avian virus, adapted to replicate efficiently in humans. In 1957 and 1968, reassortment events led to, respectively, the 'Asian influenza' H2N2 virus and the 'Hong Kong influenza' H3N2 virus. The 'Asian influenza' H2N2 virus acquired three genetic segments from an avian species (a haemagglutinin (H), a neuraminidase (N) and a polymerase (PB1) gene). The 'Hong Kong influenza' H3N2 virus acquired two genetic segments from an avian species (H and PB1). Future pandemic strains could arise through either mechanism. Figure adapted, with permission, from REF. 7 © (2005) Massachusetts Medical Society.

d'après De Clercq, Nature 2006 (14)

La principale mesure de prévention préconisée lors des gripes saisonnières est la vaccination des populations présentant un risque de complications (15). Cependant l'efficacité de la vaccination est actuellement discutée. Elle serait de 59% [IC 95% 51 - 67%] chez les adultes de 18 à 65 ans, et reste plus incertaine dans les autres catégories d'âge notamment chez les personnes âgées (16). Elle varie de plus en fonction des années et des types et sous-types viraux. Plusieurs raisons peuvent conduire à cette efficacité modeste du vaccin antigrippal, comme l'évolution permanente des virus grippaux (17) ou encore l'immunosénescence diminuant la capacité des personnes âgées à produire des anticorps en réponse au vaccin (18). Lors d'une pandémie grippale un vaccin ne pourrait être développé et utilisé qu'au bout de plusieurs mois.

Les agents antiviraux constituent dès lors une approche thérapeutique complémentaire. A ce jour, seuls les inhibiteurs de la neuraminidase (INA), oseltamivir et zanamivir, sont utilisables. Les adamantanes (amantadine et rimantadine) ne sont plus recommandés en raison de l'existence de résistances virales (19). Mais l'efficacité des INA est également discutée. Ils réduiraient le délai de guérison des symptômes d'une journée chez les patients atteints d'une grippe virologiquement confirmée (20-25). La durée moyenne des symptômes serait de 4 jours chez les patients recevant des INA et de 5 jours sans traitement. Ils diminueraient la survenue de complications respiratoires liées à la grippe chez les patients sains et à risque, la survenue des pneumopathies et de façon générale la prescription de traitements antibiotiques (20-27). Ils permettraient enfin de diminuer l'excrétion virale nasale ce qui pourrait limiter la propagation de la maladie (28). Compte tenu de ces données, les INA sont recommandés par les autorités sanitaires chez les patients à risque de complications ayant une suspicion de grippe (29, 30). Les effets secondaires observés les plus fréquents sont bénins à type de troubles digestifs et de réactions cutanées (25). Des cas de troubles neuro-psychiatriques ont été déclarés, mais n'ont pu être imputés aux antiviraux (31-33).

Malgré ces études et ces recommandations, la prescription des INA en cas de syndrome grippal reste controversée. Certains articles récents remettent en cause les études publiées et mettent en

avant le fait que les populations étudiées ne comportaient que peu de personnes à risque, en particuliers de personnes âgées, et que le niveau de preuve concernant l'effet des antiviraux sur la mortalité et les hospitalisations est faible (21). Par ailleurs il existerait un biais de publication, sous-entendant que les études ne démontrant pas d'impact des antiviraux n'ont pas toutes été publiées (20, 23, 34). Dans ce contexte de fortes controverses, la thèse proposée ici a pour objectif de mieux comprendre la place qui devrait être réservée aux antiviraux (INA) dans le traitement de la grippe saisonnière et pandémique.

2 Objectifs

Cette thèse a pour objectif de mieux comprendre la place qui devrait être réservée aux inhibiteurs de la neuraminidase (INA) dans le traitement de la grippe saisonnière et pandémique. Afin de répondre à cette question, plusieurs travaux ont été menés dans le cadre de cette thèse. Ils ont porté sur les aspects détaillés ci-dessous.

1) Efficacité d'une bithérapie associant deux INA dans le traitement de la grippe

Ce travail a été réalisé dans le cadre de la participation active à deux essais cliniques sur l'efficacité d'une bithérapie antivirale dans le traitement de la grippe saisonnière, en vue d'une possible pandémie grippale à venir :

- Essai Combina : association d'une bithérapie oseltamivir + zanamivir par rapport à une monothérapie par oseltamivir seul. Résultats publiés (35)
- Essai Bivir : association d'une bithérapie oseltamivir + zanamivir par rapport à une monothérapie par oseltamivir ou zanamivir seul. Résultats publiés (36).

2) Facteurs associés à la réponse clinique et virologique chez les patients grippés lors d'un traitement par INA en monothérapie

A partir des données de l'essai Bivir, une étude ancillaire a été réalisée pour analyser les facteurs associés à la réponse clinique et virologique chez les patients traités par oseltamivir ou zanamivir pour une grippe de type A. Résultats publiés (37).

3) *Efficacité d'un traitement associant deux INA pour limiter la transmission de la grippe par les patients infectés*

A partir des données de l'essai Bivir, une seconde étude ancillaire a été réalisée pour analyser l'effet de la bithérapie oseltamivir + zanamivir sur la transmission de la grippe dans les foyers, par rapport à une monothérapie oseltamivir ou zanamivir seul. Résultats publiés (38).

4) *Prescription et délivrance des INA lors de la pandémie grippale survenue en 2009 et lors des épidémies de grippe saisonnière suivantes*

Ce travail a porté sur l'étude de la prescription des INA en soins de premier recours chez les patients vus en consultation pour un syndrome grippal et la délivrance par les officines de ville. Il a étudié les déterminants de cette prescription et son évolution pendant et après la pandémie grippale survenue en 2009. Plusieurs bases de données disponibles en France métropolitaine ont été analysées. Résultats publiés (39).

3 Etats des lieux des connaissances sur les antiviraux antigrippaux

3.1 Histoire

Les travaux concernant les antiviraux antigrippaux ont commencé dans les années 1950. Ils sont nés de la recherche sur les anticancéreux suite à la découverte du concept d'analogues métaboliques inhibiteurs. Cette découverte a permis l'avènement des anti-métabolites, substances entrant en compétition avec les substrats naturels requis pour la survie des micro-organismes. Ces produits sont principalement des analogues nucléotidiques et nucléosidiques. Ces progrès ont été accélérés par les avancées de la biologie moléculaire, qui permirent de séquencer les génomes des virus de petites tailles (maximum 300 gènes). L'analogue nucléosidique issu de ces recherches, actif sur le virus de la grippe, fut la ribavirine synthétisée en 1972, sous le nom de Virazole[®], qui inhibe la réplication de plusieurs virus (VRS, adénovirus, virus Influenza A et B, ..) par un mode d'action complexe et mal élucidé (40 , 41). Les études n'ont montré aucun bénéfice thérapeutique par voie générale de cet antiviral, avec un risque d'anémie transitoire et d'hyperbilirubinémie (42). Lors de l'administration par aérosol, une défervescence plus rapide et une baisse du temps de portage viral nasal ont été démontrées initialement (43 , 44). Mais les études suivantes ont mis en évidence une activité antivirale faible malgré la défervescence (45, 46).

Les adamantanes sont les premiers antiviraux anti-grippaux à avoir été utilisés. L'activité inhibitrice de l'amantadine sur le virus de la grippe fut découverte en 1964. Ce médicament, anti-parkinsonien à l'origine, est un inhibiteur de la protéine M2 des virus de la grippe A. Son utilisation est limitée pour plusieurs raisons : l'inefficacité sur les virus de la grippe B (dépourvus de protéine M2), le développement de résistances, et une tolérance médiocre (47). Les résistances aux adamantanes sont dues à des mutations ponctuelles entraînant une modification de la protéine M2 au niveau du site de liaison des inhibiteurs (48, 49). Les adamantanes ne sont plus recommandés en raison de l'augmentation de la proportion de virus résistants (19). En mai 2013 la Haute Autorité de Santé a

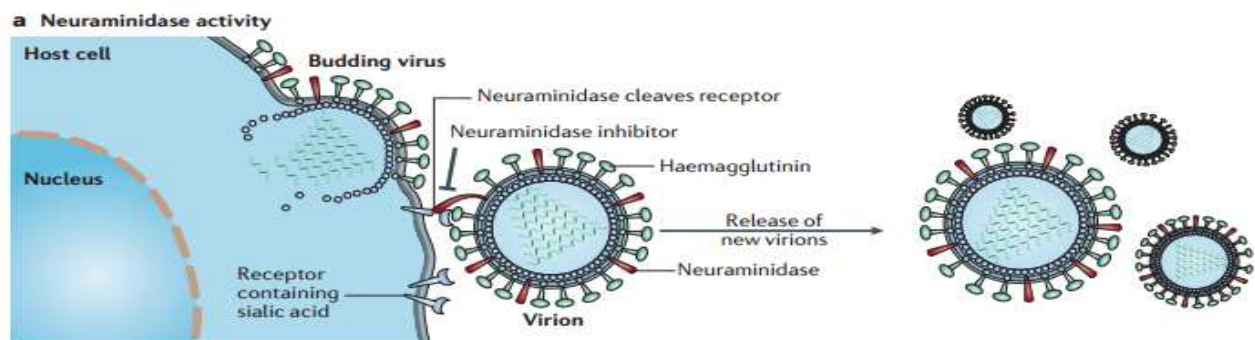
déclaré insuffisant le service médical rendu par l'amantadine dans le traitement des syndromes grippaux (50). La rimantadine utilisée de façon similaire fut retirée du marché en France en 1993.

En 1969 fut découvert le premier INA, le DANA (2-désoxy-2,3-didéhydro-N-acétylneuraminique) (51). La détermination de la structure cristalline de la neuraminidase et de son complexe avec l'acide sialique, fut réalisée dans les années 1980 (1). L'action des INA, qui s'exerce sur les virus A et B, est 100 à 1 000 fois supérieure à celle de l'amantadine (47). Les INA actuellement disponibles en France ont obtenu une autorisation de mise sur le marché en 1993 pour le zanamivir et en 1997 pour l'oseltamivir.

3.2 Mécanisme d'action des INA

La neuraminidase (ou N-acetyl-neuraminyl-hydrolase) est une sialidase. Elle est l'une des glycoprotéines de surface des virus de la grippe. Elle agit en fin de réplication en clivant le pont entre les particules virales néoformées et la membrane de la cellule infectée au niveau de résidus d'acide sialique. Ce clivage libère dans l'organisme des virus néoformés (1).

Les INA sont des analogues de l'acide sialique. Ils inhibent, par compétition spécifique, l'action de la neuraminidase en bloquant le site actif de l'acide sialique (1, 14). Il s'ensuit une entrave au relargage des virions qui s'agrègent à la surface de la cellule hôte par liaison de leur hémagglutinine aux résidus d'acide sialique non clivés. La dissémination virale s'en trouve ainsi diminuée, graphe ci-dessous.



d'après De Clercq, Nature 2006 (14)

3.3 Commercialisation des INA

3.3.1 Le zanamivir

Le zanamivir (Relenza[®]) est administré sous forme unidose de 5 mg en poudre à inhaler par la bouche à l'aide d'un appareil appelé Diskhaler[®]. Il est indiqué dans le traitement curatif de la grippe à partir de 5 ans à la dose de deux inhalations (2 x 5 mg) deux fois par jour pendant 5 jours, soit une dose quotidienne totale inhalée de 20 mg (52). La licence fut déposée par le laboratoire GlaxoSmithKline en 1990. Le zanamivir fut approuvé par la FDA (Food and Drug Administration) pour la commercialisation aux Etats-Unis en 1999. L'autorisation de mise sur le marché fut obtenue le 26 juillet 1999 en France.



La distribution dans les officines de ville a été suspendue en 2004 en France pour approvisionner l'EPRUS (Etablissement de Préparation et de Réponse aux Urgences Sanitaires) à la demande de la Direction Générale de Santé, en préparation d'une pandémie grippale. Depuis, le produit n'est plus commercialisé et les traitements actuellement délivrés par les pharmacies sont les reliquats des stocks de l'EPRUS lors la pandémie A(H1N1)pdm2009. Il n'est pas prévu de le redistribuer en ville dans l'immédiat compte tenu du stock et de la durée de conservation du zanamivir (7 ans). Il

appartient à l'EPRUS de le mettre à disposition selon le circuit de son choix en fonction des besoins de santé publique (53).

Depuis janvier 2013, le zanamivir administré par perfusion intraveineuse possède une autorisation temporaire d'utilisation (zanamivir 10 mg/ml) réservée à l'usage hospitalier (54). Il est destiné au traitement de certaines formes graves d'infection par le virus de la grippe pour des patients hospitalisés ne pouvant pas être inclus dans les essais cliniques. Ce traitement est destiné à des patients qui n'ont pas répondu aux antiviraux antigrippaux par voie inhalée ou orale, ou pour lesquels une voie d'administration autre que la voie injectable n'est pas possible, ou à des patients infectés avec un virus de la grippe résistant à d'autres agents antiviraux et pour lesquels un traitement par zanamivir inhalé n'est pas approprié. La posologie usuelle pour les adolescents de plus de 50 kg et pour tous les adultes (âge \geq 18 ans) présentant une fonction rénale normale est de 600 mg deux fois par jour pendant 5 jours. La prolongation du traitement au-delà de 5 jours peut être envisagée.

3.3.2 L'oseltamivir

L'oseltamivir (Tamiflu[®]) est administré sous forme de gélule (30 mg, 45 mg et 75 mg) ou de suspension buvable (6 mg/ml et 12 mg/ml) (55). Il est indiqué dans le traitement curatif de la grippe à partir de 1 an lors des épidémies saisonnières et chez le nourrisson en cas de pandémie grippale. La posologie d'oseltamivir recommandée est de 75 mg deux fois par jour pendant 5 jours par voie orale pour les adolescents (âgés de 13 à 17 ans) et les adultes.



Il fut développé par Gilead Sciences et le laboratoire Roche, la licence fut déposée en 1996 et les études commencèrent en 1997. Il est maintenant commercialisé uniquement par le laboratoire Roche (56). La FDA a autorisé sa commercialisation en 1999 aux Etats-Unis (57, 58). L'autorisation de mise sur le marché a été obtenue en France en juin 2002.

La Nouvelle-Zélande est le premier pays à avoir autorisé, à partir de 2007, la vente de l'oseltamivir sans ordonnance. Un premier bilan a été publié en 2012 (59), montrant que la délivrance de l'oseltamivir sans ordonnance n'a pas été associée à une augmentation des résistances, n'a pas eu pour conséquence la baisse de la vaccination antigrippale, n'a pas entraîné d'effet de stockage par la population, et suivait la dynamique épidémique. La proportion d'oseltamivir délivré sans ordonnance est restée modeste, 11% durant la pandémie A(H1N1)pdm09 et moins de 10% lors des deux épidémies saisonnières qui ont suivi.

3.3.3 Le peramivir

Le peramivir, administré par perfusion intraveineuse, a été développé par le laboratoire pharmaceutique américain BioCryst Pharmaceuticals. Il n'est pas commercialisé en France.

Il a été autorisé aux États-Unis de façon exceptionnelle et temporaire par la FDA le 23 octobre 2009, alors qu'il était toujours en cours d'essai de phase III, dans le contexte de la pandémie grippale A(H1N1)pdm09 (60, 61). La possibilité de prescription concernait les patients hospitalisés pour une infection avérée ou suspectée de grippe A(H1N1)pdm09, dans le cas où les traitements autorisés s'avéraient inactifs ou impossibles. Cette disposition d'urgence a été abrogée le 23 juin 2010. Le peramivir est actuellement disponible depuis 2010 au Japon (Rapiacta) et en Corée du Sud (PeramiFlu).

3.4 Efficacité des INA

Les critères d'efficacité étudiés pour les INA, dans le cadre de syndromes grippaux ou de grippe virologiquement confirmée concernent : la durée des symptômes, la durée du portage viral, le taux d'hospitalisations et de complications, le nombre de décès et le recours secondaire aux antibiotiques.

Trois publications récentes ont fait une synthèse de l'efficacité des antiviraux antigrippaux, deux méta-analyses et une revue de la littérature. L'une des limites majeures des deux méta-analyses présentées a été le faible nombre, dans les essais sélectionnés, des sujets de plus de 65 ans ou présentant un autre facteur de risque de grippe compliquée, diminuant la puissance des analyses réalisées ou ne les permettant pas dans ces sous-groupes.

Méta-analyse de Dobson *et al* (25)

Cette méta-analyse a inclus 12 études « publiées ou non » comparant l'oseltamivir à un placebo de façon contrôlée, randomisée, en double aveugle chez l'adulte.

- Durée des symptômes :
 - Chez des adultes présentant un syndrome grippal depuis moins de 36 h, l'analyse en intention de traiter (ITT) mettait en évidence une diminution de la durée des symptômes de 17,8 heures en moyenne chez les patients traités par oseltamivir. La diminution était de 25,2 heures en analysant seulement les personnes ayant une grippe confirmée.
 - L'analyse en sous-groupe chez les patients à risque montrait, en analysant les personnes ayant une grippe confirmée, une tendance à la baisse non significative de la durée des symptômes (tableau ci-dessous).

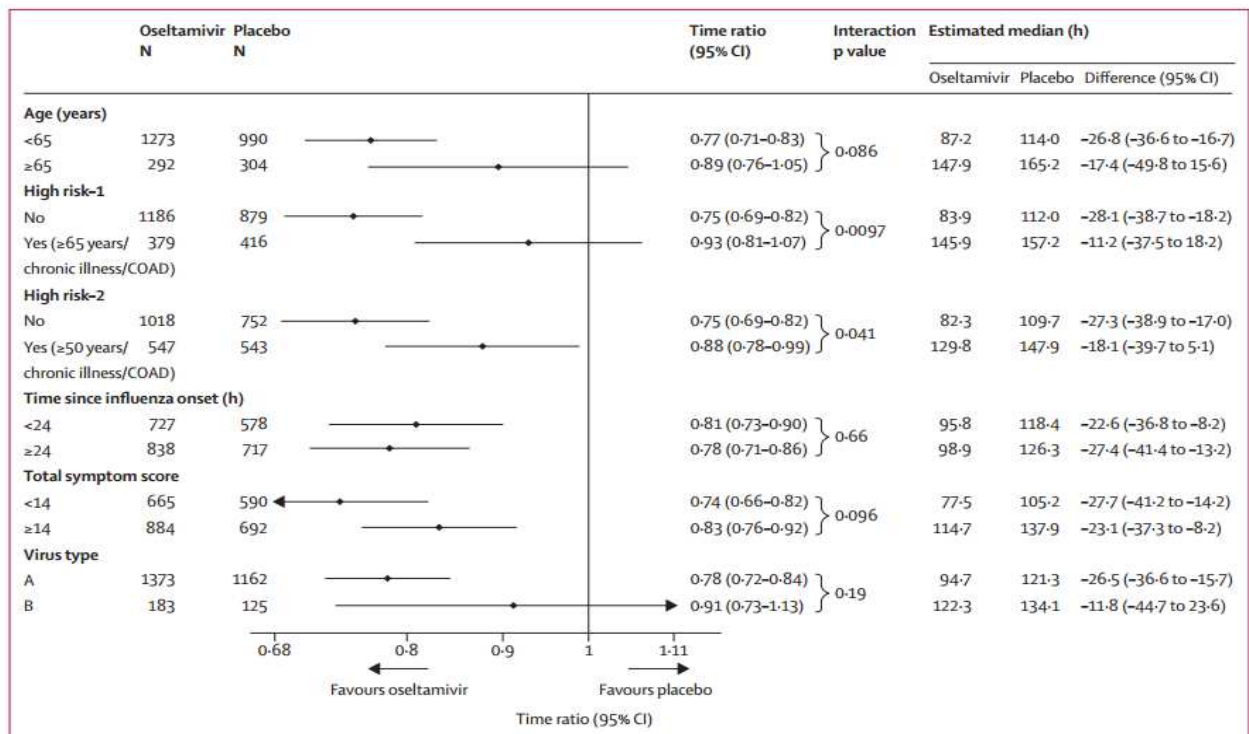


Figure 3: Subgroup analyses for time to alleviation of all symptoms in the intention-to-treat infected population
COAD=chronic obstructive airways disease. Estimated median (h)=estimated median time to alleviation of all symptoms from accelerated failure time model adjusted for trial. Diff (95% CI)=the difference in the estimated medians with bootstrap 95% CI.

d'après Dobson et al, Lancet 2015 (25)

- Complications :
 - Chez l'adulte, en analysant les personnes ayant une grippe confirmée, le risque de complications de type infections respiratoires basses nécessitant un traitement par antibiotique était réduit (4,2% (65/1544) dans le groupe oseltamivir par rapport à 8,7% (110/1263) dans le groupe placebo ; risque relatif (RR) = 0,56 [IC95% : 0,42 à 0,75] ; différence de risque (RD) = -3,8% [IC95% : -2,2% à -5%]). Il en était de même pour les pneumopathies (RR = 0,4 [IC95 : 0,19 à 0,84]).
 - Chez les patients à risque de grippe compliquée, toujours en analysant les personnes ayant une grippe confirmée, le risque de complications de type infections respiratoires basses nécessitant un traitement par antibiotique était réduit (12,1% (45/371) dans le groupe oseltamivir par rapport à 17,9% (72/403) dans le groupe placebo ; RR = 0,70 [IC95% : 0,49 à 0,98]).

- Hospitalisations :
 - Chez l'adulte, en analysant les personnes ayant une grippe confirmée, le risque d'hospitalisation quelle que soit la cause était réduit (0,6% (9/1591) dans le groupe oseltamivir par rapport à 1,7% (22/1302) dans le groupe placebo ; RR = 0,37 [IC95% : 0,17 à 0,81] ; RD = -1,1% [IC95% : -0,3% à -1,4%]).
 - L'analyse en sous-groupe, en analysant les personnes ayant une grippe confirmée, montrait une tendance à la baisse non significative.

Méta-analyse d'Ebell *et al* (20)

Cette méta-analyse a inclus 8 études publiées et 3 non publiées comparant l'oseltamivir à un placebo de façon contrôlée, randomisée, en double aveugle chez l'adulte.

- Durée des symptômes :
 - Chez des adultes présentant un syndrome grippal depuis moins de 36 h, il était mis en évidence une diminution de la durée des symptômes de 20,7 heures en moyenne chez les patients traités par oseltamivir (25,4 heures chez les patients ayant une grippe confirmée).
 - Il n'y avait pas de réduction significative de la durée des symptômes chez les personnes âgées (plus de 65 ans), et chez les patients souffrant d'une pathologie chronique, traités par oseltamivir.
- Complications :
 - Chez l'adulte ayant une grippe confirmée, le risque de pneumopathie était réduit (0,5% (9/1654) dans le groupe oseltamivir par rapport à 1,6% (18/1092) dans le groupe placebo ; différence de risque (RD) -0.9% [IC95% : -1.7% à -0.1%] ; nombre de sujet à traiter (NST) = 111), ainsi que le taux de complications nécessitant

un traitement par antibiotique en incluant « bronchite, otite, sinusite et pneumopathie » (RD = -2,8% [IC95% : -0,6 à -4,9] ; NST = 36).

- Hospitalisations :
 - Dans l'ensemble de la population (avec et sans facteur de risque et quel que soit l'âge), aucune diminution des hospitalisations n'a été montrée chez les patients présentant un syndrome grippal sans confirmation virologique. Aucune analyse n'était disponible pour les patients présentant une grippe confirmée.

Revue de la littérature de Michiels *et al* (21)

Les critères d'inclusion étaient « des revues de la littérature incluant essentiellement des essais randomisés, publiées après 2006, en français, anglais, allemand ou flamand ; présentant une analyse réalisée en intention de traiter avec le calcul d'odds ratio ou de risque relatif ». Cette revue, qui se basait sur 9 études, montrait :

- chez les adultes sains (62, 63) : i) qu'un traitement par INA permettait un gain de moins d'une journée sur la disparition des symptômes ; ii) que l'oseltamivir permettait un retour à une activité normale environ un jour et demi plus tôt et une demi-journée plus tôt pour le zanamivir ; iii) une seule des deux études montrait une diminution de la prescription d'antibiotique (63).
- chez les enfants (63) : i) qu'un traitement par INA permettait un gain de moins d'une journée sur la disparition des symptômes ; ii) que l'oseltamivir permettait un retour à une activité normale plus d'un jour plus tôt, non retrouvé pour le zanamivir ; iii) que le traitement par zanamivir diminuait le recours aux antibiotiques.
- chez les personnes âgées (63) : qu'il n'y avait pas d'effet démontré des INA.

- chez les sujets à risque de complications : i) que l'oseltamivir pouvait permettre un retour à une activité normale deux jours plus tôt, mais ce résultat n'avait qu'un faible niveau de preuve ; ii) que le zanamivir pouvait prévenir la survenue de bronchite (63).

Autres études complémentaires

En complément de ces publications, une étude menée chez des patients diabétiques présentant un syndrome grippal montrait une réduction du nombre d'hospitalisations chez les patients traités par oseltamivir et une diminution du nombre de complications respiratoires hors pneumopathie (décompensation d'asthme, détresse respiratoire,...) (64).

Par ailleurs, il a été montré que la durée du portage viral diminuait chez les patients traités par oseltamivir (28). De même, il a été mis en évidence une tendance à la diminution de la transmission intrafamiliale en cas d'utilisation de l'oseltamivir, sans apporter de conclusion définitive (65).

Le nombre de décès était trop faible dans les études pour permettre d'évaluer l'impact des INA sur la mortalité.

Peu de données sont disponibles sur l'efficacité du zanamivir par voie intraveineuse (66-68).

La commission de transparence de la Haute Autorité de Santé (HAS) a de son côté conclu en 2011 que (69) :

- le service médical rendu (SMR) par l'oseltamivir dans le traitement curatif de la grippe, lorsqu'il est débuté dans un délai de 48h après l'apparition des symptômes, en période d'épidémie ordinaire de grippe ou en période de pandémie, est faible chez :
 - les sujets ayant une forme clinique de grippe jugée sévère par le médecin ou compliquée d'emblée,
 - les sujets ayant des facteurs de risque particuliers, dont la femme enceinte.
- le SMR dans le traitement curatif de la grippe est insuffisant dans les autres situations.

3.5 Effets indésirables des INA

3.5.1 Le zanamivir

Les études précédant l'autorisation de mise sur le marché du zanamivir ont mis en évidence des effets indésirables graves dans de rares cas (moins de 1 patient sur 10 000) : réactions anaphylactiques et un risque de bronchospasme qui contre-indiquent son administration aux patients présentant une maladie respiratoire sous-jacente (asthme, broncho-pneumopathie chronique obstructive, ...) (70). Ces études retrouvent, comme effets plus fréquents (présents chez plus de 1,5% des sujets traités), des effets bénins à type de troubles digestifs et de céphalées (71).

Le suivi post-commercialisation a relevé des cas graves, mais rares, de toxidermie et des cas isolés de troubles neuropsychiatriques. Les effets les plus fréquents étaient bénins à type de troubles digestifs (27, 71).

3.5.2 L'oseltamivir

Une méta-analyse récente, qui a inclus 12 études « publiées ou non » comparant l'oseltamivir à un placebo de façon contrôlée, randomisée, en double aveugle chez l'adulte, a montré que la prise d'un traitement par oseltamivir augmentait le risque de nausée (9,9% dans le groupe oseltamivir par rapport à 6,2% dans le groupe placebo ; risque relatif (RR) = 1,6 [IC95% : 1,29 à 1,99] ; différence de risque (RD) = 3,7% [IC95% : 1,8% à 6,1]), et de vomissement (8% dans le groupe oseltamivir par rapport à 3,3% dans le groupe placebo ; RR = 2,43 [IC95% : 1,83 à 3,23] ; RD = 4,7% [IC95% : 2,7% à 7,3%]). Il n'était pas montré de différence en ce qui concernait les troubles neurologiques, psychiatriques ou les effets indésirables graves. Les résultats détaillés sont présentés ci-dessous dans un tableau issu de l'article.

	Number of events		Overall risk ratio (95% CI)	p value	Placebo group risk (%)*	Oseltamivir group risk (%)†	Risk difference (95% CI)
	Oseltamivir (n=2401)	Placebo (n=1917)					
All adverse events	1033	819	0.97 (0.91 to 1.04)	0.41	42.7	41.5	-1.2% (-4.0 to 1.8)
Serious adverse events	21	22	0.79 (0.43 to 1.47)	0.46	1.1	0.9	-0.2% (-0.7 to 0.5)
Gastrointestinal disorders	574	370	1.21 (1.07 to 1.36)	0.0019	19.3	23.3	4.0% (1.4 to 6.9)
Nausea	247	118	1.60 (1.29 to 1.99)	<0.0001	6.2	9.9	3.7% (1.8 to 6.1)
Vomiting	201	63	2.43 (1.83 to 3.23)	<0.0001	3.3	8.0	4.7% (2.7 to 7.3)
Diarrhoea	147	147	0.75 (0.60 to 0.95)	0.016	7.7	5.8	-1.9% (-3.1 to -0.4)
Cardiac disorders	13	20	0.49 (0.25 to 0.98)	0.043	1.0	0.5	-0.5% (-0.8 to -0.0)
Infections and infestations	231	217	0.84 (0.70 to 1.00)	0.049	11.3	9.5	-1.8% (-3.4 to -0.0)
Injury and poisoning	15	4	3.37 (1.08 to 10.47)	0.036	0.2	0.7	0.5% (0.0 to 2.0)
Respiratory, thoracic, and mediastinal disorders	158	143	0.74 (0.60 to 0.93)	0.0081	7.5	5.5	-1.9% (-3.0 to -0.6)
Neurological disorders	124	93	1.00 (0.76 to 1.30)	0.97	4.9	4.8	-0.0% (-1.2 to 1.5)
Psychiatric disorders	11	13	0.62 (0.26 to 1.45)	0.27	0.7	0.4	-0.3% (-0.5 to 0.3)
Additional analyses in influenza- infected participants‡							
Neurological disorders	91	73	0.95 (0.70 to 1.29)	0.76	5.6	5.4	-0.3% (-1.7 to 1.6)
Psychiatric disorders	10	9	0.81 (0.31 to 2.08)	0.65	0.7	0.6	-0.1% (-0.5 to 0.7)
Nausea	172	85	1.60 (1.24 to 2.07)	0.0003	6.5	10.5	3.9% (1.6 to 7.0)
Vomiting	155	41	3.00 (2.11 to 4.26)	<0.0001	3.2	9.5	6.3% (3.5 to 10.3)
Additional analyses in participants without influenza§							
Nausea	75	33	1.67 (1.12 to 2.49)	0.011	5.3	8.9	3.6% (0.7 to 7.9)
Vomiting	46	22	1.49 (0.90 to 2.46)	0.12	3.6	5.3	1.7% (-0.4 to 5.2)

Events=number of participants who had one or more events. *Placebo group risk is calculated using all trials (including trials with no outcomes in each group). †Oseltamivir group risk and risk difference (95% CI) obtained by applying overall risk ratio and 95% CI to pooled placebo group risk. ‡n=1590 in the oseltamivir group and n=1299 in the placebo group. §n=811 in the oseltamivir group and n=618 in the placebo group

Table 2: Meta-analyses findings for key on treatment adverse events in the safety population, by treatment received

d'après Dobson et al, Lancet 2015 (25)

La surveillance des effets indésirables des antiviraux a été particulièrement active lors de la pandémie A(H1N1)pdm09 survenue en 2009. Le bulletin de surveillance de l'AFSSAPS lors de cette épidémie fait état de 211 signalements d'effets indésirables, dont 68 cas graves, pour environ 1 122 281 boîtes d'oseltamivir délivrées entre le 1er octobre 2009 et le 26 mars 2010. Ces cas comprenaient notamment 11 cas de troubles du comportement et de l'humeur (confusion, délire, hallucinations), 11 cas de réactions anaphylactiques (œdème de Quincke, urticaire géant), 8 cas de toxidermie, 6 cas de troubles neurologiques (crises convulsives), des troubles respiratoires (7 cas), et des atteintes hépatiques cytolytiques (4 cas) (72).

Les effets rares, mais graves, ayant alerté l'opinion internationale étaient neuropsychiatriques, de fréquence indéterminée, à type d'hallucination, de troubles du comportement pouvant aller jusqu'à la mise en danger des patients (31-33). Ces effets neuropsychiatriques sont mentionnés dès 2005 par

l'Agence Européenne du Médicament, qui concluait à l'absence de lien de causalité prouvé, mais à la nécessité d'un suivi rapproché (73). En 2007, l'EMA a demandé qu'une information spécifique soit apportée aux professionnels de santé précisant que « Convulsions, niveau de conscience altéré, comportement anormal, hallucinations et délire ont été rapportés lors de l'administration de Tamiflu, conduisant dans de rares cas à une blessure accidentelle. Les patients, en particulier les enfants et les adolescents, doivent être étroitement surveillés, et un professionnel de santé doit être contacté immédiatement si le patient montre des signes de comportement inhabituel » (74). Une enquête auprès du laboratoire Roche a été mise en place par l'EMA en octobre 2013, à la suite des accusations concernant le non-respect des procédures de pharmacovigilance concernant l'oseltamivir (75). Le rapport issu de cette enquête, réalisé en novembre 2013, concluait à une absence de remise en question de la balance bénéfice-risque de ce médicament (76).

3.6 Recommandations concernant la prescription des INA

3.6.1 Recommandations actuelles

Au niveau international, l'Organisation Mondiale de la Santé a recommandé, lors de la pandémie A(H1N1)pdm09 en 2009, l'utilisation des INA en cas de grippe initialement grave, ou lors de la présence d'une pathologie sous-jacente chez un patient présentant un syndrome grippal (77). L'administration devait se faire le plus tôt possible sans attendre de confirmation virologique. Depuis l'OMS n'a pas émis de nouvelle recommandation à ce sujet.

Aux Etats-Unis, le CDC (Centers for Disease Control and Prevention) recommande lors des épidémies saisonnières l'utilisation curative et préventive des INA chez les sujets i) présentant un syndrome grippal avec des signes de gravité ou ii) à risque de grippe compliquée : grossesse ou post-partum, obésité (IMC supérieur à 40), présence d'une pathologie chronique sous-jacente, immunodépression, âge inférieur à 2 ans ou supérieur à 65 ans, indiens d'Amérique ou patients originaires d'Alaska (29). Une administration la plus précoce possible est recommandée.

L'Agence Européenne du Médicament (EMA) a réaffirmé en novembre 2013 la positivité de la balance bénéfico-risque de l'oseltamivir chez les patients à risque de grippe compliquée (76).

En France, le Haut Conseil de Santé Publique (HCSP), dans son dernier avis concernant l'utilisation des INA en date du 9 novembre 2012, recommande leur prescription dans des conditions précises en traitement curatif et préventif lors des épidémies de grippe saisonnière. Les conditions de prescription sont détaillées ci-dessous (30).

Recommandations du HCSP sur l'utilisation des antiviraux en traitement curatif (2012)

Prescription d'un antiviral antigrippal

- lors des épidémies de grippe saisonnière en période de circulation virale,
- pour les personnes présentant un syndrome grippal
 - o jugées à risque de complications, âgés de 1 an et plus y compris les femmes enceintes, ciblées par la vaccination (détaillé ci-dessous) ;
 - o présentant une grippe grave d'emblée ou dont l'état général s'aggrave selon l'appréciation du médecin ;
 - o dont l'état justifie une hospitalisation pour grippe.
- le plus précocement possible après le début des symptômes sans attendre le résultat du test de confirmation virologique du diagnostic s'il a été réalisé

Ces critères sont valables quel que soit le statut vaccinal du patient.

En complément des conditions de prescription des INA, les recommandations françaises concernant la vaccination contre les virus grippaux saisonniers sont détaillées ci-dessous. Elles sont issues du calendrier vaccinal 2014 publié par le Ministère de la Santé.

Recommandations générales

La vaccination contre la grippe est recommandée chaque année pour les personnes âgées de 65 ans et plus.

Recommandations particulières

La vaccination est recommandée chez :

- les femmes enceintes, quel que soit le trimestre de la grossesse ;
- les personnes, y compris les enfants à partir de l'âge de 6 mois, atteintes des pathologies suivantes :
 - affections broncho-pulmonaires chroniques répondant aux critères de l'ALD 14 (asthme et BPCO) ;
 - insuffisances respiratoires chroniques obstructives ou restrictives quelle que soit la cause, y compris les maladies neuromusculaires à risque de décompensation respiratoire, les malformations des voies aériennes supérieures ou inférieures, les malformations pulmonaires ou les malformations de la cage thoracique ;
 - maladies respiratoires chroniques ne remplissant pas les critères de l'ALD mais susceptibles d'être aggravées ou décompensées par une affection grippale, dont asthme, bronchite chronique, bronchiectasies, hyper-réactivité bronchique ;
 - dysplasies broncho-pulmonaires⁸ ;
 - mucoviscidose ;
 - cardiopathies congénitales cyanogènes ou avec une HTAP et/ou une insuffisance cardiaque ;
 - insuffisances cardiaques graves ;
 - valvulopathies graves ;
 - troubles du rythme graves justifiant un traitement au long cours ;
 - maladies des coronaires ;
 - antécédents d'accident vasculaire cérébral ;
 - formes graves des affections neurologiques et musculaires (dont myopathie, poliomyélite, myasthénie, maladie de Charcot) ;
 - paraplégies et tétraplégies avec atteinte diaphragmatique ;
 - néphropathies chroniques graves ;
 - syndromes néphrotiques ;
 - drépanocytoses, homozygotes et doubles hétérozygotes S/C, thalasso-drépanocytose ;
 - diabètes de type 1 et de type 2 ;
 - déficits immunitaires primitifs ou acquis (pathologies oncologiques et hématologiques, transplantations d'organe et de cellules souches hématopoïétiques, déficits immunitaires héréditaires, maladies inflammatoires et/ou auto-immunes recevant un traitement immunosuppresseur), excepté les personnes qui reçoivent un traitement régulier par immunoglobulines ; personnes infectées par le VIH quel que soit leur âge et leur statut immunovirologique (cf. *tableau 3.5*) ;
 - maladie hépatique chronique avec ou sans cirrhose ;
- les personnes obèses avec un indice de masse corporelle (IMC) égal ou supérieur à 40 kg/m², sans pathologie associée ou atteintes d'une pathologie autre que celles citées ci-dessus ;
- les personnes séjournant dans un établissement de soins de suite ainsi que dans un établissement médico-social d'hébergement quel que soit leur âge ;

3.6.2 Controverses autour de ces recommandations

Ces dernières années, les antiviraux antigrippaux et la grippe en général ont été au centre de débats scientifiques, politiques et économiques (78). La question soulevée était invariablement celle des liens entre les firmes pharmaceutiques et les instances de santé nationales et internationales, par le biais des comités d'experts.

La gestion de la pandémie grippale A(H1N1)pdm09 en 2009 fut largement critiquée. Une enquête menée par le Conseil européen pointait du doigt le manque de transparence concernant la prise des décisions, l'abus d'influence des laboratoires pharmaceutiques et l'inadéquation des mesures prises (79, 80). Selon ces travaux, il en résultait des dépenses considérables, l'exposition des populations à un risque sanitaire lié à des vaccins et des traitements antiviraux insuffisamment éprouvés et un

climat de crainte instauré à tort, responsable d'une crise de confiance des populations vis-à-vis des experts. Ces inquiétudes furent relayées par la presse scientifique avec au premier plan l'éditorial du *British Medical Journal* en juin 2010 s'adressant à l'OMS et s'interrogeant sur le bien-fondé des recommandations et les conflits d'intérêt de certains de ses membres, ayant conduit à l'achat et au stockage d'une très grande quantité d'INA et de vaccins en particulier en France et au Royaume-Uni (81). L'OMS a répondu à ces accusations en affirmant que les décisions avaient été prises indépendamment de tout intérêt commercial et que les conflits d'intérêt inhérents à toute organisation internationale avaient été maîtrisés par la volonté de création d'un comité indépendant (82). Le Sénat français, dans le rapport de la commission d'enquête sur la pandémie grippale A(H1N1)pdm2009, souligne des recommandations inappropriées, corrigées spontanément par les acteurs de terrain qui avaient mis en place une réponse plus adaptée à la réalité de la maladie (78). Les conclusions de ce rapport déploraient une mauvaise gestion de la pandémie grippale par les instances de santé, qui risquait de créer dans la population une défiance durable.

Depuis ces doutes perdurent. Malgré les recommandations actuelles concernant l'utilisation d'antiviraux antigrippaux, de nombreuses voix s'élèvent contre leur prescription. Au niveau international, plusieurs articles mettent en avant un bénéfice clinique faible et des effets secondaires non négligeables (83, 84). Si la diminution de la durée des symptômes a été démontrée dans plusieurs études, l'effet sur la prévention des complications et la diminution de la transmission est encore débattue et nécessiteraient l'analyse des données non publiées (62). Au niveau national, ces questions sont relayées par des revues médicales très suivies par les médecins prescripteurs (85). Les soupçons concernant le faible niveau de preuve de l'efficacité des antigrippaux ont été alimentés par les réticences du laboratoire Roche à rendre publiques les études réalisées (86, 87). Une enquête a été mise en place par l'EMA en 2013 à la suite des accusations concernant le non-respect des procédures de pharmacovigilance concernant l'oseltamivir (75). Le rapport concluait à une absence de remise en question de la balance bénéfice-risque de ce médicament (76).

4 Travaux de recherche

4.1 Efficacité d'une bithérapie antivirale associant deux INA comparée à une monothérapie dans le traitement de la grippe

Ce travail a été réalisé dans le cadre de la participation active à deux essais cliniques sur l'efficacité d'une bithérapie antivirale dans le traitement de la grippe saisonnière, en vue de la survenue hypothétique d'une pandémie grippale.

Trois raisons incitaient à évaluer l'efficacité et la tolérance d'une association d'antiviraux dans le traitement de la grippe. La première est d'ordre épidémiologique, une meilleure efficacité démontrée d'une telle association, en particulier en termes de réduction de la durée de la période contagieuse, pourrait être une information importante en cas de pandémie et de nature à reconsidérer la stratégie de l'usage des antiviraux afin de prévenir le plus efficacement possible la diffusion d'une nouvelle souche. La seconde raison part de l'hypothèse plausible que, lors de l'utilisation intensive de ces produits, le taux de résistances à l'un des deux antiviraux (voire aux deux) devienne préoccupant, et de nature à menacer leur utilisation. L'émergence de résistances pourrait poser de sérieux problèmes, notamment au cours de la première vague pandémique lorsque le vaccin risque de ne pas être disponible. L'absence d'expérience de l'emploi d'une association de ces antiviraux, tant en terme d'efficacité que de tolérance, alors que l'on pourrait être conduit à envisager le recours à une telle association risque de conduire à des attitudes irrationnelles. La troisième raison relève du traitement de la grippe saisonnière, en particulier chez les personnes âgées et fragiles lorsque le vaccin n'a pas été administré ou se montre inefficace. Ces personnes, mais aussi toutes celles pour lesquelles ces deux produits sont actuellement indiqués en monothérapie par l'autorisation de mise sur le marché, pourraient bénéficier d'une association d'antiviraux si elle s'avérait plus efficace et aussi bien tolérée que leur utilisation en monothérapie. Quoique leurs mécanismes d'action soient proches, il n'y a pas d'objection de principe à l'emploi de ces deux produits en association, dans le respect de leurs modalités d'administration. D'autant

plus qu'ils s'administrent par deux voies différentes, l'une systémique orale, l'autre inhalée, endo-laryngée.

4.1.1 Essai Combina : bithérapie (oseltamivir + zanamivir) comparée à l'oseltamivir seul dans le traitement de la grippe (article 1)

Jusqu'en 2007, les souches résistantes aux INA étaient exceptionnelles, et il était supposé que leur émergence était liée au mésusage des INA (doses et durée insuffisantes). Mais à partir de l'hiver 2007-2008, un nombre significatif des souches A(H1N1) circulant en Europe ont présenté une résistance naturelle à l'oseltamivir (88), liée à la présence d'une mutation (H274Y ou H275Y) au niveau de la neuraminidase (89). En revanche il n'y avait pas de résistance au zanamivir. Cette situation a souligné la nécessité de stratégies alternatives dans le traitement contre la grippe, comme par exemple l'association de l'oseltamivir à un autre antiviral (90, 91).

Dans ce contexte, en parallèle de l'essai Bivir détaillé ultérieurement dans ce manuscrit, l'essai Combina avait pour but d'apporter des premières réponses sur l'efficacité antivirale et la tolérance de l'association oseltamivir-zanamivir (O + Z) en bithérapie par rapport à l'oseltamivir en monothérapie (O). Cet essai clinique, conçu en 2008-2009, a été réalisé au cours de la pandémie grippale due au virus A(H1N1)pdm09 en 2009-2010. Les inclusions prévues concernaient 30 adultes de 18 à 64 ans souffrant d'un syndrome grippal, dont le début des symptômes dataient de moins de 42 h et testés positifs à la grippe A. Les patients inclus étaient randomisés en deux groupes de traitement : (O + Z) ou (O). Ils avaient un lavage nasal à J0, avant le début du traitement, puis tous les jours jusqu'au 4^{ème}, ainsi qu'un prélèvement nasal à J5 et J7.

L'analyse a été possible pour 24 patients, 12 dans le bras (O + Z) et 12 dans le bras (O). La charge virale moyenne a diminué d'environ 1 log₁₀ cgeq (copies équivalent génome) / µl par jour quel que soit le groupe de traitement. Il n'a pas été observé de différence significative entre les groupes de traitement pour la durée de résiliation des symptômes. Tous les traitements ont été bien tolérés. Aucune résistance à l'oseltamivir n'a été détectée chez les patients des deux groupes de traitement.

Ce travail a permis d'apporter des premières informations sur l'efficacité et la tolérance d'une bithérapie oseltamivir + zanamivir dans le traitement de la grippe, toutefois la taille de l'échantillon fut trop limitée pour que l'étude soit pleinement informative. Peu de travaux avaient été faits jusque-là sur l'utilisation d'une bithérapie associant deux INA dans le traitement de la grippe. Une étude non comparative, réalisée au Danemark également durant la pandémie de 2009, a étudié l'impact chez 21 patients hospitalisés en réanimation d'une bithérapie par oseltamivir (150 mg deux fois par jour par sonde naso-gastrique) et zanamivir (50 mg deux fois par jour par nébulisateur). Avec un taux de mortalité de 23,8% (5/21) avant la sortie d'unité de réanimation et de 28,5% (6/21) à 90 jours, les auteurs ne concluaient pas à un apport de la bithérapie (92). Dans une autre étude à Hong-Kong durant la pandémie, chez 7 patients hospitalisés en réanimation et mis sous ECMO, 3 furent traités par bithérapie oseltamivir + zanamivir sans impact évident du traitement antiviral (93). Si des études supplémentaires s'avéraient nécessaires pour confirmer ou infirmer l'intérêt de cette association, il était important de noter que depuis 2009 les souches H1N1 circulantes ne présentaient qu'exceptionnellement une résistance à l'oseltamivir (94-96).

Les résultats de cette étude ont été publiés dans Antiviral Research (35) :

- Escuret V, Cornu C, Boutitie F, Enouf V, Mosnier A, Bouscambert-Duchamp M, Gaillard S, Duval X, **Blanchon T**, Leport C, Gueyffier F, Van Der Werf S, Lina B. Oseltamivir–zanamivir bithérapie compared to oseltamivir monotherapy in the treatment of pandemic 2009 influenza A(H1N1) virus infections. Antiviral Res. 2012 November 2012;96:130-7



Oseltamivir–zanamivir bitherapy compared to oseltamivir monotherapy in the treatment of pandemic 2009 influenza A(H1N1) virus infections

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ABSTRACT

Background: The emergence of oseltamivir resistance in 2007 highlighted the need for alternative strategies against influenza. To limit the putative emergence of resistant viruses this clinical trial aimed to evaluate the antiviral efficacy and tolerability of oseltamivir–zanamivir (O + Z) bitherapy compared to oseltamivir monotherapy (O). This clinical trial was designed in 2008–2009 and was conducted during the A(H1N1) influenza virus pandemic in 2009–2010. The A(H1N1)pdm09 viruses were reported to be sensitive to oseltamivir and zanamivir but resistant to amantadine.

Methods: During the pandemic phase in France, adults with influenza-like illness for less than 42 h and who tested positive to influenza A were randomised into treatment groups: (O + Z) or (O). Patients had a nasal wash at day 0, before the beginning of treatment and daily at days 1 to 4. They also had a nasal swab at days 5 and 7 to check for the negatification of viral excretion. Virological response was assessed using the GAPDH adjusted M gene quantification.

Results: Analysis was possible for 24 patients, 12 in the (O + Z) arm and 12 in the (O) arm. The mean viral load decreased at around $1 \log_{10}$ cgeq/ μ l per day regardless of allocated treatment group. We could not detect any significant difference between treatment groups in the duration needed to alleviate symptoms. All treatments were well tolerated. No oseltamivir-resistant H275Y NA mutated virus has been detected in patients of both treatment groups.

Conclusions: The sample size of our study is too limited to be fully informative and we could not detect whether combination therapy (O + Z) improves or reduces the effectiveness of oseltamivir in the treatment of influenza A(H1N1)pdm09 virus infection in community patients. Additional studies are needed to improve the antiviral treatment of patients infected with influenza virus.

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1. Introduction

The emergence of oseltamivir-resistance in 2007 and the rapid worldwide spread of the pandemic influenza A(H1N1)pdm09 virus

have highlighted the need for effective novel antiviral approaches against influenza.

The neuraminidase inhibitors (NAIs) oseltamivir and zanamivir are the recommended antiviral agents against influenza A and B viruses. Amantadine was one of the first antiviral agents used against influenza A viruses. In the last decade there has been a substantial worldwide increase in amantadine-resistance, starting with the seasonal influenza A(H3N2) viruses (Deyde et al., 2007).

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When the study was designed, seasonal influenza A(H1N1) viruses were resistant to oseltamivir but mostly sensitive to amantadine. However, as a consequence of the emergence of the A(H1N1)pdm09 virus that carried the S31N mutation in M2 (Da-wood et al., 2009), all currently circulating human influenza A viruses are resistant to amantadine (WHO, 2011).

In the winter of 2007–2008, seasonal oseltamivir-resistant A(H1N1) viruses related to the A/Brisbane/59/2007 (H1N1) variant emerged and disseminated in individuals who were not under oseltamivir treatment (Lackenby et al., 2008; Meijer et al., 2009). However, the emergence of these viruses bearing the H275Y NA mutation had a major impact, showing that A(H1N1) viruses may develop the H275Y NA mutation and that the oseltamivir could not be used to treat infected patients. With the displacement of seasonal A(H1N1) by the A(H1N1)pdm09 viruses, all human influenza viruses were sensitive to oseltamivir and zanamivir. However, in order to limit the putative emergence of resistant clones in the context of a A(H1N1) pandemic when the use of NAIs is large, it was important to test possible alternative strategies. Theoretically, antivirals in combination could improve antiviral efficacy, reduce the duration of symptoms, and lower the risk of emergence of anti-viral resistance.

The *in vitro* combination of NAIs may be antagonistic because these agents target the same binding pocket in the neuraminidase. However, different routes of administration, orally for oseltamivir and by inhalation for zanamivir, could induce a pharmacologically interesting interaction *in vivo*. During the 2008–2009 season when A(H3N2) virus was predominantly circulating, a clinical trial conducted in France confirmed that the oseltamivir–zanamivir bitherapy was antagonistic (Duval et al., 2010). However, these results may be different for A(H1N1) and A(H3N2) viruses because neuraminidases N1 and N2 show structural differences (Russell et al., 2006). Moreover, in the context of the pandemic it was interesting to study if the results found with the A(H3N2) virus would be confirmed or not with the A(H1N1)pdm09 virus.

The randomised trial was then conducted during the A(H1N1)pdm09 virus pandemic in France, to compare the oseltamivir–zanamivir (O + Z) combination therapy with the oseltamivir monotherapy (O), in terms of antiviral efficacy, resolution of symptoms, tolerability, and the prevention of oseltamivir-resistance emergence.

2. Patients, material and methods

This phase II clinical trial was a multicentered, randomised, and unblinded study of two parallel groups.

2.1. Study population

The study targeted adult out-patients of both sexes, aged 18 to 64 years, with influenza-like illness (ILI) for less than 42 h, not vaccinated against influenza in the year of the study and who tested positive for influenza A (QuickVue® Influenza A+B test). Patients also had to be free from chronic diseases, have medical insurance, and give informed written consent. Exclusion criteria were pregnancy (a pregnancy test was performed before inclusion), lactation, lack of effective contraceptive methods, ongoing chronic obstructive pulmonary disease (COPD), asthma, renal failure, epilepsy, confusional state, hallucinations, severe uncontrolled psychotic or neurotic state, depression with antidepressant treatment, congestive cardiac insufficiency, peripheral oedema, orthostatic hypotension and a hypersensitivity to one component of the study drugs. Use of drugs like nasal topics, corticosteroids, immunosuppressive drugs, neuroleptics or antiemetics was not permitted during the study.

Patients were recruited by general practitioners in the community (in Lyon and Paris, France) during the peak circulation of the influenza A(H1N1)pdm09 virus.

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Lyon Ethics Committee (Comité de Protection des Personnes Lyon B) on 9 September 2009.

2.2. Study protocol

Randomisation was performed after checking eligibility, obtaining patient consent, and collecting baseline data. A permuted-block algorithm was used for randomisation. Concealed allocation was performed by calling the coordination centre. Patients were randomised into two groups according to the antiviral treatment they received: the oseltamivir–zanamivir (O + Z) bitherapy or the oseltamivir (O) monotherapy.

Oseltamivir (Tamiflu®) was administered orally at the recommended dosage of 75 mg, two times a day for 5 days (150 mg per day). Zanamivir (Relenza®) was administered by inhalation with the Diskhaler system, at the recommended dosage of two inhalations of 5 mg, two times a day for five days (20 mg per day). For the combination therapy both drugs were given concomitantly: there was no more than a few minutes of delay between the oral administration of oseltamivir and the inhalation of zanamivir. Oseltamivir was provided for free by Roche SA. Zanamivir was purchased from GlaxoSmithKline. Drugs were packaged by Creapharm SA.

Specimens were collected at patients' homes by a study nurse. Specimens consisted of a nasal wash within two hours after the first visit (H0), and every 24 h until 96 h after treatment start. The nasal washes were obtained by instilling 2.5 ml of physiological saline solution in each nostril and then aspirating the nasal secretions with a silicone pipe connected to a vacuum pump. To ensure good viral conservation, the viral transport medium (Sigma Virocult®, Medical Wire & Equipment Co.) was aspirated and mixed with the nasal wash. Then, the mixture was kept at 4 °C during transport to the virological laboratory. Subsequent specimens consisted of nasal swabs (Virocult®, Medical Wire & Equipment Co) performed on days 5 and 7. We changed from nasal washes to nasal swabs to alleviate the sampling performed in patients; we first assumed that the viral load would be near zero on these days and nasal swabs were done to check for the negatization of viral load.

Baseline data included the patient's medical history and influenza symptoms. Follow-up data included the evolution of symptoms, and compliance to treatment (assessed by pill count by number of study days). A follow-up visit was performed on day 6 to assess a potential carry-over effect.

Data were collected on Case Report Forms by the investigators, and entered into a database using Clininfo SA software (Clininfo SA, 99 rue de Gerland, 69007 Lyon, France).

2.3. Laboratory procedures: virological analysis

Nasal washes and nasal swabs were added to a viral medium culture for a final volume of at least 1.5 ml. Then the samples were divided into aliquots and frozen at –80 °C. All the samples for an individual were then tested in a same assay run for quantification.

RNA was extracted from 200 µl of nasal wash without antibiotics using the automated NucliSens easyMAG system (Biomerieux). Elution of the extracted nucleic acids was performed in 70 µl.

Influenza A virus was detected and quantified using a real time reverse transcription quantitative polymerase chain reaction (rt RTqPCR) on the influenza A M gene as described previously (Duchamp et al., 2010). The results were expressed in log₁₀ copies of RNA genome equivalent/µl of nasal wash or nasal swab (abbreviated

\log_{10} cgeq/ μ l). In order to control sample quality (presence of cells in the sample), a rt RTqPCR was performed on the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) intracellular gene (Wong et al., 2005; Duval et al., 2010) and an adjustment was made for the amount of GAPDH in the sample and the mean GAPDH for all samples. The GAPDH quantification gave comparable cell numbers results between groups with a mean Ct of 25.07 ± 3.01 ($5.06 \log_{10}$ cgeq/ μ l) and 25.78 ± 2.61 ($4.69 \log_{10}$ cgeq/ μ l) for (O) and (O + Z) groups respectively. The GAPDH adjusted M gene values were obtained by dividing the M gene value by the ratio (GAPDH value/GAPDH mean for all values). The results were expressed in \log_{10} cgeq/ μ l. The final statistical analysis was performed considering the initial and the GAPDH adjusted M gene quantification values (Duval et al., 2010). As the results were similar and in order to avoid any confusion, we will present only the viral load corresponding to the GAPDH adjusted M gene quantification values.

The influenza A virus subtyping was performed on the first sample (nasal wash) received for each patient using a RT-PCR specifically targeting the N1 segment of A(H1N1)pdm09 virus (primers and probes are available upon request at grippe@pasteur.fr).

The one-step allelic discrimination real-time RT-PCR used on A/Brisbane/59/2007 (H1N1) – related viruses (Carr et al., 2008) was adapted and carried out on A(H1N1)pdm09 viruses. This RT-PCR uses two probes, which specifically detect the presence of a His (H) or Tyr (Y) in position 275 of N1 for A(H1N1)pdm09 viruses. The oligonucleotide primers (900 nM final concentration, Eurogentec) were as follows: N1SWFor (sense): 5'-CAG GCC TCA TAC AAG ATC TTC AGA-3' and N1SWRev (antisense): 5'-CAC TAG AAT CAG GAT AAC AGG AGC-3'. The two allele specific probes (200 nM final concentration, Applied) with 5'dyes and 3' minor groove binding non fluorescent quenchers (MGBNFQ) were as follows: the oseltamivir sensitive probe N1SWH275 VIC – 5'-CTC ATA GTG ATA ATT A-MGBNFQ and the oseltamivir resistant probe N1SWH275 FAM – 5'-CCT CAT AGT AAT AAT TA-MGBNFQ. We used 5 μ l of RNA eluate in a 25 μ l reaction volume with the SuperScriptIII Platinum One-Step qRT-PCR system (Invitrogen) on Applied Biosystems 7500 with the following cycling parameters: 50 °C for 15 min, 95 °C for 15 s, and 60 °C for 40 s for 45 cycles. The capacity of this RT-PCR to detect H275Y NA mutated viruses was confirmed using two oseltamivir-resistant viruses isolated by cell culture from patients admitted in intensive care unit and treated by oseltamivir. The IC_{50} for oseltamivir of these isolates, determined by fluorometric NA inhibition assay using the MUNANA substrate (Sigma–Aldrich) as described previously (Ferraris et al., 2005), were 232 nM and 178 nM compared to a mean of 0.46 ± 0.23 nM for 192 sensitive A(H1N1)pdm09 influenza viruses isolated in 2009–2010 (Escuret et al., 2011). Sequencing of these isolates confirmed that their oseltamivir-resistance was due to the H275Y NA mutation. As in previous studies, we amplified a 112-bp cDNA fragment from isolates with a sequence confirmed for H275 or Y275 with the Qiagen one-step RT-PCR kit. These amplicons were purified using a PCR purification kit (Qiagen) and quantified on a Nanodrop (ND-1000 spectrophotometer). We generated standard curves with serial dilutions of quantified amplicons. Assays were performed in triplicate for each target. Linear curves were obtained over the range of 10^1 to 10^8 copies, the slopes were -3.35 and -3.33 for H275 and Y275 respectively with correlation coefficients >0.99 for both targets. The sensitivity of this RT-PCR was 10 copies for the detection of the H275Y NA mutation (20/20 reactions detected) and was between 10 and 100 copies for the detection of the wild type virus (17/20 reactions detected for 10 copies). Mixed populations of wild-type and H275Y NA mutated influenza A(H1N1)pdm09 viruses could be detected using this allelic discrimination RT-PCR. At least 10% of H275Y NA mutated virus could be detected in a mixed viral population.

Frozen aliquots of nasal samples that were detected positive for influenza by the M gene rt RTqPCR were inoculated in MDCK (Madin Darby Canine Kidney) cells (from ATCC, product N° CCL-34). Cells were cultured in a growth medium: Eagle's Minimum essential Medium (EMEM, Biowhittaker 12–125F) with L-Glutamine 1% (200 mM; Biowhittaker 17–605E), Penicillin–Streptomycin 2% (25000 U/ml and 25000 μ g/ml; Biowhittaker 17–719R) and SVF 2%. For the virus culture the medium was EMEM with L-Glutamine 1%, Penicillin–Streptomycin 2% and trypsin TPCK 2 μ g/ml (Boehringer Mannheim 109819).

The presence of virus in cell culture was assessed by the M gene rt RTqPCR performed on dilutions (1/1000) of supernatant collected at day 4 after inoculation. However when the Ct was high (Ct ≥ 38) and could correspond to a dilution of the inoculum we did a second passage in cell culture. The virus presence was tested by fluorometric NA activity assay with MUNANA substrate in the 2nd passage supernatant as previously described (Ferraris et al., 2005).

2.4. Sample size

The sample size was calculated using the one-step Fleming method (Fleming, 1982).

Our hypotheses were based on the results of previous studies (Hayden et al., 1999; Treanor et al., 2000). The first controlled trial on oseltamivir efficacy was conducted in 69 healthy adult volunteers inoculated intranasally with influenza A/Texas/36/1991 (H1N1) virus. There were 4 treatment groups with oseltamivir: 20 ($n = 15$), 100 ($n = 14$), 200 mg twice daily ($n = 14$), 200 mg once daily ($n = 13$) and 1 placebo group ($n = 13$). Administration began at 28 h after inoculation and continued for 5 days. The median duration of viral excretion estimated in cell culture was reduced from 107 h in the placebo group (13 patients) to 58 h in the combined oseltamivir treated patients (56 patients) (Hayden et al., 1999). In the study conducted in naturally influenza-infected patients, the patients were treated by oseltamivir 75 mg twice daily ($n = 124$), or oseltamivir 150 mg twice daily ($n = 121$) or placebo ($n = 129$). The median duration of illness before study was similar in all 3 treatment groups (26, 24 and 27 h in the placebo, oseltamivir 75 mg and 150 mg groups, respectively). The proportion of patients shedding virus at each time point were similar in all 3 groups: 15% and 72% of patients had a negative viral excretion estimated in cell culture after 24 h and 72 h respectively, whether they were treated by oseltamivir or placebo (Treanor et al., 2000). We then hypothesised that 50% and 80% of the patients would have a negative nasal wash in cell culture at 48 and 72 h respectively with an oseltamivir monotherapy according to the results of these previous studies (Hayden et al., 1999; Treanor et al., 2000).

Comparisons between cell culture and the rt RT-qPCR on the M gene showed that samples with less than 3 \log_{10} cgeq/ μ l generally do not grow in cell culture. This threshold was defined after analysis of 405 nasal specimens collected between the 1-09-2009 and the 31-12-2009 for routine surveillance in south of France in the frame of the GROG (*groupes régionaux d'observation de la grippe*) network, detected positive by rt RT-qPCR for influenza A M gene and A(H1N1)pdm09 and inoculated for viral cell culture. Briefly, on a total of 405 samples, 268 and 137 samples had a positive and negative viral isolation in cell culture respectively. We compared the results given by different thresholds (between 5.23 and 0.85 \log_{10} cgeq/ μ l) with the observed results of viral cell culture. We could then define proportions of true positive (when viral load was $>$ threshold with viral isolation), false positive (when viral load was $>$ threshold with no viral isolation), false negative (when viral load was $<$ threshold with viral isolation) and true negative (when viral load was $<$ threshold with no viral isolation). After analysis of the results, the threshold of 3 \log_{10} cgeq/ μ l gave the best compro-

mise with a sensitivity of 0.95 (which means that 95% of infectious samples have a viral load $>3 \log_{10}$ cgeq/ μ l); a positive predictive value of 0.76 (which means that there is a probability of 76% that the sample is infectious if its viral load is superior to $3 \log_{10}$ cgeq/ μ l) and a negative predictive value of 0.80 (which means that there is a probability of 80% that the sample is not infectious if its viral load is inferior to $3 \log_{10}$ cgeq/ μ l).

The objective was to demonstrate that the combinations of antiviral drugs would have a 48-h efficacy compared with the 72-h efficacy of oseltamivir alone. Therefore, bitherapies would be considered effective if the percentage of patients treated by bitherapies with an influenza A viral load under $3 \log_{10}$ cgeq/ μ l (the M gene) is 80% at 48 h.

With $\alpha = 5\%$, power = 80%, with P_0 = maximum probability of inefficacy fixed at 50%, P_a , the minimum probability of inefficacy fixed at 80%. The following formulae give the number of patients needed per group S:

$$N = \frac{(z1 - \beta(pa(1 - pa))1/2 + z1 - \alpha(p0(1 - p0))1/2)/(pa - p0))^2}{S \geq [Np0 + (z1 - \alpha(Np0(1 - p0))1/2)^2 + 1}$$

The minimum required sample size was 15 people per arm, but we aimed at recruiting 20 per arm. We assumed that 25% of patients would not meet inclusion criteria. Patients with missing samples were not replaced.

2.5. Efficacy end points

The primary criterion was the percentage of patients with a viral load under $3 \log_{10}$ cgeq/ μ l. This was quantified 48 h after treatment start using a rt RTqPCR on the influenza A M gene, adjusted to the GAPDH gene.

Other criteria included the evolution profile of the influenza viral load, the duration of influenza symptoms, the safety of the bitherapies, and the occurrence of oseltamivir-resistance.

2.6. Statistical analysis

The statistical analysis was performed according to the intention to treat principle, i.e. all patients were analysed in the treatment group they were allocated in. However, missing data were not replaced.

The percentages of patients with an influenza A viral load under $3 \log_{10}$ cgeq/ μ l at 48 h were calculated and compared between oseltamivir-zanamivir bitherapy and the oseltamivir monotherapy using a χ^2 test. They were then compared to the 80% theoretical threshold using an exact test based on the binomial distribution. The viral load decrease was estimated and compared between treatment groups using a multilevel linear regression model accounting for repeated measurements over time.

The analysis was performed considering the time from the beginning of treatment or time from the beginning of symptoms (data not shown) and conducted to similar results.

The median delay for alleviating all symptoms was defined as the time between drug initiation and symptom alleviation. Symptom alleviation was defined as the first day during which all the studied symptoms (chills, aches, headache, fatigue, cough, pharyngitis, sore throat or nasal congestion, expectoration, otitis, digestive disturbance) were absent or mild. It was compared between groups using a Kruskal–Wallis test. For patients with no symptom alleviation at the last visit, the delay was considered to be seven days (corresponding to the last visit).

3. Results

3.1. Patients

Between 1 October 2009 and 18 January 2010, 25 out-patients were recruited, 20 in the Lyon area and 5 in the Paris area. Out of these, 12 and 13 patients were randomised into the (O + Z) and (O) groups respectively. The virus was not amplified with the specific M gene RTqPCR in one patient's nasal sample, even though he had first a positive diagnosis for influenza A by rapid testing. A total of 24 patients were analysed, 12 in each group of treatment. All the patients had a proven infection with the A(H1N1)pdm09 influenza virus as the N1 gene specific of A(H1N1)pdm09 virus was detected positive on the first nasal wash of each patient. We could not detect any significant differences in the demographic and clinical characteristics of the patients between treatment groups before treatment (Table 1). Most patients presented general constitutional symptoms such as chills, aches, fatigue, headache and cough. Other symptoms (pharyngitis, sore throat or nasal congestion, expectoration, otitis, or digestive disturbance) were less frequent.

3.2. Primary criterion

At inclusion, all patients had more than $3 \log_{10}$ cgeq/ μ l in their nasal wash. After two days of treatment, only 1 (8.3%) patient in each group had a viral load under $3 \log_{10}$ cgeq/ μ l (Table 2). The percentage of patients under the threshold of $3 \log_{10}$ cgeq/ μ l was far below the 80% rate expected in the (O + Z) group. After using logistic regression to analyze the proportion of patients under the threshold, we did not identify any significant differences between treatment groups. We obtained interpretable cell culture results for 9 (75%) and 7 (58.3%) patients, in the (O + Z) and (O) treatment groups, respectively. After two days of treatment 5 (55.6%) and 2 (28.6%) patients had a negative influenza virus cell culture in the (O + Z) and (O) groups respectively (Table 3). We could not detect any significant difference between the treatment groups but 8 (88.9%) and 3 (42.9%) patients had a negative influenza virus in cell culture after 3 days of treatment in the (O + Z) and (O) groups respectively (Table 3).

Table 1
Demographic and clinical characteristics of the patients at inclusion before treatment.

	Osetamivir + Zanamivir	Osetamivir
<i>Demographic characteristics</i>		
N	12	12
Male sex ratio (n, %)	4 (33.3)	6 (50.0)
Age (years)		
Mean (SD)	35.9 (10.6)	32.8 (12.5)
(min–max)	(21.9–55.2)	(19.1–51.7)
<i>Delay from the beginning of symptoms (hours)</i>		
Mean (SD)	25.0 (13.5)	23.9 (9.4)
<i>Moderate or severe clinical symptoms</i>		
Highest temperature (°C)		
Mean (SD)	38.9 (0.4)	39.2 (0.4)
<i>Constitutional symptoms</i>		
Chills and/or sweats (n, %)	10 (83.3)	9 (75.0)
Aches (n, %)	11 (91.7)	10 (83.3)
Fatigue (n, %)	11 (91.7)	11 (91.7)
Headache (n, %)	12 (100.0)	8 (66.7)
<i>Respiratory symptoms</i>		
Cough (n, %)	9 (75.0)	12 (100.0)
Pharyngitis (n, %)	4 (33.3)	5 (41.7)
Sore throat or nasal congestion (n, %)	7 (58.3)	6 (50.0)
<i>Other symptoms</i>		
Expectoration (n, %)	3 (25.0)	1 (9.1)
Otitis (n, %)	1 (8.3)	0 (0.0)
Digestive disturbance (n, %)	1 (8.3)	3 (25.0)

Table 2

Number of patients with a viral load above $3 \log_{10}$ cgeq/ μ l according to treatment group and time of sampling.

Days of treatment	Treatment		p Value ^a (O + Z) versus (O)
	(O + Z) (n = 12)	(O) (n = 12)	
<i>Number of patients with viral load above the $3 \log_{10}$ cgeq/μl threshold (%)</i>			
Day 0	12 (100.0%)	12 (100.0%)	
Day 1	11 (91.7%)	11 (91.7%)	
Day 2	11 (91.7%)	11 (91.7%)	1.00
Day 3	10 (83.3%)	8 (66.7%)	
Day 4	6 (50.0%)	4 (33.3%)	
Day 5	0/11 ^b (0.0%)	4/11 ^b (36.4%)	
Day 7	0/11 ^b (0.0%)	2/11 ^b (18.2%)	

(O + Z) means oseltamivir–zanamivir bitherapy, (O) means oseltamivir monotherapy.

At days 0–4 specimens consisted in nasal washes. At days 5 and 7 specimens were nasal swabs performed in order to control the negativation of viral excretion.

^a Statistical analysis was performed considering the number of patients with viral load under the $3 \log_{10}$ cgeq/ μ l threshold.

^b Samples were missing for some patients at days 5 and 7 and percentages were calculated out of 11 patients.

3.3. Mean viral load decrease based on treatment group

At day 0, the mean viral load was between 6.4 and 6.6 \log_{10} cgeq/ μ l according to the group. These mean viral loads decreased regularly in the two groups to reach between 2.6 and 2.8 \log_{10} cgeq/ μ l after four days of treatment. The mean viral load decreased regularly and similarly between the treatment groups. The mean viral load decreased 0.96 \log_{10} per day and 0.94 \log_{10} per day in the (O + Z) and (O) groups respectively. There was no significant difference between the two groups (Table 4).

3.4. Resolution of symptoms of influenza-like illness during treatment

The median duration of illness before inclusion was of 24 h whatever the group of treatment. There was no significant difference in the median delay for symptom alleviation between groups, with 4 days in the (O + Z) group versus 5.5 days in the (O) group.

3.5. Adverse events

Treatment-related adverse events were reported for 3 and 1 patients in the (O + Z) and (O) groups, respectively. These adverse

Table 3

Number of patients with a positive influenza virus culture according to treatment group and time of sampling.

Days of treatment	Treatment	
	(O + Z) (n = 9 ^a)	(O) (n = 7 ^a)
<i>Number of patients with a positive influenza virus in cell culture (%)</i>		
Day 0	9 (100.0%)	7 (100.0%)
Day 1	7 (77.8%)	5 (71.4%)
Day 2	4 (44.4%)	5 (71.4%)
Day 3	1 (11.1%)	4 (57.1%)
Day 4	0 (0.0%)	1 (14.3%)
Day 5	0 (0.0%)	0/6 ^b (0.0%)
Day 7	0/8 ^b (0.0%)	0/6 ^b (0.0%)

(O + Z) means oseltamivir–zanamivir bitherapy, (O) means oseltamivir monotherapy.

^a These results are for a subset of patients with a day 0 sample positive for influenza and with cell culture results available for all the samples received.

^b Samples at days 5 and 7 were missing for some patients. We could suppose that these missing samples would have been negative as the viral excretion was already negative for these patients at least one day before.

events were headaches, nausea, vomiting, and gastralgia in the (O + Z) group and nightmares in the (O) group.

Two severe adverse events were notified during the study but only one was judged to be oseltamivir related. It was a post prandial gastralgia successfully treated by lansoprazole.

3.6. Emergence of resistance to oseltamivir

We tested the presence of the H275Y NA mutation responsible for oseltamivir-resistance with an allele-specific rt RT-PCR in order to detect a possible emergence of oseltamivir-resistance. This test was performed on the nasal wash after 3 days of treatment (or 2 days of treatment when the detection of the M gene was negative at day 3) for all patients and on all samples for patients presenting a low viral load decrease. The mean Ct detecting the presence of an H in position 275 of the NA was 32.4 ± 5.44 and 34.6 ± 4.07 for samples after 3 days of treatment (or 2 days of treatment in three cases) from patients treated by (O) monotherapy or (O + Z) combination therapy respectively. With this approach we did not detect any H275Y NA mutation in the patients treated by (O + Z) combination therapy or (O) monotherapy.

4. Discussion

This clinical trial aimed to study the antiviral efficacy and tolerability of oseltamivir–zanamivir (O + Z) combination therapy compared to oseltamivir monotherapy (O) and was conducted during the A(H1N1)pdm09 virus pandemic in 2009–2010. To our knowledge, our study is the first one to evaluate the antiviral efficacy of this combination therapy *in vivo* on the A(H1N1)pdm09 virus.

We hypothesised that a combination of antiviral agents may lead to more rapid viral clearance and to reduction in the emergence of resistant viral strains. Our hypotheses were the extrapolations of previous studies' results (Hayden et al., 1999; Treanor et al., 2000) that are briefly described in material and methods section. We hypothesised from these studies that around 15%, 50% and 80% of patients should have a negative viral excretion after 24, 48 and 72 h of treatment by oseltamivir monotherapy. We then expected that 50% and 80% of the patients would be detected negative by influenza virus cell culture and would have a viral load under the threshold of $3 \log_{10}$ cgeq/ μ l after 48 h of oseltamivir monotherapy and oseltamivir–zanamivir bitherapy respectively. The results we obtained after viral cell culture for a few samples are similar to those of previous studies albeit the low number of included patients prevents extensive interpretation. However, it is only after 3 days of treatment, and not 2 days as expected, that nearly 43% and 89% of patients had a negative viral excretion with oseltamivir monotherapy or oseltamivir–zanamivir bitherapy respectively.

If we consider the results of the M gene amplification, after 48 h of treatment, patients with a viral load under $3 \log_{10}$ cgeq/ μ l were only 8.3% in both the (O + Z) and (O) groups respectively. In our study, the detection of viral excretion was performed by real-time RTqPCR. When samples were collected in patients presenting less than three days after symptom onset, viral culture and RT-PCR gave comparable results for the detection of A(H1N1)pdm09 virus (Cheng et al., 2010). In our hands, there was a probability of 80% that the A(H1N1)pdm09 viral culture was negative when the viral load estimated by M gene quantification was under the threshold of $3 \log_{10}$ cgeq/ μ l. Previous studies estimated viral load by tissue culture-infective doses and discrepancies between tissue culture-infective doses and real time RTqPCR could explain differences between the hypotheses and the observed results. There is a large difference between the expected and observed percentages of patients with a viral load under the threshold of $3 \log_{10}$ cgeq/ μ l.

Table 4
Mean viral load decrease from day 0 and day after day according to treatment group.

		Treatment		p Value (O + Z) versus (O)
		(O + Z) (n = 12)	(O) (n = 12)	
<i>Mean viral load decrease (SD)</i>				
Decrease from day 0 ^a				
	Day 0–1	1.30 (0.98)	1.05 (0.70)	
	Day 0–2	2.58 (1.05)	2.16 (1.55)	
	Day 0–3	3.18 (1.23)	2.73 (2.12)	
	Day 0–4	3.79 (1.56)	3.76 (1.89)	
Decrease day after day ^b				
	Day 0–1	1.30 (0.98)	1.05 (0.70)	
	Day 1–2	1.28 (0.78)	1.11 (1.20)	
	Day 2–3	0.68 ^c (0.51)	0.58 (1.03)	
	Day 3–4	0.59 ^c (1.09)	1.02 (1.65)	
	Estimated slope	0.96 (0.12)	0.94 (0.12)	0.90

(O + Z) means oseltamivir–zanamivir bitherapy, (O) means oseltamivir monotherapy.

Viral load is expressed in (M gene adjusted to GAPDH) log₁₀ cgeq/μl of nasal wash.

^a Mean viral load decrease from day 0 was calculated by subtracting the viral load at day 1, 2, 3 or 4 to the viral load at day 0 for each patient in each group. Then a mean and a standard deviation (SD) was calculated for each day (1 to 4) and for each patient group.

^b Mean viral load decrease day after day was calculated by subtracting the viral load at day 1, 2, 3, or 4 to the viral load of the day before. Then a mean and a SD was calculated for each day (day 0 to 1; day 1 to 2, etc.) and for each patient group.

^c For one patient in the (O + Z) group the nasal wash received at day 3 had no GAPDH gene detected and could not be interpreted. The calculation of mean viral load decrease between days 2 and 3 and between days 3 and 4 was then obtained considering 11 instead of 12 patients.

Because of this we think the evolution profile of influenza viral load and the occurrence of oseltamivir-resistance are better criteria for evaluating antiviral efficacy.

We could not detect any significant difference between treatment groups in the mean viral loads, the evolution of the viral loads, the delay for symptom alleviation and the occurrence of adverse events. The included population corresponds to the targeted population (age, sex, free of concomitant disease) as well as the symptom duration before treatment. However, the absence of significant difference could be due to the low number of patients included in our study that represents only 80% of the minimum expected number of patients (a minimum of 15 evaluable patients per group were expected).

Our study was conducted in healthy adults infected with influenza A(H1N1)pdm09 virus and we could not find any difference in treatment efficacy between the oseltamivir–zanamivir combination and oseltamivir alone. In the randomised placebo-controlled trial conducted in adults with 2008–2009 seasonal influenza A(H3N2) virus infection, the oseltamivir–zanamivir combination appeared less effective than oseltamivir monotherapy (Duval et al., 2010). In a study conducted *in vitro* on the A(H1N1)pdm09 virus, the combination of zanamivir and OC or peramivir showed additivity to moderate antagonism against influenza A(H1N1)pdm09 virus (Nguyen et al., 2010). Due to the low number of patients we were probably not able to perform analyses of viral load profiles powerful enough to detect small differences between treatment groups.

Our study was performed in healthy adults. However, the optimization of the antiviral treatment is important for critically ill patients. A study was conducted in Denmark in 21 patients admitted in intensive care unit (ICU), infected with influenza A(H1N1)pdm09 virus and treated with oseltamivir (150 mg twice a day) via a nasogastric tube and zanamivir (50 mg twice a day) administered in a soluble form for intravenous administration by a nebulizer in the inspiratory ventilator tube. The treatment was started more than 48 h after the first symptoms appeared and could explain a low efficiency of the antiviral treatment for these patients: 76% and 47.6% had a positive RT-PCR for A(H1N1)pdm09 at 7 and 10 days after the treatment start respectively. The mortality in these patients was high despite the combined antiviral treatment which could not prevent the development of acute lung injury (Petersen et al., 2011).

One major strength of our study is the close patient monitoring and virological analysis with nasal washes or swabs performed nearly every day for seven days and RTqPCR which allowed for a good estimation of viral decrease under treatment. We observed a regular decrease around 1.0 log₁₀ cgeq/μl every day in both treatment group. When comparing our results with those of the previous trial conducted on A(H3N2), we observed a similar decrease of 2 log₁₀ cgeq/μl in the viral load after two days of treatment despite some differences between trials, based on the type of neuraminidase and the duration of viral load follow-up (Duval et al., 2010).

We did not detect any H275Y NA mutation responsible for oseltamivir-resistance in both groups of treatment. The risk of oseltamivir-resistance emergence was low. This may be due to the fact that our population was adult and that influenza symptoms were mild. Oseltamivir-resistance emergence is preferentially observed in the context of prolonged viral excretion often associated with a paediatric population (Whitley et al., 2001; Kiso et al., 2004; Tamura et al., 2011), immunosuppression (Ison et al., 2006; Tramontana et al., 2010; Hill-Cawthorne et al., 2010), or severe cases (WHO, 2010; Petersen et al., 2011).

There are some limitations to our study. First, the low number of enrolled patients prevented us from detecting small differences between treatment groups. We decided to monitor patients closely for seven days to detect any possible oseltamivir-resistant influenza viruses. It was very difficult to include more patients with such a close follow-up. However, if combination therapy had a marked benefit in term of antiviral efficacy as expected in our first hypothesis, this could have been shown even with the few enrolled patients. We decided not to continue the study for a second flu season because we could not detect any significant difference and previous studies and *in vitro* data were unable to detect any advantage with the combinations therapies tested either. Secondly, our trial was conducted in adult outpatients that are at a low risk for oseltamivir-resistance. Close patient monitoring allowed us to detect possible oseltamivir-resistant viruses that occurred late in the course of treatment. However, it was difficult to implement our clinical trial in children, immunocompromised patients or those with a severe presentation making them at a higher potential risk of developing oseltamivir-resistant variants.

Our study could not detect whether the combination therapy (O + Z) improves or reduces the effectiveness of oseltamivir in

clinical practice for the treatment of influenza A(H1N1)pdm09 virus infection in an out-patient setting.

Alternative antiviral treatments to oseltamivir monotherapy are still needed against influenza infections mostly in severely ill and/or immunocompromised patients (Petersen et al., 2011). The triple combination of oseltamivir carboxylate, amantadine, and ribavirin was shown to be synergistic *in vitro* for amantadine- or oseltamivir-resistant strains (Nguyen et al., 2010), was superior to double combinations and single agents at suppressing resistance emergence *in vitro* (Hoopes et al., 2011), and was synergistic for A(H5N1) and A(H1N1)pdm09 virus infection in mice (Nguyen et al., 2012). Clinical studies are in progress to identify the potential benefits of this triple combination in humans (Nguyen et al., 2012). New antiviral agents, like favipiravir or DAS181 are promising as new influenza antiviral agents with novel mechanisms of action (Boltz et al., 2010).

5. Conclusions

Due to the limited sample size we could not detect any therapeutic benefit in using oseltamivir–zanamivir bitherapy compared to oseltamivir monotherapy for ambulatory patients infected with influenza A(H1N1)pdm09 virus. Other studies are necessary to confirm this result and to improve the antiviral treatment of patients infected with influenza, especially for those who are immunocompromised and severely ill.

Role of the funding source

The funder had no role in study design, in the collection, analysis and interpretation of data, in the writing of the report and in the decision to where to submit the paper for publication.

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A.M. has membership in the ministry of health advisory board on influenza, was involved in epidemiological studies partially or fully granted by Roche and GSK and declares travel grants from Roche for participation in scientific meetings.

X.D. had a conference invitation from GSK and lecture fees from Roche and Gilead.

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B.L. declares potential conflicts of interests with Roche, GSK, BMS, and BioCryst. B.L. is a member of the scientific board of the GEIG and of the ESWI Board of Directors.

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4.1.2 Essai Bivir : bithérapie (oseltamivir + zanamivir) comparée à l'oseltamivir ou au zanamivir seul dans le traitement de la grippe (article 2)

Dans le contexte décrit précédemment d'émergence de résistance naturelle à l'oseltamivir (88, 89) et de préparation au risque pandémique, il est apparu opportun de déterminer s'il existait un bénéfice virologique (durée d'excrétion virale) et clinique (durée des symptômes) à l'utilisation d'une bithérapie associant deux INA. Ce travail de recherche a été réalisé dans le cadre d'un projet hospitalier de recherche clinique (PHRC). En parallèle de l'essai Combina, un essai plus large a été mis en place. L'objectif était de comparer l'efficacité d'un traitement par une bithérapie oseltamivir + zanamivir en comparaison de chaque monothérapie.

Cet essai en double aveugle a été mené en médecine générale en France au cours de la saison hivernale 2008-2009. Les inclusions concernaient les patients adultes vus en consultation et présentant un syndrome grippal depuis moins de 36h avec un test de diagnostic rapide positif pour la grippe. Les personnes incluses étaient randomisées dans l'un des trois bras de l'essai : oseltamivir + zanamivir (OZ), oseltamivir + un placebo inhalé (O), ou zanamivir + un placebo par voie orale (Z) pendant 5 jours. L'efficacité du traitement était évaluée sur un critère de jugement principal virologique (proportion de patients avec une charge virale négative au deuxième jour de traitement (J2)), et un critère de jugement secondaire clinique sur la durée des symptômes. Il était initialement prévu l'inclusion de 900 patients au cours de deux épidémies de grippe saisonnière (2008/2009 et 2009/2010). Mais en raison de l'émergence de la pandémie grippale due au virus A(H1N1)pdm09 en avril 2009 en Amérique du Nord (12), et sa propagation dans le monde entier dans les mois qui ont suivi, le comité indépendant de l'essai a demandé que soit mis fin à l'essai au bout d'un an et que les résultats soient analysés plus tôt que prévu, au regard de l'impact possible des conclusions en matière de gestion de traitement antiviral pendant la pandémie.

Au total, 541 patients ont été inclus. Dans l'analyse en intention de traiter menée chez les 447 patients atteints à J0 d'une grippe de type A confirmée par RT-PCR, respectivement 46%, 59%, et

34% dans les bras OZ (n = 157), O (n = 141), et Z (n = 149) avaient une RT-PCR négative, avec une différence significative pour les comparaisons OZ/O et OZ/Z (respectivement -13,0% [IC95% : -23,1 à -2,9] et + 12,3% [IC95% : 2,39 à 22,2]). La durée moyenne de disparition des symptômes était de 4, 3 et 4 jours (+1,0 [IC95% : 0,0 à 4,0] et + 0,0 [IC95% : -3,0 à 3,0]). Quatre événements indésirables graves ont été observés. Les nausées et/ou vomissements avaient tendance à être plus fréquents dans le bras de la bithérapie (OZ, n = 13; O, n = 4 et Z, n = 5 patients, respectivement).

Chez les adultes présentant une grippe, majoritairement de type A(H3N2), un traitement en association oseltamivir + zanamivir semblait moins efficace que l'oseltamivir en monothérapie, et faiblement plus efficace que le zanamivir en monothérapie. Ce résultat, important pour la prise en charge des patients présentant une forme particulièrement grave de grippe saisonnière et pour la préparation d'une future pandémie, semblait robuste au regard des points suivants : i) il a été obtenu lors d'un essai en double aveugle, ii) il existait une concordance entre les résultats cliniques et virologiques, et iii) il a été retrouvé dans les différents sous-groupes de l'étude (les 541 patients inclus, les 447 ayant présenté une grippe confirmée et les 382 ayant présenté une grippe confirmée et ayant bien suivi le traitement prescrit). Contrairement à ce qui avait été évoqué sur la complémentarité possible de l'oseltamivir et du zanamivir, qui interagissent de façon différente avec la neuraminidase (1), une étude in vitro a mis en avant le peu de synergie qui existait entre ces deux molécules (97), expliquant probablement les résultats retrouvés dans l'essai Bivir. Chez l'homme, en dehors des essais Combina et Bivir, seules deux études non comparatives ont été réalisées en réanimation lors de la pandémie A(H1N1)pdm09 sans montrer d'intérêt de la bithérapie oseltamivir-zanamivir (92, 93). Aucun autre essai randomisé n'a été réalisé à ce jour chez l'homme pour tester cette association. Seul un essai canadien récent chez la souris est disponible (98). Il concluait que l'association oseltamivir + zanamivir avait une efficacité comparable à celle du zanamivir seul, mais supérieure à celle de l'oseltamivir. Des questions étaient toutefois posées sur le dosage de zanamivir utilisé dans cette étude et l'impossibilité de dire qu'il pouvait être considéré

comme identique à celui utilisé chez l'homme. Le zanamivir était de plus utilisé en intranasal et non en aérosol. Un tableau de synthèse sur les études qui ont évalué l'association oseltamivir – zanamivir est présenté ci-dessous. Ainsi, malgré le potentiel théorique de la réduction de l'émergence de la résistance aux antiviraux, la faible efficacité de cette combinaison observée à ce jour n'appelle pas à son utilisation en pratique clinique.

Tableau de synthèse des études réalisées sur l'efficacité de l'association de deux INA dans le traitement de la grippe

Etude	Contexte	Type	Bras de traitement	Virus grippal étudié	Nombre de sujets
Nguyen JT et al (97)	In vitro	Etude comparative	Différentes associations : oseltamivir, zanamivir, peramivir, amantadine, ribavirine	A(H1N1)pdm09	-
Pizzorno A et al (98)	Souris	Essai randomisé	oseltamivir-zanamivir vs. oseltamivir ou zanamivir seul	A(H1N1)pdm09 et A(H3N2)	48
Chan KKC et al (93)	Homme en réanimation	Etude descriptive	oseltamivir-zanamivir et oseltamivir seul	A(H1N1)pdm09	7
Petersen E et al (92)	Homme en réanimation	Etude descriptive	oseltamivir-zanamivir	A(H1N1)pdm09	21
Escuret V et al (35)	Homme en médecine générale	Essai randomisé	oseltamivir-zanamivir vs. oseltamivir seul	A(H1N1)pdm09	24
Duval X et al (36)	Homme en médecine générale	Essai randomisé	oseltamivir-zanamivir vs. oseltamivir ou zanamivir seul	A(H3N2)	541

Les résultats de cette étude ont été publiés dans PLOS Medicine (36) :

- Duval X, van der Werf S, **Blanchon T**, Mosnier A, Bouscambert-Duchamp M, Tibi A, Enouf V, Charlois-Ou C, Vincent C, Andreoletti L, Tubach F, Lina B, Mentre F, Leport C. Efficacy of oseltamivir-zanamivir combination compared to each monotherapy for seasonal influenza: a randomized placebo-controlled trial. PLoS Med. 2010;7(11):e1000362.

Efficacy of Oseltamivir-Zanamivir Combination Compared to Each Monotherapy for Seasonal Influenza: A Randomized Placebo-Controlled Trial

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Abstract

Background: Neuraminidase inhibitors are thought to be efficacious in reducing the time to alleviation of symptoms in outpatients with seasonal influenza. The objective of this study was to compare the short-term virological efficacy of oseltamivir-zanamivir combination versus each monotherapy plus placebo.

Methods and Findings: We conducted a randomized placebo-controlled trial with 145 general practitioners throughout France during the 2008–2009 seasonal influenza epidemic. Patients, general practitioners, and outcome assessors were all blinded to treatment assignment. Adult outpatients presenting influenza-like illness for less than 36 hours and a positive influenza A rapid test diagnosis were randomized to oseltamivir 75 mg orally twice daily plus zanamivir 10 mg by inhalation twice daily (OZ), oseltamivir plus inhaled placebo (O), or zanamivir plus oral placebo (Z). Treatment efficacy was assessed virologically according to the proportion of patients with nasal influenza reverse transcription (RT)-PCR below 200 copies genome equivalent (cgeq)/ μ l at day 2 (primary outcome), and clinically to the time to alleviation of symptoms until day 14. Overall 541 patients (of the 900 planned) were included (OZ, $n = 192$; O, $n = 176$; Z, $n = 173$), 49% male, mean age 39 years. In the intention-to-treat analysis conducted in the 447 patients with RT-PCR-confirmed influenza A, 46%, 59%, and 34% in OZ ($n = 157$), O ($n = 141$), and Z ($n = 149$) arms had RT-PCR < 200 cgeq/ μ l (-13.0% , 95% confidence interval [CI] -23.1 to -2.9 , $p = 0.025$; $+12.3\%$, 95% CI 2.39 – 22.2 , $p = 0.028$ for OZ/O and OZ/Z comparisons). Mean day 0 to day 2 viral load decrease was 2.14, 2.49, and 1.68 \log_{10} cgeq/ μ l ($p = 0.060$, $p = 0.016$ for OZ/O and OZ/Z). Median time to alleviation of symptoms was 4.0, 3.0, and 4.0 days ($+1.0$, 95% CI 0.0 – 4.0 , $p = 0.018$; $+0.0$, 95% CI -3.0 to 3.0 , $p = 0.960$ for OZ/O and OZ/Z). Four severe adverse events were observed. Nausea and/or vomiting tended to be more frequent in the combination arm (OZ, $n = 13$; O, $n = 4$; and Z, $n = 5$ patients, respectively).

Conclusions: In adults with seasonal influenza A mainly H3N2 virus infection, the oseltamivir-zanamivir combination appeared less effective than oseltamivir monotherapy, and not significantly more effective than zanamivir monotherapy. Despite the theoretical potential for the reduction of the emergence of antiviral resistance, the lower effectiveness of this combination calls for caution in its use in clinical practice.

Trial registration: <http://www.ClinicalTrials.gov> NCT00799760

Please see later in the article for the Editors' Summary.

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Competing Interests: XD has had a conference invitation from GSK and lecture fees from Roche and Gilead. AM has membership in the ministry of health advisory board on influenza; involvement in some epidemiological studies partially or fully granted by Roche and GSK, and travel grants from Roche for participation in scientific meetings. SVDW has had a conference invitation from GSK; research grant from GSK on unrelated subject; joined patent from institution with GSK on unrelated subject; travel grants for meetings from GSK; contribution to clinical trial financed by Roche; member of the advisory committee on influenza of the French ministry of health; is a member of ESWI; is a member of the scientific committee of the GEIG; and is vice-president of the GROG network. FM received fees from Roche, preclinical pharmacokinetic department, for a course on MONOLIX in December 2008. BL has had paid consultancy and board membership (Roche, GSK, Novartis, BioCryst, MedImmune), has had research grants from Roche and Sanofi-Pasteur, and had received travel grants and honoraria for speaking or participation at meetings (Roche, Sanofi-Pasteur).

Abbreviations: cgeq, copies genome equivalent; CI, confidence interval; IQR, interquartile range; ITT, intention to treat; NIC, National Influenza Centre; O, oseltamivir; OZ, oseltamivir-zanamivir combination; RT, reverse transcription; SD, standard deviation; Z, zanamivir.

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Introduction

Neuraminidase inhibitors (oseltamivir [O], zanamivir [Z]) are thought to be efficacious as compared to placebo in outpatients with uncomplicated seasonal influenza [1–6], both clinically in terms of reduction in duration of symptoms, as well as in terms of a reduction in viral shedding. In 2008, they were considered an important strategy to limit the impact of an influenza pandemic both individually, by reducing morbidity and mortality, and collectively, by slowing spread of the virus to allow time for vaccine production, the cornerstone of influenza control [2–4,7]. It was hypothesized that the widespread use of a single antiviral might result in the emergence of resistant strains whose subsequent spread could dramatically reduce the effectiveness of antiviral therapy. The combination of two antiviral agents, if well tolerated, and if producing at least additive antiviral activity, theoretically offers several advantages: reducing disease severity, viral shedding, and viral excretion period, thereby also lowering the attack rate and risk of selection of resistant viruses, specifically in individuals with prolonged viral shedding, such as immunocompromised patients [8,9]. Indeed, mathematical modelling showed a reduction in risk of emergence of resistant strains during early phases of a pandemic, associated with use of two antivirals as compared to single antiviral therapy [9]. Finally, another theoretical advantage of combining two drugs would be to ensure optimal treatment of all circulating influenza virus types, subtypes, or variants, as susceptibility of influenza viruses has been shown to vary, and seasonal H1N1 viruses naturally resistant to oseltamivir, which remain susceptible to zanamivir, emerged in 2008 [10]. Among antivirals active against influenza virus, the combination of neuraminidase inhibitors is attractive, because both compounds are licensed for seasonal influenza, they are delivered to the respiratory tract by distinct means (directly through a diskhaler for zanamivir, after gastrointestinal absorption and hepatic metabolism for oseltamivir), and key mutations associated with resistance are different for each drug. However, negative interactions cannot not be ruled out owing to the possible competition between these two drugs, which target the same binding pocket in the neuraminidase.

In 2006, in the context of pandemic planning, we designed a double-placebo randomized controlled trial in patients presenting with seasonal influenza-like illness to compare the oseltamivir-zanamivir combination to each of the monotherapies plus placebo. The trial was conducted in France, during the winter of 2008–2009. Because of the emergence of the pandemic 2009 (H1N1) virus in humans, in April 2009 in North America, and its subsequent worldwide spread, the independent data-monitoring committee requested that we terminate the trial early and analyze the results earlier than planned, given the possible impact of the results on antiviral treatment management during the pandemic [11].

Methods

Patients

From January 7th to March 15th 2009 (period of the winter 2008–2009 influenza epidemic in France), we enrolled throughout France adults 18 y old and older who consulted their general practitioner within 36 h of influenza symptoms onset (following the first influenza symptoms reported by the patient), with a temperature greater than or equal to 38°C (reported or observed by the practitioner), one or more respiratory symptoms (cough, sore throat), one or more general symptoms (headache, dizziness, myalgia, sweats and/or chills, fatigue), and a positive nasal rapid

test for influenza A (Clearview Exact Influenza A & B) performed by the practitioner. Enrolment of women required a negative urine pregnancy test. Exclusion criteria were vaccination against influenza during the 2008–2009 season, recent exacerbations of chronic obstructive pulmonary disease (COPD), asthma or severe chronic disease, previous history of depression, and prior inclusion in this trial. Prior to inclusion, patients gave informed written consent. The protocol was approved by the Ethics Committee of Ile de France 1 (Texts S1 and S2).

Study Procedures

At enrolment (day 0), a nasal swab for virological analysis was performed using a standard operating procedure (sample kit plus instructional video). Patients were allocated to treatment by a randomization list, with an arm ratio of 1:1:1, balanced by practitioner. A computer random number generator was used to select random permuted blocks of size 3. This randomisation code was given to the central hospital pharmacy that prepared blinded treatment units in conformity with good manufacturing practices (GMP). Each general practitioner received six treatment units and was told to distribute them by order of inclusion of his patients in the trial. Allocation was concealed through the similarity of all the containers and the impossibility for the GP to identify the treatment arm when opening the container. The three treatments were (1) oseltamivir capsule for oral use plus inhaled zanamivir, (2) oseltamivir plus inhaled placebo, (3) zanamivir plus oral placebo. Oseltamivir dosage was 75 mg orally twice daily; zanamivir dosage was 10 mg by oral inhalation using the commercialized GlaxoSmithKline Diskhaler, twice daily. Active drugs and placebo were kindly provided by Roche and Glaxo-SmithKline laboratories. A visiting nurse performed a nasal swab for virological analysis on day 2. Patients returned to their general practitioner at day 7 for a follow-up examination, and were contacted by phone on day 14. Patients, general practitioners assigning the patients, and outcome assessors (practitioners, virologists, patients), were blinded to treatment assignment throughout the study and statisticians until the end of the analysis.

Virological Analysis

Nasal swabs placed into a transport medium (Virocult, Elitech) were transported at 4°C by special courier to the nearest National Influenza Centre (NIC) (Hospices Civils de Lyon, Lyon, or Pasteur Institute, Paris, France). Upon arrival, the swab samples were eluted into 2 ml of transport medium, processed for real-time reverse transcription (RT)-PCR analyses and inoculated onto MDCK cells for virus isolation and subsequent subtyping using a standard hemagglutination inhibition assay. For RT-PCR analyses, RNA extraction from 200 µl of specimen was performed using the QIAmp virus RNA mini kit (Qiagen) with RNA elution into a final volume of 60 µl. All real-time RT-PCR assays were performed in a final volume of 15 µl with 5 µl RNA, 0. µM of each primer, 0.2 µM probe, and 0.8 µl enzyme mix (SuperScriptIII platinum one-step quantitative RT-PCR system, Invitrogen). Type A influenza virus RNA was detected by a real-time RT-PCR targeting the conserved matrix gene using GRAM/7Fw (5'-CTTCTAACCGAGGTGCGAAACGTA-3') and GRAM/161Rv (5'-GGTGACAGGATTG GTCTTGTCTTTA-3') primers and GRAM probe/52/+ (5'[Fam]-TCAGGCC CCTCAA-AGCCGAG-[BHQ-1]3') probe. The quality of the specimens was assessed by real-time RT-PCR targeting the GAPDH cellular gene [12]. Amplification was performed on a LightCycler 480 (Roche Diagnostics) (NIC, Pasteur Institute, Paris) or an ABI 7500 (Applied Biosystems) (NIC, Lyon). Cycling conditions are available

upon request. Quantified synthetic RNA transcripts corresponding to the M and GAPDH genes were used as controls in parallel [13].

To take into account the variability in the quantity of cells collected by nasal swab, we calculated a normalized influenza viral load for each specimen; this normalized viral load was defined as the ratio of the M RT-PCR and GAPDH RT-PCR multiplied by the average GAPDH RT-PCR at day 0 to express results in copies of genome equivalent/ μl (cgeq/ μl). The virological response was defined as a normalized viral load below 200 cgeq/ μl at day 2. This threshold was determined according to results on specimens from patients with a positive influenza A rapid test from winter 2008–2009 included in the French influenza surveillance network (GROG), and analysed by the 2NICs, and because it resulted in 5% false positive and 5% false negative results with respect to virus isolation (Table S1). It was validated by the independent data-monitoring committee prior to any analysis. Sensitivity of the qRT-PCR was assessed using serial dilutions of quantified synthetic transcripts corresponding to the target genes (Text S3). To assess comparability of the data between the two NICs, specimens were exchanged showing excellent concordance. The threshold of the qRT-PCR used was set well above the limit of detection.

Clinical Response

Oral temperature was recorded and severity of seven symptoms (nasal stuffiness, sore throat, cough, muscle aches, tiredness or fatigue, headache, and feverishness) was rated by the patient twice daily (morning and evening) up to day 5 and then once daily on a four-point scale (0, none; 1, mild; 2, moderate; 3, severe) [2–4,14]. The time to resolution of illness was defined as time from study drug initiation to time of symptom alleviation. Symptom alleviation was defined as the first 24-h period during which the above seven symptoms were absent or only mild as previously described [3,4]. Influenza-related clinical events were defined as incidence of a secondary complication (such as pneumonitis or otitis) independently of any antibiotic initiation, and/or occurrence of exacerbation of a preexisting chronic disease. Patients reported treatment compliance using a self-administered questionnaire; full compliance during the day 0 to day 2 period was considered when 100% of planned drug intakes had been completed.

Endpoints, Analyzed Populations

The primary efficacy endpoint was the proportion of patients with RT-PCR < 200 cgeq/ μl on day 2 of treatment. Given the viral shedding kinetics in patients with seasonal influenza receiving neuraminidase inhibitors, the day 2 virological endpoint was considered to be best suited to measurement of virological effects [2,4]. Other endpoints were (1) the decrease of \log_{10} viral load between days 0 and 2 in the patients with confirmed influenza A on day 0 and available samples both at days 0 and 2; (2) the time to resolution of illness; (3) the number of patients with alleviation of symptoms at the end of treatment (day 5); (4) the symptoms score at the end of treatment; (5) the incidence of secondary complications of influenza such as otitis, bronchitis, sinusitis, pneumonia, and the use of antibiotics; (6) the occurrence of adverse events in all participants having received at least one dose.

According to the protocol, the intention-to-treat (ITT) analysis was performed on two populations: (1) all enrolled patients (primary objective), and (2) enrolled patients with an influenza A virus infection confirmed by RT-PCR on day 0 (influenza A-infected population).

Sample Size and Statistical Analysis

Sample size evaluation assumed that virological response was obtained in 70% of patients in the oseltamivir-zanamivir arm,

compared to 55% in each of monotherapy arms on the basis of the extrapolation of the results of previous trials [2,4]. Samples of 300 subjects per arm had 90% power to detect this difference, with a two-sided test and a type I error of 0.025 because of the two tests (factoring 20% lack of follow-up). Proportions of response at day 2 were compared between the combination therapy arm (OZ) and each monotherapy arm (O or Z) separately using two tests with a type I error of 0.025 because of the two tests. Patients without a day 2 sample were considered treatment failures. Mean decreases of \log_{10} viral load were compared using a *t*-test in patients who had both day 0 and day 2 samples assuming a value of 0.5 cgeq/ μl when RT-PCR was negative. For clinical endpoints nonparametric tests were used. Times to resolution of illness and symptoms score at the end of treatment were compared using Wilcoxon tests. If time to symptom alleviation was missing it was imputed to be 14 d, i.e., the end of the trial; 95% confidence intervals (95% CIs) of median differences were estimated by bootstrap. Probability of symptoms alleviation versus day of treatment was estimated using the Kaplan Meier method and was compared between groups with the log-rank test. Proportions of clinical events and of patients with alleviation of symptoms were compared using Fisher's exact tests. As an exploratory analysis, 95% CIs for differences of response between the two monotherapies were also estimated. All analyses were performed using SAS software, version 9.1 (SAS Institute).

Early Study Termination

Recruitment was interrupted on March 15th 2009 at the end of the epidemic period in France after the enrolment of the first 541 patients and prior to the emergence of the 2009 H1N1 pandemic later in 2009. A second recruitment period was planned for the next winter (i.e., 2009–2010) to reach the number of 900 patients required. However, due to the emergence of the 2009 H1N1 pandemic virus, the independent data-monitoring committee, without any prior knowledge of the results, recommended terminating the trial and conducting an early analysis on May 6th 2009. This decision was based firstly on the need to rapidly provide results on efficacy and tolerance of combined oseltamivir-zanamivir therapy, and secondly, on the inadvisability of pooling the results of the two winters, one with a seasonal virus (winter 2008–2009, mainly H3N2) and the other one with a novel pandemic virus of a different subtype (winter 2009–2010).

Results

Patients

Out of the 900 patients initially planned, a total of 541 patients were enrolled by 145 general practitioners. They were randomly assigned to oseltamivir plus zanamivir (OZ, $n = 192$), oseltamivir plus inhaled placebo (O, $n = 176$), zanamivir plus oral placebo (Z, $n = 173$) (Figure 1). Mean age was 39 y (standard deviation [SD] = 13), 49% were male, 14% had preexisting chronic diseases, mean fever was 38.2°C (SD = 0.8). The mean duration of illness before enrolment was 25 h (SD = 10). Other characteristics of patients appeared well balanced in the three arms (Table 1). The rate of fully compliant patients was not significantly different among the three arms (84% for the OZ arm, 88% for the O arm, and 85% for the Z arm).

Virological Samples

Out of the 541 enrolled patients, 447 (83%) had a RT-PCR laboratory confirmation of influenza A virus infection on the day 0 specimen, with a mean viral load of 4.38 \log_{10} cgeq/ μl (interquartile range [IQR] 3.75–5.30). All the day 0 specimens were GAPDH RT-PCR positive with a mean value of 3.88 \log_{10} copies/ μl .

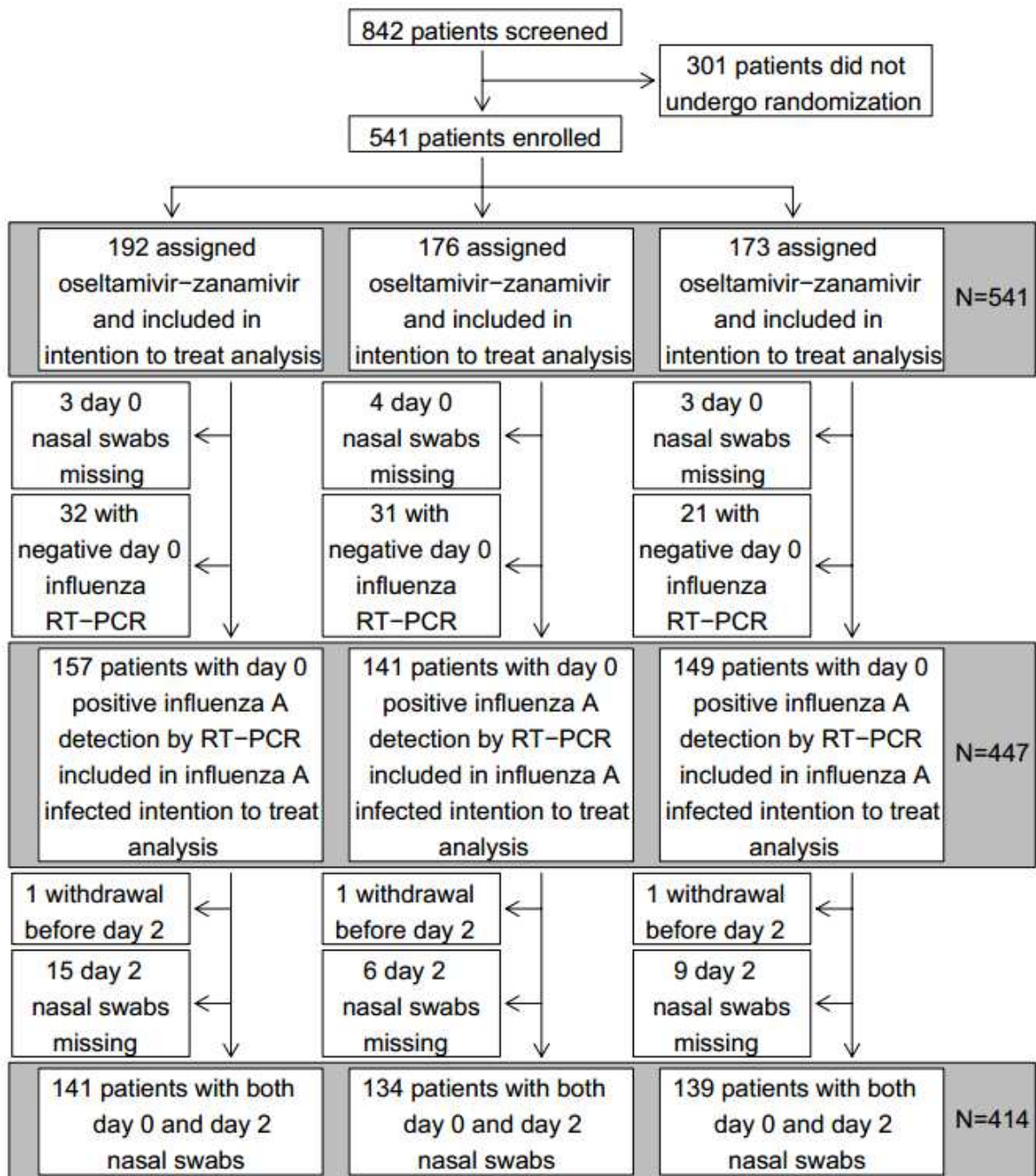


Figure 1. Trial flow chart.
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Virological Endpoints

Primary endpoint. In the ITT analysis, considering the 541 enrolled patients with positive influenza A rapid test, the proportion of patients with a RT-PCR <200 cgeq/μl on day 2 of treatment was 52.6% in the oseltamivir-zanamivir arm, 62.5% in the oseltamivir monotherapy arm ($p = 0.055$, for the OZ versus

O comparison, treatment effect comparison: -9.9% , [95% CI -19.9 to 0.2]), and 40.5% in the zanamivir monotherapy arm ($p = 0.020$, for the OZ versus Z comparison; treatment effect comparison: $+12.1\%$, [95% CI 2.02 - 22.3]) (Table 2).

In the ITT analysis, considering the 447 influenza RT-PCR-confirmed patients, the proportions were 45.9% in the oseltamivir-

Table 1. Characteristics of the 541 patients enrolled in the study and of the 447 influenza A-infected patients according to treatment arms.

Patients	Characteristics	Combined Oseltamivir and Zanamivir	Oseltamivir Plus Placebo	Zanamivir Plus Placebo
All patients included in the study n=541 (%)		n=192 (35.5%)	n=176 (32.5%)	n=173 (32.0%)
	Age (y): mean (SD)	38.7 (13.2)	39.5 (13.1)	39.9 (13.8)
	[Age range]	[18.3–73.2]	[18.1–76.3]	[18.0–84.2]
	n male (%)	91 (47.6%)	92 (52.3%)	86 (49.7%)
	n smoker (%)	34 (17.8%)	25 (14.2%)	26 (15.0%)
	n comorbidities (%)	27 (14.1%)	27 (15.3%)	23 (13.3%)
	n fever at enrolment ≥38°C (%)	123 (69.9%)	118 (73.3%)	117 (75.5%)
	n initiation of treatment ≤24 h after onset of symptoms (%)	92 (47.9%)	85 (48.3%)	101 (58.4%)
	Symptoms score per patient ^a			
	Mean (SD)	15.2 (2.8)	14.9 (3.2)	15.1 (3.2)
	% of maximal score: mean (SD) ^b	72.4% (13.4)	71.0% (15.2)	72.1% (15.4)
Influenza A-infected patients n=447 (%)		n=157 (35.1%)	n=141 (31.6%)	n=149 (33.3%)
	Age (y): mean (SD)	38.7 (13.2)	39.5 (13.0)	40.1 (14.1)
	[Age range]	[18.3–73.2]	[18.1–76.3]	[18.0–84.2]
	n male (%)	76 (48.7%)	73 (51.8%)	77 (51.7%)
	n smoker (%)	22 (14.1%)	15 (10.7%)	20 (13.4%)
	n comorbidities (%)	21 (13.4%)	20 (14.2%)	20 (13.4%)
	n fever ≥38°C at enrolment (%)	101 (67.8%)	95 (70.9%)	104 (75.9%)
	n initiation of treatment ≤24 h after onset of symptoms (%)	72 (45.9%)	68 (48.2%)	86 (57.7%)
	Symptoms score per patient ^a			
	Mean (SD)	15.6 (2.7)	15.3 (3.2)	15.5 (3.1)
	% of maximal score: mean (SD) ^b	74.2% (12.8)	72.7% (15.2)	73.8% (15.0)
	Influenza virus subtype			
	H1N1	9 (5.7%)	5 (3.5%)	7 (4.7%)
	H3N2	136 (86.6%)	130 (92.2%)	129 (86.6%)
	Not determined	12 (7.6%)	6 (4.3%)	13 (8.7%)

^aSum of the severity of the seven day 0 influenza symptoms (feverishness, nasal stuffiness, sore throat, cough, muscle aches, tiredness-fatigue, and headache) using a four-point scale [2,14].

^bThe score is expressed as a percentage of the maximal score of 21.

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zanamivir arm, 58.9% in the oseltamivir monotherapy arm ($p=0.025$ for the OZ versus O comparison; treatment effect comparison: -13.0% , [95% CI -23.1 to -2.9]), and 33.6% in the zanamivir monotherapy arm ($p=0.028$ for the OZ versus Z comparison; treatment effect comparison: $+12.3\%$, [95% CI 2.39 – 22.2]) (Table 2). The same trends were observed in the 382 fully compliant influenza A-infected patients (Table S3), and in the 395 patients with H3N2 infection with proportions of 42.4% in the oseltamivir-zanamivir arm, 58.6% in the oseltamivir monotherapy arm, and 30.3% in the zanamivir monotherapy arm.

Other virological endpoints. In the 414 influenza RT-PCR confirmed patients with both day 0 and day 2 available specimens, the day 2 to day 0 decrease was $2.14 \log_{10}$ cgeq/ μ l in the oseltamivir-zanamivir arm, $2.49 \log_{10}$ cgeq/ μ l in the oseltamivir monotherapy arm, ($p=0.060$ for the OZ versus O comparison; treatment effect comparison -0.35 , [95% CI -0.8 to 0.07]), and $1.68 \log_{10}$ cgeq/ μ l in the zanamivir monotherapy arm ($p=0.016$ for the OZ versus Z comparison; treatment effect comparison: $+0.46$, [CI 95% 0.03 – 0.9]) (Table 2).

Clinical Endpoints

The median time to resolution of illness in the 541 enrolled patients was 3.5 d in the oseltamivir-zanamivir arm, 3.0 d in the oseltamivir monotherapy arm ($p=0.015$ for the OZ versus O comparison; treatment effect comparison: $+0.5\%$, [95% CI 0.0 – 1.5]), and 4.0 d in the zanamivir monotherapy arm ($p=0.78$ for the OZ versus Z comparison; treatment effect comparison: -0.5 , [95% CI -1.0 to 0.5]) (Table 3). In the 447 influenza A-infected patients, this figure was 4.0 d in the oseltamivir-zanamivir arm, 3.0 d in the oseltamivir monotherapy arm ($p=0.018$ for the OZ versus O comparison; treatment effect comparison: $+1.0$, [95% CI 0.0 – 4.0]), and 4.0 d in the zanamivir monotherapy arm ($p=0.96$ for the OZ versus Z comparison; treatment effect comparison: $+0.0$, [95% CI -3.0 to 3.0]). Figure 2 presents the time to resolution of illness in the 447 patients, also showing by the log-rank test a significantly shorter time in the oseltamivir monotherapy arm. The time to resolution of illness was significantly shorter in patients with day 2 viral load below 200 cgeq/l (3.5 d) than in patients with viral load above 200 cgeq/l (7 d; $p=0.0014$). The

Table 2. Virological and clinical response according to treatment arms in the 541 enrolled patients, between day 0 and day 2 (ITT analysis).

Type of Response	Virological and Clinical Response Variables	Combined Oseltamivir and Zanamivir	Oseltamivir Plus Placebo	O+Z versus O		Zanamivir Plus Placebo	O+Z versus Z		O versus Z
				p-Value	Difference [95% CI]		p-Value	Difference [95% CI]	
	<i>n</i> patients	192	176			173			
Virological	Primary virological endpoint								
	Day 2 influenza RT-PCR<200 cgeq/μl (% patients)	52.6%	62.5%	0.055	-9.9% [-19.9 to 0.2]	40.5%	0.020	+12.1% [2.02-22.3]	+22.0% [12.1-32.0]
Clinical	Time to resolution of illness in days (median, IQR)	3.5 [2.5-14]	3.0 [2-7]	0.015	+0.5 [0.0-1.5]	4.0 [2.5-14]	0.78	-0.5 [-1.0 to 0.5]	-1.0 [-1.5 to -0.5]
	<i>n</i> (%) of patients with alleviation of symptoms at end of treatment	111 (57.8%)	122 (69.3%)	0.023	-11.5% [-21.3 to -1.7]	100 (57.8%)	1.00	+0.0% [-10.1 to 10.1]	+11.5% [1.7-21.3]
	Symptoms score at end of treatment (median, IQR)	3 [2-5]	2 [1-4]	0.0006	+1.0 [0.0-1.0]	3 [1-6]	0.79	+0.0 [-1.0 to 0.0]	-1.0 [-2.0 to -1.0]
	<i>n</i> (%) of patients with clinical event during treatment	26 (13.5%)	15 (8.5%)	0.14	+5.0% [-1.3 to 11.4]	23 (13.3%)	1.00	+0.3% [-6.7 to 7.2]	-4.8% [-11.2 to 1.6]
	Initiation of antibiotics	17 (8.9%)	10 (5.7%)	—	—	13 (7.5%)	—	—	—
	Pneumonia	2 (1.0%)	1 (0.6%)	—	—	0 (0.0%)	—	—	—
	Other	21 (10.9%)	14 (8.0%)	—	—	22 (12.7%)	—	—	—

*Exploratory analysis.
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median symptoms score at day 5 (end of treatment) was 3 in the oseltamivir-zanamivir arm, 2 in the oseltamivir monotherapy arm ($p = 0.013$ for the OZ versus O comparison; treatment effect comparison: +1, [95% CI 0.0-1.0]), and 3 in the zanamivir monotherapy arm ($p = 0.93$ for the OZ versus Z comparison; treatment effect comparison: +0.0, [95% CI -1.0 to 0.0]) (Tables 2 and 3). Other clinical outcomes showed similar trends (Tables 2, 3, and S3).

Tolerance

Four serious adverse events occurred during the study, one of which was considered unrelated to study drugs (acute bacterial pneumonia at day 3 in a patient receiving oseltamivir-zanamivir combination). Two adverse events also occurred in patients receiving the oseltamivir-zanamivir combination: severe headaches leading to interruption of therapy and facial oedema following the first administration, disappearing within 24 h postdrug interruption. The remaining patient experienced repeated vomiting after oseltamivir monotherapy drug administration. All four patients completely recovered.

Other nonserious adverse events reported in more than 1% of the total population were in the OZ, O, and Z arms, respectively, nausea and/or vomiting (in 13, 4, and 5 patients), diarrhoea (in 2, 1, and 5 patients), and rash (in 1, 2, and 2 patients).

Discussion

This large publicly funded clinical trial examined the effect of combination neuraminidase inhibitor antiviral therapy in influenza, as compared to each monotherapy plus placebo. It showed that, during the prepandemic winter of 2009 with a predominance of H3N2 viruses (more than 85%) in France, the oseltamivir-zanamivir combination seemed less effective than oseltamivir monotherapy, and not significantly more effective

than zanamivir monotherapy in adults with seasonal influenza A virus infection.

Analysis of the different antiviral regimens' efficacy was based on a primary virological endpoint, which we hypothesized could be a sensitive and a more specific indicator than a primary clinical endpoint. Clinical endpoints, used as primary endpoints in previous studies, were used as secondary endpoints in the present study [1,5]. Clinical endpoints, which are based on a global assessment of both general (mainly immunologically linked) and respiratory (mainly virologically linked) symptoms, are probably not the best way to monitor the virological effect of treatment, because clinical symptoms are not exclusive to influenza. We thus considered that a difference in viral shedding rate would be the best indicator of the virological effects of combined therapy, and consequently a valuable surrogate. Our initial hypothesis was that the combination of two antivirals may reduce the rate of resistant virus emergence (for a naturally susceptible pandemic virus and a nonimmune population). In addition, we hypothesized that for cases of infection with susceptible seasonal influenza viruses, this could not be easily shown, owing first to the rarity of this phenomenon in adults, and second, to the necessity of monitoring virus excretion for several days, whereas for cases of influenza due to H1N1 viruses, which are naturally resistant to oseltamivir, the question was not relevant. Given the viral shedding kinetics in patients with seasonal influenza receiving neuraminidase inhibitors, the day 2 virological endpoint was considered to be best suited to quantify virological effects. The 200 cgeq/l threshold was chosen, as it was the best compromise in terms of specificity and sensitivity as compared to standard culture. Of note, the same trends were observed when a 100 cgeq/l or a 1,000 cgeq/μl cut-off was used to define virological success (Table S2). Furthermore, the study was designed to be statistically two-sided to take into account the possibility that the combination would perform worse than either

Table 3. Virological and clinical response according to treatment arms in the 447 influenza A-infected patients between day 0 and day 2 (ITT analysis).

Type of Response	Virological and Clinical Response Variables	Combined Oseltamivir and Zanamivir	Oseltamivir Plus Placebo	O+Z versus O		Zanamivir Plus Placebo	O+Z versus Z		O versus Z
				p-Value	Difference [95% CI]		p-Value	Difference [95% CI]	
	<i>n</i> patients	157	141			149			
Virological	Primary virological endpoint								
	Day 2 influenza RT-PCR<200 cgeq/μl (%)	45.9%	58.9%	0.025	-13.0% [-23.1 to -2.9]	33.6%	0.028	+12.3% [2.39-22.2]	+25.3% [15.5-35.2]
	<i>n</i> patients	141	134			139			
	Other virological endpoints in patients with available day 0 and day 2 nasal swabs (n=414)								
	Mean (SD) viral load at day 0 (log ₁₀ cgeq/μl)	4.36 (1.36)	4.57 (1.32)			4.34 (1.37)			
Mean (SD) viral load at day 2 (log ₁₀ cgeq/μl)	2.22 (1.12)	2.08 (1.17)			2.66 (1.35)				
Mean (SD) viral load decrease between day 0 and 2 (log ₁₀ cgeq/μl)	2.14 (1.54)	2.49 (1.52)	0.060	-0.35 [-0.8 to 0.07]	1.68 (1.68)	0.016	+0.46 [0.03-0.9]	+0.81 [0.4-1.3]	
	<i>n</i> patients	157	141			149			
Clinical	Time to resolution of illness in days (median, IQR)	4.0 [2.5-14]	3.0 [2-7]	0.018	+1.0 [0.0-4.0]	4.0 [2.5-14]	0.96	+0.0 [-3.0 to 3.0]	-1.0 [-4.0 to 0.0]
	<i>n</i> (%) of patients with alleviation of symptoms at end of treatment	87 (55.4%)	95 (67.4%)	0.043	-12.0% [-21.8 to -2.1]	84 (56.4%)	0.91	-1.0% [-11.1 to 9.2]	+11.0% [1.1 to 20.9]
	Symptoms score at end of treatment (median, IQR)	3 [2-5]	2 [1-4]	0.013	+1.0 [0.0-1.0]	3 [1-6]	0.93	+0.0 [-1.0 to 0.0]	-1.0 [-2.0 to -0.5]
	<i>n</i> (%) of patients with clinical event during treatment	19 (12.1%)	10 (7.1%)	0.17	+5.0% [-1.0 to 11.0]	18 (12.1%)	1.00	+0.02% [-6.6 to 6.7]	-5.0% [-11.0 to 1.0]
	Initiation of antibiotics	14 (8.9%)	7 (5.0%)	—	—	10 (6.7%)	—	—	—
	Pneumonia	2 (1.3%)	1 (0.7%)	—	—	0 (0.0%)	—	—	—
	Other	15 (9.6%)	9 (6.4%)	—	—	17 (11.4%)	—	—	—

*Exploratory analysis.
doi:10.1371/journal.pmed.1000362.t003

drug alone because of the theoretical concern of antagonism at the receptor level.

The oseltamivir-zanamivir combination seemed, both virologically and clinically, significantly less effective than the oseltamivir monotherapy. This result seems robust because (1) it was found using a double-blind placebo methodology, (2) there was overall concordance both among virological endpoints, and between virological and clinical endpoints, (3) it was confirmed over the three different subgroups of subjects included in the global population (541 enrolled patients, 447 influenza A-infected patients, 382 influenza A-infected and fully compliant patients). This lower clinical and virological response to the combination may suggest a negative effect of zanamivir on oseltamivir, as in the absence of interactions the effect of the combination should at least be additive [15]. A negative interaction at the level of binding at the catalytic pocket of the neuraminidase is an explanation that should be further investigated in vitro for both seasonal H3N2 and H1N1 viruses. Recent in vitro data showing the lack of synergy between oseltamivir and zanamivir, and some antagonism at higher concentrations of zanamivir on pandemic H1N1 2009 virus, are in agreement with this hypothesis [16]. Furthermore, contrary to oseltamivir, which upon digestive absorption needs to

be metabolized, thus delaying arrival of the active drug at the infection site (*t*_{max} = 4 h), inhaled zanamivir is delivered directly to the primary site of influenza virus replication. The hypothesis that zanamivir is more likely to occupy the catalytic pocket first, thus preventing the action of oseltamivir, must be tested. According to this hypothesis, the combination would be largely reduced to a zanamivir monotherapy.

Whereas the results of the primary virological endpoints indicated a superiority of the oseltamivir-zanamivir combination to zanamivir monotherapy, clinical results were not significantly different, suggesting that oseltamivir adds essentially nothing to zanamivir monotherapy. This view is concordant with the above hypothesis of the predominant catalytic site occupation by zanamivir when the combination is administered.

As an exploratory analysis, oseltamivir showed a significantly higher clinical and virological efficacy as compared to zanamivir. This finding could be the consequence of a suboptimal treatment regimen in the zanamivir arm, since the IC50 values for the A(H3N2) viruses of the 2008–2009 season were 2- to 3-fold higher for zanamivir as compared to oseltamivir, but remained within the range for susceptible strains (GROG surveillance; NICs, unpublished data). The virological result is confirmed by the longer time

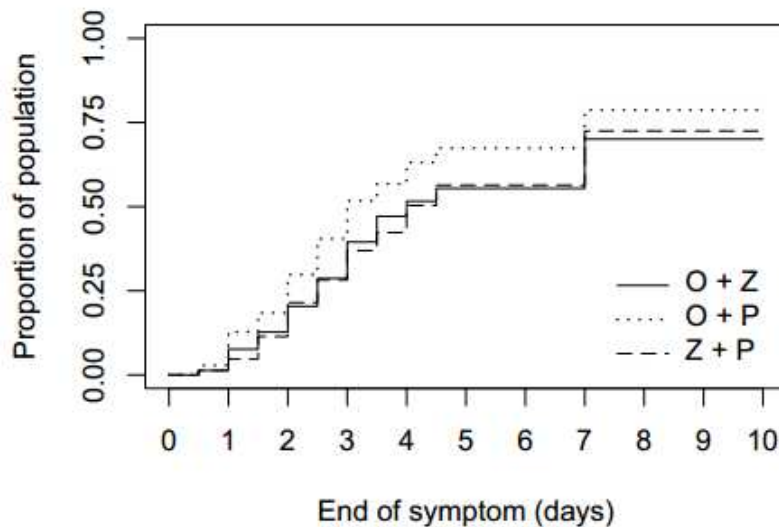


Figure 2. Proportion of the 447 influenza A-infected patients with alleviation of symptoms when treated with combined oseltamivir-zanamivir (plain line), oseltamivir plus placebo (dotted line), or zanamivir plus placebo (dashed line). Log-rank test for oseltamivir-zanamivir versus oseltamivir-placebo: $p=0.025$ and for oseltamivir-zanamivir versus zanamivir-placebo: $p=0.036$. Alleviation of symptoms defined by the presence of no symptoms of nasal stuffiness, sore throat, cough, muscle aches, tiredness-fatigue, feverishness, and headache or only mild ones, for at least 24 h. doi:10.1371/journal.pmed.1000362.g002

to alleviation of the influenza symptoms in patients receiving zanamivir.

As the present study was conducted before the 2009 H1N1 pandemic during an influenza season where A(H3N2) viruses predominated, the impact of prepandemic seasonal A(H1N1) oseltamivir-resistant viruses on the results is expected to be negligible. We observed the same trends after excluding patients infected with seasonal H1N1 from our population. Of note, A(H3N2) viruses are to date sensitive to both drugs. It remains to be determined to what extent the present results can be extrapolated to susceptible viruses of other subtypes, e.g., H1N1, and in particular to the pandemic H1N1 2009 virus, which displays significant differences in the catalytic pocket of the neuraminidase [17], or to a mixed viral season with H3N2 and H1N1 cocirculating viruses.

We must acknowledge several limitations to our study. First, this preliminary analysis was conducted on a partial set of data after enrolment of 541 patients instead of the 900 initially planned. However, it is highly unlikely that the lower response of the combination as compared to oseltamivir would have been reversed if all originally planned 900 patients had been enrolled. Second, as previous randomized clinical trials had shown the superiority of each monotherapy as compared to placebo in terms of time to symptom alleviation and viral shedding, it was decided, on the basis of ethical reasons, that the study would not comprise a double placebo arm. Third, the proportion of patients with unavailable viral swab on day 2 was higher in the combination arm. As the missing value equals failure, this may have biased the results in the combination arm towards reduced performance. Indeed, in the analysis of the 414 patients with available day 0 and day 2 nasal swabs, the same trends were observed. Fourth, the virological response was assessed only in one site (nose) and at one time (day 2), which prevents extrapolation of the results to the entire virological response over time and throughout the respiratory tract. However, clinical endpoints completed the picture, giving information on the overall response. Fifth, as

mentioned above, day 2 sampling was chosen to show the virological effect. However, this is probably not the best moment to look for resistance emergence induced by drug selective pressure, as it has been shown to occur later in the course of treatment [18–20]. Nevertheless, we looked for neuraminidase inhibitor resistance using a standard fluorimetric test in the 65 patients with day 2 positive viral culture; none of them carried a resistant virus, except for one patient infected with an H1N1 virus resistant to oseltamivir but susceptible to zanamivir, as were all H1N1 viruses circulating during the study period. However, the absence of resistance at day 2 does not rule out any further (post-day 2) resistance selection. Finally, this trial was conducted in adult outpatients, which prevents any extrapolation of the results to adults with severe presentation necessitating hospitalisation, and to children, who usually have more prolonged viral shedding. We chose the outpatient adult population because it seemed to be the most homogenous and the easiest in which to test our hypothesis.

Despite the theoretical potential for the reduction of the emergence of antiviral resistance, the lower efficiency of the oseltamivir-zanamivir combination found in this study calls for caution in its use in clinical practice. Thus, also considering the superiority of oseltamivir monotherapy over zanamivir monotherapy observed in this trial, oseltamivir should be the recommended primary anti-influenza treatment during influenza seasons with predominant H3N2 viruses naturally susceptible to oseltamivir. These results would need to be confirmed for the 2009 H1N1 pandemic virus and in the coming years, for future circulating influenza viruses.

Supporting Information

Table S1 Proportion of false positive and false negative results for various viral load thresholds compared to viral isolation in the sample of GROG patients.

Found at: doi:10.1371/journal.pmed.1000362.s001 (0.04 MB DOC)

Table S2 Virological response in the 447 influenza A infected patients for thresholds of 100 and 1000 cgeq/ μ L.

Found at: doi:10.1371/journal.pmed.1000362.s002 (0.04 MB DOC)

Table S3 Virological and clinical response according to treatment arms in the 382 influenza A infected patients and fully compliant between day 0 and day 2 Intention to treat analysis.

Found at: doi:10.1371/journal.pmed.1000362.s003 (0.06 MB DOC)

Text S1 Methods. Choice of threshold for virological endpoint and sensitivity analysis.

Found at: doi:10.1371/journal.pmed.1000362.s004 (0.03 MB DOC)

Text S2 Trial protocol.

Found at: doi:10.1371/journal.pmed.1000362.s005 (0.59 MB PDF)

Text S3 CONSORT checklist.

Found at: doi:10.1371/journal.pmed.1000362.s006 (0.22 MB DOC)

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Author Contributions

ICMJE criteria for authorship read and met: SvdW TB AM AT VE CCO CV LA FT BL FM CL. Agree with the manuscript's results and conclusions: XD SvdW TB AM MBD AT VE CCO CV LA FT BL FM CL. Designed the experiments/the study: XD SvdW TB AM VE CCO LA BL FM CL. Analyzed the data: XD SvdW TB AM CV LA FT BL FM CL. Collected data/did experiments for the study: XD SvdW TB AM MBD VE CCO FT BL FM CL. Enrolled patients: CCO. Wrote the first draft of the paper: XD SvdW TB BL FM CL. Contributed to the writing of the paper: XD SvdW TB AM MBD AT VE CCO LA FT BL FM CL. Enrolled investigators and took part in the epidemiological surveillance which gave the go-ahead to the study: AM. Responsible for data monitoring: FT. Obtained funding: FM CL.

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Editors' Summary

Background. In the last few years, use of the neuraminidase inhibitors, oseltamivir and zanamivir, has been considered a key strategy for limiting the impact of influenza both in individuals (by reducing morbidity and mortality) and collectively (by slowing the virus' spread to buy time for vaccine production, the cornerstone of influenza control). However, there are concerns that widespread use of a single antiviral drug may lead to resistant strains, which could dramatically reduce its effectiveness in future. Theoretically, if well tolerated, and if producing at least additive antiviral activity, the combination of two antiviral agents could offer several advantages such as reducing disease severity and reducing the viral shedding period, which in turn could lead to lower infection rates and reduced resistance especially in immunocompromised patients. Importantly, combining two drugs could ensure optimal treatment of all types of circulating influenza virus and subtypes or variants. The combination of two neuraminidase inhibitors is feasible as both oseltamivir and zanamivir are licensed for seasonal influenza and have different key mutations associated with resistance to each drug.

Why Was This Study Done? As yet, there have been no robust randomized controlled trials that compare the effectiveness of monotherapy with either oseltamivir or zanamivir with the effectiveness of a oseltamivir-zanamivir combination. Such a study would be important for influenza pandemic planning.

What Did the Researchers Do and Find? The researchers conducted a randomized, placebo-controlled trial within 145 general practitioners throughout France during the seasonal influenza epidemic in 2008–2009. Adults who visited their general practitioner with symptoms of an influenza-like illness for less than 36 hours and who had a positive influenza A rapid test were randomized to one of three arms: (1) oral oseltamivir 75 mg twice daily plus zanamivir 10 mg by inhalation twice daily, (2) oral oseltamivir 75 mg twice daily plus inhaled placebo, or (3) zanamivir 10 mg by inhalation twice daily plus oral placebo. The effects of the drugs or combination of drugs was assessed virologically, by looking at the proportion of patients with nasal influenza reverse transcription (RT)-PCR below a particular level on day 2 of treatment. Clinical measures of effectiveness included the time to resolution of illness, the number of patients with alleviation of symptoms at the end of treatment, and the incidence of secondary complications of influenza such as

otitis, bronchitis, sinusitis, and pneumonia. In the study, patients, general practitioners, and outcome assessors were all blinded to treatment assignments. Due to the emergence of the H1N1 pandemic in 2009, the study's independent data-monitoring committee requested that the researchers terminate the trial early and analyze the results earlier than planned.

541 patients (of the 900 planned) were enrolled in the study (192 in group 1; 176 in group 2; and 173 in group 3) of whom 447 were infected with influenza A. Overall the oseltamivir-zanamivir combination was both virologically and clinically significantly less effective than the oseltamivir monotherapy. In addition, the clinical effects of the oseltamivir-zanamivir combination on time to resolution of symptoms were not significantly different from that of zanamivir monotherapy, suggesting that oseltamivir does not add clinical benefit to zanamivir monotherapy.

What Do These Findings Mean? The results of this study essentially show that in France during the Winter of 2009 pre-pandemic (of which 85% was due to of H3N2 virus), in adults with seasonal influenza A virus infection, the combination of oseltamivir and zanamivir was less effective than oseltamivir monotherapy and not significantly more effective than zanamivir monotherapy. These results call for caution in the use of the oseltamivir-zanamivir combination in treatment of adult outpatients. In addition, as the clinical and virological effects of oseltamivir monotherapy over zanamivir monotherapy were superior in this trial, oseltamivir should be the recommended treatment during influenza seasons with predominant H3N2 viruses. However, the results of this study should be confirmed in the coming years on future circulating influenza viruses.

Additional Information. Please access these Web sites via the online version of this summary at <http://dx.doi.org/10.1371/journal.pmed.1000362>.

- Wikipedia has information on H3N3 influenza A virus (note that Wikipedia is a free online encyclopedia that anyone can edit; available in several languages)
- The World Health Organization has a global alert and response site on seasonal influenza
- Patient UK provides information about antivirals for influenza
- Answers.com has information about oseltamivir and about zanamivir

4.2 Facteurs associés à la réponse clinique et virologique chez les patients grippés lors d'un traitement par INA en monothérapie (article 3)

Dans le cadre de l'essai Bivir, une étude ancillaire a été réalisée pour analyser les facteurs associés à la réponse clinique et virologique chez les patients traités par oseltamivir ou zanamivir pour une grippe de type A. L'association oseltamivir-zanamivir n'ayant pas démontré son intérêt, il n'a pas été étudié les déterminants de la réponse clinique et virologique à cette bithérapie. Connaître ces facteurs pourrait permettre aux cliniciens d'identifier les patients les plus à risque de ne pas répondre correctement au traitement et donc nécessitant un suivi plus attentif. Chez les patients traités par oseltamivir, les facteurs associés à la réponse clinique identifiés dans des études précédentes étaient un âge plus important, une température élevée, un délai allongé entre le début des symptômes et la mise en route du traitement, et le type de virus grippal (99-103). Aucune étude n'avait été menée jusque-là pour évaluer les facteurs qui influencent la réponse au zanamivir.

La réponse à l'oseltamivir et au zanamivir a été étudiée respectivement chez 141 et 149 patients porteurs d'une grippe A inclus dans l'essai Bivir (essai décrit précédemment comparant l'association oseltamivir- zanamivir à l'oseltamivir et au zanamivir en monothérapie dans le traitement de la grippe (36)). Les réponses clinique et virologique ont été évaluées respectivement sur le temps de disparition des symptômes et sur le taux de patients avec une charge virale J2 <200 cgeq/ μ L (déterminée par RT-PCR à partir de prélèvements nasaux). Les déterminants de la réponse au traitement ont été identifiés par une analyse multivariée (modèle de Cox et régression logistique).

Le temps moyen de disparition des symptômes était de 3 [2-7] et 4 [2,5-14] jours respectivement pour l'oseltamivir et le zanamivir. A J2, 59% et 34% des patients avaient une charge virale <200 cgeq/ μ L. Pour l'oseltamivir, la réponse clinique était associée au sexe féminin (HR : 0,53 (IC95% 0,36-0,79)), à une charge virale à J0 ≥ 5 log cgeq/ μ L (HR : 0,63 (0,43-0,93)), à un score de symptômes à J0 >14 (HR: 0,47 (0,32-0,70)), et à l'initiation d'une antibiothérapie à J0 (HR : 0,30 (0,12-0,76)) ; la réponse virologique était associée au sexe féminin (OR : 0,45 (0,21-0,96), à une

charge virale à J0 $\geq 5 \log \text{ cgeq}/\mu\text{L}$ (OR : 0,40 (0,20-0,84)) et à une mauvaise observance à J2 (OR : 0,31 (0,10-0,98)). Pour le zanamivir, la réponse virologique était associée à un âge ≥ 50 ans et à l'initiation d'une antibiothérapie (respectivement OR : 0,29 (0,10 – 0,85) et OR : 4,24 (1,07 à 17,50)).

Les liens entre la réponse au traitement par INA et l'observance, le sexe et l'intensité de la maladie n'avaient jamais été montrés jusqu'ici. Le fait d'avoir utilisé des données recueillies lors d'un essai clinique, avec une collecte très organisée et systématique, a sans doute facilité leur détection. Le lien entre le sexe et la réponse clinique et virologique à l'oseltamivir pourrait être dû aux caractéristiques pharmacologiques de ce médicament. A l'inverse du zanamivir, qui est un composant directement actif, l'oseltamivir est une pro-drogue qui nécessite d'être convertie en oseltamivir caboxylate par une estérase hépatique pour de devenir un métabolite actif (104). Une hypothèse pour expliquer cette moindre efficacité de l'oseltamivir chez la femme serait une moindre efficacité de ce processus hépatique (105). Il n'était pas noté de différence significative d'observance entre les hommes et les femmes que ce soit pour l'oseltamivir ou le zanamivir. L'observance en cas de traitement par INA fait l'objet d'une étude en cours. Ce travail, réalisé à partir des données Bivir, est décrit dans la suite de ce manuscrit dans la partie « Projets en cours ». Le sexe, comme les autres facteurs associés à la réponse au traitement retrouvés dans cette étude, paraissent facilement identifiables par le clinicien lors de sa consultation et la mise en route du traitement. L'utilisation de ces résultats pourraient permettre une identification des patients les plus à risque de répondre moins bien au traitement, nécessitant une attention particulière de la part du médecin.

Les résultats de cette étude ont été publiés dans *Clinical Microbiology and Infection* (37) :

- **Blanchon T**, Mentré F, Charlois-Ou C, Dornic Q, Mosnier A, Bouscambert M, Carrat F, Duval X, Enouf V, Leport C; Bivir Study Group. Factors associated with clinical and virological response in patients treated with oseltamivir or zanamivir for influenza A during the 2008-2009 winter. *Clin Microbiol Infect.* 2013 Feb;19(2):196-203.

Factors associated with clinical and virological response in patients treated with oseltamivir or zanamivir for influenza A during the 2008–2009 winter

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Abstract

Oseltamivir or zanamivir are effective in outpatients with seasonal influenza; however, factors associated with response have been incompletely described. During the 2008/2009 epidemic, in a randomized trial for influenza A-infected outpatients, clinical (time to alleviation of flu-related symptoms) and virological (rate of patients with day 2 nasal viral load <200 cgeq/ μ L) responses to oseltamivir or zanamivir were assessed and associated factors were determined using multivariate analysis. For oseltamivir (141 patients) and zanamivir (149 patients) median times to alleviation of symptoms were 3 and 4 days, respectively; 59% and 34% had virological response. For oseltamivir, a lower clinical response was associated with female gender (HR, 0.53; 95% CI, 0.36–0.79), baseline symptoms score >14 (HR, 0.47; 0.32–0.70), viral load ≥ 5 log cgeq/ μ L (HR, 0.63; 0.43–0.93), and initiation of antibiotics (HR, 0.30; 0.12–0.76); a lower virological response was associated with female gender (OR, 0.45; 0.21–0.96), baseline viral load ≥ 5 log cgeq/ μ L (OR, 0.40; 0.20–0.84) and days 0–2 incomplete compliance (OR, 0.31; 0.10–0.98). For zanamivir, virological response was associated with age ≥ 50 years (OR, 0.29; 0.10–0.85) and initiation of antibiotics at baseline (OR, 4.24; 1.07–17.50). Factors associated with lower response to neuraminidase inhibitors in outpatients appeared to be easily identifiable during routine clinical examination and, when appropriate, by nasal sampling at baseline. The unknown association between gender and response to oseltamivir was not explained by compliance.

Keywords: Epidemiological factors, gender, neuraminidase inhibitors, seasonal influenza, treatment outcome

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Introduction

In influenza-infected patients, recent systematic reviews have shown that neuraminidase inhibitors reduce the median time

of symptom alleviation in adults and children by approximately 0.5 day [1–3]. Beyond reducing the duration of the disease, antivirals have a variable impact on reducing the viral nasal shedding [4–7].

In 2009 for pandemic A(H1N1) influenza, the World Health Organization recommended the use of neuraminidase inhibitors, oseltamivir or zanamivir, for the treatment of patients with confirmed or strongly suspected influenza infection, when clinical presentation was severe or for patients in higher risk groups [8]. However, factors influencing the clinical and virological responses, which may help

physicians to detect patients who will get the lowest benefit from treatment and have to be particularly followed-up, have been incompletely analysed. Several factors associated with the clinical response in patients receiving oseltamivir were identified in a few studies as: age, high body temperature, delay from onset to treatment start, influenza virus type and infection with an oseltamivir-resistant A(H1N1) virus [9–13]. No specific study has been conducted to evaluate factors influencing the response to zanamivir.

To address these questions, we analysed the data from a double-blind randomized controlled trial performed during seasonal influenza. This trial (Bivir) conducted in France during the A(H3N2) 2008/2009 epidemic, compared the effectiveness of an oseltamivir-zanamivir combination with each of the monotherapies plus placebo. As this trial found an oseltamivir-zanamivir combination to be less effective than oseltamivir monotherapy [14], we chose to analyse data collected only from patients treated with a WHO recommended regimen (i.e. oseltamivir or zanamivir monotherapy). A better understanding of these factors influencing response to neuraminidase inhibitors would provide important insights into the use of antivirals in future seasonal epidemics or pandemics.

Methods

Recruitment and follow-up of participants

The present study is a secondary analysis of data collected in the Bivir trial, a community-based randomized trial, conducted between 7 January and 15 March 2009 (period of the influenza epidemic in France during the winter 2008–2009), reported in detail elsewhere [14]. Briefly, patients were adults over 18 years old who consulted their general practitioner within 36 h of onset of influenza symptoms and had a positive nasal rapid test for influenza A. Exclusion criteria were: vaccination against influenza during the 2008–2009 season; recent exacerbation of COPD; previous history of depression; and known hypersensitivity to neuraminidase inhibitors. Patients gave informed written consent to participate in the study. The protocol was approved by the Ethics Committee of Ile de France I.

At enrollment (day 0), a nasal swab for virological analysis was performed by the general practitioner before initiation of treatment. In the present study, only day 0 PCR documented influenza A-infected patients, allocated to one of the two oseltamivir or zanamivir monotherapy arms out of three arms of the Bivir trial, were analysed. Oseltamivir (Roche, Bale, Switzerland) dosage was 75 mg orally twice daily; zanamivir dosage was 10 mg by oral inhalation using the commercialized GlaxoSmithKline Diskhaler[®] (GlaxoSmithKline,

Philadelphia, PN, USA), twice daily. The first drug administration was performed in the presence of the general practitioner after the patient had been given instructions on capsule intake and diskhaler use. Treatments were thereafter self-administered twice daily for 5 days. A self-administered questionnaire was given to the patient for self-evaluation of symptoms and notification of drug intake twice daily. A nurse visited the patients on day 2, performed a nasal swab for virological analysis between the 4th and 5th drug intake, and collected data on any adverse event. Patients returned to their general practitioner 2 days (day 7) after completion of treatment for follow-up examination and to report any adverse event. Patients were also contacted by phone on day 14 to collect data on any further adverse events.

Statistical analysis

As in the main analysis of the Bivir trial [14], clinical response was assessed as the time to alleviation of influenza-related symptoms and virological response as the rate of patients with, at day 2, a normalized nasal viral load determined by RT-PCR below 200 cgeq/ μ L [14].

Factors associated with clinical or virological response were studied separately for oseltamivir and zanamivir by performing univariate and then multivariate analysis, using Cox regression for the clinical response and logistic regression for virological response. The following explanatory variables were studied: gender, age, smoking status, delay from onset of any symptom and start of treatment, baseline symptoms score, baseline fever, baseline physical signs (defined as conjunctival hyperaemia, erythematous throat, congestive eardrum, abnormal chest auscultation, or other), presence at baseline of at least one co-morbidity, or one clinical complication, or initiation of antibiotics, type of influenza virus, baseline normalized viral load, and full compliance between day 0 and day 2 (defined as having perfectly taken up the prescribed treatment during the first 2 days of the trial).

From the univariate analyses results, a multivariate model was built with all variables with p-values <0.10 and then a backward selection approach was used. Then, for each clinical or virological response outcome, a model with all variables remaining in the model either for oseltamivir or for zanamivir, was constructed in order to compare the results of the two drugs with similar adjustments. All analyses were performed using SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

Results

Clinical and virological responses were assessed, respectively, in the 141 and 149 influenza A-infected outpatients random-

ized to the oseltamivir and zanamivir monotherapy arms. At baseline, for oseltamivir and zanamivir, respectively, mean age was 39.5 and 40.1 years, 52% and 52% were male, and 92% and 87% had H3N2 virus (Table 1). Median times to alleviation of symptoms were 3 days (interquartile range (IQR), 2–7) and 4 days (IQR, 2.5–14), respectively. At day 2, 59% and 34% of patients had a viral load <200 cgeq/ μ L, and 88% and 85% of the patients had full compliance for days 0–2. A complete description of the patient's characteristics is detailed in the main article of the trial [14].

Clinical response

For oseltamivir, in univariate analysis, explanatory variables with *p*-values <0.10 were gender, baseline symptoms score >14, baseline normalized viral load ≥ 5 log cgeq/ μ L, initiation of antibiotics at baseline, and days 0–2 compliance (Table 2). In the multivariate analysis, a less favourable clinical response was associated with female gender (HR, 0.53; 95% CI, 0.36–0.79), baseline symptoms score >14 (HR, 0.47; 0.32–0.70), baseline normalized viral load ≥ 5 log cgeq/ μ L (HR, 0.63; 0.43–0.93), and initiation of antibiotics at baseline (HR, 0.30; 0.12–0.76) (Table 2). Figure 1 presents the time to alleviation of symptoms in the 141 patients treated with oseltamivir according to sex and days 0–2 compliance.

For zanamivir, in univariate analysis, explanatory variables with *p*-values <0.10 were baseline symptoms score >14 and presence of physical signs (Table 2). In the multivariate analysis, the clinical response was not associated with any explanatory variable (Table 2).

Virological response

For oseltamivir, in univariate analysis, explanatory variables with *p*-values <0.10 were gender, baseline normalized viral load ≥ 5 log cgeq/ μ L and compliance between day 0 and day

2 (Table 3). In the multivariate analysis, a less favourable virological response was associated with female gender (OR, 0.45; 0.21–0.96), baseline normalized viral load ≥ 5 log cgeq/ μ L (OR, 0.40; 0.20–0.84) and days 0–2 incomplete compliance (OR, 0.31; 0.10–0.98) (Table 3).

For zanamivir, in univariate analysis, explanatory variables with *p*-values <0.10 were age ≥ 50 , baseline normalized viral load ≥ 5 log cgeq/ μ L, initiation of antibiotics at baseline and days 0–2 compliance (Table 3). In the multivariate analysis, a less favourable virological response was associated with age ≥ 50 years (OR, 0.29; 0.10–0.85), while a more favourable virological response was associated with an initiation of antibiotics at baseline (OR, 4.24; 1.07–17.50) (Table 3).

Discussion

In the present study, various factors appeared to be associated with the clinical and/or virological responses to neuraminidase inhibitors in the context of the 2008/2009 seasonal influenza, mainly due to H3N2 viruses. Most were clinically relevant data such as: gender, age, baseline score of symptoms, prescription of antibiotics, and compliance. One item of virologically relevant information, nasal viral load at baseline, appears to be independently associated with the response. Different associations of these factors were found depending on the antiviral drug and on whether the clinical or virological response was considered.

Three types of factors were associated with either the clinical or the virological response to oseltamivir: compliance, gender and intensity of the disease, none of these having been previously reported in the literature, possibly because they were not tested. It is a hypothesis that the particular context of a therapeutic trial, with systematic recording of

TABLE 1. Baseline characteristics of the 290 influenza A-infected patients enrolled in the study according to treatment arms

	Osetamivir, n = 141	Zanamivir, n = 149
Age (years), mean (SD)	39.5 (13.0)	40.1 (14.1)
[Age range]	[18.1; 76.3]	[18.0; 84.2]
Female, n (%)	68 (48.2)	72 (48.3)
Smoker, n (%)	15 (10.7)	20 (13.4)
Comorbidities, n (%)	20 (14.2)	20 (13.4)
Fever $\geq 38^{\circ}\text{C}$ at enrollment, n (%)	95 (70.9)	104 (75.9)
Initiation of treatment ≤ 24 h after onset of symptoms (%)	68 (48.2)	86 (57.7)
Time of initiation of treatment after onset of symptoms (hours), mean (SD)	25.4 (10.7)	24.4 (10.8)
Symptoms score per patient ^a , mean (SD)	15.3 (3.2)	15.5 (3.1)
% of maximal score ^b , mean (SD)	72.7 (15.2)	73.8 (15.0)
Influenza virus subtype, n (%)		
H1N1	5 (3.5)	7 (4.7)
H3N2	130 (92.2)	129 (86.6)
Not determined	6 (4.3)	13 (8.7)
Viral load (log cgeq/ μ L), mean (SD)	4.5 (1.33)	4.3 (1.43)

^aSum of the severity of the seven day 0 influenza symptoms (feverishness, nasal stuffiness, sore throat, cough, muscle aches, tiredness-fatigue, and headache) using a four-point scale [5].

^bThe score is expressed as a percentage of the maximal score of 21.

TABLE 2. Association of various factors^a with less favourable clinical response to oseltamivir or to zanamivir in 290 influenza A-infected patients (univariate and multivariate analysis)

	Oseltamivir (n = 141)				Zanamivir (n = 149)			
	n	Median time to alleviation of flu-related symptoms (IQR)	Hazard ratio (95% CI)	p-value	Median time to alleviation of flu-related symptoms (IQR)	Hazard ratio (95% CI)	p-value	
Female	68	4.25 (2.50–14.00)	0.57 (0.39–0.83)	0.0036	4.00 (2.75–14.00)	0.93 (0.64–1.36)	0.72	
Male	73	3.00 (2.00–4.00)	0.50 (0.34–0.74)	0.0004	4.50 (2.50–14.00)	0.70 (0.47–1.05)	0.085	
Baseline symptoms score >14 ^b	90	4.00 (2.50–14.00)	0.70 (0.48–1.02)	0.0066	3.00 (2.00–7.00)	0.82 (0.55–1.22)	0.33	
Baseline symptoms score ≤14	49	2.00 (1.00–3.50)	0.40 (0.16–0.97)	0.043	7.00 (3.50–14.00)	1.40 (0.71–2.76)	0.34	
Baseline viral load ≥5 log copies/mL	61	4.00 (2.00–14.00)	1.02 (0.47–2.19)	0.97	4.00 (2.00–14.00)	0.45 (0.21–0.99)	0.046	
Baseline viral load <5 log copies/mL	80	3.00 (2.00–5.75)	0.56 (0.29–1.08)	0.086	2.50 (2.00–7.00)	0.75 (0.42–1.31)	0.30	
Institution of antibiotics at baseline	11	14.00 (3.00–14.00)			4.00 (2.00–14.00)			
No institution of antibiotics at baseline	130	3.00 (2.00–7.00)			4.50 (2.50–14.00)			
Physical signs	133	3.00 (2.00–7.00)			4.50 (2.50–14.00)			
No physical sign	8	5.75 (1.50–7.00)			2.75 (2.00–3.00)			
Days 0–2 incomplete compliance	17	7.00 (1.50–14.00)			7.00 (2.00–14.00)			
Days 0–2 full compliance	124	3.00 (2.00–7.00)			4.00 (2.50–14.00)			

^aOther explanatory variables (including age, smoking status, delay from onset of any symptoms and start of treatment, baseline fever, presence of at least one clinical complication at baseline, type of influenza virus) were not significantly associated with oseltamivir or zanamivir clinical response in univariate analysis (p-values < 0.10).
^bSymptom score calculated, respectively, for 139 and 145 patients in oseltamivir and zanamivir arms.

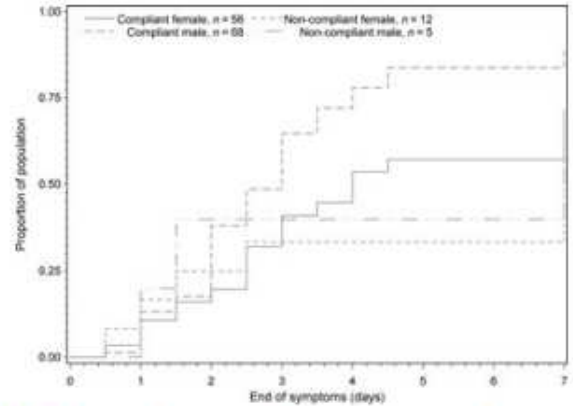


FIG. 1. Proportion of the 141 influenza A-infected patients with alleviation of symptoms when treated with oseltamivir plus placebo according to sex (female dark lines, male grey lines) and days 0–2 compliance (full compliance continuous lines, incomplete compliance dotted lines). Alleviation of symptoms defined by the presence of no symptoms of nasal stuffiness, sore throat, cough, muscle aches, tiredness-fatigue, feverishness, and headache or only mild ones, for at least 24 h.

detailed clinical and virological data, may have favoured their detection. The lower response in less compliant patients is expected, as previously reported in chronic diseases [15–17], although less frequently assessed in acute situations of infection. It confirms, if necessary, the clear efficiency of oseltamivir. In contrast, the less favourable both clinical and virological responses in women are not usually described. In contrast to what has been suggested in some other studies [18,19], the effect of gender in the present study was not found to be associated with compliance (no interaction with compliance in the model and no significant difference between men and women for compliance, data not shown) (Fig. 1). Similar results were obtained in another study, which found that male gender and low adherence to treatment were negatively and independently associated with hypertension control [20]. The role of endocrinological characteristics has been advocated to influence the immune response [21,22]; it cannot be here investigated due to the lack of such information available in the database of the trial. The fact that gender was not associated with the response to zanamivir may suggest that its impact on the response to oseltamivir is specific to some pharmacological characteristics that differ between the two drugs. Indeed, unlike zanamivir, which is delivered as the active compound, oseltamivir is delivered as an inactive prodrug. After oral administration, oseltamivir is readily absorbed from the gastrointestinal tract and extensively converted in the liver to the active metabolite, oseltamivir carboxylate, by an esterase [23]. One

TABLE 3. Association of various factors^a with less favourable virological response to oseltamivir or to zanamivir in 290 influenza A-infected patients (univariate and multivariate analysis)

	Zanamivir (n = 149)																
	Osetamivir (n = 141)			Univariate analysis			Multivariate analysis			Nasal viral load <200 cgeq/mL n (%)			Univariate analysis			Multivariate analysis	
	n	Nasal viral load <200 cgeq/mL n (%)	odds ratio (95% CI)	p-value	odds ratio (95% CI)	p-value	odds ratio (95% CI)	p-value	odds ratio (95% CI)	p-value	n	Nasal viral load <200 cgeq/mL n (%)	odds ratio (95% CI)	p-value	odds ratio (95% CI)	p-value	
Female	68	34 (50.00)	0.49 (0.25–0.97)	0.040	0.45 (0.21–0.96)	0.040	72	26 (36.11)	1.25 (0.63–2.47)	0.52	72	26 (36.11)	1.18 (0.56–2.46)	0.66			
Male	73	49 (67.12)					77	24 (31.17)			77	24 (31.17)					
Age ≥50 ^b	35	21 (60.00)	1.06 (0.49–2.32)	0.88	1.58 (0.64–3.97)	0.32	36	5 (13.89)	0.25 (0.09–0.70)	0.0075	112	44 (39.29)	0.42 (0.19–0.91)	0.028	0.29 (0.10–0.85)	0.023	
Age <50	106	62 (58.49)					51	11 (21.57)			98	39 (39.80)			0.44 (0.19–1.01)	0.052	
Baseline viral load ≥5 log cgeq/mL	61	29 (47.54)	0.44 (0.22–0.87)	0.018	0.40 (0.20–0.84)	0.015	98	39 (39.80)	3.87 (1.08–13.91)	0.038	11	7 (63.64)	0.27 (0.08–0.96)	0.043	4.24 (1.07–17.50)	0.040	
Baseline viral load <5 log cgeq/mL	80	54 (67.50)					11	7 (63.64)			138	43 (31.16)					
No initiation of antibiotics at baseline	11	4 (36.36)	0.37 (0.10–1.32)	0.13	0.27 (0.06–1.14)	0.074	11	7 (63.64)			22	3 (13.64)					
Initiation of antibiotics at baseline	130	79 (60.77)					22	3 (13.64)			127	47 (37.01)					
Days 0–2 incomplete compliance	17	5 (29.41)	0.25 (0.08–0.74)	0.013	0.31 (0.10–0.98)	0.046											
Days 0–2 full compliance	124	78 (62.90)															

^aOther explanatory variables (including smoking status, delay from onset of any symptoms to start of treatment, baseline symptoms score, baseline fever, baseline physical signs, presence of at least one comorbidity, presence of at least one clinical complication at baseline, type of influenza virus) were not significantly associated with oseltamivir or zanamivir virological response in univariate analysis (p-values > 0.10).

^bAge calculated for 148 patients in zanamivir arms.

hypothesis could thus be a gender difference during the hepatic process of oseltamivir transformation into the active compound, which seems to be more active in men compared with women, as suggested in one previous publication [24]. In an animal model, with another drug, indinavir, such an influence of the differential status of an enzyme involved in the metabolism of the drug has been described between men and women [25]. Another hypothesis for the lower clinical response to oseltamivir might be that women would declare a higher intensity of symptoms in the course of the disease [26]. However, such a hypothesis would not explain the influence of gender on the virological component of the response. Whether the severity of the symptom at baseline was different in men and women was assessed in the study and found not to be different (data not shown). If this association is confirmed by other studies, it might lead to the proposition that dosage of oseltamivir should be adjusted in women, to achieve similar efficacy as in men. This could prove of importance in patients with complicated or severe influenza, including when i.v. administration is needed. This study also found that the more severe the disease, as suggested by symptom score and viral load at baseline, the lower the clinical and virological responses in patients treated with oseltamivir. This corroborates previous results showing that higher fever was associated with a lower response rate [9,27]. Even if it seems logical, this had not been clearly demonstrated for virological determinants, maybe also because quantitative assessment of viral load by real-time RT-PCR has been introduced only recently and has not been used extensively in such a trial design.

It is noteworthy that a limited number of studies have assessed specifically which factors were associated with the response to zanamivir. In the present study, no variables were found to influence significantly the clinical response to zanamivir. The only factor associated with a poor virological response to zanamivir was older age. The effect of age has already been reported for clinical response to oseltamivir in two previous studies, but not for zanamivir. In one study, oseltamivir appeared clinically less effective in young children, between 0 and 6 years, compared with patients aged 16–64 years [28]. In the other study, which included children between 1 and 12 years, a similar effect was observed [27]. Because patients younger than 18 were not included in the Bivir trial, this association with younger age cannot be assessed in our study. Whether older people in the Bivir trial would have had more difficulties in using the Diskhaler device required for delivery of zanamivir remains a question.

Initiation of antibiotics at baseline was the only factor for which the association was in the opposite direction for oseltamivir and zanamivir. Such a factor has not been studied in

previous publications, and the limited number of patients given antibiotics in our study makes interpretation of these data hazardous. One could suggest that for oseltamivir response it might be considered as a proxy for the intensity of the disease at baseline, as were symptom scores and viral load. It is less easy to explain the positive effect of antibiotics at baseline on the response to zanamivir. It may be more directly related to the antibacterial effect; a hypothesis would be that, in reducing the possible bacterial co-infection, antibiotic treatment would allow more efficient local diffusion and action of the antiviral drug.

We must recognize several limitations to this study. The presence of a control placebo group might have been helpful to distinguish factors associated with the natural history of the influenza infection and those truly linked to the response to antiviral treatment. This choice of a no placebo-placebo group in the Bivir trial was based on the following reasons: (i) it is proved that neuraminidase inhibitors reduce the median time of symptom alleviation in adults and children by approximately 0.5 days, and have a variable impact on reducing the viral nasal shedding; and (ii) neuraminidase inhibitors are recommended in France to treat patients with risk factors. Another limitation is the sample size of our study, which was relatively small, and could be responsible for a lack of detection of some associations. The present study was a secondary analysis of data collected in a community-based randomized trial, and the number of patients included corresponds to the number of patients included in the oseltamivir or zanamivir arms of the Bivir therapeutic trial. The counterpart is that it allowed a parallel assessment of the same factors, which were recorded prospectively for the two drugs in exactly similar conditions. It could explain why some factors already known as influencing the response of oseltamivir have not been found in our study. For instance 'Time from the onset of any symptoms to the start of treatment', demonstrated in several studies [9,29,30], has been tested as variable factor in our study, but was not associated with the response for oseltamivir or zanamivir. The limitation is that subjects in the Bivir trial were adults older than 18 years who consulted their general practitioner and were included quickly after the onset of the disease, within 36 h of influenza symptoms onset.

Factors associated with a lower response to oseltamivir or zanamivir appeared to be easily identifiable during routine clinical examination and, when appropriate, by nasal sampling at baseline. The association between gender and response to oseltamivir, unknown until now, was not linked to the effect of compliance. If it is confirmed, other hypotheses remain to be investigated.

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Conflict of Interest

Thierry Blanchon has received funds for travel for a scientific conference from Roche, is a member of the scientific committee of the GEIG. France Mentré has received funds from Roche, preclinical pharmacokinetic department, for a course on MONOLIX in December 2008. Anne Mosnier has membership of the ministry of health advisory board on influenza, involvement in some epidemiological studies partially or fully funded by Roche and GSK, has received funds for travel for participation in scientific meetings from Roche, and is a member of the scientific committee of the GEIG. Fabrice Carrat is a member of the ministry of health advisory board on influenza and the national public health council, and participated in scientific boards for Novartis and GSK. Xavier Duval has received funds for travel for a scientific conference from GSK and Roche, and lecture fees from Roche and Gilead. Vincent Enouf has membership of the ministry of health advisory board on influenza and has received lecture fees from DANONE. Catherine Lepout is associated expert to the national public health council. The other authors have no conflict of interest to declare.

Author Contribution

Designed the experiments/the study: TB, FM, CCO, QD, AM, MB, FC, XD, VE, CL. Analyzed the data: TB, FM, QD, XD, VE, CL. Collected data/did experiments for the study: TB, FM, CCO, AM, MB, XD, VE, CL. Wrote the first draft of the paper: TB, FM, QD, XD, VE, CL. Contributed to the writing of the paper: TB, FM, CCO, QD, AM, MB, FC, XD, VE, CL. Obtained funding: FM CL.

Transparency Declaration

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che clinique, AOM 06060 and AOM 08209). The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix

Bivir Study Group membership

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Invited members (informed of study design but did not participate in any decision-making study discussions; supplied drugs and placebos): Eid Z (GSK), Peurichard C (GSK), Pecking M (Roche), Dantin S (Roche), Gysembergh-Houal A (AP-HP).

Independent data-monitoring committee (advice on trial conduct and interruption): Chêne G, Hannoun C, Vittecoq D.

Monitoring and statistical analysis: Boucherit S, Dornic Q, Quintin C, Hoffman I, Vincent C, and Atlanstat, Studypharm clinical research organizations.

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4.3 Transmission de la grippe par les sujets grippés traités par INA (article 4)

S'il a été montré que la durée du portage viral diminuait chez les patients traités par oseltamivir (28), l'efficacité des INA pour réduire la transmission de la grippe lorsqu'ils sont utilisés comme traitement chez les patients grippés reste débattue (65, 106-108). Dans le cadre de l'essai Bivir (essai contrôlé randomisé en double aveugle étudiant l'efficacité de la combinaison oseltamivir + zanamivir par rapport à l'oseltamivir et au zanamivir en monothérapie, décrit ci-dessus (36)), une étude ancillaire a été réalisée pour analyser l'effet de la bithérapie oseltamivir + zanamivir sur la transmission de la grippe dans les foyers, par rapport à une monothérapie oseltamivir ou zanamivir seul.

Dans cette étude, les taux de syndromes grippaux secondaires survenus dans les trois bras de l'essai, chez les contacts familiaux des patients inclus dans l'essai (cas index) et ayant une grippe confirmée, ont été comparés. Les foyers dans lesquels au moins un cas de syndrome grippal avait été rapporté dans les 15 jours précédant l'inclusion du cas index ont été exclus. Un cas secondaire était défini par la survenue chez une personne contact d'un syndrome grippal (fièvre accompagnée d'une toux) dans les 7 jours suivant l'inclusion du cas index. Les caractéristiques du foyer, comme la taille du ménage et la survenue d'un syndrome grippal, ont été enregistrées par le médecin investigateur à l'inclusion et au 7^{ème} jour de traitement (J7) du cas index lors d'une visite de suivi.

Au total, 543 contacts familiaux de 267 patients index ont été inclus dans l'étude, dont 466 avaient un formulaire de suivi rempli. Un syndrome grippal a été rapporté chez 58 contacts (12,5%) sans différence significative entre les trois groupes ($p = 0,07$). Lors de l'analyse limitée aux 232 contacts des 136 patients index dont la première prise de traitement (INA) était survenue dans les 24 heures suivant l'apparition des symptômes, 14 cas secondaires ont été observés chez les 81 patients du bras oseltamivir (15%), 14 chez les 95 patients du bras zanamivir (15%) et 2 chez les 56 patients du bras oseltamivir + zanamivir (4%) ($p = 0,05$).

Le fait d'avoir observé une différence lorsque l'analyse a été limitée aux cas index pour lesquels leur syndrome grippal avait débuté moins de 24 avant leur inclusion dans l'essai, alors qu'elle n'était pas observée pour l'ensemble de patients de l'essai Bivir, est en adéquation avec les données de la littérature. Des résultats précédemment publiés suggèrent que, pour limiter la transmission de la grippe au sein du foyer, le traitement antiviral doit être initié dans les 24 premières heures de la maladie (65, 106-108). Par contre la plus grande efficacité de la bithérapie oseltamivir + zanamivir pour réduire la transmission de la grippe semble en contradiction avec les résultats de l'essai Bivir, qui montraient que le traitement par oseltamivir seul était plus efficace que la bithérapie oseltamivir + zanamivir (36). L'une des explications avancées serait que la bithérapie pourrait avoir, dans les premières heures, une cinétique de décroissance virale plus rapide que les deux monothérapies. Cette hypothèse, qui n'a pas pu être vérifiée pour le moment, nécessiterait d'être évaluée dans des études ultérieures.

La principale limite des résultats présentés est que le diagnostic de grippe des cas secondaires n'a pas pu être confirmé virologiquement. L'effet supérieur de la bithérapie, contradictoire avec les résultats princeps de l'essai et obtenu à partir d'une analyse en sous-groupe, doit ainsi être interprété avec prudence.

Les résultats de cette étude ont été publiés dans Antiviral Therapy (38) :

- Carrat F, Duval X, Tubach F, Mosnier A, Van der Werf S, Tibi A, **Blanchon T**, Leport C, Flahault A, Mentré F; BIVIR study group. .Effect of oseltamivir, zanamivir or oseltamivir-zanamivir combination treatments on transmission of influenza in households. Antivir Ther. 2012;17(6):1085-90.

Short communication

Effect of oseltamivir, zanamivir or oseltamivir–zanamivir combination treatments on transmission of influenza in households

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Background: The effectiveness of neuraminidase inhibitors to reduce transmission when used as treatment in influenza-infected patients remains debated.

Methods: In a prespecified analysis of a blinded randomized controlled trial on the efficacy of oseltamivir–zanamivir combination therapy versus oseltamivir and zanamivir monotherapy conducted during the 2008–2009 seasonal influenza epidemic, we compared the rate of secondary illness in household contacts of influenza-positive index patients between arms. Secondary illness was defined as occurrence in contacts of fever plus cough within 7 days from randomization of index patients. Analyses were conducted according to the delay between patients' onset of symptoms and intervention.

Results: A total of 543 household contacts of 267 index patients were included, of which 466 had

follow-up assessment. A secondary illness was reported in 58 (12.5%) contacts with no significant difference between arms overall ($P=0.07$). When the analysis was limited to the 232 contacts of 136 index patients with first treatment intake within 24 h of onset of symptoms, a lower rate of secondary illness was reported in the combination therapy arm (2 of 56 [4%]) than in the oseltamivir arm (14 of 81 [17%]; $P=0.014$) and the zanamivir arm (14 of 95 [15%]; $P=0.031$). Multivariate analysis accounting for intra-household correlation confirmed these findings. **Conclusions:** Our analysis suggests a greater effectiveness of the combination therapy to reduce transmissibility when given to the index patient within 24 h of onset of symptoms. As the finding was obtained from a subgroup analysis, it should be interpreted with caution.

Introduction

Prevention of influenza transmission in households is recognized as a means of reducing spread of influenza. Neuraminidase inhibitors (nebulized zanamivir [Relenza[®]; Glaxo Wellcome] and oral oseltamivir [Tamiflu[®]; Hoffmann-La Roche]) demonstrated clear efficacy on susceptibility when used as postexposure prophylaxis in household contacts [1]. By contrast, the effectiveness of neuraminidase inhibitors to reduce infectivity and subsequent onward transmission when used as treatment in symptomatic infected patients remains debated [2–5].

During winter 2008–2009, a large blinded randomized trial comparing the efficacy of oseltamivir–zanamivir combination therapy to oseltamivir monotherapy and zanamivir monotherapy was conducted in France [6]. The trial showed that oseltamivir–zanamivir combination therapy was less effective than oseltamivir monotherapy and not more effective than zanamivir monotherapy for achieving virological and clinical end points. In a prespecified secondary analysis, we compared the rate of secondary illness in household contacts of influenza-positive patients between arms and assessed factors associated with secondary illness.

Methods

A complete description of the trial is described by Duval *et al.* [6]. Briefly, the trial was conducted in 47 centres in France and included patients 18 years or older who sought medical advice within 36 h of the onset of influenza symptoms and who were tested positive with a rapid influenza test (Clearview[®] Exact Influenza A & B; Inverness Medical, Cologne, Germany) between 7 January to 15 March 2009. The predominant epidemic strain was A/Brisbane/10/2007 (H3N2)-like influenza virus. At enrolment, a nasal swab was collected and the patient was allocated to one of the three treatment arms: oseltamivir orally 75 mg twice daily plus inhaled placebo (O), zanamivir 10 mg inhaled daily plus oral placebo (Z) and oseltamivir 75 mg orally twice daily plus zanamivir 10 mg inhaled (OZ). The primary end point in the trial was the proportion of patients with reverse transcription (RT)-PCR viral load <200 copies of genome equivalent (cgeq)/ μ l on a nasal sample collected at day 2 during a nurse visit. Symptoms and other characteristics, including size of the household and presence of any flu-like illness in household members were recorded by the practitioner from the patient at inclusion and at day 7 during a follow-up visit. Patients with negative or missing baseline influenza RT-PCR measurement were excluded from the analyses. A household contact was defined as a subject living in the same house as the randomized patient. Households in

which ≥ 1 contact reported influenza symptoms during the previous 15 days before inclusion of the patient in the trial were excluded. A secondary illness was defined as fever plus cough occurring between inclusion and the follow-up visit in a household contact.

Statistics

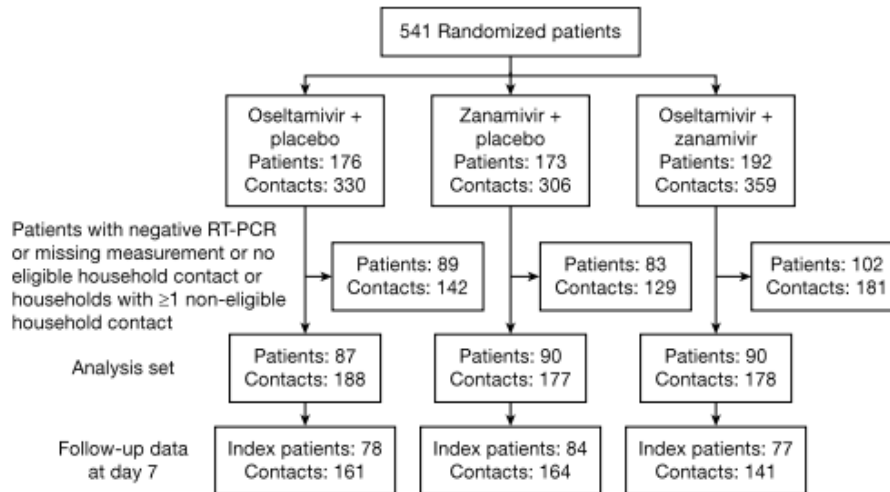
The comparison of secondary illness rates between arms was a prespecified analysis. *Post hoc* subgroup analyses were conducted by taking into account the time between onset of symptoms in the patient and allocation to intervention or by considering only secondary illness that occurred after a minimum time lag after allocation to intervention, as these factors were shown to influence the effectiveness of preventive measures [7,8]. Household contacts with missing follow-up data were discarded from the analyses. The χ^2 test or the Kruskal–Wallis test was used for global comparisons across the three arms. In case of a significant test, pairwise comparisons were performed. We used an alternating logistic regression model with an exchangeable log odds ratio to test the association between the treatment arm and secondary illness and to identify other factors associated with secondary illness in household contacts [9]. Potential factors were included based on a *P*-value <0.20 in univariate analysis and were then selected in a multivariate analysis using a backward procedure. For all analyses, a *P*-value of 0.05 was considered as statistically significant. Analyses were performed using SAS[®] version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

The analysis set included 267 index patients and 543 household contacts (Figure 1). Characteristics of index patients or household contacts at inclusion (day 0) were well balanced across arms (Table 1). At day 2, the proportion of index patients with a viral load <200 cgeq/ μ l was lower in the Z arm compared with the OZ arm (33% versus 54%; *P*=0.007) but did not differ between the O arm and the OZ arm (59% versus 54%; *P*=0.51).

Follow-up status was available in 466 household contacts, of whom 232 were contacts of 136 index patients with first treatment intake within 24 h of onset of symptoms. A secondary illness was reported in 58 (12.5%) contacts with no significant difference between arms overall (Table 2). When the allocation to intervention occurred within 24 h of the onset of symptoms in the index patient, the rate of secondary illness differed between the three arms (*P*=0.0499) and pairwise comparisons showed a lower rate in the OZ arm (2 of 56 [4%]) than in the O arm (14 of 81 [17%]; *P*=0.014) and the Z arm (14 of 95 [15%]; *P*=0.031). The median time between inclusion and secondary illness onset was 3 days (range 0–7). In total, 14 (25%) secondary

Figure 1. Participant flow chart



RT, reverse transcription.

Table 1. Characteristics of 267 treated index patients and their 543 household contacts

Characteristic	Oseltamivir	Zanamivir	Oseltamivir + zanamivir	P-value
Patients, <i>n</i>	87	90	90	-
Age	-	-	-	0.56
18–44 years, <i>n</i> (%)	63 [72]	57 [63]	60 [67]	-
45–64 years, <i>n</i> (%)	19 [22]	29 [32]	26 [29]	-
≥ 65 years, <i>n</i> (%)	5 (6)	4 (4)	3 (3)	-
Unknown, <i>n</i> (%)	0 (0)	0 (0)	1 (1)	-
Male gender, <i>n</i> (%)	42 (48)	48 (53)	43 (48) ^a	0.74
Smokers, <i>n</i> (%)	10 (11)	11 (12)	8 (9) ^a	0.77
Timeframe ≤ 24 h, <i>n</i> (%) ^b	43 (49)	54 (60)	39 (43)	0.08
Mean symptom score at day 0 (sd) ^c	15.3 (2.9)	15.3 (3.2)	15.3 (2.8)	0.99
Severe cough at day 0, <i>n</i> (%) ^d	27 (31)	23 (26)	28 (31)	0.69
Mean log ₁₀ viral load at day 0, cgeq/ μ l (sd)	4.61 (1.33)	4.41 (1.47)	4.16 (1.43)	0.12
Mean number of contacts (sd)	2.16 (1.04)	1.97 (1.00)	1.98 (1.03)	0.34
Mean log ₁₀ viral load at day 2, cgeq/ μ l (sd) ^e	2.15 (1.25)	2.72 (1.31)	2.17 (1.13)	0.002
Viral load < 200 cgeq/ μ l at day 2, <i>n</i> (%) ^e	48 (59)	29 (33)	45 (54)	0.002
Observance (100%) at day 2, <i>n</i> (%)	76 (87)	75 (83)	75 (83)	0.69
Eligible household contacts, <i>n</i>	188	177	178	-
Age of eligible household contacts	-	-	-	0.16
0–4 years, <i>n</i> (%)	18 (10)	19 (11)	25 (14)	-
5–17 years, <i>n</i> (%)	50 (27)	55 (31)	41 (23)	-
18–44 years, <i>n</i> (%)	53 (28)	60 (34)	65 (37)	-
45–64 years, <i>n</i> (%)	50 (27)	35 (20)	37 (21)	-
≥ 65 years, <i>n</i> (%)	9 (5)	7 (4)	7 (4)	-
Unknown, <i>n</i> (%)	8 (4)	1 (1)	3 (2)	-
Vaccinated eligible household contacts, <i>n</i> (%) ^f	18 (8)	11 (5)	14 (7)	0.45

^aOne missing. ^bTimeframe between onset of symptoms to intervention. ^cPatient self-report at day 0, defined as the ratio of the sum of the severity (0= none, 1= mild, 2= moderate and 3= severe) of seven influenza symptoms to a maximum of 21. ^dCough with patient self-report severity of 3. ^eThe number of participants missing data in oseltamivir: 6, zanamivir: 6 and oseltamivir–zanamivir combination: 7. ^fThe number of participants missing data in oseltamivir: 9, zanamivir: 16 and oseltamivir–zanamivir combination: 8.

Table 2. Comparison of secondary illness rates in 466 household contacts with follow-up data across arms

Subgroups	Household contacts, n	Oseltamivir, n/total (%)	Zanamivir, n/total (%)	Oseltamivir plus zanamivir, n/total (%)	P-value (overall)
All subgroups	466	23/161 (14)	25/164 (15)	10/141 (7)	0.0676
Time to intervention ≤ 24 h	232	14/81 (17)	14/95 (15)	2/56 (4)	0.0499
Excluding secondary illness the day of inclusion ^a	228	13/80 (16)	11/92 (12)	2/56 (4)	0.0711
Excluding secondary illness the day of inclusion and the day after inclusion ^a	226	12/79 (15)	10/91 (11)	2/56 (4)	0.0962
Time to intervention > 24 h	234	9/80 (11)	11/69 (16)	8/85 (9)	0.4491
Excluding secondary illness the day of inclusion	233	8/79 (10)	11/69 (16)	8/85 (9)	0.3996
Excluding secondary illness the day of inclusion and the day after inclusion	225	7/78 (9)	6/64 (9)	6/83 (7)	0.8787

^aAlso excludes one secondary illness in the oseltamivir arm without information on the delay between inclusion and onset of symptoms.

illnesses occurred the day of or the day after inclusion. No difference between the treatment arms was revealed when these illnesses were excluded.

In the multivariate analysis of the 466 contacts, secondary illness was associated with severe cough in the index patient (OR versus not severe 2.08 [95% CI 1.06, 4.08]; $P=0.03$) and no other covariate was significant. When the analysis was restricted to the 232 household contacts of index patients allocated to intervention within 24 h of onset of symptoms, secondary illness was associated with younger age in the index patient (OR per year increase 0.95 [95% CI 0.91, 0.98]; $P=0.005$), and was increased in case of monotherapy by comparison with combination therapy (O arm versus OZ arm OR 5.29 [95% CI 1.08, 25.9]; $P=0.04$ and Z arm versus OZ arm OR 4.60 [95% CI 0.97, 21.7]; $P=0.05$).

Discussion

When the index patient was treated within 36 h of onset of symptoms, no significant difference was shown between oseltamivir, zanamivir or oseltamivir plus zanamivir concerning the secondary illness rates in patients' household contacts. However, when the analysis was restricted to treatment initiated < 24 h from the onset of symptoms, the combination of oseltamivir plus zanamivir was found to be associated with a lower secondary illness rate than oseltamivir or zanamivir monotherapy.

This finding was surprising, as the trial found superior virological and clinical effectiveness with oseltamivir [6] – and, consequently, a decrease of secondary illness in the O arm compared to the two other arms was expected. A selection bias was unlikely since we did not find any difference regarding various virological end points between influenza-positive patients selected in the household contact analysis and those not selected (Additional file 2).

Among other explanations for our findings, a false-positive finding by inflation of statistical type I error in a *post hoc* subgroup analysis cannot be eliminated.

However, the fact that we observed significant differences when the intervention could be applied quickly in the presumably first influenza-infected patient is in favour of a lack of a false-positive result. Indeed, studies of antiviral treatment to limit influenza transmission within the household suggested that effectiveness could be obtained only when the intervention was applied within 1 day of first symptoms in the index patient [4]. Based on these findings, we hypothesize that the combination therapy might have a different kinetic pattern with faster antiviral activity than each standalone monotherapy, leading to a more rapid decline in viral load and acting on transmission when it can be prevented, that is, during the first 24 h of symptoms. This hypothesis does not contradict with the trial findings, but is not supported by direct data, thus highlighting the need for pharmacodynamic studies of combined neuraminidase inhibitor therapy.

Our secondary analysis has other limitations. First, secondary illness in household contacts was not virologically confirmed and was simply reported without any objective measurement of fever by the patient during a medical follow-up visit, and therefore may have been subject to lack of specificity and recall bias. Second, our study did not capture non-febrile respiratory illnesses or asymptomatic influenza infections. Therefore, the rate of febrile secondary illness underestimated the rate of secondary influenza infections. However, assuming that the proportion of febrile illnesses among all influenza infections was comparable across the study arms, the differences in secondary influenza infection rates between the combination and monotherapy arms would be higher than those reported for febrile illnesses. Third, some household contacts with febrile illnesses may have been infected from sources outside the household; this proportion has been estimated at 23% during the H1N1-2009 pandemic [10]. Fourth, 77 (14%) household contacts were lost to follow-up and a bias caused by unequal distribution of unmeasured confounders between arms

cannot be excluded. Fifth, the trial did not include an untreated placebo–placebo arm, therefore does not allow assessment of the absolute decrease of secondary illness rates using neuraminidase inhibitors.

It is worthy of note, however, that the rates of febrile secondary illnesses reported in the O or Z monotherapy arms (9–17%) were similar to those reported in a non-pharmaceutical intervention study conducted in the same setting and during the same influenza season (16%) [11]. Secondary, febrile illness rates were also in the range of values reported in the literature during seasonal epidemics [3,4] and the risk reduction of household transmission was of similar magnitude to the reduction reported with early zanamivir treatment in a retrospective survey during the H1N1-2009 pandemic [5]. Finally, the strength of our analysis was that data was collected during a blinded randomized trial, and was therefore less subject to differential bias across arms.

Considering all household contacts, we found that the severity of cough in the index patient was associated with an increased risk of secondary illness in household contacts, which may be associated with a greater amount of infectious droplets generated during intense coughing [12]. We also found that younger age in the index patient was associated with more transmission, which is consistent with findings from other household studies [5,13,14].

Our analysis suggests a greater effectiveness of the combination therapy to reduce transmissibility when given to the index patient within 24 h of onset of symptoms. As the finding was obtained from a subgroup analysis, it should be interpreted with caution and warrants further study on the topic.

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Disclosure statement

FC is member of the French Ministry of Health advisory board on influenza and the National Public Health Council, and participated in scientific boards for Novartis and GSK. XD has received funds for travel for scientific conference from GSK and Roche, and lecture fees from Roche and Gilead. AM has membership in the French

Ministry of Health advisory board on influenza, involvement in some epidemiological studies partially or fully funded by Roche and GSK, has received funds for travel for participation in scientific meetings from Roche and is member of the scientific committee of the Groupe d'Expertise et d'Information sur la Grippe (GEIG). TB has received funds for travel for a scientific conference from Roche, and is a member of the scientific committee of the GEIG. FM received funds from Roche, Preclinical Pharmacokinetic Department, for a course on MONOLIX in December 2008. CL is an associated expert to the National Public Health Council. All other authors declare no competing interests.

Additional files

Additional file 1: A list of the members of the BIVIR study group can be found at http://www.intmedpress.com/uploads/documents/AVT-11-SC-2282_Carrat_Add_file1.pdf

Additional file 2: Supplementary table 1, which displays a comparison of baseline characteristics between the 267 index patients selected in the study and 274 patients not selected but included in the trial, can be found at http://www.intmedpress.com/uploads/documents/AVT-11-SC-2282_Carrat_Add_file2.pdf

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4.4 Prescription et délivrance des INA lors de la pandémie grippale survenue en 2009 et lors des trois épidémies de grippe saisonnière entre 2010 et 2013 (article 5)

Dans un contexte de forte controverse autour de l'utilisation des antiviraux antigrippaux, cette étude avait pour objectif d'évaluer la prescription et la délivrance des INA et d'étudier les déterminants de leur prescription, en médecine de ville entre 2009 et 2013.

Ce travail a été entrepris grâce aux collaborations nées de l'essai Bivir. Il a été effectué à partir de l'analyse de deux bases de données de médecine de ville, issues des réseaux Sentinelles et Grog avec respectivement 38 024 et 1854 patients inclus, et d'une base de données d'officines de ville, issue du panel IMS-Health rapportant l'activité de 60% des pharmacies de ville de France métropolitaine.

Le taux de prescription d'INA a été estimé à 61,1% chez les patients grippés présentant un facteur de risque vus en consultation de médecine générale durant la pandémie A(H1N1)pdm09, et en moyenne à 25,9% durant les trois épidémies de grippe qui ont suivi. Les facteurs associés à la prescription d'INA étaient l'existence d'une pathologie chronique chez les sujets de moins de 65 ans (OR=14,85; IC95%=13,00-16,97) et chez ceux de 65 ans et plus (OR=7,54 ; 5,86-9,70), la grossesse (OR=10,63; 7,67-15,76), l'obésité (OR=4,67 ; 3,50-6,22), le fait d'avoir consulté durant la pandémie A(H1N1)pdm2009 (OR=3,19 ; 2,93-3,48), et avoir 65 ans ou plus sans pathologie chronique (OR=1,35; 1,04-1,74). Le nombre de boîtes d'antiviraux délivrées en pharmacie durant la pandémie A(H1N1)pdm09 a été de 835 pour 100 000 habitants, et en moyenne de 275 pour 100 000 habitants durant les trois épidémies de grippe qui ont suivi.

Bien que la recommandation de traiter les patients à risque présentant un syndrome grippal soit consensuelle au niveau international (29, 30), cette étude montre une baisse progressive du taux de prescription des INA chez ces patients, atteignant moins de 20% durant l'épidémie de grippe 2012/2013. Cette réticence des médecins à appliquer systématiquement ces recommandations a également été montrée récemment aux Etats-Unis (109, 110). Plusieurs raisons peuvent expliquer

ces résultats : les interrogations persistantes sur l'efficacité des antiviraux antigrippaux (20, 21, 23, 34), la possibilité d'effets indésirables graves (31), le manque de transparence des laboratoires pharmaceutiques (86, 87) ou encore les suspicions de conflits d'intérêt des experts de la grippe (78). La chute impressionnante entre le taux de prescription durant la pandémie de 2009 et les trois épidémies qui ont suivi reflète peut-être également le sentiment chez les professionnels de santé que la grippe saisonnière est moins grave que les épisodes pandémiques. Cette impression est contredite des données récentes montrant que la mortalité due à la grippe a été inférieure lors de la pandémie que lors des épidémies ultérieures (données de l'Institut de Veille Sanitaire en cours de publication). Ces résultats montrent la nécessité d'adapter les messages de santé publique, si l'on veut qu'ils soient écoutés par les professionnels de santé. L'HAS explique ainsi que "les inhibiteurs de la neuraminidase pourraient avoir un intérêt de santé publique tout particulier lorsque la couverture vaccinale n'est pas assurée ou non optimale (situation pandémique et indisponibilité précoce des vaccins, situation épidémique de non adéquation entre souche virale vaccinale et souche circulante)" (69). C'est ce message qu'a désiré passer récemment la DGS en France, suite aux suspicions d'une efficacité limitée du vaccin antigrippal lors de l'épidémie de grippe (111).

Les résultats de cette étude ont été publiés dans Antiviral Therapy (39) :

- **Blanchon T**, Geffrier F, Turbelin C, Daviaud I, Laouénan C, Duval X, Lambert B, Hanslik T, Mosnier A, Leport C. Use of neuraminidase inhibitors in primary health care during pandemic and seasonal influenza between 2009 and 2013. *Antivir Ther.* 2015 Feb 17.

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Antiviral Therapy

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Original article

Use of neuraminidase inhibitors in primary health care during pandemic and seasonal influenza between 2009 and 2013

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Abstract

Background: In a context of controversy about influenza antiviral treatments, this study assessed primary health care physicians' prescription of neuraminidase inhibitors (NIs) in France during pandemic and seasonal influenza between 2009 and 2013.

Methods: This observational study, using data recorded in three national databases, estimated the rate of NIs' prescription among influenza like-illness (ILI) patients seen in GPs' and paediatricians' consultations, and determined factors associated with this prescription according to a multivariate analysis. NIs' delivery by pharmacists was also evaluated.

Results: Rates of NIs' prescription were estimated to 61.1% among ILI patients with a severe influenza risk factor seen in GPs' consultation during the A(H1N1)pdm2009 pandemic versus an average rate of 25.9% during the three following seasonal influenza epidemics. Factors associated with NIs' prescription were a chronic disease in patients under 65 years (OR, 14.85; 95%CI, 13.00-16.97) and in those aged 65 and older (OR, 7.54; 5.86-9.70), an age ≥ 65 years in patients without chronic disease (OR, 1.35; 1.04-1.74), a pregnancy (OR, 10.63; 7.67-15.76), obesity (OR, 4.67; 3.50-6.22), and a consultation during the pandemic A(H1N1)pdm2009 (OR, 3.19; 2.93-3.48). The number of antiviral treatments delivered by pharmacists during the A(H1N1)pdm2009 pandemic was 835 per 100 000 inhabitants, and an average of

275 per 100 000 inhabitants during the three following seasonal influenza epidemics.

Conclusions: Although physicians seem to follow the recommended indications for NIs in primary health care practice, this study confirms the low rate of NIs prescription to ILI patients with a severe influenza risk factor, especially during seasonal epidemics.

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Running head: Outpatient influenza antiviral treatment

Introduction

Influenza is an acute infectious respiratory disease, which can lead to serious and life-threatening complications, especially in specific populations such as the elderly and patients with a chronic disease. In France, seasonal epidemics are associated with 700,000 to 4,800,000 consultations for influenza like-illness (ILI) (1-8% of the general population) [1,2], and with 0 to 24 deaths per 100,000 inhabitants [3]. Although vaccination remains the most effective prophylactic intervention for influenza infection in the population at risk of complications [4,5], its effectiveness is estimated only around 60% in adults between 18 and 65 years old, and less certain in the other age groups including the elderly [6–8].

Antiviral treatments thus constitute a complementary prophylactic and therapeutic approach. NIs are recommended by French public health authorities [9], as in most other countries [10], to treat, as soon as possible, ILI patients i) at risk of influenza complications, defined by patients with at least one of the following characteristics: age \geq 65 years old, chronic underlying disease, obesity defined as BMI \geq 40, or pregnancy; ii) who is hospitalized; iii) or has severe, complicated, or progressive illness. Currently only neuraminidase inhibitors (NIs) (oseltamivir and zanamivir) may be prescribed, amantadanes are no longer recommended because of viral resistance [11]. They reduce the time for alleviation of symptoms in patients with a virologically confirmed influenza infection by one day [12,13,14,15]. They decrease the occurrence of respiratory complications related to influenza in healthy patients and those at risk, the occurrence of pneumonia, and generally the prescription of antibiotics [12,13,14-16]. Finally, they reduce nasal shedding which could limit the spread of the disease [17].

Despite the results of these studies and the recommendations, the effectiveness of NIs to reduce infectivity and transmission, when used as treatment for symptomatic infected patients, remains questioned. The latest reviews and meta-analysis challenge the literature [13]. They highlight the fact that the populations studied did not specifically include patients with a severe influenza risk factor, as the elderly, and that the level of evidence regarding the effect of NIs on mortality and hospitalization was low. There might be a publication bias, suggesting that studies showing no impact of antivirals have not been published [12,15,18]. To better understand how physicians apply recommendations in this debated context, the present study was designed to describe NIs' prescriptions by GPs and paediatricians in France during the last pandemic A(H1N1)pdm2009 and the three seasonal epidemics that followed.

Methods

In the follow-up to the BIVIR clinical trial [19,20], an observational study of NIs' prescriptions has been conducted using three French national databases: i) French GPs' Sentinel network database to study NIs' prescription in ILI patients seen in general practitioners (GPs) consultations, ii) GROG network database to study NIs' prescription in ILI patients seen in GPs' and paediatricians' consultations ≤ 48 h after onset of symptoms, iii) and IMS Health database to study NIs' delivery in pharmacies.

French GPs' Sentinel network database

French GPs' Sentinels network [21], focused on infectious diseases, consists of 1,298 general practitioners (GPs), 2.1% of all GPs with a liberal or mixed activity in France. Sentinels GPs' characteristics are comparable to those of all French GPs regarding regional distribution, proportion in rural practice, type of practice and distribution of main clinical skills [22]. Sentinel physicians transmit every week by Internet the number of patients seen in consultation for ILI. ILI was defined as a sudden onset of fever over 39°C with myalgia and respiratory symptoms (cough, sore throat). For each case, data collected by GPs were age, sex, influenza vaccination for the current season, prescription of antiviral treatment, chronic underlying disease without any specification, obesity, pregnancy and if a hospitalisation was requested. The date of onset of symptoms was not reported.

GROG network database

GROG network [23], focused on influenza and respiratory virus, consists of 526 representative physicians (391 GPs and 114 paediatricians) distributed over the French territory. Physicians transmit every week by Internet the number of patients seen in consultation for ILI, with the same definition as Sentinel physicians. They are asked to perform, every week, a clinical questionnaire only for the first ILI case seen in consultation ≤ 48 h after onset of symptom, reporting the same data than Sentinel physicians.

Inclusion criteria for Sentinel and Grog databases

All cases reported by Sentinel physicians from July 1, 2009 to June 30, 2013 were included in this study, corresponding to four influenza epidemic seasons, 2009/2010, 2010/2011, 2011/2012, 2012/2013. From January 1, 2009 to June 30, 2013, influenza epidemic periods were determined by applying a periodic regression model on weekly ILI incidence rates below a cut-off value [1]. All cases reported by Grog physicians during these four epidemics periods were included in this study

Pharmacists' database

Private IMS-Pharmastat network consists of 14,000 representative pharmacies distributed over the French territory (60% of pharmacies), excluding hospital pharmacies. All medication sales are collected weekly. Data are aggregated into therapeutic classes from the Anatomical Classification of the European Pharmaceutical Marketing Research Association (EphMRA) [24] and an estimation of medication sales in France, out of hospital, is calculated each week. From January 1, 2009 to June 30, 2013, sales of influenza antivirals agents (class J05B4=oseltamivir and zanamivir), measured in boxes (10 pills per box), were included in the study.

Statistical analysis

For the two physicians' databases, a descriptive analysis was performed on all variables collected for included cases. A new variable named "Presence of at least one severe influenza risk factor" was created, defined by patients with at least one of the following characteristics: age ≥ 65 years old, chronic underlying disease, obesity defined as BMI ≥ 40 , or pregnancy. To prevent a misinterpretation of our results, the rate of NIs' prescription was analysed for the whole study population, but particularly for cases with the presence of at least one severe influenza risk factor.

Factors associated with NIs' prescription were studied by performing univariate and then multivariate logistic regression. The following explanatory variables were analysed: age, sex, influenza vaccination, chronic underlying disease, obesity, pregnancy, pandemic vs. influenza season, hospitalisation requested at the end of the consultation, and seen in consultation by a GP or a paediatrician. A composite variable was created from "age" and "chronic underlying disease" due to a strong interaction (data not shown) between these two variables. From the univariate analyses results, a multivariate model was built with all variables with p-values <0.20 and then a backward selection approach was used. Analysis were performed in three populations: i) all ILI cases, ii) ILI cases seen in consultation ≤ 48 h after onset of symptoms, iii) women between 15 and 50 years old.

Medication sales as influenza antivirals agents were analysed weekly during the study period. A reference period of July (year n) to June (year n +1) was chosen to estimate the rate of boxes delivered during the influenza epidemic period.

All analyses were performed with the R software (version 2.13.2; R Development Core Team 2011).

Results

Over the four influenza epidemics analysed herein, Sentinel GPs reported 35,188 ILI cases distributed in 11,067 ILI cases during the A(H1N1)pdm2009 pandemic, 7,809 during the seasonal influenza epidemic in 2010-2011, 4,984 in 2011-2012 and 11,328 in 2012-2013. Mean age of patients was 26 years and 48.9% (n=16,923) of them were men. Patients having at least one severe influenza risk factor represent 8.9% (n=2,866) of the population. Detailed characteristics of cases are given in Table 1. Over the same influenza epidemic periods, GROG physicians reported 1,854 ILI cases seen in consultation ≤ 48 h after onset of symptoms. Among them, 48.2% (n=894) were included by a GP, and 51.8% (960) by a paediatrician. Mean age was 12 years (22.7 years for GPs' patients and 2.4 years for paediatricians' patients) and 51.7% (n=950) were men (Table 1).

NIs' prescription

For the whole study period, rate of NIs' prescription among ILI patients seen in Sentinel GPs' consultation during influenza epidemics was 8.8% (n=2845) (Table 2). The rate of NIs' prescription among ILI patients with at least one severe influenza risk factor was 33.4% (n=919) (Table 2); 61.1% during the A(H1N1)pdm2009 pandemic (n=410), 34.5% during the seasonal influenza in 2010-2011 (n=176), 23.6% in 2011-2012 (n=146) and 19.6% in 2012-2013 (n=187). The rate of NIs' prescription

among ILI patients without any severe influenza risk factor was 6.4% (n=1863) (Table 2); between 12.3% during the A(H1N1)pdm2009 pandemic (n=991) and 3.2% in 2012-2013 (n=314). During influenza epidemics, 66.7% of NIs' prescriptions were to patients without any severe influenza risk factor. Oseltamivir represented 99.9% of NIs prescription (n=2793), zanamivir 0.1% (n=4).

Rate of NIs' prescription among ILI patients seen in GROG GPs' consultation ≤ 48 h after onset of symptom during influenza epidemics was 12.8% (n=114), and 32% (n=32) among ILI patients with at least one severe influenza risk factor (Table 2). These data are respectively of 11.1% (n=106) and 40% (n=14) among ILI patients seen in GROG paediatricians' consultation ≤ 48 h after onset of symptom.

During the study period, 6670 ILI cases were declared by Sentinel GPs outside of the epidemic periods. Among these cases, 487 (7.5%) had a NIs' prescription. Patients receiving NI outside of the epidemic periods were older (mean age 34.8 vs 29.5, $p < 0.001$) and had more frequently obesity (22.6% vs 12.5%, $p = 0.003$) than those during the epidemic periods. There was no difference for the other characteristics (sex, seasonal influenza vaccination, presence of at least one severe influenza risk factor, chronic underlying disease and pregnancy) (data not shown).

Factors associated with NIs' prescription

In multivariate analysis, factors associated with NIs' prescription during influenza epidemics were a chronic disease in patients under 65 years (OR, 14.85; 95% CI, 13.00-16.97) and in those aged 65 and older (OR, 7.54; 5.86-9.70), an age ≥ 65 years in patients without chronic disease (OR, 1.35; 1.04-1.74), obesity (OR, 4.67; 3.50-6.22), and a consultation during the pandemic A(H1N1)pdm2009 (OR, 3.19; 2.93-3.48) versus the other three seasons (Table 3). Pregnancy was also associated with NIs' prescription in women population aged between 15 and 50 years old (OR, 10.63; 7.17-15.76) (Table 4). Among ILI patients seen in consultation ≤ 48 h after onset of symptoms, same factors were associated with NIs' prescription (data not shown). The medical speciality of the physician (GP or paediatrician) was not associated with NIs' prescription.

NIs' delivery by pharmacists

Between January 2009 and June 2013, the weekly number of NIs boxes delivered in France by pharmacists followed strictly the dynamics of influenza epidemics (Figure 1a), and the GPs NIs' prescription (figure 1b). This volume delivered fluctuated according to epidemics with a maximum during the pandemic A(H1N1)pdm2009 (n=524,773 or 835.5/100 000 inhabitants), and a lower level during seasonal epidemics in 2010-2011 (n=181,005 or 286.6/100 000), 2011-2012 (n=104 624 or 164.7/100 000), and 2012-2013 (n=238,777 or 373.9/100 000). Zanamivir represented only 157 boxes in 2009-2010, 72 in 2010-2011, 10 in 2011-2012 and 35 in 2012-2013, other boxes delivered were oseltamivir.

Over a reference period of July (year n) to June (year n +1), the rate of boxes delivered outside the epidemic periods was 4.8% in 2009-2010, 14.3% in 2010-2011, 23.1% in 2011-2012 and 8.9% in 2012-2013 (Figure 3), corresponding respectively to 26649 NIs' boxes in 2009-2010, 30266 in 2010-2011, 31393 in 2011-2012 and 23186 in 2012-2013. Considering a margin of 3 weeks before and 3 weeks after the official dates of influenza epidemics, the rate of NIs' prescription outside this

"large epidemic period" was 2.4% in 2009-2010, 4.7% in 2010-2011, 12.4% in 2011-2012 and 3.8% in 2012-2013, corresponding respectively to 13282, 9869, 16825 and 10040 boxes.

Discussion

In a context of controversy about prescription of influenza antiviral treatments [25,26], this study established in France an inventory of NIs' prescription and delivery in primary health care practice during seasonal epidemics and pandemic between 2009 and 2013.

As previously reported [27–29], the population which consult a GP or a paediatrician for an influenza like-illness, included in our study, was relatively young and healthy. A minority of patients included in our study received influenza vaccination. One of the reasons is that influenza vaccination, as NIs' prescription, is recommended in France only for patients at risk of severe influenza, who represent a minority of ILI cases seen in GPs consultation during influenza epidemics. Our results are consistent with those (5-14.5%) reported in European countries [27,28].

Recommendations regarding NIs' prescription in the treatment of ILI patients at risk of influenza complication are consensual, both at the national and international level [9,10]. However, this study showed a low and decreasing NIs' prescription rate in targeted patients. This reluctance of physicians to prescribe NIs to all ILI patients with a severe influenza risk factor and follow strictly NIs' recommendation in primary health care practice has been studied recently in United States [30,31]. In this study, only 195 of 1021 (19%) of ILI participants who met high-risk criteria received an antiviral prescription. These low NIs' prescription rates can be explained by different factors. Concerns remain about the NIs' effectiveness [12,13,15,18], serious side effects are evocated [32], and pharmaceutical companies are suspected of a lack of transparency [33,34]. Moreover, some doubts about public influenza expertise have been mentioned with a perception of conflicts of interest between experts and pharmaceutical industry [35]. At last, French primary care practitioners are very concerned in advices coming from independent educational medical reviews, which during the whole study period expressed doubts about oseltamivir effectiveness and interest [36]. The gap between the rate of NIs' prescription among high risk ILI cases seen in consultation by GPs in France during the 2009 pandemic and the following seasonal epidemics could reflect the physicians' perception that seasonal epidemic are less severe, that an unknown influenza virus which emerge during a pandemic could have a higher morbidity and mortality, and that a NIs' treatment has to be prescribed more frequently during influenza pandemic.

However, in the present study when NIs were prescribed, there was a good concordance between physicians' practices and the recommendations. A clear association between NIs' prescription and the presence of at least one severe influenza risk factor which reflects the recommendation of the public health authorities [9,10]. The delivery of NIs by pharmacists strictly followed the dynamics of influenza epidemics. Respiratory specimens are not collected routinely in GPs' or pediatricians' offices for influenza testing in France. Furthermore, influenza laboratory confirmations were not available for ILI cases included in the present study. Although shown in a recent article [30], the association between NIs' prescription and influenza confirmation could not be tested.

Another important point is that 6.4% of ILI patients without any severe influenza risk factor but seeking physician consultation received NIs. It is difficult to know whether these prescriptions adhere to the recommendations or not; these latter specify that NIs have to be prescribed for ILI patients with severe symptoms or according to the discretion of the physician [9,10]. Regardless, these prescriptions for patients without severe influenza risk factors represented a high proportion (66.7%) of all NIs' prescriptions during influenza epidemic periods since 2009, and should be explored. Another intriguing result found in this study is the existence of NIs' prescription outside the epidemic periods. The recommendations stipulate that NIs are to be prescribed only during the circulation period of influenza virus. French official epidemic periods, used in our study, are defined by a statistical method [1], which does not forcibly reflect the circulation of influenza virus. It is possible that the perception of the influenza epidemic periods was quite different for the physicians. However, even if we take a margin of 3 weeks before and 3 weeks after the official dates, NIs' prescriptions outside this "large epidemic period" were still found. These results are difficult to explain, and the reasons for such prescription should be explored in subsequent studies.

We must recognize several limitations to this study. The fact that we use pre-existent physicians' database did not permit the collection of more precise biomedical, psychosocial and socio-demographic data about ILI patients, which could be associated to NIs' prescription. In the same way, we did not have data about physicians' characteristics. As expected [29], the rate of hospitalizations requested by GPs among ILI cases seen in primary health care consultations was low. The fact that it is an infrequent event could explain the lack of power to show an association between hospitalization and NIs' prescription, although it is a specific factor in the French NIs' recommendations. Pharmacist's database was limited to the number of boxes delivery without any clinical or social information about patients who received NIs. Conversely this study presented the advantage of the large number of subjects included in the physicians' databases. Inclusion of the whole ILI patients seen in Sentinel GPs' consultations during the epidemic period avoided a risk of a selection bias. The use of two different and complementary physicians' databases, showing close results, reinforces our conclusions. Another advantage of the study's design is the use of physicians' and pharmacists' databases, which permit to present data about prescription and delivery.

Although physicians seem to follow recommended indications for NIs' antiviral use in primary health care practice, this study confirms their hesitation to prescribe NIs to all ILI patients with a severe influenza risk factor. It would be useful to try to understand the reasons of the gap between experts' recommendations and physicians' practices. A prospective study would better assess biomedical, psychosocial and socio-demographic factors associated with NIs' prescription. Considering this reluctance from GPs to prescribe NI systematically to ILI patients with a severe influenza risk factor, it could be interesting to modulate our public health messages. Improvement of adherence to NIs' prescription might be focused among those for whom influenza vaccination is less effective. For example, when the early estimate suggests that the influenza vaccine has low effectiveness against circulating influenza, as against the (H3N2) viruses circulating this year [37].

Contributors

Designed the experiments/the study: TB, FG, CT, ID, CL, XD, BL, TH, AM, CL ; Analysed the data: TB, FG, CT, CL ; Collected data/did experiments for the study: TB, FG, ID, BL, AM ; Wrote the first draft of the paper: TB, FG, CL ; Contributed to the writing of the paper: TB, FG, CT, ID, CL, XD, BL, TH, AM, CL.

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Figures

Figure 1a: Number of boxes of oseltamivir delivered per 100 000 inhabitants per week in France between 2009 and 2013, weekly incidence of ILI cases seen in general practice.

*Shaded periods represent influenza epidemic periods determined by applying a periodic regression model on weekly ILI incidence rates below a cut-off value [1]

Figure 1b: Number of cases included in the study who received a NIs' prescription in and outside of the influenza epidemic periods in the Sentinel GPs database.

* Shaded periods represent influenza epidemic periods determined by applying a periodic regression model on weekly ILI incidence rates below a cut-off value [1]

Table 1: Description of ILI cases included between 2009 and 2013, 35188 cases seen in GPs' consultation (French GPs' Sentinels database) and 1854 seen in GPs' and paediatricians' consultation ≤ 48 h after onset of symptoms (GROG network database)

	French GPs' Sentinels database	GROG network database	
	Included by GPs N=35188	Included by GPs N=894	Included by Paediatricians N=960
Age, Median [Quartile]	21 [9 ; 40]	15 [6 ,37]	2 [1 ,3]
< 15 yo, n (%)	14003 (39.8)	438 (49.0)	960 (100)
Male, n (%)	16923 (48.9)	451 (50.7)	499 (52.6)
Seasonal influenza vaccinated, n (%)	1413 (4.1)	49 (5.5)	16 (1.7)
Presence of at least one severe influenza risk factor*, n (%)	2866 (8.9)	101 (13.7)	35 (4.3)
≥ 65 yo, n (%)	1413 (4.0)	34 (3.8)	0
Chronic underlying disease, n (%)	1487 (4.2)	75 (8.4)	35 (3.6)
Obesity, n (%)	309 (0.9)	7 (0.8)	0
Pregnancy, n (%)	121 (0.3)	2 (0.2)	0

* Presence of a severe influenza risk factor was defined by patients with at least one of the following characteristics: age ≥ 65 years old, chronic underlying disease, obesity defined as BMI ≥ 40 , and pregnancy

Table 2: NIs' prescription among ILI cases included between 2009 and 2013, 35188 cases seen in GPs' consultation (French GPs' Sentinels database) and 1854 seen in GPs' and paediatricians' consultation ≤ 48 h after onset of symptoms (GROG network database)

		2009/2010	2010/2011	2011/2012	2012/2013	Total
Sentinelles GPs' database * N=35188	NIs' prescription, n (%)	1440 (16.1)	493 (6.5)	402 (8.2)	510 (4.6)	2845 (8.8)
	among ILI patients with at least one influenza risk factor***, n (%)	410 (61.1)	176 (34.5)	146 (23.6)	187 (19.6)	919 (33.4)
	among ILI patients without any influenza risk factor***, n (%)	991 (12.3)	309 (4.4)	249 (5.9)	314 (3.2)	1863 (6.4)
Grog GPs database ** N=894	NIs' prescription, n (%)	61 (16.8)	15 (8.3)	14 (11.5)	24 (10.7)	114 (12.8)
	among ILI patients with at least one influenza risk factor***, n (%)	17 (43.6)	3 (21.4)	6 (26.1)	6 (25)	32 (32)
	among ILI patients without any influenza risk factor***, n (%)	27 (15.3)	11 (6.6)	8 (8.1)	18 (9.3)	64 (10.1)
Grog paediatricians' database ** N=960	NIs' prescription, n (%)	57 (15.7)	18 (9.2)	16 (9.8)	15 (6.4)	106 (11.1)
	among ILI patients with at least one influenza risk factor***, n (%)	9 (50)	5 (55.6)	0 (0)	0 (0)	14 (40)
	among ILI patients without any influenza risk factor***, n (%)	35 (17.2)	13 (7.0)	16 (10.1)	14 (6.2)	78 (10.1)

* All ILI cases seen in consultation

** ILI cases seen in consultation ≤ 48 h after onset of symptoms

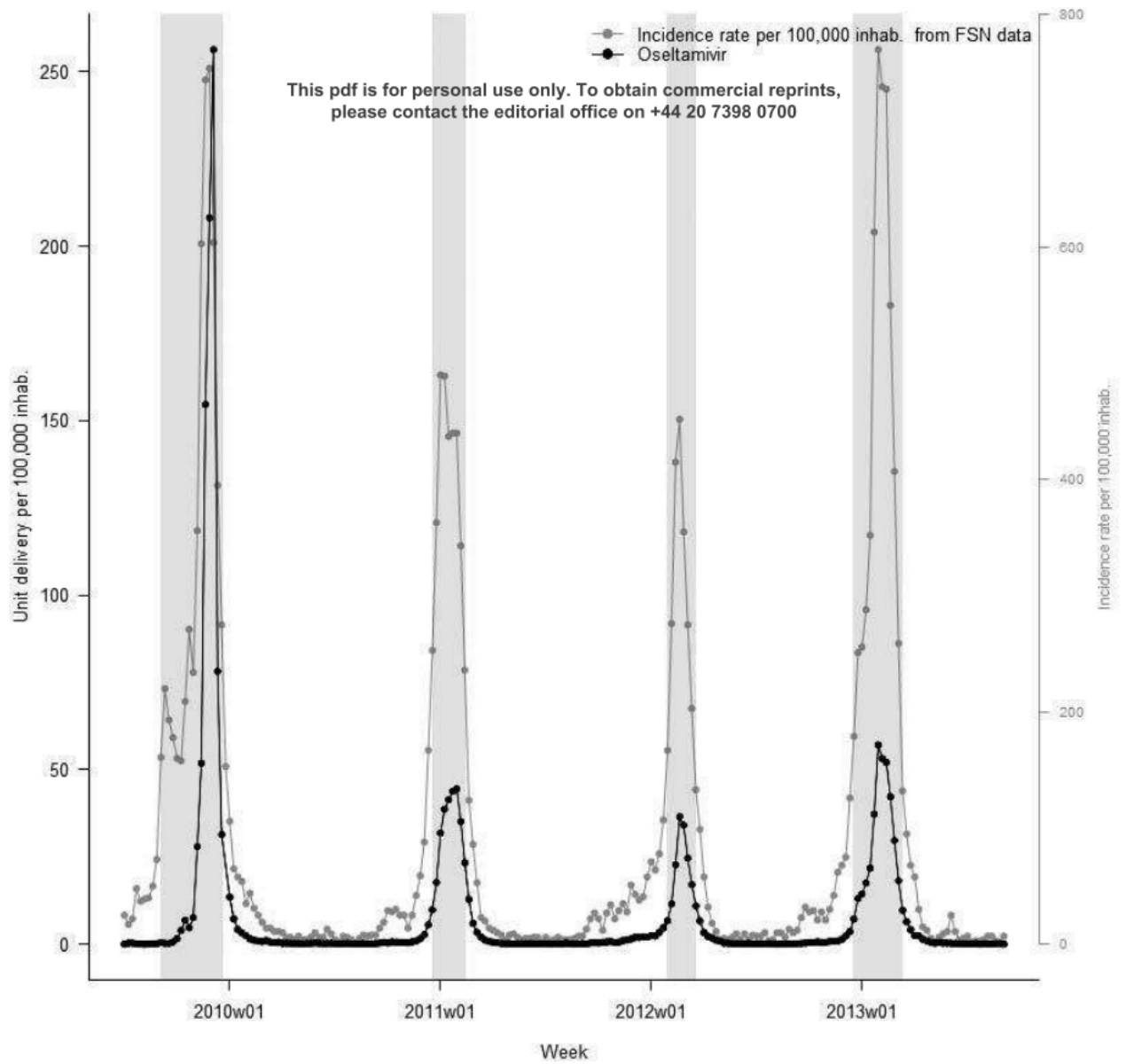
*** Presence of a severe influenza risk factor was defined by patients with at least one of the following characteristics: age ≥ 65 years old, chronic underlying disease, obesity defined as BMI ≥ 40 , and pregnancy

Table 3: Factors associated with NIs' prescription during influenza epidemics since 2009 among 32,458 ILI patients seen in GPs' consultation (univariate and multivariate analysis)

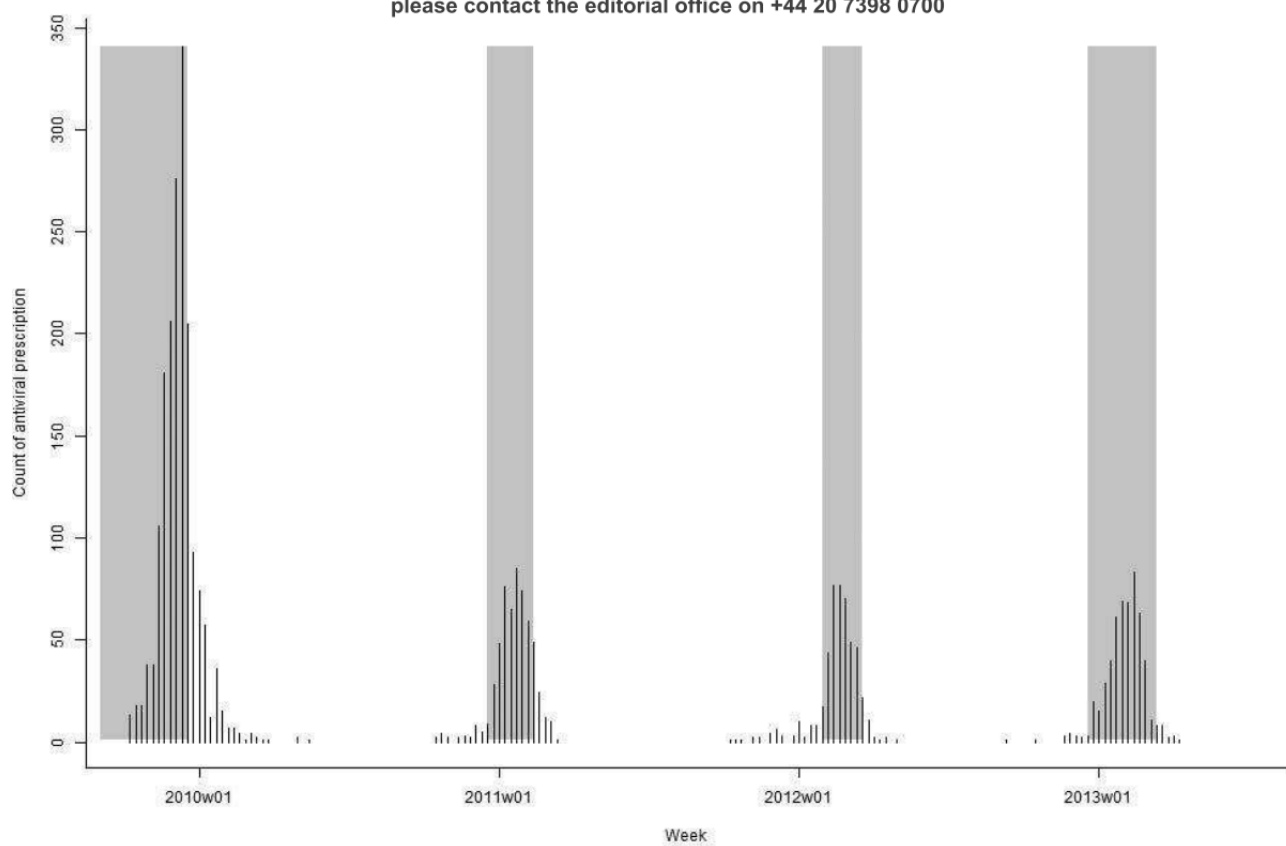
Variable	N	Treated with NIs n (%)	Univariate analysis				Multivariate analysis N= 31,154			
			OR	OR (IC 95%)	p	OR	OR (IC 95%)	p		
Male	15,657	1,419 (9.1)	1.08	[1.01 ; 1.17]	0.047	-				
Female	16,289	1,374 (8.4)								
Age ≥65 yo with chronic underlying disease	327	105 (32.1)	6.46	[5.10 ; 8.18]	<0.001	7.54	[5.86 ; 9.70]	<0.001		
Age ≥65 yo without chronic underlying disease	986	70 (7.1)	1.04	[0.81 ; 1.34]	0.737	1.35	[1.04 ; 1.74]	0.023		
Age <65 yo with chronic underlying disease	1,134	607 (53.5)	15.72	[13.87 ; 17.82]	<0.001	14.85	[13.00 ; 16.97]	<0.001		
Age <65 yo without chronic underlying disease	29,640	2,023 (6.8)								
Seasonal influenza vaccinated	1,363	262 (19.2)	2.64	[2.29 ; 3.04]	<0.001	-				
Not seasonal influenza vaccinated	30,620	2,533 (8.3)								
Obesity	303	126 (41.6)	7.73	[6.13 ; 9.75]	<0.001	4.67	[3.50 ; 6.22]	<0.001		
No obesity	31,793	2,681 (8.4)								
Hospitalisation requested	131	17 (13.0)	1.55	[0.93 ; 2.58]	0.094	-				
No hospitalisation requested	31,352	2,757 (8.8)								
Pandemic A(N1N1)2009	8,918	1,440 (16.1)	3.03	[2.81 ; 3.28]	<0.001	3.19	[2.93 ; 3.48]	<0.001		
Seasonal influenza epidemic	23,540	1,405 (6.0)								

Table 4: Factors associated with the NIs' prescription during influenza epidemics since 2009 among 8008 women aged between 15 and 50 yo seen in GPs' consultation for ILI (univariate and multivariate analysis)

Variable	N	Treated with NIs n (%)	Univariate analysis			Multivariate analysis N= 7 200		
			OR	OR (IC 95%)	P	OR	OR (IC 95%)	P
Pregnant	116	53 (45.7)	8.53	[5.87 ; 12.40]	<0.001	10.63	[7.17 ; 15.76]	<0.001
Not pregnant	7084	636 (9.0)						
Seasonal influenza vaccinated	169	42 (24.9)	3.27	[2.28 ; 4.70]	<0.001	-		
Not seasonal influenza vaccinated	6977	641 (9.2)						
Chronic underlying disease	244	133 (54.5)	13.79	[10.57 ; 18.00]	<0.001	12.86	[9.70 ; 17.05]	<0.001
No chronic underlying disease	6956	556 (8.0)						
Obesity	91	39 (42.9)	7.45	[4.88 ; 11.38]	<0.001	6.56	[4.02 ; 10.68]	<0.001
No obesity	7109	650 (9.1)						
Hospitalisation requested	23	4 (17.4)	1.95	[0.66 ; 5.75]	0.225	-		
No hospitalisation requested	7023	684 (9.7)						
Pandemic A(N1N1)2009	1877	339 (18.1)	3.09	[2.64 ; 3.63]	<0.001	3.05	[2.57 ; 3.62]	<0.001
Seasonal influenza epidemic	5395	357 (6.6)						



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5 Etudes en cours

5.1 Etudes des facteurs associés à la participation des médecins à un essai clinique lors d'une épidémie de grippe

Les travaux à partir des données recueillies durant l'essai Bivir se poursuivent. Une étude, en cours de finalisation, vise à mieux comprendre les facteurs influençant la participation des médecins à un essai clinique au cours d'une épidémie grippale. La littérature rapporte plusieurs facteurs limitant l'inclusion dans les essais menés en médecine ambulatoire, comme l'âge, le mode d'exercice, le type de mise en place de l'étude, le manque de temps, et la peur que l'enquêteur s'immisce dans la relation médecin-patient (112-116). Toutefois aucune étude de ce type n'a été menée au cours d'une épidémie comme la grippe, occasionnant un afflux de patients et donc des difficultés supplémentaires pour les médecins d'inclure des participants dans un essai. Afin d'y répondre, un questionnaire complémentaire a été envoyé aux médecins de l'essai Bivir, pour mieux connaître leurs caractéristiques socio-démographiques, mais également leurs pratiques professionnelles, leurs formations médicales et scientifiques, et leurs motivations. L'étude a été proposée à l'ensemble des médecins qui avaient donné leur accord pour participer à l'essai Bivir, qu'ils aient ou non inclus finalement au moins un patient. Au total 276 médecins ont été contactés et 160 (58%) ont rempli le questionnaire. Des analyses sont en cours pour étudier les facteurs associés à une participation active ou non à l'essai. Ce travail est en cours de finalisation.

5.2 Observance de la prescription d'un INA chez les patients grippés

Une seconde étude, toujours issue des données de l'essai Bivir, est également en cours concernant le respect par les patients de la prescription d'un INA dans le traitement de la grippe. Jusqu'à présent les travaux menés sur ce thème ont porté essentiellement sur la prise d'un INA en prophylaxie durant la pandémie A(H1N1)pdm09 (117-120). Pour répondre à cette question, ont été étudiés dans les trois bras de l'essai (oseltamivir+zanamivir, oseltamivir+placebo et

zanamivir+placebo) les facteurs de non observance au traitement antiviral prescrit par les médecins généralistes chez des patients présentant un syndrome grippal et un test rapide de grippe positif. L'observance était mesurée à partir des auto-questionnaires remplis par les patients à chaque prise de traitement, complétée par l'information sur le nombre de gélules rendues à la fin de l'essai lorsqu'elle était disponible. En référence aux données publiées dans les traitements antibiotiques pour des infections aiguës, ont été classés comme observants les patients ayant pris au moins 80% du traitement prescrit. Ce travail a fait l'objet d'une présentation à la RICAI en novembre 2014 et la soumission d'une publication est prévue prochainement.

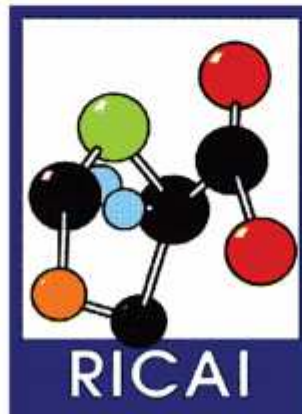
Les résultats de cette étude ont été présentés lors du dernier congrès de la RICAI en novembre 2014 à Paris (121) :

- Flicoteaux R, Protopopescu C, Tibi A, Duval X, Lina B, van der Werf S, **Blanchon T**, Mosnier A, Chevret S, Leport C. Facteurs de non observance à une bi-thérapie anti-grippale : études des données d'un essai randomisé dans le cadre de la grippe saisonnière. RICAI; Paris. 2014.

Sessions orales

Oral sessions

Vendredi 28 novembre
Friday, November 28



Facteurs de non observance à une bi-thérapie anti-grippale : études des données d'un essai randomisé dans le cadre de la grippe saisonnière. RICAI 2014. Présentation orale 192.

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En ambulatoire, l'inobservance est l'un des obstacles à l'efficacité des traitements prescrits. Nous avons étudié les facteurs de non observance à une combinaison oseltamivir (O) et zanamivir (Z) prescrite par des médecins généralistes à des patients présentant un syndrome grippal et un test rapide de grippe positif. Les patients ont été randomisés dans 3 bras : oseltamivir 75 mg deux fois par jour plus zanamivir deux fois par jour pendant 5 jours (OZ), oseltamivir et placebo de zanamivir (Opz), placebo d'oseltamivir et zanamivir (poZ). L'observance était mesurée par auto-questionnaires remplis à chaque occasion de traitement et analyse des retours pharmacie lorsqu'ils étaient disponibles. En références aux données publiées dans les traitements antibiotiques pour des infections aiguës, nous avons classés les patients comme observants si au moins 80% du traitement prescrit était pris. Les déterminants de l'observance ont été étudiés après imputation multiple afin de tenir compte du biais liés aux données manquantes. Les médecins généralistes participants à l'étude ont inclus 541 patients; parmi eux 32 (5,9%) étaient non-observant au traitement oral et 42 (7,8%) avait des données manquantes ne permettant de conclure sur l'observance ; pour le traitement inhalé les résultats étaient respectivement de 44 (8,1%) et 50 (9,2%). La différence de l'observance suivant le mode d'administration était significative (p = 0,0009). La non-observance, à la fois au traitement oral et au traitement inhalé, était associée à une durée d'évolution des symptômes élevée avant traitement, une charge virale faible au moment de la mise en route du traitement, et l'appartenance au bras de traitement OZ. Outre le profil clinico-virologique de l'infection grippale, l'observance au traitement au cours d'un essai thérapeutique en double aveugle peut-être influencée par la prise de 2 principes actifs dans l'un des bras de traitement comparativement aux bras n'en comprenant qu'un seul.

6 Perspectives

6.1 Etudes des déterminants de la prescription d'un antiviral dans le traitement de la grippe saisonnière en médecine ambulatoire

Comme il a été montré dans le cadre de cette thèse, si les INA sont recommandés dans la prise en charge de la grippe saisonnière chez les personnes à risque (29, 30), il existe des réticences de la part des médecins à appliquer systématiquement ces recommandations (39). Cette première étude s'est toutefois appuyée sur trois bases de données existantes, dont les informations avaient été collectées dans un but différent de ce travail. Il n'a ainsi pas été possible de disposer d'informations biomédicales, psychosociales et socio-démographiques précises sur les patients inclus. De même, peu d'informations sur les caractéristiques des médecins participants étaient disponibles.

Une seconde étude est en cours de réflexion afin de mieux comprendre les facteurs multidimensionnels associés à la prescription d'un antiviral dans le traitement de la grippe. Ce travail se déroulerait en deux étapes. Une première étude qualitative serait menée auprès d'un petit nombre de médecins et de patients grippés (environ 25) afin de faire ressortir les facteurs mis en avant par les uns et les autres pour expliquer la prescription ou non d'un antiviral antigrippal. La seconde étude, quantitative, s'appuierait sur un questionnaire élaboré à partir des résultats de l'enquête qualitative. Cette dernière sera menée au cours d'une épidémie de grippe saisonnière sur un échantillon représentatif de médecins généralistes, et de leurs patients grippés.

Un groupe de plusieurs partenaires a déjà été constitué avec des compétences dans les domaines de l'anthropologie, de la sociologie, de l'épidémiologie, de la thérapeutique et de la recherche clinique pour mener à bien cette étude (Département des Sciences Humaines et Sociales de la Faculté de médecine de Brest, EHESP, UMR 1337 Université Paris-Diderot Inserm et UMR-S 1136 UPMC Inserm). Ce travail a été proposé une première fois à un appel d'offre de l'ANSM il y a deux ans sans succès pour le moment. Suite à la publication en 2015 de la première étude, le protocole devrait être revu prochainement et soumis dans les mois à venir à un nouvel appel d'offre.

7 Discussion générale

Ce travail de thèse a été entrepris dans le contexte de la préparation à une potentielle pandémie grippale, juste avant celle survenue en 2009 due au virus A(H1N1)pdm09. A ce moment se posait les questions de l'évolution de la prise en charge de la grippe suite à l'émergence de souches résistantes aux INA (88), et de l'intérêt d'associer plusieurs antiviraux (90, 91).

7.1 Infériorité de la bithérapie oseltamivir-zanamivir : recommandation de non utilisation

La première conclusion majeure a été de montrer à travers deux essais cliniques, Combina et Bivir, la moindre efficacité de l'association de l'oseltamivir et du zanamivir dans le traitement des patients grippés, comparée à une monothérapie par oseltamivir. Ce résultat, constaté en utilisant une méthodologie en double aveugle contre placebo, est concordant en fonction des critères virologiques et cliniques étudiés, et confirmé dans les différents sous-groupes de patients (inclus, grippés, et grippés et compliants). Au regard des résultats de l'essai Bivir, diffusés aux autorités de santé dès le printemps 2009, il a été vivement déconseillé durant la pandémie A(H1N1)pdm09 de prescrire l'oseltamivir en association avec le zanamivir.

Dans les suites de l'essai Bivir, peu d'études se sont intéressées à l'association des INA dans le traitement de la grippe (synthèse des travaux réalisées dans le tableau page 37). Deux études ont été effectuées en laboratoire, l'une in vitro montrait peu de synergie entre l'oseltamivir et le zanamivir (97), et l'autre chez la souris ne montrait pas de différence d'efficacité entre le zanamivir seul et la bithérapie oseltamivir-zanamivir, l'oseltamivir seul étant moins efficace que la bithérapie (98). Chez l'homme trois études cliniques ont été publiées suite à la pandémie A(H1N1)pdm09 avec un nombre très limité de sujets, l'une en médecine générale (35) et les deux autres en réanimation (92, 93). Leurs résultats n'ont apporté aucun élément motivant l'utilisation de l'association oseltamivir-zanamivir dans le traitement de la grippe. L'inefficacité de cette association mériterait d'être

intégrée en France aux recommandations officielles du HCSP sur l'utilisation des antiviraux chez les patients grippés. Ce d'autant que les résultats préliminaires d'une étude en cours tendent à monter que l'observance serait diminuée en cas de prescription de l'association oseltamivir-zanamivir par rapport à une monothérapie de l'un des deux antiviraux (121), et que les risques d'effets secondaires pourraient être augmentés lors de cette bithérapie (36). Si on peut se poser la question de l'intérêt d'études supplémentaires pour confirmer ou infirmer l'intérêt de cette association, il est important de noter que depuis 2009 les souches H1N1 circulantes ne présentent qu'exceptionnellement une résistance à l'oseltamivir (94-96).

Bien que l'oseltamivir et le zanamivir interagissent différemment vis-à-vis du site d'action de la neuraminidase (1), l'inefficacité de leur association est le signe d'un antagonisme entre ces deux molécules. Ceci confirme l'urgence du développement de nouveaux antiviraux avec un mode d'action différent des INA. Plusieurs antiviraux sont actuellement en cours de développement ciblant différentes étapes du cycle viral (photo ci-dessous). Un tableau de synthèse des antiviraux commercialisés et en cours de développement est présenté ci-dessous.

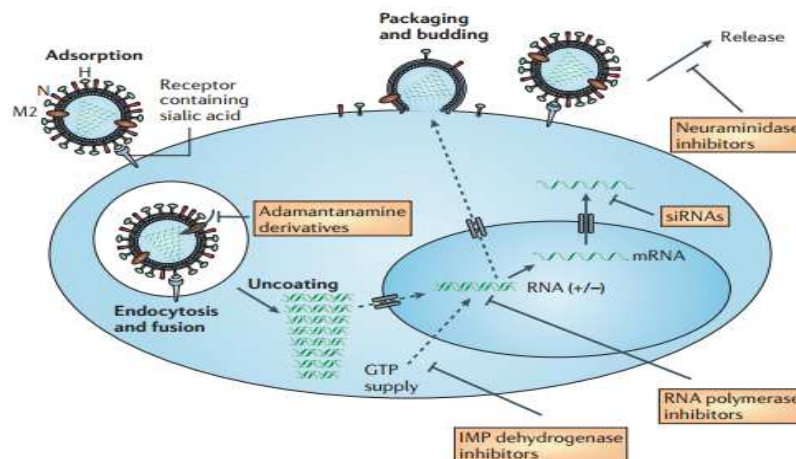


Figure 2 | Inhibition of the influenza-virus replication cycle by antiviral agents. After binding to sialic-acid receptors, influenza virions are internalized by receptor-mediated endocytosis. The low pH in the endosome triggers the fusion of viral and endosomal membranes, and the influx of H⁺ ions through the M2 channel releases the viral RNA genes in the cytoplasm. Adamantan(amine) derivatives block this uncoating step. RNA replication and transcription occur in the nucleus. This process can be blocked by inhibitors of inosine 5'-monophosphate (IMP) dehydrogenase (a cellular enzyme) or viral RNA polymerase. The stability of the viral mRNA and its translation to viral protein might be prevented by small interfering RNAs (siRNAs). Packaging and budding of virions occur at the cytoplasmic membrane. Neuraminidase (N) inhibitors block the release of the newly formed virions from the infected cells. Figure adapted with permission from REF. 8 © (2004) Macmillan Magazines Ltd. H, haemagglutinin.

d'après De Clercq, Nature 2006 (14)

Tableau : synthèse des antiviraux antigrippaux commercialisés ou en développement (phases 2 et 3)

Molécule	Classe thérapeutique	Voie d'administration	Stade de développement	Commentaires
rimantadine	inhibiteur de la protéine de canal à proton M2	per os	AMM abrogée	
amantadine	inhibiteur de la protéine de canal à proton M2	per os	AMM en France	non recommandée suite aux résistances du virus A(H3N2) et A(H1N1)pdm09 Pas d'activité sur le virus grippal B
oseltamivir	INA	per os	AMM en France	recommandé (30)
oseltamivir	INA	IV	développement arrêté	
zanamivir	INA	inhalé	AMM en France	recommandé (30)
zanamivir	INA	IV	ATU en France	réservé à l'usage hospitalier
peramivir	INA	IV	en cours de développement	ATU aux USA entre 2009 et 2010 AMM au Japon et en Corée du sud
laninamivir	INA	inhalé	en cours de développement	AMM au Japon
TCAD (combinaison amantadine + ribavirine + oseltamivir)	inhibiteur de la protéine de canal à proton M2 + analogue nucléosidique + INA	per os	développement arrêté	
T-705 ou favipiravir	inhibiteur de l'ARN polymérase	per os	en cours de développement	
DAS181 ou fludase	Inhibiteur de l'attachement et de la fusion	inhalé	en cours de développement	protéine recombinante permettant le clivage à la surface de l'épithélium respiratoire des acides sialiques, inhibant l'attachement du virus à la cellule
nitazoxanide	thiazolides	per os	en cours de développement	bloque la maturation de l'hémagglutinine virale

7.2 INA en monothérapie : l'oseltamivir en première intention

Bien que l'essai Bivir ne possédait pas de bras placebo sans traitement antiviral, il a permis de confirmer l'efficacité de l'oseltamivir en montrant un portage viral plus faible à J2 et une durée plus courte de la maladie dans le bras oseltamivir seul par rapport aux bras oseltamivir-zanamivir et zanamivir seul (36). C'est également le premier essai à comparer en double aveugle avec placebo l'efficacité de l'oseltamivir par rapport au zanamivir, démontrant la supériorité du premier dans la pratique clinique. Malgré des données controversées dans la communauté scientifique, ces résultats renforcent les recommandations que l'oseltamivir reste le traitement de première intention chez les patients grippés présentant un facteur de risque de grippe compliquée (30).

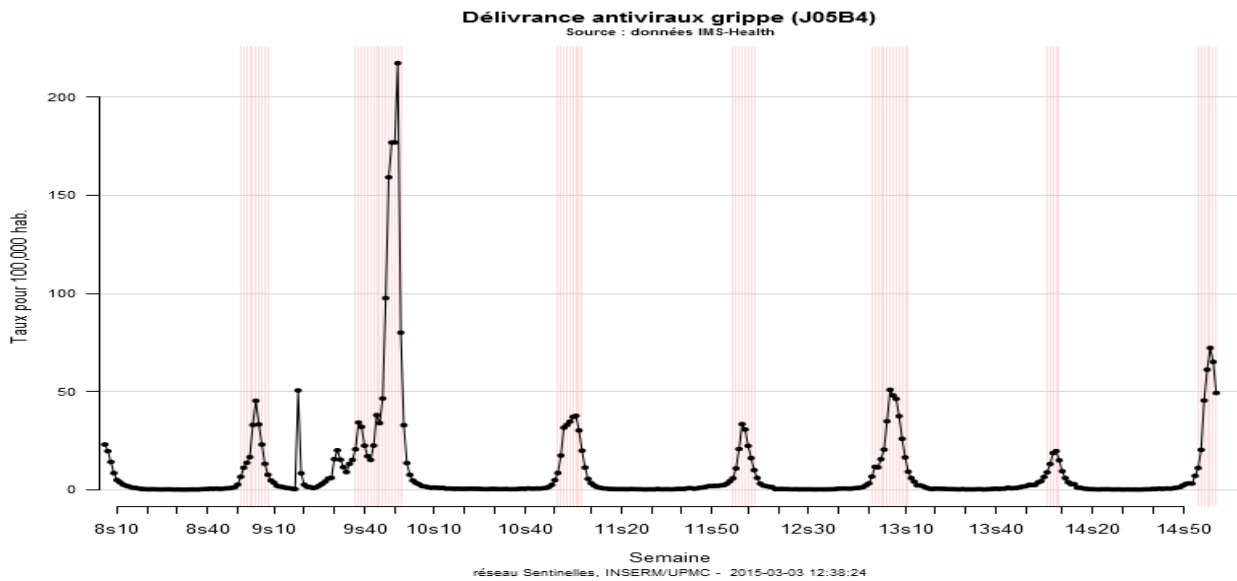
Le lien entre la réponse au traitement par oseltamivir et le sexe n'avait jamais été montré jusqu'ici (37). Cette relation possible mériterait d'être étudiée de façon plus approfondie, car pourrait nécessiter une adaptation de la posologie chez la femme si elle était confirmée. Il est toutefois important de noter que des essais récents n'ont pas montré d'efficacité supérieure d'une double dose d'oseltamivir par rapport à la dose actuellement recommandée (75 mg deux fois par jour pendant 5 jours) (122, 123). Ces résultats appuient le besoin de développer des antiviraux n'utilisant pas la même voie métabolique que l'oseltamivir qui nécessite d'être converti en oseltamivir caboxylate par une estérase hépatique pour devenir un métabolite actif (104). Cette étape pourrait expliquer cette moindre efficacité de l'oseltamivir chez la femme (105).

La deuxième partie de cette thèse, qui s'est intéressée aux pratiques médicales, a confirmé les réticences des médecins de ville à prescrire de l'oseltamivir chez tous les patients grippés avec un facteur de risque qu'ils voient en consultation. Cette réticence a également été montrée récemment aux Etats-Unis (110). Plusieurs facteurs peuvent expliquer ce phénomène, tels que les interrogations toujours en cours sur l'efficacité réelle des INA (20, 21, 23, 34), la possibilité d'effets secondaires graves (31), les soupçons du manque de transparence des sociétés pharmaceutiques et de non-publication d'études ne montrant pas d'impact des INA (86, 87), ou encore la perception par la

population générale de conflits d'intérêt possibles entre les experts et l'industrie pharmaceutique (78). Ce qui a conduit des chercheurs à évoquer le fait que la prise en charge des patients pourrait être améliorée (109).

Ces travaux sur la prescription et la délivrance des antiviraux ont été l'occasion en France de mettre en place des collaborations entre différents réseaux collectant des données de façon continue et l'écriture de programmes d'analyse capables d'étudier en temps réel l'évolution des pratiques médicales vis à vis des INA et leur délivrance. Cette recherche appliquée a permis de répondre fin février 2015, au cours de la dernière épidémie de grippe, aux interrogations du Haut Conseil de la santé publique (HCSP) suite à la saisine de la Direction générale de la santé relative à la priorisation de l'utilisation des antiviraux. Cette saisine survenait dans un contexte de tensions notables dans l'approvisionnement du marché en oseltamivir qui pouvait évoluer vers une rupture de stock. Il a ainsi pu être constaté que le taux de prescription des INA en médecine générale restait proche de ceux des années passées (7,5% des patients grippés vus en consultation depuis le début de l'épidémie 2014-2015), mais que, cette épidémie touchant un nombre important de personnes, les volumes délivrés par les officines de ville étaient plus élevés que lors des quatre dernières épidémies saisonnières (figure ci-dessous). Il est dommage que de telles informations n'aient pas pu être disponibles pour le versant hospitalier, tout particulièrement lors d'une épidémie comme celle-ci où les hospitalisations ont été plus nombreuses qu'habituellement. Seule la société IMS-health a pu fournir des informations sur la délivrance hospitalière d'oseltamivir. Ces données étant mises à jour mensuellement, il n'était toutefois pas possible d'avoir une bonne visibilité de la situation, ce qui aurait pu aider la prise de décision des autorités de santé.

Figure : Evolution hebdomadaire des délivrances d'oseltamivir par les officines de villes en France métropolitaine depuis 2008



7.3 La recherche sur la grippe un modèle pour la préparation de la recherche concernant de nouvelles maladies infectieuses émergentes ?

Les différents travaux présentés dans le cadre de cette thèse démontrent la possibilité de développer la recherche clinique en médecine de ville et d'y mener des essais thérapeutiques randomisés. Ceci paraît particulièrement intéressant pour des pathologies comme la grippe prises en charge dans la très grande majorité des cas en dehors de l'hôpital. Le travail en cours sur les facteurs associés à la participation des médecins généralistes à des essais cliniques durant une épidémie de grippe devrait permettre d'améliorer l'organisation de ce type d'études dans les années à venir. L'implication de la médecine générale dans une approche pluridisciplinaire avec différents groupes de travail mis en place lors de ces projets de recherche (médecins de ville et hospitaliers, épidémiologistes, microbiologistes, Centres nationaux de référence, pharmaciens, chercheurs en sciences humaines et sociales) a permis une vision globale de la problématique posée par les antiviraux antigrippaux. La constitution de partenariats solides avec des chercheurs ayant appris à travailler ensemble a rendu possible l'apport en temps réel de certaines réponses aux autorités de santé lors de la

pandémie grippale A(H1N1)pdm09 et la réalisation de nouveaux projets de recherche durant cette pandémie (124). Il pourrait être intéressant de réfléchir à la transposition d'une organisation de ce type à la préparation à de nouvelles maladies infectieuses émergentes dans les années à venir. C'est ce qu'essaye de développer le groupe de Coordination Opérationnelle du Risque Epidémique et Biologique infectieux (COREB) dans le cadre d'un partenariat entre la Société de Pathologie Infectieuse de Langue Française (SPILF) et la DGS.

7.4 Conclusion

Si de nouveaux antiviraux antigrippaux sont en cours d'évaluation (14), seuls les INA sont actuellement commercialisés et recommandés pour le traitement des patients grippés présentant un facteur de risque. Il apparaît donc nécessaire de préciser dans les recommandations officielles de ne pas utiliser l'association oseltamivir-zanamivir et rappeler les résultats des dernières études démontrant l'efficacité des INA en monothérapie. Il serait également important de continuer le travail entrepris afin de confirmer ou d'infirmer la possible diminution de l'efficacité des INA chez la femme. Enfin mieux comprendre les freins à la prescription des INA, aussi bien du côté du médecin que du côté du patient permettra d'adapter les messages de santé publique dans les années à venir. Les études en préparation dans la suite de cette thèse, que ce soit celle sur l'observance ou celle sur les déterminants à la prescription, devraient permettre d'avancer dans cette voie.

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RESUME

Malgré les nombreuses études menées et les recommandations consensuelles au niveau international, la prescription des inhibiteurs de la neuraminidase (INA) en cas de syndrome grippal reste controversée. Cette thèse avait pour objectif de mieux comprendre la place qui devrait leur être réservée dans le traitement de la grippe saisonnière et pandémique.

Afin de répondre à cette question, plusieurs travaux ont été menés et ont montré :

- la moindre efficacité de l'association oseltamivir + zanamivir par rapport à l'oseltamivir seul dans le traitement des patients grippés pour réduire la durée de la maladie et le portage viral naso-pharyngé, alors qu'un effet inverse a été observé sur la transmission de la grippe lorsque la bithérapie était administrée dans les 24 heures suivant l'apparition des symptômes ;
- l'association de facteurs liés aux caractéristiques du patient et de l'infection virale avec la réponse clinique et virologique lors d'un traitement par oseltamivir ou zanamivir, avec en particulier une possible efficacité de l'oseltamivir diminuée chez la femme ;
- les hésitations des médecins à appliquer systématiquement les recommandations concernant la prescription des INA en cas de syndrome grippal chez les patients à risque de grippe compliquée, en particulier lors des épidémies saisonnières.

Au vu de ces résultats et dans l'attente de nouveaux traitements en cours d'évaluation, il apparaît particulièrement important de continuer le travail entrepris, afin de mieux comprendre les freins à la prescription des INA et d'améliorer leur utilisation dans les années à venir.