Master Protocol:

ADaptive ASsessment of TReatments for influenzA: A phase 2 multi-centre adaptive randomised platform trial to assess antiviral pharmacodynamics in early symptomatic influenza infection (AD ASTRA)

Short title: A phase 2 trial comparing antiviral treatments in early symptomatic influenza

Acronym: AD ASTRA

OxTREC Ref: 6-23

Trial Registration Number: NCT05648448

Protocol number: VIR22003

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Investigator's contributions:

NJW conceived of the study and initiated the study design with other investigators.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committee and Regulatory Authorities unless authorised to do so.

Investigator Agreement and Declaration of Interests

"The undersigned has read and understood the trial protocol detailed above and:

- agrees to conduct the trial in compliance with the protocol.
- agrees to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.
- declare no conflict of interest, according to the current version of the Declaration of Helsinki"

Dr William Schilling	……..	……19 Nov 2025……
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1. SYNOPSIS

Study title	ADaptive ASsessment of TReatments for influenzA: A phase 2 multi-centre adaptive randomised platform trial to assess antiviral pharmacodynamics in early symptomatic influenza infection (AD ASTRA)
Protocol no	VIR22003
Rationale	Quantitative evidence and comparison of antiviral activity in patients with influenza is required to inform therapeutic guidelines, and prescribing and purchasing decisions
Study design	Randomised, open label, controlled, group sequential, adaptive platform trial

Inclusion and Exclusion criteria	**Inclusion criteria**
	• Patient understands the procedures and requirements and is willing and able to give informed consent for full participation in the study
	• Adults, male or female, aged 18 to 60 years at time of consent.
	• Early symptomatic Influenza (A or B); at least one reported symptom of influenza (including fever, history of fever, myalgias, headache, cough, fatigue, nasal congestion, rhinorrhoea and sore throat) within 4 days (96 hours)
	• Influenza positive by rapid antigen test OR a positive RT-PCR test for influenza viruses within the last 24hrs with a Ct value of <30
	• Able to walk unaided and unimpeded in activities of daily living (ADLs)
	• Agrees and is able to adhere to all study procedures, including availability and contact information for follow-up visits
	
	Exclusion criteria
	• Taking any concomitant medications or drugs which could interact with the study medications or have antiviral activity (see Appendix 4)
	• Presence of any chronic illness/condition requiring long term treatment or other significant comorbidity (see Appendix 4)
	• BMI ≥35 Kg/m^2
	• Clinically relevant laboratory abnormalities discovered at screening
	  ○ Haemoglobin <10g/dL
	  ○ Platelet count <100,000/uL
	  ○ ALT > 2x ULN
	  ○ Total bilirubin >1.5 x ULN
	  ○ eGFR <70mls/min/1.73m^2
	• For females: pregnancy, actively trying to become pregnant or lactation (women on OCP are eligible to join)
	• Contraindication to taking, or known hypersensitivity reaction to any of the proposed therapeutics (see Appendix 4)
	• Currently participating in another interventional influenza or COVID-19 therapeutic trial
	• Clinical evidence of pneumonia- e.g. shortness of breath, hypoxaemia, crepitations (imaging not required)
	• Known to be currently co-infected with SARS-CoV-2 (i.e. confirmed with positive ATK or RT-PCR)
	• Received live attenuated influenza virus vaccine within 3 weeks prior to study entry

Planned sample size	Continuously running group sequential adaptive platform trial. There is no fixed sample size. This study aims to enrol 1500 patients.
Planned study period	3 years for total duration of trial and 7 days for individual patient's involvement

Interventions	The platform trial will assess available antiviral drugs currently licensed in different countries for treatment of influenza. These include: **Oseltamivir, Peramivir, Zanamivir, Laninamivir, Baloxavir, Favipiravir, Oseltamivir PLUS Baloxavir, Oseltamivir PLUS Favipiravir, Favipiravir PLUS Baloxavir** And those with antiviral activity against influenza demonstrated in pre-clinical studies e.g. **Molnupiravir**	
Control	Controls will receive no antiviral treatment (although the current standard of care, local hospital supportive treatment will remain the same e.g. antipyretics, anti-tussives, antihistamines etc as per the treating Physician's judgement)	
Rationale	Many antivirals are licensed for the treatment of influenza in patients at high risk of severe disease, oseltamivir being the most widely studied and used. However, there are insufficient direct comparisons of *in vivo* antiviral effect to inform prescribing and guidelines and assist National policies and purchasing	
	Objectives	**Endpoint**
Primary	1. To evaluate Influenza antiviral efficacy in-vivo (accelerated viral clearance relative to the no study drug arm). This is a superiority comparison. 2. To compare Influenza antiviral efficacy with current best antiviral treatment option (accelerated viral clearance relative to the positive control arm). This is a non-inferiority or superiority comparison.	Rate of viral clearance- estimated from the $\log_{10}$ viral density derived from qPCR of standardised duplicate oropharyngeal swabs/saliva taken daily from baseline (day 0) to day 5 for each therapeutic arm compared with the contemporaneous no antiviral treatment control/ positive control
Secondary	To characterise the determinants of viral clearance in early influenza infection e.g. contribution of baseline serology, influenza type/subtype, prior vaccination, host genetics	Rate of viral clearance
	To determine optimal dosing regimens for drugs with evidence of antiviral activity	Rate of viral clearance
	To compare time to symptom resolution and fever duration with respect to no treatment	The following comparisons will be made: • Time to resolution of fever • Area Under the Curve of recorded temperature • Time to resolution of symptoms
	To determine the effects of drugs on the development of drug resistant viral mutants between interventions and no treatment arm	Number of mutations known to confer resistance in detectable virus at later timepoints
Tertiary	Characterise the relationship between viral clearance and hospitalisation for clinical reasons	Hospitalisation for clinical reasons up to day 7
	Characterise the relationship between viral clearance and development of influenza complications including bronchitis, sinusitis, otitis media and pneumonia requiring antibiotics	Development of influenza-related complications including bronchitis, sinusitis, otitis media and pneumonia requiring antibiotics, up to day 7

2. ABBREVIATION

ADLs	Activities of daily living
AE	Adverse event
AR	Adverse reaction
ARI	Acute Respiratory Infection
COVID-19	Coronavirus disease of 2019
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTSG	Clinical Trials Support Group
DSMB	Data Safety Monitoring Board
EC	Ethic Committee
EDTA	Ethylenediaminetetraacetic acid
FBC	Full blood count
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IL	Interleukin
IM	Intramuscular
IMP	Investigational Medicinal Product
IV	Intravenous
LFT	Liver function test
MORU	Mahidol Oxford Tropical Medicine Research Unit
OxTREC	Oxford Tropical Research Ethics Committee
PD	Pharmacodynamic
PI	Principle Investigator
PK	Pharmacokinetics
PPE	Personal Protective Equipment
RT-qPCR	Real-time quantitative polymerase chain reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SOP	Standard Operating Procedure
TNF	Tumor Necrosis Factor
U&E	Urea and Electrolytes
VTM	Viral transport medium
WHO	World Health Organisation

3. BACKGROUND AND RATIONALE

Epidemiology

Before the COVID-19 pandemic, annual seasonal influenza epidemics (mainly associated with influenza A/H3N2, influenza A/H1N1 pandemic 2009 and influenza B strains), were estimated by the WHO to cause ~1 billion infections, 3-5million cases of severe illness and 290,000-650,000 deaths each year. Influenza is therefore a significant cause of global morbidity and mortality (1). Global influenza incidence was low in 2020 attributed to the implementation of COVID-19-associated non-pharmaceutical interventions (NPIs) such as travel restrictions, social distancing, mask-wearing and enhanced hand-hygiene. Late 2021 saw a resurgence in influenza cases as use of NPIs to reduce COVID-19 transmission declined (2). We now face a situation where both influenza and SARS-CoV-2 viruses can be expected to co-circulate for the foreseeable future. Upcoming influenza seasons may be compounded in severity as population-level immunity has waned in the face of reduced exposure. In this context, it remains essential to ensure we are well prepared to deal with future influenza outbreaks while resources and attention have been diverted to COVID-19. Each year influenza viruses with genetic origins in East Asia spread westwards across the world. In addition, sporadic zoonotic infections with novel influenza A viruses continue to pose a pandemic threat with potentially devastating consequences. Currently a highly pathogenic "bird-flu" H5N1 is causing large numbers of deaths in wild birds and poultry. Fortunately, human cases have been rare as the virus is currently not well adapted to human hosts. Since 1918, when the 'Spanish Flu' killed an estimated 25-50 million people (true figures may be as high as 60 million), new highly pathogenic influenza strains have posed a perennial threat. The conditions which allowed another respiratory illness, COVID-19, to spread across the world i.e., increased population density and travel, increase the risks of further pandemic acute respiratory illnesses, of which Influenza is the most likely candidate.

Vaccines

Vaccination is currently the most effective method for preventing and reducing the severity of influenza infection, but ongoing rapid mutation within the immunogenic haemagglutinin surface glycoprotein necessitates annual revaccination. When vaccines strains are well-matched to circulating strains, clinical efficacy reaches ~60% but is generally lower in high-risk groups for severe illness, and is variable from year to year with waning of the protective response (3). In addition, uncertainty regarding future seasonal influenza circulation due to COVID-19-associated disruptions to normal seasonal patterns and resultant difficulties in predicting circulating stains, will further challenge influenza vaccine efficacy (2). Since vaccine-induced immunity is suboptimal, short-lived and will not protect against pandemic strains, there is a strong rationale for identifying effective antiviral drugs with activity against a wide range of influenza subtypes and strains, for use in those at high-risk of severe illness.

Influenza viral kinetics

In most cases, influenza is a short-lived infection with an incubation period of approximately 2 days. Viral shedding data from volunteer challenge studies have formed the basis of the standard influenza virus kinetic model. For influenza A infection, this is characterised by an initial phase of rapid viral replication with a peak in viral load occurring 1-3 days post infection, followed by a non-linear decline to undetectable levels over the subsequent 3-5 days (4). The trajectory of symptoms largely correlates with these dynamics; abrupt onset of systemic and respiratory symptoms coincides with the peak in viral load followed by gradual clinical improvement as viral load decreases, with systemic symptoms subsiding first. In comparison, influenza B infections may result in a more prolonged period of viral shedding with a less prominent peak occurring prior to symptom onset (5). These viral kinetics are based on viral load data derived from viral culture, which is a less sensitive method of viral detection compared to RT-PCR (6). Recent studies of naturally acquired influenza infection using RT-PCR to quantify viral loads report symptom and viral shedding patterns consistent with the culture observations, the main difference being detection of virus for a longer period of time (up to 7 days post symptom onset) with RT-PCR (7,8). The elderly and those with comorbidities may experience even longer periods of active viral replication beyond those documented in healthy individuals (9).

Analysis of cytokine responses in experimental influenza A infection demonstrated an early peak in IL-6 and INF-a levels, correlating with peak viral titres and symptom scores. This suggests a role for these cytokines in symptom formation and host defence in influenza. TNF-a responses peaked later in the course of infection when viral shedding and symptoms were subsiding (10).

Severe influenza

Although the majority of influenza infections are uncomplicated, a minority of patients (particularly those >65 years, children <5 years and those with comorbidities) will experience progression to severe disease including pneumonia (primary viral or secondary bacterial), acute respiratory distress syndrome (ARDS), multi-organ failure and sometimes death (3). In these later stages of disease ongoing deleterious inflammatory processes predominate, resulting in the immunopathology seen (11).

Anti-influenza drugs

Several classes of antiviral drugs are approved in different countries for the treatment of influenza infection in those at risk of severe disease: neuraminidase inhibitors (five days of oral oseltamivir, single dose of intravenous peramivir, five days of inhaled zanamivir, single dose of inhaled laninamivir), an endonuclease inhibitor (single dose of oral baloxavir), a polymerase inhibitor (five days of oral favipiravir) and a haemagglutinin inhibitor (five days of oral umifenovir) (3).

The neuraminidase inhibitors (NAIs) are the most commonly used class of influenza antivirals, differing by availability and routes of administration. Of these, oseltamivir is the most widely studied, used and stockpiled in case of future pandemics. Early randomised controlled trials of oseltamivir versus placebo in both uncomplicated acute influenza and experimental influenza demonstrated that oseltamivir administered within 48 hours reduces the duration and severity of symptoms (12-15). Three out of four of these licensing trials showed statistically significant reductions in viral titres (12,13,15) with oseltamivir, although one study did not demonstrate any difference in viral shedding at any time point (14). A recent re-analysis of median viral shedding curves from experimental influenza infection trials showed that, in addition to reducing peak viral loads, oseltamivir increases the rate of viral clearance after the peak. However, this study showed that 20-40% of oseltamivir treated volunteers continued shedding virus as did placebo-treated controls, suggesting treatment failure. This study also demonstrated that in the oseltamivir treated group, the rate of viral clearance was slower for influenza B than influenza A infections (16). This is supported by other studies which have shown reduced clinical efficacy (in reducing fever duration) of oseltamivir in treating influenza B infection compared with influenza A infections (16-18). Inhaled zanamivir has demonstrated superior efficacy with regards to this endpoint in treating influenza B infections (18,19). Thus, although there is evidence for antiviral efficacy of oseltamivir, the data are variable and there are clear differences in efficacy in treating different influenza subtypes.

The clinical effectiveness of oseltamivir in ameliorating symptoms and possibly in reducing complications is considered greatest when the drug is started as soon as possible after illness onset, preferably within 48 hours (20,21) i.e., when viral replication is at its peak. This suggests that accelerating viral clearance reduces subsequent pathology, a hypothesis supported by studies demonstrating that viral clearance correlates with symptom resolution and may be associated with a shorter duration of hospitalisation (22,23). A 48 hour window for treatment initiation, which is recommended for some anti-influenza drugs, is based on the trials which recruited healthy, non-hospitalised individuals. Viral replication patterns and the efficacy of antiviral drugs in severe influenza have not been adequately studied so evidence supporting antiviral use in later stages of the disease is lacking. However, one study of hospitalised patients with influenza infection found high viral RNA concentrations even beyond the first two days of illness. In this context, oseltamivir started within 4 days of symptom onset was associated with accelerated viral clearance when compared with no treatment, and viral RNA clearance was associated with a shorter duration of hospitalisation (9). This suggests there may be antiviral and clinical efficacy of oseltamivir even beyond the recommended 48 hour window, where there is evidence of prolonged viral replication.

Data regarding oseltamivir's effectiveness in reducing mortality and viral complications is not robust, as studies involved previously healthy individuals in whom complication and mortality rates are low (23). Oseltamivir is currently the only antiviral recommended by WHO for the treatment of individuals at high-risk of severe influenza illness. It is acknowledged this advice is based on evidence considered low-quality for critical outcomes.

Several other neuraminidase inhibitors have been developed and all have been individually compared with oseltamivir in clinical trials. However, no trial has compared all of the neuraminidase inhibitors together. A recent network meta-analysis of RCTs compared the relative efficacy of all NAIs in reducing the duration of influenza symptoms using surface under the cumulative ranking curve (SUCRA); in adults and children,

peramivir (SUCRA = 82.6%), zanamivir (SUCRA = 64%) and oseltamivir (SUCRA = 55.1%) were the three top-ranking drugs for this endpoint (24). Direct high quality RCTs with head-to-head comparisons of all NAIs are required to establish relative antiviral efficacies.

Baloxavir is a newer influenza antiviral which, in phase 3 trials of healthy and high-risk outpatients, has shown superior virological efficacy compared with oseltamivir (significantly faster declines in viral titres) but similar efficacy to oseltamivir in ameliorating symptoms (25). Efficacy with regards to other clinical outcomes including reducing complications is unclear.

Favipiravir, currently only licensed in Japan for the treatment of novel or re-emerging influenza viruses, has demonstrated significant reductions in viral titres and shortened duration of viral shedding in two phase 3 randomised placebo-controlled trials of uncomplicated influenza, but evidence regarding symptom alleviation was inconsistent. This was attributed to considerable inter-subject variability in pre- and post-dose favipiravir plasma concentrations over time. Optimal dose regimens for favipiravir require further study. These trials did not compare the antiviral or clinical efficacy of favipiravir to other influenza antivirals (26).

Despite the availability of a large number of antivirals to treat influenza, only one of these is recommended by the WHO and this recommendation is based on low-quality evidence for clinical outcomes. Although some clinical trials of new antivirals have included oseltamivir as a comparator arm, direct comparisons of antiviral and clinical efficacy between the multiple available antivirals are lacking. This comparative information is important for guideline development and aiding purchasing and prioritisation decisions when several options are available. In addition, *in vivo* synergy testing of combinations of antivirals will also be important when considering the potential for development of antiviral resistance, particularly in those patients with prolonged viral replication and in the circumstances that more virulent strains appear. Combination antiviral therapies can offer synergistic, and therefore more effective, rapid and multi-mechanistic inhibition of viral replication and thus accelerated viral clearance, with implications for improved clinical outcomes, transmission dynamics as well as reducing risk of development of resistance.

More data regarding antiviral efficacy against different influenza types and subtypes is required, allowing for development of alternative treatment regimens. However large sample sizes would be required for a platform study comparing the impact of multiple antivirals on clinically meaningful outcomes in patients with early uncomplicated influenza. A study comparing the impact of different antivirals on *in vivo* viral clearance is an efficient method for rapidly comparing antiviral efficacy, as demonstrated for anti-SARS-CoV-2 drugs by the ongoing PLATCOV trial (27).

Assessing viral clearance

PLATCOV is an ongoing multi-country platform adaptive trial currently being conducted in Thailand, Brazil, Laos and Nepal through which we have developed and validated a platform for rapid quantitative and comparative *in vivo* assessment of antiviral effects in low-risk patients with uncomplicated COVID-19 and high viral burdens. The primary outcome of this trial is the rate of viral clearance measured as the slope of the $\log_{10}$ oropharyngeal viral clearance curve. This was shown to be a uniformly better endpoint in terms of increased study power compared to the widely used and reported time to viral clearance. The assessment is of the antiviral effect *in vivo*, as distinct from the outcome on the clinical course of the infection. The results are applicable to all stages of disease where viral replication occurs. This trial has so far shown that remdesivir accelerates the rate of viral clearance in early symptomatic COVID-19 and that ivermectin does not have measurable antiviral activity.

This validated platform is being adapted for *in vivo* assessment of antiviral activity in other acute respiratory viruses, including seasonal and pandemic influenza.

3.1. Proposal

In this randomised, open-label, controlled, group sequential adaptive platform trial, we will assess and compare the performance of currently licensed interventions with activity against influenza viruses, and those with potential activity demonstrated in pre-clinical and early clinical studies relative to each-other and the control (no antiviral treatment).

Many antivirals for use against influenza are available but only oseltamivir is recommended by the WHO. Some clinical trials of antiviral drugs have used oseltamivir as a comparator arm and compared clinical and virological endpoints but direct, standardised comparison of *in vivo* antiviral effects between multiple available antivirals

is lacking. There is also a lack of information regarding synergistic properties of antiviral combinations which may have relevance for those with prolonged viral replication.

By comparing each intervention to the no study drug arm, we can demonstrate that the method does identify active compounds. We will then compare the antiviral effects across interventions.

This information will aid prescribers, and guideline developers, and inform healthcare systems in making purchasing and prioritisation decisions.

The key metric assessed is the rate of viral clearance.

3.2. Pharmacodynamic Assessment

For the PLATCOV trial, we used prospectively collected serial viral load data from 46 individuals infected with SARS-CoV-2 and an accompanying pharmacodynamic model to simulate different trial designs for putative antiviral drugs using either time-to-clearance or rate-of-clearance as the primary trial endpoint. This model was then tested on data from a small pilot study of 24 patients with early symptomatic COVID-19 who were randomised to either ivermectin or placebo. Rate-of-clearance was shown to be a uniformly better endpoint in terms of type 2 error (i.e., results in increased power) than the widely used and reported time-to-clearance. Rate of decline in oropharyngeal qPCR estimates of viral density measured daily also allows shorter and more efficient evaluation of viral clearance. These simulations showed that this trial design could identify an effective therapy rapidly in the setting of a platform trial and quickly discard ineffective therapies. The available data suggests that the elimination kinetics of influenza virus from the pharynx are very similar to those for SARS-CoV-2. The initial results of the PLATCOV study demonstrated that regular oropharyngeal swabbing from day 0 to day 7 well-characterised the rate of viral clearance, and was well-tolerated. Although some previously published influenza studies with virologic endpoints have suggested a shorter duration of shedding for influenza viruses compared to SARS-CoV-2, when RT-PCR has been used instead of viral culture, influenza RNA has been detected up until day 7 (7,8). Without data to change the sampling schedule used for PLATCOV (SARS-CoV-2), we initially opted for the same sampling schedule and methodology for AD ASTRA (Influenza viruses), to be reviewed and updated after the first interim analysis of 50 patients recruited. A review of the qPCR data for the first 17 patients, with assignment concealed, indicated that oropharyngeal swabbing from day 0 to day 5 would be optimal to characterise viral clearance (as many were undetectable at D6 and D7). Swabbing on day7 was maintained considering secondary outcomes to measure emergence of viral mutations (see section 4).

4. OBJECTIVES AND ENDPOINTS

	Objectives	Endpoint	Timepoint(s) of the evaluation of this endpoint (if applicable)
Primary	1. To evaluate Influenza antiviral efficacy in-vivo (accelerated viral clearance relative to the no study drug arm). This is a superiority comparison. 2. To compare Influenza antiviral efficacy with current best antiviral treatment option (accelerated viral clearance relative to the positive control arm). This is a non-inferiority or superiority comparison.	Rate of viral clearance- estimated from the log_{10} viral density derived from qPCR of standardised duplicate oropharyngeal swabs/saliva taken daily from baseline (day 0) to day 5 for each therapeutic arm compared with the contemporaneous no antiviral treatment control/positive control.	Days 0-5
Secondary	To characterise the determinants of viral clearance in early influenza infection e.g. contribution of baseline serology, influenza type/subtype, prior vaccination, host genetics	Rate of viral clearance	Days 0-5
	To determine optimal dosing regimens for drugs with evidence of antiviral activity	Rate of viral clearance	Days 0-5
	To compare time to symptom resolution and fever duration with respect to no treatment	The following comparisons will be made: • Time to resolution of fever • Area Under the Curve of recorded temperature • Time to resolution of symptoms	Days 0-7
	To determine the effects of drugs on the development of drug resistant viral mutants between interventions and no treatment arm	Number of mutations known to confer resistance in detectable virus at later timepoints	Days 0-7
Tertiary	Characterise the relationship between viral clearance and hospitalisation for clinical reasons	Hospitalisation for clinical reasons up to day 7	Days 0-7
	Characterise the relationship between viral clearance and development of influenza complications including bronchitis, sinusitis, otitis media and pneumonia requiring antibiotics	Development of influenza-related complications including bronchitis, sinusitis, otitis media and pneumonia requiring antibiotics, up to day 7	Days 0-7

5. STUDY DESIGN

The study is a randomised, open label, controlled, adaptive platform trial that will be conducted in low-risk patients with influenza, recruited from outpatient acute respiratory infection clinics (ARIs) or through other approved facilities, or by patient self-referral to the study site. These will be adults 18-60 years old with early symptomatic influenza and without co-morbidities.

After obtaining fully informed consent, we will recruit adult patients with early symptomatic influenza (within 4 days (96 hours) since the reported onset of symptoms), who are positive by an influenza rapid antigen test

OR a positive RT-PCR test for influenza within the last 24 hours with a Ct value less than 30. Patients will be included who can be followed up reliably for 7 days.

The primary pharmacodynamic measure in this study is the rate of viral clearance (expressed as the slope of a fitted regression to the linear segment of the serial standardised oropharyngeal sample qPCR densities from 0 to day 5) following treatment. Each site will include a no antiviral treatment control arm consisting of patients not receiving any antiviral treatment, although local hospital supportive treatment will remain unchanged for the patients including antipyretics, antitussives, antihistamines and other required medications, in the clinical judgement of the treating physician.

5.1. Interventions

- The list of intervention influenza antivirals is Oseltamivir, Peramivir, Zanamivir, Laninamivir, Baloxavir, Favipiravir, a combination of Oseltamivir PLUS Baloxavir, and combination of Oseltamivir PLUS Favipiravir and a combination of Favipiravir PLUS Baloxavir. The interventions will be chosen in order of priority as well as local feasibility at sites (availability of drugs, local EC and regulatory approvals)
- Antivirals with *in vitro* activity against influenza viruses/activity in early clinical trials: Molnupiravir

At any given time in the study, it is possible that not all intervention arms are available. Randomisation ratios will be uniform across all available treatments and the control arm in each site, although the no study drug arm will have a minimum randomisation of 20%.

Randomisation will be continuously monitored by MORU. A positive influenza rapid antigen test will be used as the main inclusion criteria in patients with symptoms of influenza illness. Viral load is proportional to the severity of symptoms (8). In order to assess viral clearance dynamics accurately over time initial high viral densities are required.

Initially we aim to identify interventions that accelerate viral clearance by a minimum of 20% for the futility/success assessments relative to the no study drug arm, as in the current PLATCOV study (see section 10.4 and appendix 3). This threshold and the sampling schedule will be reviewed at the first interim analysis of 50 participants. This analysis will be open - thereafter the investigators will be blinded to the viral clearance results and interim analyses will be conducted as described in appendix 3.

Any intervention that shows an acceleration of viral clearance, defined as a greater than 90% probability of accelerating viral clearance by more than 20%, or if there is evidence of accelerated viral clearance from other studies, may be selected for inclusion in a nested pharmacokinetic-pharmacodynamic study. In this nested study, frequent blood sampling will allow assessment of the relationship between plasma drug concentrations and viral clearance. The design of the pharmacokinetic study depends on the known pharmacokinetic properties of each drug. In each case, no more than 10 blood samples (20ml) will be taken. Any intervention that meets the futility endpoint (less than 10% probability of accelerating viral clearance by more than 20%) will be dropped from the study. New arms can be added at any time during the trial. Decisions concerning success and futility will be made sequentially, first at the pre-specified interim analysis to be conducted after 50 patients are enrolled, and thereafter (blinded to the investigators) interim analysis will be conducted as described in appendix 3. The nested intensive PK-PD substudy may be triggered if the probability that viral clearance is accelerated by more than 20% goes above 90% or if there is evidence of accelerated viral clearance from other studies (e.g. oseltamivir).

5.2. Randomisation

Randomisation involves randomising the participants once. All drugs currently available and being used in the study may be included in this i.e.,:

Oseltamivir OR Peramivir OR Zanamivir OR Laninamivir OR Baloxavir OR Favipiravir OR Molnupiravir OR Oseltamivir PLUS Baloxavir OR Oseltamivir PLUS Favipiravir OR Favipiravir PLUS Baloxavir OR no study drug.

6. PATIENT IDENTIFICATION AND RECRUITMENT

6.1. Study Patients

Adult patients with early symptomatic influenza. The subject population includes those at low risk for complications and morbidity from influenza (as defined by WHO).

6.2. Inclusion Criteria

- Patient understands the procedures and requirements and is willing and able to give informed consent for full participation in the study
- Adults, male or female, aged 18 to 60 years at time of consent
- Early symptomatic Influenza (A or B); at least one reported symptom of influenza (including fever, history of fever, myalgias, headache, cough, fatigue, nasal congestion, rhinorrhoea and sore throat) within 4 days (96 hours)
- Influenza positive by rapid antigen test OR a positive RT-PCR test for influenza viruses within the last 24hrs with a Ct value of <30
- Able to walk unaided and unimpeded in activities of daily living (ADLs)
- Agrees and is able to adhere to all study procedures, including availability and contact information for follow-up visits

6.3. Exclusion Criteria

The patient may not enter the study if ANY of the following apply:

- Taking any concomitant medications or drugs which could interact with the study medications or have antiviral activity (see Appendix 4)
- Presence of any chronic illness/condition requiring long term treatment or other significant comorbidity (see Appendix 4)
- BMI ≥35 Kg/m^2
- Clinically relevant laboratory abnormalities discovered at screening
    - Haemoglobin <10g/dL
    - Platelet count <100,000/uL
    - ALT > 2x ULN
    - Total bilirubin >1.5 x ULN
    - eGFR <70mls/min/1.73m^2
- For females: pregnancy, actively trying to become pregnant or lactation (women on OCP are eligible to join)
- Contraindication to taking, or known hypersensitivity reaction to any of the proposed therapeutics (see Appendix 4)
- Currently participating in another interventional influenza or COVID-19 therapeutic trial
- Clinical evidence of pneumonia- e.g. shortness of breath, hypoxaemia, crepitations (imaging not required)
- Known to be currently co-infected with SARS-CoV-2 (i.e. confirmed with positive ATK or RT-PCR)
- Received live attenuated influenza virus vaccine within 3 weeks prior to study entry

7. STUDY ENROLMENT AND PROCEDURES

Recruitment of patients will be from outpatient influenza/ARI clinics or through other approved facilities, or by patient self-referral to the study site.

Patients with a positive influenza rapid antigen-detection test OR a positive PCR test for influenza within the last 24 hours with a Ct value of less than 30 will be enrolled in the study after providing fully informed written consent. In each study site there will be a designated research ward for study procedures. In accordance with local and national infection control guidelines the initial assessments will be conducted in a designated research ward set-up to manage influenza patients. Patients will receive care in either an inpatient or outpatient setting, in accordance with local and national guidelines.

All recruited patients will be visited at their home or place of residence, or be seen in the designated clinical trials unit or hospital ward. All staff will wear appropriate personal protective equipment (PPE), in accordance

with local hospital guidelines. For home visits a trained member of staff will perform the swabbing (and may ask the patient to give a saliva sample).

Participants will be asked if they have been in the study before.

The patient will undergo a physical examination and blood sampling for routine haematology and biochemistry and other baseline investigations.

After enrolment into the study, the patient will be randomised to one of the study arms. Further details of randomisation process will be provided.

An oropharyngeal swab will be obtained initially 1 time in duplicate (i.e. total 2 swabs, one from the left tonsil, one from the right tonsil) (baseline) by the study team using a standard operating procedure (SOP) after a full explanation is provided to the patient and before receiving the first dose of the study drug. An optional set of swabs may also be collected at this time (baseline). Swabs will be taken in duplicate again 1 hour after receiving the study drug, or 1 hour after the first swabs for those who be randomised to no study drug. To minimise patient discomfort, all influenza qPCRs will be performed on eluates from standard oropharyngeal swabs taken in duplicate as opposed to nasopharyngeal sampling.

In addition, some patients may be asked to have an extra set of swabs taken between day 0 – day 5 either by study personnel or self-swabbing following standardised study self-swab instructions and/or give a sample of saliva (see section 7.1).

For oral and inhaled study drugs, the first dose will be administered and observed at the research ward/hospital. In the case of inhaled study drugs, training will be provided on proper inhaler technique. Subsequent doses will be administered on the research ward in cases of patients admitted. In the case of outpatients, either participants will self-administer the medication at their home/current residence and record the timing of this in a diary card, or they will administer the medication under the supervision of study staff when they attend the research facility for daily oropharyngeal swabbing, or in some cases they may be supervised administering the medication at their home/current residence (if multiple doses, then one of the doses per day will be confirmed in this case). In case a study drug requires parenteral administration, this will be provided under supervision by the study personnel in the research ward, or hospital, or the patient's home.

7.1. Virological Sampling

On Day 0, oropharyngeal swabs will be taken initially 1 time in duplicate (one swab each tonsil) before receiving the first dose of the study drug (baseline). An optional set of swabs may also be collected at this time (baseline). Swabs will be taken in duplicate again 1 hour after receiving the study drug, or 1 hour after the first swabs for those who be randomised to no study drug.

From Day 1 to Day 5, and again on Day 7, serial oropharyngeal swabs will be taken in duplicate (one swab each tonsil) according to the sample schedule. The swabs will be taken from the back of the throat (oropharynx; tonsillar fossa) by trained study personnel according to the SOP abiding by PPE measures. Each swab will be placed in 3ml of viral transport medium (VTM) (e.g. Copan UTM® 330C), snapped off and sealed.

In addition, the study team may ask some participants to have an extra set of swabs taken between day 0 – day 5. Either the study personnel will be asked to take this extra set, or the participant will be asked to take it themselves as a self-swab, according to a set of given instructions. The purpose of this extra swab set (between day 0 – 5) is to assess whether an additional set of values would be beneficial for analysis of viral clearance. The purpose of self-swabs is to assess and validate whether self-swabbing will provide qPCR data of comparable quality to that carried out by study personnel, if standardised instructions are provided and adhered to. If the result is positive, this could reduce inconvenience to participants and reduce loss to follow up.

In addition, some patients may be asked to give a sample of saliva between day 0 and 5, either by spitting in a tube, or by sucking on a swab tip until it is saturated. The volume of saliva will be standardised. The purpose of saliva collection is to assess and validate a method of determining the viral density in the oropharynx in a less invasive manner, or supplement the results of the oropharyngeal swabs to improve the characterisation of viral clearance.

Swabs scheduled for days 6 and 14 will be removed, while those on days 7 will be retained to better characterise potential viral rebound. Consequently, the study will assess viral clearance rates up to day 5, rather than day 7 as previously done. The inclusion of additional early samples is expected to enhance the characterisation of the initial viral decline. At least once daily sampling will be performed by the study personnel who will place the swabs in the transport medium, and document the time. The samples will be kept cool, and subsequently stored at -80°C as detailed in the SOP. Transport times will be recorded, as well as the time at which the swab is frozen at -80°C. Viral genomes from the throat swabs will be quantitated by RT-qPCR, according to published methods (28). Each observed value will be recorded along with the RNAseP $\log_{10}$ density (representing human cell numbers), if available. The measurements for the patient over time will be used to estimate the rate of viral clearance under a Bayesian hierarchical linear mixed effects model.

The concentration of urea in the VTM will also be measured and compared with the serum urea concentration in the baseline biochemistry sample. This comparison enables determination of the extracellular fluid content in the eluate volume of the oropharyngeal samples. The urea concentration in throat fluid is the same as in serum, so that the dilution factor of the throat sample in the VTM fluid can be easily calculated (29).

All samples positive for influenza will be typed and subtyped to determine the effect of type/subtype on viral load and response to treatment. This will be done using whole genome sequencing technology or specific PCRs to determine subvariants.

7.2. Recruitment

Potential patients with a positive influenza test obtained during routine screening, or those with symptoms in keeping with influenza even if they do not have the results of their influenza test or have not been tested yet may be contacted by the research group. Alternatively, they may contact the research group for participation in the study. Each will be provided with a written participant information sheet.

7.3. Screening and Eligibility Assessment

Eligibility assessment will occur at the point of screening. If, based on the inclusion and exclusion criteria, the patient is eligible and willing to complete the full study, they will be included into the study after informed consent is obtained.

7.4. Informed Consent

Written and verbal versions of the participant information and informed consent will be presented to the patients detailing the exact nature of the study; what it will involve for the patient; the implications and constraints of the protocol; and the known side effects of the medicines under evaluation and any risks involved in taking part. It will be stated clearly that the patient is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The patient will be allowed as much time as required to consider the information as long as they remain eligible and within the time-frame for recruitment into the study, and the opportunity to question the investigator or other independent parties to decide whether they will participate.

Written informed consent will then be documented by a patient with the dated signature of the person who presented and obtained the informed consent. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the site PI. A copy of the signed informed consent will be given to the patient. The original signed form will be retained at the study site.

7.5. Baseline Assessments*

A physical exam, vital signs, oxygen saturation, symptom review and basic demographics will be recorded.

Oropharyngeal viral swabs will be taken by the study personnel 1 time in duplicate (a left sided and right sided swab i.e., two swabs in total) at baseline. An optional set of swabs may also be collected at this time (baseline). Some participants will be asked if they agree to have saliva (see section 7.1) collected in addition to oropharyngeal swabs. If they agree, they will have a saliva specimen taken at this point.

A total of 16ml of venous blood for study testing will be taken at this point.

Administration of the therapeutic may be delayed until the results of the FBC and biochemistry will be available (although these will ideally be available within 1 hour of sample collection i.e., before randomisation), to determine if there is any contraindication.

If these bloods are not available in this timeframe, then the patient can be safely enrolled, randomised and given their first dose, and can be excluded prior to their next dose if laboratory abnormalities resulting in a contraindication are present.

If an FBC and biochemistry has been taken in the previous 24hrs, the results of these can be used to determine whether there is a contraindication.

One of the baseline blood tests will be used for host genetic tests. This will allow investigation of potential genetic signatures associated with differential immune responses to influenza infection. Additionally, in those antivirals shown to accelerate viral clearance, genetic determinants of drug response (pharmacogenomics) may prove essential to optimised dosing of drugs and thus management of early influenza.

Laboratory tests at baseline

- Blood sample for
  - FBC and differential count
  - Biochemistry (including U&Es, creatinine, LFTs)
  - Serology (antibody responses)
  - Drug level
  - Host genotyping to assess determinants of the therapeutic response
- Oropharyngeal swab in duplicate, up to 2 times and possibly a saliva sample (see section 7.1).

*See appendix 1 for detailed schedule of activities

7.6. Days of Study

7.6.1. Randomisation D0

After enrolment, the patient will be randomised to one of the study arms using an online randomisation application which is managed by the trial statistician. The number of arms at a site can be limited by the approval and availability of the study drug at that site - there will be a minimum of 2 arms per site. The randomisation ratios will be uniform for all available interventions (e.g. 1:1:1) but randomisation to the no antiviral treatment control arm (no antiviral treatment arm) will be fixed at a minimum of 20% throughout the study. The study team will use a web-app to randomise as different sites may have different drugs. Only designated study staff will be authorised to access the web-app for patient allocation and it will be password protected. The drug allocations are not blinded for the patient for practical reasons. For patients randomised to an active drug, the first treatment dose (hour 0) will be supervised by study team after viral swabs (and possibly a saliva sample see section 7.1) are taken for qPCR (taken in duplicate) (see appendix 1 for schedule of activities). The patient will then be observed for a minimum of 1 hour in the ward. Oropharyngeal swabs will be taken again by the study personnel in duplicate at hour 1 (H1) for all participants.

For the therapeutic arms for which intensive PK has been triggered, allocated patients will stay for extra blood tests for drug monitoring levels, according to the schedule in the drug-specific appendix 2.

Body temperature recorded up to two times daily by study staff or participant (information collected in a diary and reviewed by the study personnel).

7.6.2. Day 1-Day 5 and Day 7*

The following variables will be assessed daily:

- Vital signs, eligibility check, and assessment of treatment (taking of medications will be confirmed by the study personnel and the time recorded. In participants who are outpatients, for medications taken more than once daily, the patient will record the time non-observed doses are taken in a diary card).
- Oropharyngeal swab for qPCR taken from each tonsil (two swabs) and possibly a saliva sample (see section 7.1) once a day by the study personnel (time taken recorded).

- Temperature recorded up to two times daily by study staff or participant (information collected in a diary and reviewed by the study personnel daily).
- Study personnel completes a brief symptom checklist each day.
- Blood samples will be taken (Day 3 and Day 7) for later pharmacokinetic analyses and FBC and biochemistry, to assess potential drug adverse effects. Blood samples for serology testing will be taken on Day 7.

  Remark: In addition, some participant if agreeable will be asked to take an extra set of swab (two swabs, one from each tonsil) between day 0 – day 5 either by study personnel or self-swab at home according to a set of given instructions. The purpose of this extra swab set (between day 0 – 5) is to assess whether an additional set of values would be beneficial for analysis of viral clearance. The purpose of self-swabs is to assess and validate whether self-swabbing will provide qPCR data of comparable quality to that carried out by study personnel, if standardised instructions are provided and adhered to. If the result is positive, this could reduce inconvenience to participants and reduce loss to follow up.

*****See appendix 1 for detailed schedule of activities

7.7. Intensive Pharmacokinetic Sampling:

For those study drugs that are shown to be effective antivirals in other studies or meet the success criteria of the study (defined as greater than 90% probability of accelerating viral clearance by more than 20% relative to the no study drug arm), we will then conduct intensive pharmacokinetic (PK) sampling for further patients who are enrolled into that arm. The exact sampling schedule will depend on the study drug characteristics (see appendix 2), but in general the patient will stay at the ward for the remainder of the day to have further blood tests performed. A cannula will be inserted so that blood can be drawn without the need for repeated venepuncture. A further blood test will be performed the next day, either at the ward or at their home if feasible. The volume of blood from this will not exceed 20 mls.

7.8. Management of Patients Who Become Ill

During the study, although this is unlikely, the patient may deteriorate clinically from their influenza or may develop a new intercurrent illness or potentially a side-effect related to the study medication.

All patients who develop difficulty with activities of daily living, shortness of breath or worsening of other symptoms, will be assessed initially by the study personnel. All of their vital signs will be measured, including oxygen saturations. They will be brought to the clinic research physician for further assessment. Clinical deterioration in this study may be due to several factors including progression of influenza illness, development of complications e.g. pneumonia, co-infection with other respiratory viruses, adverse reaction to medications, amongst others. Thus, a thorough clinical assessment will be performed by the study physician and we will take advice from infectious diseases experts. Based on this assessment, they may be referred to hospital, re-examined the next day, or asked to update the research team by mobile phone frequently on their well-being. The Physician may decide to repeat study oropharyngeal swabs in duplicate if symptoms of influenza recur, for the duration of the illness, to characterise symptomatic viral rebound or lack of response to treatment.

If a patient is referred to hospital, a clinical assessment will be made by the hospital physician and a diagnosis made. The decision to stop study drug will depend on that diagnosis and be made between the hospital team and the study PI.

The hospital physicians will have the responsibility for patient care but the research team will continue to follow the progress of the patients in hospital. Treatment for influenza severe enough to warrant hospitalisation will follow national guidelines. For those patients where treatment is changed (i.e., a new treatment is started for clinical reasons) an extra set of swabs in duplicate (and possibly a saliva sample see section 7.1) should be collected prior to the initiation of the new treatment (i.e. 2 sets of swabs will be taken in duplicate). This will only occur if the patient is within the first 7 days of the study.

The D0, D3, and D7 blood samples will be tested for FBC and biochemistry (e.g. U&Es, LFTs) to assess for drug-related adverse effects or abnormalities which require a potential dose adjustment or stopping the therapeutic or clinical intervention. The responsibility for evaluating and acting upon the results of blood tests will belong to the study team at the local site. They will use their clinical judgement in the assessment of the patient, further

tests and onwards referral. At any point of the study, extra laboratory tests may be conducted on the patient if felt to be clinically indicated by the research team, based on symptoms or previous laboratory abnormalities.

7.9. Sample Handling and Retention

Samples will be transferred to designated testing facilities, where they will undergo testing in accordance with best practice laboratory measures and safety procedures.

Oropharyngeal swabs (and possibly a saliva sample see section 7.1) will be processed using validated quantitative Real-Time quantitative Polymerase Chain Reaction (qPCR) to detect influenza, according to the study SOP. A urea measurement will be measured on each and compared to the baseline blood concentration. Swabs will be tested in duplicate.

The samples will be retained as per University of Oxford and local site regulations. Consenting patients may rescind their consent up until the completion of the study. Unless requested the samples collected prior to date of withdrawal will be retained for study analysis.

Samples, including those for PK, may be transferred to MORU or other designated testing facilities outside the site country, with appropriate material transfer agreements (MTA) and associated approvals prior to shipment.

Some of the leftover blood samples will be stored and may be used for further studies in the scope related to susceptibility or response to the influenza infection, and treatments. Blood samples will be labelled with a unique number and initials but not with the patient name. Any additional testing apart from what indicated in this study will be tested only after permission by the IRB/IEC.

8. DISCONTINUATION/WITHDRAWAL OF PATIENTS FROM STUDY

Patients may choose to stop treatment and/or study assessments but may remain on study follow-up. Patients may also withdraw their consent, meaning that they wish to withdraw from the study completely. In the case of withdrawal from both treatment and active follow up the following options for a tiered withdrawal from the study would apply:

a) Patients withdraw from the study but permit data and samples obtained up until the point of withdrawal to be retained for use in the study analysis. No further data or samples would be collected after withdrawal;

b) Patients withdraw from active follow-up and further communication but allow the trial team to continue to access their medical records and any relevant hospital data that is recorded as part of routine standard of care; i.e., CT-Scans, blood results and disease progression data etc.

c) Patients withdraw but do not allow data and samples collected to be used.

In addition, the Investigator may **discontinue a patient from the trial treatment** at any time if the Investigator considers it necessary for any reason including, but not limited to:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures
- Disease progression which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures

The reason for discontinuation and/or withdrawal will be recorded in the Case Report Form. qPCR data from patients withdrawn from the study will still be analysed if at least three distinct timepoints are available for the estimation of a clearance slope. The sample size is adaptive so there is no need to replace withdrawn patients.

Consenting patients may rescind their consent up until the completion of the study. Unless requested, data and samples collected prior to the date of withdrawal will be retained in the study database and analysis.

8.1. Definition of End of Study

The end of study is the date of the 7 day follow up visit of the last enrolled patient.

9. SAFETY REPORTING

9.1. Definitions

Adverse Event (AE)	Any untoward medical occurrence in a patient to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a patient to an investigational medicinal product which is related to any dose administered to that patient. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation§ • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the patient or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. Hospitalisation is defined as an unplanned, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. The patient must be admitted overnight, a short stay of several hours to receive treatment is not considered hospitalisation. If a patient is admitted overnight or longer for social/economic or isolation reasons and is otherwise medically stable, this does not constitute a SAE. Other examples of visits to a hospital facility that are not considered hospitalisation are: Emergency room visits, outpatient surgery, pre-planned or elective procedures for a pre-existing condition (as long as that condition has not deteriorated while on trial treatment or brought forward because of worsening symptoms) and for the purpose of this study, being hospitalised for isolation.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

9.2. Causality

The relationship of each adverse event to the trial medication must be determined by a medically qualified individual according to the following definitions:

Definitely related: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

Probably related:	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Possibly related:	There is some evidence to suggest a causal relationship (e.g., because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the patient's clinical condition, other concomitant treatments).
Unlikely to be related:	There is little evidence to suggest there is a causal relationship (e.g., the event did not occur within a reasonable time after administration of the trial medication), or there is another reasonable explanation for the event (e.g., the patient's clinical condition, other concomitant treatment).
Not related:	There is no evidence of any causal relationship.

9.3. Procedures for Recording Adverse Events

A symptom checklist will be performed daily from D0 until D5 with a final follow-up assessment on D7 to aid in the identification of adverse events. The severity of adverse events will be assessed following the Common Terminology Criteria for Adverse Events (CTCAE) v5.0:

1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening, 5 = fatal.

AEs occurring in patients from enrolment and during trial participation that are observed by the Investigator or reported by the patient with severity **grade of 3 (severe) or higher** will be recorded on the CRF, whether or not attributed to trial medication.

If drug combinations are assessed, the team will record all clinical AEs of severe grade (**grade 3 or higher**) for those arms which have been previously assessed in humans and shown to be safe and well-tolerated e.g., Oseltamivir PLUS Favipiravir, Oseltamivir PLUS Baloxavir. All clinical AEs for other combinations e.g., Favipiravir PLUS Baloxavir will be recorded **grade of 1 or higher**. This will be important to monitor the safety and tolerability of combinations in direct comparison to the individual drugs.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the patient's removal from treatment. A patient may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the patient must undergo an end of trial assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

9.4. Grading of Laboratory Abnormalities

Abnormal laboratory findings detected during the study or are present at baseline and worsen following the taking of study medication will be reported as AEs or SAEs. If found to be abnormal the values will be graded according to the CTCAE v5.0. AEs and SAEs of severity grade 3 or higher will be recorded in the CRF and will be followed up until grade 2 or below, returned to baseline, or deemed to be permanent. For laboratory results that are not available in the CTCAE v5.0, the site investigator should make a determination as to whether or not the laboratory abnormality is clinically significant. If the site investigator believes a laboratory abnormality is clinically significant, it should be reported as an adverse event and a grade should be identified to the best of their ability.

9.5. Reporting Procedures for Serious Adverse Events

All SAEs detected by the site investigator must be reported to the AD ASTRA safety team within 24 hours of site awareness. The Serious Adverse Event CRF documenting the SAE should be emailed to AD ASTRA safety team.

The AD ASTRA safety team will inform the DSMB within 10 days of initial notification of the SAE and keep the DSMB updated as needed.

Further follow-up reports should be submitted, if required, until the SAE is resolved, is deemed stable/ permanent or results in death. A final status should be determined for any SAEs ongoing at the study end date.

The site PI must also report the SAEs to the local ethics committee and the regulatory authority in accordance with local requirements.

9.5.1 Expectedness

Expectedness will be determined according to the Investigators' Brochure/Summary of Product Characteristics.

9.6. Data Safety Monitoring Board (DSMB)

An independent Data Safety and Monitoring Board (DSMB) will be set up consisting of qualified volunteers with the necessary knowledge of clinical trials. The DSMB will receive summary reports from MORU as defined per charter or per ad-hoc request, prior to each meeting. All data reviewed by the DSMB will be in the strictest confidence. A DSMB charter will outline its responsibilities and how it will operate.

An interim report will be prepared by the Trial Statistician for the pre-specified interim analysis. In case of safety concerns, additional information or formal interim analyses can be requested by the DSMB.

The DSMB will meet formally at the following timepoints:

- after the first 50 patients have been accrued into the study
- At additional time-points as indicated by the DSMB after their review, if deemed necessary

All DSMB recommendations will be communicated to site PIs. The site PI will be responsible for submitting the written DSMB summary reports with recommendations as applicable to local/ national ethics committees and other applicable groups.

10. STATISTICS AND ANALYSIS

10.1. Overview of Adaptive Study Design and Overall Approach

The statistical plan for AD ASTRA is based on our COVID-19 platform trial, PLATCOV. The final statistical analysis of the AD ASTRA data will be according to the most recent signed version of the Statistical Analysis Plan (SAP). Below is a description of the general principles of the statistical method and analysis, with current examples from the SAP.

The initial results of the PLATCOV study demonstrated that regular oropharyngeal swabbing from day 0 to day 7 well-characterised the rate of viral clearance. Although some previously published influenza studies with virologic endpoints have suggested a shorter duration of shedding for influenza viruses compared to SARS-CoV-2, when RT-PCR has been used instead of viral culture, influenza RNA has been detected up until day 7 (7,8). As such, without data to justify a change to the PLATCOV (SARS-CoV-2) methodology, we opted for the same sampling schedule, methodology and thus power calculations for AD ASTRA (Influenza viruses), with a plan to review and update after the first interim analysis of 50 patients recruited.

An analysis of the PCR data for the first 400 patients with concealed assignment indicated a day 0 to 5 sampling schedule would be optimal to characterise viral clearance. However, patients will continue to be swabbed from D0-D5 and again D7 to allow for detection of viral mutants conferring resistance, and for closer follow-up of patients from a clinical perspective.

Clinical trials with adaptive designs are increasingly being used in research, including in such successful and prominent COVID-19 studies as the RECOVERY study. In addition, the FDA and EMA supply guidance on studies with these designs. They do not have fixed sample sizes as conventional RCTs. The number of patients recruited depends on the results (i.e., they are adaptive). The benefits of an adaptive sample size are increased efficiency and ethical considerations (i.e., they can both stop ineffective arms, and identify effective arms earlier, thereby limiting numbers in ineffective arms compared to fixed sample number studies). Because of these advantages this type of study is likely to become more prevalent, but it is important that the study is well-designed in advance, that the adaptive design is appropriate for the overall study design, and has mechanisms in place to control type I error.

As in the PLATCOV trial, the main pharmacodynamic endpoint is the rate of viral clearance, estimated from serial qPCR measurements in each patient. The measured end point is the slope of the initial log linear decline in qPCR estimated viral densities. The clearance rate will be correlated with the presence of symptoms and possibly other clinical endpoints such as duration of hospitalisation. As this is a multi-centre trial, populations may differ between sites with respect to key covariates. Therefore, the main analytic approach will be to fit a hierarchical Bayesian model to the serial qPCR measurements (using default weakly informative Bayesian priors to aid model fitting), whereby the baseline viral loads (intercept) and the first-order clearance rates (slope of initial decline) can vary between sites and between individuals. The viral clearance slope is also dependent on the randomised study arm. Stopping an intervention arm for futility or triggering an intensive PK-PD nested sub-study will be decided using the posterior probabilities that the arm results in an increased slope. The PK-PD nested sub-study will be also triggered if there is evidence of accelerated viral clearance from other studies.

10.2. Bayesian Hierarchical Model of Viral Clearance

At each interim analysis we will fit a series of Bayesian hierarchical (mixed effects) model to the serial viral load measurements (modelled directly on the Ct scale). The main linear model will take the following form:

$$y_{k,i,t} \sim \text{Student}(\alpha_0 + \alpha_k + \alpha_i + (\beta_0 + \beta_i + \beta_k + \beta_{T(i)})t \text{ , sigma}_2, \text{dof})$$

where $y_{k,i,t}$ is the observed $\log_{10}$ viral load for individual i at time t from site k (two values per timepoint), and T(i) is the randomised arm assigned to individual i. Sigma_2 is the measurement error (residual error). The error model is a t-distribution with degrees of freedom inferred from the data (dof). The slope of viral clearance decomposes into 4 terms: the population mean slope β_0; the site random effect β_k; the individual random effect β_i; and the treatment effect (fixed across sites and individuals) $\beta_{T(i)}$. The intercept term (baseline viral load) decomposes into 3 terms: the population intercept α_0; the site random effect α_k; and the individual random effect α_i. All comparisons are made relative to the no antiviral treatment control arm, so we set $\beta_{control} = 0$. Adjustment will be made for human RNaseP concentration. The model will treat viral load corresponding to Ct values of 40 as left censored. An equivalent non-linear model (allowing for an initial increase in viral load, followed by a linear decrease) will also be fitted to the data as a sensitivity analysis.

10.3. Time Varying Effects

Characterising and identifying time varying effects not specified in the main model is important:

- Interventions which are added later on during the trial cannot be compared directly to all data from the control arm without accounting for possible differences in population over time (as an epidemic evolves, included populations may differ in a single site over time).

We will use descriptive approaches to detect time-varying effects (residual plots over time, mean slopes in control individuals over time). If substantial time-varying effects are observed, we will add a smooth time varying spline component to the linear model. The base model will not include this for simplicity.

10.4. Sample Size Estimation, Randomisation and Statistical Considerations for the Bayesian Group Sequential Design

The platform study has two main objectives:

- Characterise antiviral efficacy of interventions relative to the no study drug arm to demonstrate that this methodology works in identifying active compounds
- Identify the antiviral with the best virological efficacy in head-to-head comparisons

The sample size is adaptive. For each intervention, the required sample size is based on pre-specified stopping rules, which use 1) a futility/success margin λ_1 for comparisons with the no-study drug arm, and 2) a non-inferiority margin λ_2 for comparisons with the positive control arm, defined as the intervention with the highest virological efficacy upon interim analyses. Each intervention arm will be stopped when it meets the stopping rules in the interim analyses, as laid out in the most recent signed version of the Statistical Analysis Plan. See below example:

1. Meets the futility criteria: if Probability[$\beta_T < \lambda_1 * \beta_0$] > 0.9;
2. Meets the success criteria: if Probability[$\beta_T > \lambda_1 * \beta_0$] > 0.9 relative to the no-study drug arm *AND* meets the non-inferiority criteria *or* the inferiority criteria: if Probability[$\beta_T > -\lambda_2 * \beta_0$] > 0.9 or Probability[$\beta_T < -\lambda_2 * \beta_0$] > 0.9 relative to the positive control arm, respectively.

The flow of interim analyses and decision rules for removing an arm from the platform or designating an arm as the new positive control is described in Appendix 3. While the negative control arm (no-study drug arm) and the positive control arm are required as contemporaneous control arms to adjust for potential temporal bias in estimating antiviral efficacies, the required sample size for these arms depends on the duration of the trial and the number of concurrent intervention arms.

The trial is continuously running group sequential adaptive platform trial. Therefore, there is no fixed sample size. If the trial starts with 5 intervention arms (one of which becomes the positive control, and one is the negative control arm - the 'no study drug' arm), all starting recruitment simultaneously, the expected maximum sample size would be approximately 1000 participants. If all intervention arms start at different time points, additional participants are required as contemporaneous negative and positive controls (enrolment is competitive). In other words, the resulting in the expected maximum patient number could exceed 1000 participants.

Therefore, across all study sites, the trial is expected to enrol approximately 1500 participants in total, depending on the number, timing, and duration of active intervention arms. Further recruitment can occur after consultation with the DSMB or TSC, if it is felt that more precision of the result is beneficial. Different arms may be assessed in different sites in the multinational platform study. Recruitment will be competitive between the sites in an adaptive manner without pre-defined numbers per site.

The most recent determinations of the futility/success margin λ_1 and the non-inferiority margin λ_2 were informed by insights gained from simulations using data from the first 500 participants enrolled in the PLATCOV trial, which has a similar study design for assessing the efficacies of SARS-CoV-2 antivirals. Details of the simulations are described in Appendix 3. The results suggested that choosing the futility/success margin λ_1 = 1.2 (20%) minimized the false positive rate to 1% in interventions with no antiviral effect, with a median sample size of 30 participants per arm (for futility/success decision). It also minimised false negative rates to less than 1% in interventions with antiviral effects of 40% and 60%, with median sample sizes of 50 and 20 participants per arm (for futility/success decision), respectively. Additional participants are required for the non-inferiority assessment.

For the non-inferiority assessment, the simulations described in Appendix 3 suggested that choosing a stringent non-inferiority margin (λ_2 = 0.9; -10%) results in earlier stopping of interventions which are clearly less effective than the positive control and later stopping for interventions with an effect close to the positive control.

This study is open label and so a centralized server-based randomization system will be used. Only designated study staff will be authorised to access the webapp for patient allocation. The webapp will be password protected. All randomisation activities will be traced within the webapp and attributed to a timestamp along with the anonymised patient study code, age and sex.

10.5. Sample Size for the Intensive PK-PD Sub-study

Any drugs with evidence of accelerated viral clearance from other studies or which meet the success endpoint (>90% probability that clearance is accelerated by more than 20% relative to control) will be included in an intensive PK-PD study. The sample size calculation for each sub-study will be drug dependent and will use the information regarding the variability in clearance in the intervention arm versus the variability in clearance in the control arm (i.e., it will be determined by the results), but based on previous experience with PLATCOV, would be capped at approximately 40 participants per arm. Larger observed variability in the intervention arm would be expected if an active intervention had variation in exposures that corresponded to variation around the near maximal effect concentration (e.g., EC_{95}).

We will use a PK simulation-based approach where we will simulate predicted drug AUC values for patients given the characteristics of the patients in each site (weight and sex) and use the observed variability in viral clearance rates to estimate the necessary sample size to infer a dose-response relationship whereby a 10% increase in AUC resulted in a 10% increase in the slope of viral clearance with 80% power and 5% type 1 error.

10.6. Final Analysis of Primary Outcome

As for PLATCOV, the final analysis will fit a series Bayesian hierarchical linear and non-linear models to the serial log_{10} viral load measurements (copies per mL) as determined in the most recent signed version of the SAP. The

primary estimates of interest are the relative changes in the rate of viral clearance for the intervention arms compared to the no study drug arm. These will be reported as their mean posterior estimates along with credible intervals. All analyses will be done using *R* using the package *RStan* along with bespoke software. All analysis code for AD ASTRA is openly available via a GitHub repository: https://github.com/jwatowatson/ADASTRA-SAP. Anonymised viral load data and key meta data (e.g., site, strata, randomised allocation) will be made available at publication of results.

11. DATA MANAGEMENT

11.1. Access to Data

Direct access will be granted to authorised representatives from the University of Oxford, local ethics committees and regulatory authorities, and any host institution for monitoring and/or audit of the study to ensure compliance with regulations. Outcome data and treatment assignment data will be made available for analysis in real time.

11.2. Data Handling and Record Keeping

Clinical study data will be recorded on CRFs and entered on to a password-protected database by the local study PI, research nurse or designee. The study database will be built in MACRO EDC, a clinical data management system that is compliant with ICH GCP and FDA 21 CFR Part 11 and will be hosted in a secure, access-restricted server in MORU. The study database will include internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

Measures will be taken to ensure non-disclosure of information that is potentially harmful to patients. Paper records (for example, patient identifiable information for the purposes of follow-up, the screening logs and signed ICFs) will be kept in locked cabinets; electronic data will only be accessible to staff with user accounts and passwords. The database contains an audit trail that keeps record of changes to data and user activity within the database. All electronic data will be stored on secure servers that are backed up daily, with weekly off-site storage.

Patient records at site will, taking into account the ability of the sites, be stored in binders in the secured access-limited room or scanned and stored electronically. The records will be retained for at least five years following completion of the study, or according to local site regulation. The study database will be retained indefinitely.

With patient's consent, clinical data and results from blood analyses stored in the database may be shared according to the terms defined in the MORU data sharing policy with other researchers to use in the future. (www.tropmedres.ac/units/moru-bangkok/bioethics-engagement/data-sharing/moru-tropical-network-policy-on-sharing-data-and-other-outputs).

12. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with relevant regulations and standard operating procedures.
The study will be conducted in compliance with this protocol, International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP) and any applicable regulatory requirement(s). Monitoring will be overseen by the MORU Clinical Trials Support Group (CTSG) according to a prespecified risk-based monitoring plan to ensure compliance to the study protocol and applicable guidelines and regulations. Biological specimens will be processed, stored and shipped in accordance with MORU SOPs.

Data validation will be performed to identify errors or discrepancies and thus ensure completeness, validity and accuracy of data.

13. ETHICAL AND REGULATORY CONSIDERATIONS

This study will be conducted in patients who would be unlikely to progress to severe illness. The investigated medications may benefit the patient; i.e., shorten the duration of symptoms or decrease their severity, but are unlikely to have a significant adverse effect on the patient's illness and subsequent health.

Funds will be set aside to cover hospital costs in the unlikely event of a drug adverse reaction.

Women who are pregnant, actively trying to become pregnant, or breast feeding will be excluded from this study as it is not known if any of the treatments being tested will have additional benefits that would outweigh any risks associated with pregnancy/breast feeding.

13.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the current revision of the Declaration of Helsinki.

13.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

13.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to OxTREC and local ethics committees for written approval.
The Investigator will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents.

13.4. Patient Confidentiality

The study staff will ensure that the patients' anonymity is maintained. The patients will be identified only by a patient ID number on all study documents and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with GDPR, which requires that personal data must not be kept as identifiable data for longer than necessary for the purposes concerned.

13.5. Expenses

Patients will be financially compensated for their time in the study, in accordance with local EC guidance and approval.

13.6. Risk

All of the treatments being tested initially (see Appendix 2 for study drugs) are generally well-tolerated with the main adverse effects related to gastrointestinal symptoms (diarrhoea, nausea and vomiting). Further information regarding specific side effects for each of the drugs used in the study can be found in Appendix 2. These are usually mild and settle without the need for medical intervention. In the case of intravenously administered treatments, there may be discomfort, bleeding or bruising of the skin at the site of needle puncture.

The risks associated with blood withdrawal during the study include local discomfort, occasional bleeding or bruising at the site of needle puncture and very rarely, infection. The risks associated with oropharyngeal swabbing are limited to slight local discomfort.

13.7. Benefits

There may be earlier resolution of symptoms in those who are randomised to certain active treatment arms, but benefit is not guaranteed. Although an individual patient may not personally benefit, this study should help future influenza patients by identifying treatments that have the best antiviral effect in early disease. Remuneration will be provided to the patients for the period they are enrolled in the study. Patients will be reimbursed for costs associated with traveling to the study site and loss of work time. The amount will be determined per local allowed guidelines and ethics committee policies.

13.8. Reporting

The PI shall submit an Annual Progress Report to OxTREC and local EC on the anniversary of the date of approval of the study. In addition, the PI shall submit an End of Study Report to OxTREC and local EC of upon the completion of the study.

13.9. Finance and Insurance

13.9.1 Funding

The trial is funded by Wellcome Trust Grant ref: 226933/Z/23/Z through the COVID-19 Therapeutics Accelerator.

13.9.2 Insurance

The University of Oxford has a specialist insurance policy in place which would operate in the event of any patient suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

13.10. Data Ownership

The data generated in this study belongs to the study group as a whole. The final database will be shared amongst the site PI and key members of the research team.

The database may be shared with researchers not directly involved in this study after the main paper has been published and in accordance with MORU guidelines on data sharing. The criteria for authorship will be consistent with the international guidelines (http://www.icmje.org/#author).

13.11. Publication Policy

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by Wellcome Trust. Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines and other contributors will be acknowledged.

The results of the study will be summarised in lay language, in both English and the language(s) commonly spoken at the study sites, and disseminated to key stakeholders, user communities and patients.

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15. Appendix 1: Schedule of Activities

Procedures	D0			D1	D2	D3	D4	D5	D7	If new Tx/ symptoms
	Pre-1st dose	H0	H1							
Screening incl. lateral flow Ag test	X									
Urine pregnancy test (female of childbearing age)	X									
Eligibility assessment	X									
Informed consent	X									
Randomisation	X									
Baseline information including basic demographics, symptoms, physical exam, vital signs, oxygen saturation	X									
Vital sign	X			X	X	X	X	X	X	
Symptom checklist			X	X	X	X	X	X	X	
Observed/ confirmed dose		X[1]		X[1]	X[1]	X[1]	X[1]			
Observation post dose			X[1]							
Temperature [2]	X			X	X	X	X	X	X	
Virology										
Oropharyngeal swabs [3, 4, 5]	X[6]		X	X	X	X	X	X	X	X
Bloods										
FBC+diff	X[7]					X			X	
Biochemistry (including U&Es, creatinine, LFTs)	X[7]					X			X	
Serology	X								X	
Drug level	X					X			X	
Host genotyping	X									

[1] Depends on dosing schedule of the drug. The first dose will be given at the designated trial facility. For drugs where further doses are required, at least one dose per day will be confirmed by the study team. The time the drug is taken will be recorded by the study team.
[2] Performed up to two times a day and self-recorded in a temperature card if non-observed.
[3] Swabbing will be conducted in different locations within the study site depending on whether the participant is an inpatient or outpatient.
[4] Taken at least once daily in duplicate by the study team (right and left tonsillar fossa). The time is recorded when swabs were taken
[5] Some participants may be asked for an additional set of bilateral swabs (two swabs: one swab from each tonsil) to be collected between day 0 to 5 either by study personnel, or by patient self-swabbing, following study standardised self-swabbing instructions, and/or a saliva sample, between the same period (day 0-5)
[6] Taken at least 1 time, up to 2 times in duplicate (up to four swabs, two from each of the right and left tonsillar fossa). The time is recorded when swabs were taken.
[7] Results will be used to detect laboratory abnormalities included in the exclusion criteria

16. Appendix 2: Study Drugs

16.1. Oseltamivir

16.1.1. Rationale

Neuraminidase inhibitors are a class of antivirals that were developed in the 1990s as a novel approach to prophylaxis and treatment of influenza. By selective binding to the active site of influenza neuraminidase, neuraminidase inhibitors block the enzyme's ability to cleave sialic acid residues on the cell surface, thus blocking release of progeny virions from infected respiratory epithelial cells and reducing viral spread in the respiratory tract.

The use of neuraminidase inhibitors, oseltamivir and zanamivir in particular, has increased dramatically since the outbreak of A/H1N1 in April 2009, partly because of the rise in amantadine/rimantadine resistance, and in the early stages of the outbreak, the lack of a vaccine.

Of the four neuraminidase inhibitors developed, oseltamivir is the most widely studied and used. It is considered the standard of care in many countries for treatment of suspected or proven influenza in seriously ill or hospitalised patients, as well as in ambulatory patients with underlying conditions associated with higher risks of influenza complications.

Oseltamivir carboxylate is the active metabolite of the pro-drug oseltamivir phosphate (Tamiflu©). Oseltamivir phosphate dissociates in the gastrointestinal tract to form oseltamivir, which is absorbed and metabolised into oseltamivir carboxylate by hepatic carboxylesterase.

Oseltamivir carboxylate inhibits influenza A and B neuraminidases in vitro. Neuraminidase enzyme IC_{50} (the half maximal inhibitory concentration) values for oseltamivir for clinically isolated influenza A ranged from 0.1nM to 1.3nM, and for influenza B was 2.6nM. Higher IC_{50} values for influenza B, up to a median of 8.5nM have been observed in published studies[1]. This is reflected in clinical data where oseltamivir has shown to be less effective in treating influenza B viral infections compared with influenza A infections[2,3].

Prospective, placebo-controlled data supporting the use of oseltamivir in the treatment of adult influenza comes from 4 published studies[4,5,6,7]. Two of these enrolled previously healthy individuals with acute uncomplicated influenza presenting within 36 hours of onset, and the other two evaluated experimentally induced influenza (one influenza A study and one influenza B study) with oseltamivir administered 24-28 hours post-inoculation. These trials consistently showed that oseltamivir reduces the duration of illness by approximately 24 hours. Three out of the four trials demonstrated statistically significant reductions in viral titers (measured as area under the curve) with oseltamivir but one study did not demonstrate a significant difference in viral shedding at any time point. A recent re-analysis of median viral shedding curves from early experimental influenza infection trials[8] showed that in addition to reducing peak viral loads, oseltamivir increases the rate of viral clearance after the peak, although more slowly in influenza B than influenza A infection; however, analysis of individual shedding curves did not reveal an impact of oseltamivir on viral decline rates. In addition, this study showed that 20-40% of oseltamivir treated volunteers continued shedding virus similarly as placebo-treated controls, suggesting treatment failure. Thus, although there is evidence for antiviral efficacy of oseltamivir, data is somewhat variable.

Clinical effectiveness in symptom alleviation is greatest when administered within 48 hours thus it is licensed for use within this time-frame. A Cochrane meta-analysis of 20 treatment trials demonstrated considerable variation in the reduction of clinical symptoms after oseltamivir treatment, ranging from 8.4 to 25.1 hours[9].
Data on the effectiveness of oseltamivir in the prevention of influenza-related complications are variable. A 2014 Cochrane review, which analysed data in the intention-to-treat (ITT) group without accounting for confirmed infection, found no decrease in the risk of hospital admission or serious complications with oseltamivir treatment[10]. A subsequent meta-analysis by Dobson and colleagues divided individuals into an ITT group and an ITT-infected (ITT-I) group, in which influenza infection was confirmed by testing. This study estimated a 44% risk reduction (relative risk 0.56 [95% CI 0.42-0.75]; p=0.0001) in lower respiratory tract complications and a 63% risk reduction (0.37 [0.17-0.81]; p=0.013) in hospital stay for the ITT-I group that received oseltamivir[11].

No RCTs of oseltamivir versus placebo have been conducted in patients hospitalised with influenza meaning evidence for its efficacy in patients with severe disease is uncertain.

Oseltamivir is the only antiviral currently recommended by the WHO for the treatment of influenza. However, evidence for these recommendations is largely based on observational studies and is acknowledged to be based on evidence considered low quality for critical outcomes including mortality[12].

During the 2007-2008 influenza season, substantial resistance to oseltamivir emerged in influenza A H1N1 viruses through a histidine to tyrosine substitution (H275Y) in the neuraminidase protein. Since the emergence of the 2009 pandemic H1N1 (H1N1pdm09) influenza A viruses and their establishment as circulating epidemic strains, resistance to neuraminidase inhibitors including oseltamivir has been uncommon. As of March 2016, 5% of influenza A H1N1pdm09 isolates were resistant to oseltamivir. Rates of oseltamivir resistance were lower than 5% in the European union, with resistance observed in less than 1% of influenza A H1N1pdm09 isolates[13].

16.1.2. Composition and dose

Each Tamiflu® 75mg hard capsule contains oseltamivir phosphate equivalent to 75mg oseltamivir
The recommended dosing schedule is 75mg of oseltamivir twice daily for five days
Tamiflu® may be taken with or without food but when taken with food, tolerability may be enhanced in some patients

We will use Tamiflu® or a high-quality generic alternative in the study.

16.1.3. Pharmacokinetic characteristics

Oseltamivir phosphate is an oral pro-drug which is cleaved by esterases in the liver, gastrointestinal tract and blood to the active oseltamivir carboxylate with good bioavailability estimated ~80%. Administration with food may delay absorption slightly but does not decrease overall bioavailability[1].
Following oral administration of oseltamivir, the time to maximum concentration of oseltamivir carboxylate is 3-4 hours and the plasma half-life ($t_{1/2}$) averages 7 to 9 hours.

Both the prodrug and parent are eliminated primarily unchanged through the kidney. Distribution is not well characterised in humans, but peak bronchoalveolar lavage levels are similar to plasma levels in humans. In humans, concentrations in sinus and middle ear fluid aspirates are similar to those in plasma[1].

No dose adjustment is required for treatment in patients with hepatic dysfunction.

Dose adjustment is recommended for adults and adolescents (13-17 years) with moderate or severe renal impairment (creatinine clearance <60ml/minute)[1]. In this study, patients with creatinine clearance <60ml/minute will not be eligible for randomisation.

<u>Pharmacokinetic sampling schedule (only if there is evidence of accelerated viral clearance):</u>

All patients will need to be admitted for 12 hours for intense sampling

The timings are:

- Pre-dose D0H0, then
- 1,2,3,4,6,9,12 and 24h post dose

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentrations

16.1.4. Toxicity

Oral oseltamivir is generally well tolerated and no serious end-organ toxicity has been found[1].
The overall safety profile of Tamiflu is based on data from 6049 adult/adolescent and 1473 patients treated with Tamiflu or placebo for influenza. In adults and adolescents, the most commonly reported adverse reactions were nausea, vomiting and diarrhoea in the treatment studies. The majority of these ARs were reported on a single occasion on either the first or second treatment day and resolved spontaneously within 1-2 days[1].

Animal studies do not indicate reproductive toxicity. Data on oseltamivir exposure of pregnant women from post-marketing reports and observational studies (more than 1000 exposed outcomes during the first

trimester) indicate no malformative nor feto/neonatal toxicity by oseltamivir. The use of Tamiflu may be considered during pregnancy if necessary[1].

16.1.5. Contraindications[1]

- Hypersensitivity to the active substance, oseltamivir carboxylate
- Hypersensitivity to any excipient:
    - *Capsule contents*: pregelatinised starch, talc, povidone, croscarmellose sodium and sodium stearyl fumarate
    - *Capsule shell*: gelatin, yellow iron oxide (E172), red iron oxide (E172), black iron oxide (E172) and titanium dioxide (E171)
    - *Printing ink*: shellac (E904), titanium dioxide (E171) FD and C Blue 2 (indigo carmine E132).

16.1.6. Cautions

The following serious adverse reactions have been rarely reported since oseltamivir has been marketed (<1/1000): anaphylactic and anaphylactoid reactions, thrombocytopenia, hepatic disorders (fulminant hepatitis, hepatic function disorder and jaundice), angioneurotic oedema, Steven-Johnson syndrome and toxic epidermal necrolysis, gastrointestinal bleeding and neuropsychiatric disorders[1].

There have been postmarketing reports, mostly from Japan, of neuropsychiatric events including abnormal behaviour, self-injury and delirium during administration of Tamiflu in patients with influenza. These reports were primarily among paediatric patients and these events have also been experienced by patients with influenza without oseltamivir treatment[1]. Estimates of frequency cannot be made but these events appear to be uncommon based on Tamiflu usage data. The FDA package insert for Tamiflu advises monitoring for signs of abnormal behaviour.

16.1.7. Drug interactions

No clinically significant drug interactions have been recognised for either drug. Neither oseltamivir or oseltamivir carboxylate interact with human cytochrome P450 enzymes which suggests that drug interactions as a result of competition with this pathway are unlikely. Oseltamivir also has low protein binding and metabolism independent of the glucuronidase systems again suggesting that clinically significant drug interactions are unlikely[1].

The FDA package insert for Tamiflu advises against administration of Tamiflu until 2 weeks following administration of the live attenuated influenza vaccine.

16.1.8. References

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16.2. Zanamivir

16.2.1. Rationale

Zanamivir is a potent, selective inhibitor of influenza A and B neuraminidase, including some influenza virus variants with NA substitutions conferring loss of susceptibility to other NAIs.

The inhibition of this enzyme is reflected in both *in vitro* and *in vivo* activity against influenza A and B virus replication, and encompasses all of the known neuraminidase subtypes of influenza A viruses. Neuraminidase inhibition occurs in vitro at very low zanamivir concentrations; the concentrations of zanamivir required to inhibit in vitro plaque formation of influenza A and B viruses by 50% in Madin-Darby canine kidney cells (MDCK) were 0.002 to 16umol/L in assays of clinical isolates[1].

In one study, the respective reported mean values of inhibitory concentrations of 50% of zanamivir and oseltamivir were 1.14 and 0.9 nmol/L for influenza A/H1N1, 2.09 and 0.73nmol/L for influenza A/H3N2, and 4.15 and 11.53 nmol/L for influenza B[2] i.e. oseltamivir exhibits less potent inhibition of influenza B virus than zanamivir in vitro.

Consistent with in vitro findings, results from mouse and ferret models demonstrate the antiviral activity of intranasal zanamivir in vivo with relatively low doses of the drug (as low as 0.05mg/kg) inhibiting viral replication[1].

Pooled data from four randomised, controlled trials of intranasal zanamivir versus placebo in experimental influenza A (H1N1) infection indicated that early treatment (zanamivir 16mg nasal drops two or six times daily beginning 26 or 32 hours after viral inoculation and continuing for 4 days) significantly reduced peak viral titres by $2\log_{10}$ $TCID_{50}$/ml and the median duration of viral shedding by 3 days compared with placebo. Early treatment was also associated with statistically significant reductions in the incidence of febrile illness[3].

In phase 3 studies involving otherwise healthy adults with laboratory-confirmed influenza A or B infection, zanamivir (10mg by inhalation twice daily for 5 days) significantly reduced the duration of symptoms of influenza by a median of 1.5 days (range 1-2.5 days). Efficacy was demonstrated when treatment is initiated within 48 hours[4,5,6,7].

Several studies in both children and adults have demonstrated superior efficacy of inhaled zanamivir compared to oral oseltamivir in reducing the duration of fever in those with influenza B infection[8,9,10]. In an observational study enrolling adults aged 9-94 years with laboratory confirmed influenza, similar efficacy of zanamivir compared to oseltamivir in reducing fever duration was reported for influenza A/H1N1 infections whereas superior efficacy of oseltamivir compared to zanamivir was seen for influenza A/H3N2 infections[9], reflecting in vitro data. These data suggest that zanamivir therapy may be more effective than oseltamivir for the treatment of influenza B infection and oseltamivir may be more effective than zanamivir for treatment of A/H3N2 infections.

In a network meta-analysis comparing the clinical effects of all four neuraminidase inhibitors as monotherapy and oseltamivir and zanamivir combination therapy, zanamivir was higher ranked (as measured by surface under the cumulative ranking, SUCRA) than oseltamivir with regards to effectiveness in reducing time to symptom alleviation compared with placebo. Based on SUCRA, zanamivir therapy was associated with the lowest risk of nausea and vomiting among the four regimens[11].

16.2.2. Composition and dose

Each pre-dispensed quantity of Relenza® inhalation powder (one blister) contains 5mg zanamivir. Zanamivir is administered directly to the respiratory tract by oral inhalation via DISKHALER®.
The recommended dose of Zanamivir for treatment of influenza in adults and children from the age of 5 years is two inhalations (2x 5mg) twice daily for five days, providing a total daily inhaled dose of 20mg[12].
Training will be provided on Diskhaler technique

16.2.3. Pharmacokinetic characteristics

Studies of orally inhaled zanamivir indicate that approximately 4-17% of the dose is systemically absorbed, with serum concentrations generally peaking within 1-2 hours. The poor absorption of the drug results in low systemic concentrations and therefore there is no significant systemic exposure to zanamivir after oral

inhalation[12]. Lung scintigraphy in healthy adult volunteers (n=11) showed that the main deposition site for inhaled zanamivir (10mg) was the oropharynx (mean 77.6%), with other lesser sites of deposition being the bronchi and lungs (mean 13.2%) and the trachea (mean 1.2%)[1].

The serum half-life of zanamivir following administration by oral inhalation ranges from 2.6 to 5.05 hours. At 12 and 24 hours post-dose, inhaled zanamivir (10mg) is retained in tracheal and bronchial epithelia at concentrations which exceed those inhibitory for influenza A and B neuraminidases. Dose proportionality of C_{max} values indicate linear pharmacokinetics[1].

Although elimination of the drug takes place largely via renal excretion, the bioavailability of a therapeutic dose of inhaled zanamivir is low and as a result there is no significant systemic exposure. Therefore, dosage adjustments in patients with renal dysfunction are not necessary with administration of zanamivir via oral inhalation[12].

Zanamivir does not inhibit the cytochrome P450 enzymes.

No dose modification is required with hepatic dysfunction[12].

16.2.4. Toxicity

General toxicity studies did not indicate any significant toxicity of zanamivir.
The tolerability of inhaled zanamivir is similar to that of placebo in otherwise healthy adults, high-risk and elderly patients, and children. In phase II and III trials of therapeutic efficacy, 33% of patients receiving zanamivir (inhaled or intranasal) and 38% of those receiving placebo reported adverse events during treatment[1]. Adverse events that occurred with an incidence ≥1.5% in treatment studies include headaches, diarrhoea, nausea, vomiting, nasal signs and symptoms, bronchitis, cough, sinusitis and dizziness[13].

No drug-related malformations, maternal toxicity or embryotoxicity were observed in pregnant rats or rabbits or their foetuses following intravenous administration of zanamivir at doses up to 90mg/kg/day.

16.2.5. Contraindications[12]

- Hypersensitivity to the active substance, zanamivir
- Hypersensitivity to any of the excipients
    - lactose monohydrate (which contains milk protein)
- Milk protein allergy
- Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine

16.2.6. Cautions

There have been rare reports of patients with previous history of respiratory disease (asthma, COPD) and very rare reports of patients without previous history of respiratory disease, who have experienced acute bronchospasm and/or serious decline in respiratory function after use of zanamivir. Many of these cases were reported during postmarketing and causality was difficult to assess. Thus, zanamivir is not recommended in individuals with underlying airways disease. Any patients experiencing such reactions should discontinue zanamivir and medical evaluation should be provided. Should zanamivir be considered appropriate for patients with asthma or COPD, the patient should be informed of the potential risk of bronchospasm and should have a fast-acting bronchodilator available[12].

Allergic-like reactions, including oropharyngeal oedema, serious skin reactions (erythema multiforme, Stevens-Johnson syndrome, Toxic epidermal necrolysis) and anaphylaxis have been reported in postmarketing experience with Relenza.

There have been postmarketing reports, mostly from Japan, of neuropsychiatric events during administration of zanamivir in patients with influenza, mainly in children and adolescents[12]. The contribution of Relenza to these events has not been established. It is advised to monitor for signs of abnormal behaviour during zanamivir treatment.

16.2.7. Drug interactions

Zanamivir is not a substrate nor does it affect cytochrome P450 enzymes. There are currently no known reported drug interactions with zanamivir and no clinically significant drug interactions are likely[12].

Because of potential interference with the live attenuated influenza vaccine (LAIV), LAIV should not be administered within 2 weeks before or 48 hours after administration of Relenza[13].

16.2.8. References

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16.3. Peramivir

16.3.1. Rationale

Peramivir is a novel neuraminidase inhibitor of influenza virus. It was approved by the Food and Drug administration in December 2014 for treatment of acute uncomplicated influenza in patients 18 years and older. It was approved for treatment of influenza in Japan in 2010. Peramivir is administered intravenously, providing an alternative access to patients unable to take medication via the oral or inhalation routes.

Peramivir is a novel cyclopentane (five vs six-membered ring structure of oseltamivir and zanamivir) with side chain substitutions (a carboxyl group, a guanidine group and lipophilic side chains), allowing higher affinity interactions between peramivir and the neuraminidase catalytic site compared to oseltamivir and zanamivir[1].

In vitro activity of peramivir has been investigated against human and avian influenza A and B viruses. These studies have shown that peramivir possesses similar or more potent in vitro activity against influenza A and B viruses than oseltamivir, zanamivir and laninamivir[2]. Peramivir shows improved in vitro activity against influenza B isolates compared to oseltamivir or zanamivir; the IC_{50} values of peramivir against influenza B were reported to be several folds lower than those of the rest of the NAIs[3,4]. Peramivir has been also demonstrated to have in vitro activity against emerging avian influenza viruses of pandemic potential including H5N1, H7N9 and H9N2[5,6].

Despite well-established efficacy of oral peramivir in animal studies, human clinical trials resulted in less success due to low oral bioavailability. Thus, further efforts were focused on exploring the parenteral route. Studies in non-human primates, ferrets and mice showed that a single IV administration of peramivir significantly reduced viral titers and clinical symptoms[2].

The efficacy of the approved peramivir regimen (600mg dose given as a single 15-30 minutes infusion) for the treatment of uncomplicated influenza within 48 hours of symptoms in otherwise healthy adults was evaluated in two large, randomised, controlled, double-blind trials[7,8]. Relative to placebo, peramivir 600mg significantly reduced the median time to alleviation of influenza symptoms (hazard ratio 0.666, adjusted p value, 0.0092). When stratifying by influenza A infection subtype, there was no significant difference in median time to alleviation of symptoms between the peramivir 600mg group and placebo in those infected with A/H1 although the median time for this outcome was shorter in those infected with A/H3 (HR 0.326, p=0.0008). The proportion of virus-positive subjects (50% tissue culture infective dose) by day 3 was significantly decreased in the peramivir 600mg group compared with placebo (25.8% versus 51.5%, p=0.0003)[7].
A single dose of peramivir was noninferior to 5 days of oseltamivir 75mg twice daily for the median time to alleviation of symptoms. Significantly more peramivir 600mg than oseltamivir recipients were afebrile 24h after treatment[8].

Available data do not support a conclusion that peramivir is effective in patients with influenza B or in those with complicated influenza.

For peramivir, less than 1% of influenza clinical isolates (including A/H1N1, A/H3N2, B/Victoria lineage and B/Yamagata-lineage) analysed by WHO Global Influenza Surveillance and Response System (GISRS), exhibited reduced inhibition or highly reduced inhibition, with this percentage being relatively consistent for each influenza season since 2009[1].

16.3.2. Composition and dose

RAPIVAB® has been approved for intravenous use by FDA since 2014.
Each vial of RAPIVAB® injection contains 200mg per 20ml (10mg per ml) as a clear, colourless solution.
In this study, participants will be given 600mg IV peramivir administered once via IV infusion for 15-30 minutes in accordance with FDA license[9]

16.3.3. Pharmacokinetics

Plasma concentrations of IV peramivir peak immediately after administration.
In healthy adult volunteers, after a single 600mg dose, peak concentrations of peramivir in pharyngeal and bronchial epithelial lining fluid exceeded the IC_{50} value for influenza. The elimination half-life of peramivir following a single IV administration of 600mg to healthy subjects is approximately 20h. Together with the slow neuraminidase dissociation rate, the prolonged half-life allows for infrequent dosing regimens.

The pharmacokinetic parameters following IV administration of peramivir showed a linear relationship between dose and exposure parameters (C_{max} and AUC). Peramivir exhibits low (<30%) binding to human plasma proteins in vitro[1].

Peramivir is not significantly metabolised by the liver in humans. Thus, dose adjustment is unnecessary in hepatic impairment[1].

The drug is not a substrate for CYP enzymes, does not affect glucuronidation and is not a substrate or inhibitor of P-glycoprotein mediated transport[2].

Peramivir is almost entirely eliminated by renal excretion with 90% of the drug excreted unchanged. In patients with moderate and severe renal impairment, exposure to peramivir upon IV administration is expected to be increased by 3.4- and 6-fold, respectively, compared to those with normal renal function. Therefore, dose adjustment based on renal function (creatinine clearance <50 ml/min) in patients with renal impairment is required.[1,9] In this study, individuals with creatinine clearance <60ml/min will be excluded.

No difference in PK has been observed by gender, and the effects of age and body weight on peramivir clearance are negligible[1].

Pharmacokinetic sampling schedule (only if there is evidence of accelerated viral clearance):

All patients will need to be admitted for 12 hours for intense sampling

The timings are:
- Pre-dose D0H0, then
- 1,2,3,4,6,9,12 and 24h post dose

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentrations

16.3.4. Toxicity

A single dose of IV peramivir was generally well tolerated in adults and paediatric patients with uncomplicated influenza participating in clinical trials.

In five randomised, double-blind, controlled trials, 1399 subjects with acute uncomplicated influenza received a single dose of RAPIVAB, administered intravenously at doses up to 600mg. Among the 664 subjects receiving RAPIVAB 600mg, the most commonly observed adverse reaction was diarrhoea occurring at a rate of 8% versus 7% in subjects receiving placebo. No subject receiving RAPIVAB experienced a serious adverse event[9].

Laboratory abnormalities occurring in ≥2% of subjects treated with RAPIVAB 600mg included low neutrophils, ALT >2.5x ULN, creatinine phosphokinase >6x ULN.

In rats, no treatment-related maternal and fetal toxicities were observed when RAPIVAB was given by IV bolus at the maximum feasible dose of 600mg/kg, representing exposures approximately 8-fold that in humans at the recommended dose. There are no adequate and well-controlled trials of RAPIVAB in pregnant women. Because animal reproduction studies are not always predictive of human response, and peramivir has been shown to cross the placenta in animal studies, RAPIVAB should only be used during pregnancy if clearly needed[9].

16.3.5. Contraindications

- Hypersensitivity to the active substance, peramivir

16.3.6. Cautions

Post-marketing evaluations under routine clinical settings mirrored the clinical trials in terms of safety in adults and paediatrics.

However, rare cases of anaphylactic reactions and serious skin reactions (including erythema multiforme, toxic epidermal necrolysis and Stevens-Johnson syndrome) have been reported with peramivir treatment[1].

There have been postmarketing reports (from Japan) of delirium and abnormal behaviour leading to injury in patients with influenza (mainly paediatric patients) who were receiving neuraminidase inhibitors, including RAPIVAB. These events appear uncommon[9].

16.3.7. Drug interactions

Given the known elimination route of peramivir, there is a low potential for interactions between peramivir and other drugs[1].

The use of live attenuated influenza vaccines is not recommended within 2 weeks before or until 48h after peramivir administration due to the theoretical risk that peramivir could reduce the immunogenicity of the vaccine[1].

16.3.8. References

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16.4. Laninamivir

16.4.1. Rationale

Laninamivir octanoate is the orally inhaled octanyl ester prodrug of laninamivir, the 7-methyoxy derivative of zanamivir. Laninamivir shares zanamivir's mechanism of action and antiviral spectrum of activity against influenza A and B viruses[1]. In vitro studies showed potent inhibitory activity to NAs of various influenza viruses, such as the seasonal H1N1, pandemic H1N1, H3N2, H5N1 and influenza B viruses, although potency was less against NA of H3N2 and influenza B viruses compared with zanamivir and oseltamivir[2]. It retains inhibitory activity against some NA variants with reduced oseltamivir susceptibility (e.g. H275Y, R293K or E119V)[1].

Laninamivir octanoate is a long-acting NAI because of its pharmacokinetic properties in the respiratory tract and its slower dissociation from influenza NAs compared to other NAIs. It is thus administered as a single intranasal dose[1].

In mice and ferret influenza infection models, a single intranasal dose of laninamivir octanoate significantly reduced virus titers compared with oral oseltamivir and placebo[3]. After a single intranasal administration of laninamivir octanoate in a mouse model of influenza infection, lung laninamivir concentrations increased soon after administration and retained a half-life of as long as 41.4h. Even at 120h post-dose, laninamivir remained in the lungs at concentrations considerably higher than the IC_{50} of laninamivir against the NA activities of various influenza viruses[4].

One phase 2 double-blind, placebo-controlled RCT compared the safety and efficacy of single inhaled 40mg and 80mg doses of laniamivir octanoate, enrolling 248 influenza-positive adult outpatients across 12 countries. Despite showing more rapid antiviral effects, neither the 40mg nor 80mg group significantly reduced the median time to alleviation of influenza symptoms compared to placebo[1].

A phase three study of adults, the Multinational Asian Clinical Research for Influenza Virus Extermination on Long-acting Neuraminidase-Inhibitor Study, was performed as a randomised double-blinded study in order to confirm the efficacy and safety of Laninamivir Octanoate administered as a single inhaled dose of 20 or 40mg compared with oral oseltamivir 75mg administered twice daily for 5 days in adult patients with influenza A or B virus infection. Non-inferiority to oseltamivir was confirmed in both the 20mg and 40mg group of laninamivir octanoate in terms of the primary endpoint which was time to alleviation of influenza illness[5].

A meta-analysis of treatment studies that had oseltamivir as a comparator arm found comparable duration of fever and symptoms between laninamivir and oseltamivir. However, laninamivir was associated with a significantly longer fever duration in those with A/H3N2 virus infection[6].

In analysing four observational studies that compared the clinical effectiveness of inhaled laninamivir to inhaled zanamivir in children, no differences were found in fever duration[7].

In Japan, this drug has been approved for influenza treatment by a single inhalation since 2010 and for prophylaxis since 2013 (commercially available as Inavir – Daichi Sankyo Co., Ltd, Tokyo) but remains investigational in other countries.

16.4.2. Composition and dose

Inavir© dry powder inhaler 20mg is manufactured by Daiichi Sankyo. It contains 20.76mg of Laninamivir Octanoate Hydrate (equivalent to 20mg of laninamivir octanoate).
In adults, Inavir© is administered as a single dose of 40mg laninamivir octanoate by inhalation (two devices)[8].
Training will be provided on inhaler technique.

16.4.3. Pharmacokinetic characteristics

In an ascending single-dose study involving healthy, male volunteers, inhaled laninamivir octoneate was administered as a single dose of 5, 10, 20, 40, 80 or 120mg. The time to maximum concentration of the pharmacologically active form of Laninamivir octoneate, laninamivir, was 4 hours. Laninamivir was slowly eliminated from the body, lasting for up to 144h after administration with a half-life of approximately 3 days. The maximum concentration of the prodrug and laninamivir after a single inhaled dose increased almost linearly with the doses tested.
Laninamivir octeonate was well tolerated by all the subjects even with high exposures attained[9].

16.4.4. Toxicity

Laninamivir octanoeate has been generally well tolerated.

In a large RCT, the most common adverse events included asthma exacerbation (5.9%), diarrhoea (3.9%) and bronchitis (3.9%), and occurred in similar proportions to oseltamivir[1].

16.4.5. Contraindications

- Hypersensitivity to the active substance, laninamivir
- Hypersensitivity to excipients
    - Lactose monohydrate
- Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine

16.4.6. Cautions

In 2017, the pharmaceuticals and medical devices agency reported 8 cases of bronchial spasm and dyspnoea (including 3 cases for which a causal relationship with the product could not be ruled out) associated with Inavir© use over the preceding 3 years in Japan; the package insert was updated in view of this information[10].

16.4.7. Drug interactions

No significant drug interactions are mentioned in the literature.

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16.5. Baloxavir

16.5.1. Rationale

Baloxavir marboxil selectively inhibits the cap-dependent endonuclease activity of the polymerase acid protein (PA) subunit of the highly conserved influenza polymerase complex, thus preventing viral mRNA transcription. It is an oral prodrug that is rapidly converted to its active form, baloxavir acid[1].

The 50% inhibition concentration (IC_{50}) of baloxavir was 1.4-3.1nmol/L for influenza viruses and 4.5-8.9nmol/L for influenza B viruses in an enzyme inhibition assay[2].

In cell culture, baloxavir acid inhibits replication of representative seasonal influenza A and B viruses, including strains resistant to neuraminidase inhibitors, and avian influenza viruses, at low nanomolar concentrations[3];

In a MDCK cell culture assay, the median 50% effective concentration (EC_{50}) values of baloxavir were 0.73nmol/L for H1N1 strains, 0.83nmol/L for H3N2 strains and 5.97nmol/L for type B strains. EC_{90} values were between 0.80-3.16nmol/L for avian subtype H5N1 and H7N9 viruses[2].

Oral baloxavir's prolonged half-life enables the use of a single dose in the treatment of uncomplicated influenza.

CAPSTONE -1 was a phase 3, placebo and oseltamivir-controlled RCT testing single, weight-based baloxavir doses (40 or 80mg) administered within 48 hours of symptoms to patients aged 12-64 years with uncomplicated influenza[4]. The study found that the median time to alleviation of influenza symptoms was 53.7 hours in baloxavir recipients compared to 80.2 hours in placebo recipients ($p<0.0001$). The median time to alleviation of influenza symptoms was similar between baloxavir and oseltamivir groups. By 1 day after initiating treatment, the reductions in nasal virus titers compared to baseline were >100 fold and >1000-fold greater in the baloxavir groups compared to the oseltamivir and placebo groups, respectively. The median duration of infectious virus detection was shorter in the baloxavir group (24 hours) than in the oseltamivir group (72 hours, $p<0.001$) and the placebo group (96 hours, $p<0.001$). In this study, therapeutic use was associated with relatively high frequencies of emergence of variants with PA substitutions (PA I38T and I38M) conferring reduced susceptibility- resistant viruses emerged in 9.7% of 370 baloxavir recipients (all infected with H3N2) within five days, associated with prolonged virus shedding and longer median time to alleviation of symptoms. The transmission fitness and pathogenicity of these variants requires assessment.

Baloxavir was approved by the FDA in 2018 for treatment of uncomplicated influenza following CAPSTONE-1. It was approved by the European Commission in 2021.

CAPSTONE-2[5] was a phase 3 oseltamivir and placebo-controlled trial of single weight-based baloxavir treatment (40 or 80mg) administered within 48 hours of symptoms in outpatients with at least one risk factor for severe disease. This RCT again showed the superior efficacy of baloxavir compared to placebo in shortening duration of symptoms. Baloxavir was associated with a significantly faster decline in infectious virus titres than were placebo and oseltamivir, a finding also observed for subgroups infected with influenza A/H3N2 or influenza B. Median time to alleviation of symptoms was similar between oseltamivir and baloxavir in analysis of all patients and those infected with influenza A H3N2 virus but was significantly shorter in the baloxavir group compared to the oseltamivir group in those with influenza B virus.

16.5.2. Composition and dose

The following information is taken from the European Medicines Compendium (EMC) summary of product characteristics for Xofluza©, [2].

Xofluza© 20mg film-coated tablets contains 20mg baloxavir marboxil.
Xofluza© 40mg film-coated tablets contains 40mg baloxavir marboxil.

The approved doses for adults and adolescents (>/= 12 years of age) are:

- Patients <80kg take a single dose of 40mg (taken as 2x20mg tablets at the same time)
- Patients >/=80kg take a single dose of 80mg (taken as 2x40mg tablets at the same time).

A single dose of baloxavir marboxil should be taken as soon as possible within 48 hours of symptom onset.
Xofluza© may be taken with or without food. It should not be taken with products that contain polyvalent cations such as laxatives, antacids or oral supplements containing iron, zinc, selenium, calcium or magnesium.

16.5.3. Pharmacokinetic characteristics

After oral administration of baloxavir marboxil, the drug is extensively converted to its active constituent, baloxavir. Following a single oral administration of 80mg of baloxavir marboxil, the time to achieve peak plasma concentrations is approximately 4 hours in the fasted stage. The absolute bioavailability of baloxavir after oral dosing has not been established[2].

In an ascending single-dose study involving healthy volunteers, baloxavir was administered up to the highest dose tested (80mg) without evident safety concerns, and it showed linear pharmacokinetic characteristics and a long plasma elimination half-life (range 49-91 hours)[1]. The prolonged plasma half-life enables the use of a single dose in the treatment of uncomplicated influenza.

In clinical studies, there were no clinically relevant differences in efficacy when baloxavir was taken with or without food[5].

Body weight is a significant covariate for baloxavir pharmacokinetics based on the population pharmacokinetic analysis. Dosing recommendations for baloxavir marboxil are thus based on body weight[2].

The effects of renal impairment on the pharmacokinetics of baloxavir marboxil or baloxavir have not been evaluated. Renal impairment is not expected to alter the elimination of baloxavir[5].

No clinically meaningful differences in the pharmacokinetics of baloxavir were observed in patients with mild or moderate hepatic impairment (Child-Pugh class A and B) compared with healthy controls with normal hepatic function. The pharmacokinetics in patients with severe hepatic impairment have not been evaluated.[2]

Based on human mass balance studies the metabolism of baloxavir is predominantly via glucuronidation (via UGT1A3 enzyme). Oxidation to sulfoxide metabolites **are mediated via CYP3A4. Baloxavir was found to inhibit CYP2B6**, **CYP2C8, and CYP3A4 enzymes in human liver microsomes.** Baloxavir has **some induction potential for CYP1A2, CYP2B6, and CYP3A4**, although with lesser potency (<20% effect of positive controls), thus the potential for inducible effects of baloxavir is low. In a DDI study considering the predominance of glucuronidation, plasma exposure of baloxavir was decreased in the presence of the pan-UGT inhibitor probenecid. This reduction of 21-25% suggests there is an unexplained effect.[2]

Baloxavir was detected in breast milk of nursing rats up to 8 hours and was undetected at 24 hours.[2]

<u>Pharmacokinetic sampling schedule (only if there is evidence of accelerated viral clearance):</u>

All patients will need to be admitted for 12 hours for intense sampling

The timings are:

- Pre-dose D0H0, then
- 1,2,3,4,6,9,12 and 24h post dose

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentrations

16.5.4. Toxicity

Nonclinical data reveal no special hazards for humans.[2]
The safety of Xofluza in adults and adolescents is based on data from 3 placebo-controlled trials in which a total of 1640 subjects received Xofluza. No specific drug-related adverse events have been identified in baloxavir studies to date[1]. Adverse events reported in at least 1% of adults and adolescents treated with Xofluza included diarrhoea, bronchitis, nausea, sinusitis and headache[6].

Baloxavir marboxil had no effects on fertility when given orally to male and female rats at doses providing exposure equivalent to 5-times the human exposure based on $AUC_{0\text{-}24hr.}$. There are no or limited data from the use of baloxavir marboxil in pregnant women. As a precautionary measure, it is preferable to avoid the use of Xofluza during pregnancy. [2]

16.5.5. Contraindications[5]

- Hypersensitivity to the active substance, baloxavir
- Hypersensitivity to any of the following excipients:
    - *Tablet core*: Lactose monohydrate, Croscarmellose sodium (E468), Povidone (K25), Microcrystalline cellulose (E460), Sodium stearyl fumarate
    - *Film-Coating*: Hypromellose, Talc (E553b), Titanium dioxide (E171)
- Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine

16.5.6. Cautions

Cases of anaphylaxis, urticaria, angioedema, and erythema multiforme have been reported in postmarketing experience with Xofluza[6].

16.5.7. Drug interactions

Based on in vitro and in vivo drug-drug interaction studies, baloxavir marboxil and baloxavir had low potential to inhibit isoenzymes of the CYP (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) or UGT families or cause relevant induction of CYP enzymes[5].

Baloxavir marboxil and baloxavir are substrates of P-glycoprotein (P-gp). A clinical DDI study confirmed that baloxavir marboxil minimally altered PK of P-gp and BCRP substrates to a degree that is not considered to have an effect on drugs that are substrates for these transporters.[2]

Avoid coadministration with dairy products, polyvalent cation-containing laxatives, antacids or oral supplements (e.g. calcium, iron, magnesium, selenium or zinc).

16.5.8. References

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16.6. Favipiravir

16.6.1. Rationale

Favipiravir is a substituted pyrazine derivative that inhibits the replication of many RNA viruses, including influenza A, B and C viruses. Once metabolised intracellularly, favipiravir acts as a purine nucleoside analogue which functions as a competitive substrate inhibitor of the RNA-dependent RNA polymerase of RNA viruses, leading to chain termination. In addition, another mechanism of antiviral action is lethal mutagenesis caused by increased guanosine to adenine mutation frequency, causing non-viable progeny viruses[1].

Favipiravir was first reported to inhibit influenza virus replication in vitro and in mice in 2002. Favipiravir inhibits seasonal influenza A and B viruses, including those resistant to adamantanes and NAIs and avian A/H5N1 and A/H7N9 viruses. It has broad spectrum RNA virus activity in vitro and inhibits a range of RNA viruses in animal models[1].

The 50% inhibitory concentration (IC50) of Favipiravir in inhibiting RdRp of influenza virus has been reported as 0.34uM. Plaque reduction experiments in MDCK cells showed the 50% effective concentration ranges from 0.013 to 3.53 ug/ml[2].

Favipiravir has shown better efficacy compared to oseltamivir in an animal model of severe lethal influenza infection where initial viral titres are high; this model corresponds to pandemic influenza in humans. In a mouse model of nonlethal influenza infection, corresponding to human seasonal influenza infection, oseltamivir and favipiravir have shown similar efficacy[3].

Clinical studies of various dose regimens have been conducted mainly in adults with acute, uncomplicated influenza. One RCT enrolling influenza-infected participants found that a twice-daily dosing regimen (1800mg BD on day 1 and 800mg BD on days 2-5) gave better antiviral and clinical effects than a thrice-daily regimen. The favipiravir 1800mg/800mg BD group demonstrated significantly faster median time to alleviation of influenza symptoms (difference of 15 hours) and viral load reductions compared with the placebo group[4].

Two placebo-controlled phase 3 RCTs tested a favipiravir regimen consisting of two separate 1800mg loading doses on day 1 followed by 800mg BD on days 2-5 in adults with uncomplicated influenza presenting within 48 hours of symptoms[5]. Both showed significant reductions in nasal infectious virus titers (as measured by RNA load area under the curve over days 1-5, and median time to cessation of virus detection) compared to placebo. One study found a significant difference of 14.4h in median time to alleviation of symptoms in the favipiravir recipients compared to placebo, whereas the other study found a non-significant difference of 6.1h. The inconsistent clinical outcomes despite significant antiviral activity is unclear. However, considerable intersubject variability in both pre-dose and postdose favipiravir concentration was seen over time so that some participants had relatively low favipiravir exposures and trough concentrations below the target of 20ug/ml, a concentration above which has been associated with clinical and antiviral effect in prior phase 2 trials. The optimal dose regimes for favipiravir need further study.

Favipiravir was approved in Japan in 2014 with an indication limited to the treatment of novel or re-emerging influenza virus infections unresponsive or insufficiently responsive to current agents. It remains investigational for influenza elsewhere. The dose regimen approved in Japan (1600mg BD for 1 day followed by 600mg BD for 4 days) is lower than that tested in the above trials.

16.6.2. Composition and dose

In this study, patients will be given 1800mg of favipiravir (nine 200mg tablets) at H0 and 1800mg of favipiravir (nine 200mg tablets) at H12. Thereafter the patient will take 800mg BD (four 200mg tablets) for a further 4 days.

16.6.3. Pharmacokinetics

Oral favipiravir reaches maximal concentrations at 2 hours post oral administration and has a half-life of 2.5-5 hours. Protein binding occurs at 54%. The active parent drug is metabolised to the inactive metabolite T-705M1 mainly by aldehyde oxidase and partially by xanthine oxidase. PK analysis of continuous doses of favipiravir in nonhuman primate models exhibit nonlinear pharmacokinetics over time, with decreasing trough levels at D4, relative to D2.

Pharmacokinetic schedule: only if there is evidence of accelerated viral clearance

All patients will be admitted for 12 hours for intense sampling. The timings are:

- Pre-dose D0, H0, then
- 1,2,3,4,6,9,12 and 24h

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentration.

Favipiravir is mainly metabolised by aldehyde oxidase, leading to the inactive metabolite T705M1, and marginally by xanthine oxidase. Most metabolites are excreted under hydroxylated forms via the kidney.[6]

16.6.4. Toxicity

A review of the safety of favipiravir from 29 studies demonstrates a favourable safety profile.
Side-effects which tend to be mild, include diarrhoea, as well as blood abnormalities including raised uric acid levels, raised liver function tests and uncommonly, decreased neutrophil counts. Early concerns regarding QTc prolongation were not born out in a study of healthy Japanese individuals using moxifloxacin as a positive control, where favipiravir showed no effect on QT intervals.

Animal models have demonstrated teratogenicity and no human studies on pregnant or lactating women have been conducted.

16.6.5. Contraindications[7]

- Women known or suspected to be pregnant and in lactating women
- Hypersensitivity to the active substance, favipiravir
- Hypersensitivity to any excipient
    - Povidone, colloidal silicon dioxide, low-substituted hydroxypropyl cellulose, crospovidone, sodium stearyl fumarate, hydroxypropyl methylcellulose, titanium dioxide, talcum, yellow iron oxide

16.6.6. Cautions

Because favipiravir is associated with dose-related increases in serum uric levels, it should be used with care in patients with gout or a history of gout, and in those with hyperuricaemia[2]

When administrating favipiravir to women of child-bearing potential, confirm a negative pregnancy test before starting the treatment. Instruct to use most effective contraceptive methods with her partner during and for 7 days after the end of the treatment[6]

Because favipiravir is distributed in sperm, men should use the most effective contraception methods during treatment and for 7 days afterwards[2].

16.6.7. Drug interactions

Favipiravir is not metabolised by cytochrome P-450 (CYP). The drug inhibits AO and CYP2C8 but does not induce CYP.

Favipiravir inhibits the formation of acetaminophen sulphate *in vitro* and *in vivo*, resulting in a small magnitude increase in acetaminophen levels. As a result, daily acetaminophen (paracetamol) should not exceed 3g/day.

16.6.8. References

1. Beigel JH et al. Influenza Therapeutics in Clinical Practice- Challenges and Recent Advances. *Cold Spring Harbor Perspectives in Medicine*. 2021;11:a038463.
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16.7. Molnupiravir

16.7.1. Rationale

Molnupiravir is a small molecular ribonucleoside prodrug of N-hydroxycytidine (NHC). It is phosphorylated intracellularly into NHC triphosphate (NHC-TP). NHC-TP incorporates into viral RNA by viral RNA polymerase causing an accumulation of deleterious errors into the viral genome. This causes "error catastrophe" by increasing the rate of mutation in the viral genome to a level lethal to the virus and causing extinction[1].

Molnupiravir was originally intended to treat alphavirus infections and at the beginning of the COVID-19 pandemic it was in pre-clinical testing for seasonal influenza. After the spread of COVID-19, the molnupiravir development program moved to the treatment of COVID-19[2].

Molnupiravir has demonstrated potent anti-influenza activity and good oral bioavailability in mice/ferrets/nonhuman primates. In a ferret model of influenza infection (either intranasally infected with pandemic 2009 H1N1 or H3N2), molnupiravir administered 24 hours post-infection reduced shed viral titers by ≥4 orders of magnitude within 24 hours of the first dose and compared favourably to prophylactically administered oseltamivir phosphate which had no significant effect on shed virus titer. Duration of fever was significantly shortened in molnupiravir treated ferrets (H1N1-infected group). This study also highlighted that molnupiravir shows antiviral effects because of its mutagenic property towards influenza viruses and suggested further clinical studies of molnupiravir for influenza treatment[3].

A phase 1 safety trial of doses up to 1600mg per day found molnupiravir was well tolerated with the incidence of adverse effects being highest in the placebo arm[4]. Another phase 1 trial also concluded molnupiravir was safe and well tolerated and advised 800mg BD for 5 days for the treatment of SARS-COV2 infection[5].

Molnupiravir has been licensed for treatment of mild-moderate COVID-19 in patients at risk of severe disease at a dose of 800mg BD for 5 days based on phase 2 and 3 trials. No phase 2 studies of molnupiravir treatment have occurred in patients with influenza.

16.7.2. Composition and dose

Each Lagevrio© capsule contains 200mg of molnupiravir.
The recommended dose for treatment of mild to moderate COVID-19 is 800mg (4 capsules) taken orally every 12 hours for 5 days.
Molnupiravir is being manufactured by Merck, Sharpe & Dohme.
We will use Lagevrio© or a high-quality generic alternative.

16.7.3. Pharmacokinetics

Molnupiravir is a prodrug metabolised to the ribonucleoside analogue N-hydroxyxytidine (NHC) and phosphorylated to the active rinonucleoside triphosphate (NHC-TP). Following twice daily oral administration of 800mg molnupiravir, median time to peak plasma NHC concentrations is 1.5 hours. The effective half-life of NHC is 3.3. hours. It can be taken with or without food.

Pharmacokinetic schedule: only if there is evidence of accelerated viral clearance

All patients will be admitted for 12 hours for intense sampling. The timings are:

- Pre-dose D0, H0, then
- 1,2,3,4,6,9,12 and 24h

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentration.

16.7.4. Toxicity

Highest dose tested in safety trials was 800mg BD, found to have a 0.9% probability of having 30% excess toxicity compared to control therefore considered safe[5].

Studies in animals have shown reproductive toxicity.

In an interim analysis of a phase 3 trial of subjects with mild to moderate COVID-19 treated with molnupiravir (n=386), the most common adverse reactions (>/=1% of subjects) reported during treatment and during 14 days

after the last dose were diarrhoea (3%), nausea (2%), dizziness (1%) and headache (1%) all of which were Grade 1 (mild) or Grade 2 (moderate).

16.7.5. Contraindications

- Hypersensitivity to the active substance, molnupiravir
- Hypersensitivity to any of the excipients:
    - *Capsules contents*: Croscarmellose sodium (E468), Hydroxypropyl cellulose (E463), Magnesium stearate (E470b), Microcrystalline cellulose (E460)
    - *Capsule shell*: Hypromellose (E464), Titanium dioxide (E171), Red iron oxide (E172)
    - *Printing ink*: Butyl alcohol, Dehydrated alcohol, Isopropyl alcohol, Potassium hydroxide, Propylene glycol (E1520), Purified water, Shellac, Strong ammonia solution, Titanium dioxide (E171)

16.7.6. Cautions

No dose adjustment needed for renal impairment however the pharmacokinetics have not been evaluated with eGFR <30ml/min, also not evaluated with hepatic impairment.

As per US FDA advice, those on molnupiravir need to be aware of avoiding pregnancy. Females of child bearing potential need to use a reliable method of contraception for the duration of the treatment, and also for 4 days after the final doe of molnupiravir. Males of reproductive potential, if they are sexually active with females of child bearing potential, should use a reliable form of contraception during the treatment and at least 3 months after the final dose.

Breast-feeding is not recommended during treatment and for 4 days after the last dose of Lagevrio.

16.7.7. Drug interactions

None identified. Molnupiravir and its metabolites are not substrates of the CYP enzymes, and are broken down into uridine and cytidine through the normal intracellular pyrimidine catabolism pathways.
Based on in vitro studies, neither molnupiravir or NHC are inhibitors or inducers of major drug metabolising enzymes or inhibitors of major drug transporters. Therefore, the potential for molnupiravir or NHC to interact with concomitant medications is considered unlikely.

16.7.8. <u>References</u>

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16.8. Combination Therapies

Rationale

Combination antiviral therapies can offer synergistic, and therefore more effective, rapid and multi-mechanistic inhibition of viral replication and thus accelerated viral clearance, with implications for improved clinical outcomes, transmission dynamics as well as reducing risk of development of resistance.

Pre-clinical in-vitro studies have shown synergy between neuraminidase inhibitors Oseltamivir, Zanamivir and Peramivir when combined with cap-dependent endonuclease inhibitor Baloxavir. Synergy was also seen between Baloxavir and RdRp inhibitor Favipiravir when used together against two strains of influenza A. (1)

16.8.1. Oseltamivir PLUS Baloxavir

16.8.1.1. Rationale

In-vitro synergy has been shown with a weighted combination index (CIwt) of 0.48 A(H1N1) 0.49 A(H3N2), as well as in mice (2, 3). Note if CIwt <1 there is synergy. In humans, a randomised parallel group double blind superiority trial of neuraminidase inhibitors (any of oseltamivir, peramivir and zanamivir) versus neuraminidase inhibitor PLUS Baloxavir was conducted, called the FLAGSTONE study. In 366 hospitalised patients >12 years with severe influenza (NEWS2 or above at baseline) no difference in time to clinical improvement (defined as median time to NEWS2) was shown between the combination arm (n=241) and the single therapy arm (n=128) (4). However, median time to negative RT-PCR was 23.9 hours for combination therapy versus 63.7 NAI suggesting added antiviral benefits not captured by the clinical endpoint. Combination therapy resulted in a rapid reduction of viral titre compared to NAI alone in hospitalised patients. Notably the sample was skewed towards late presentation (only 39% in the Baloxavir group started treatment within 48 hours of symptom onset), otherwise subjects were recruited up until day 4 after symptom onset. Around 85% of patients in each group had started NAI treatment prior to enrollment. Delineation between combination types and associated results was not carried out, making it difficult to understand any differences between each of the NAI-Baloxavir combinations and possible superiority. Another trial comparing oseltamivir monotherapy with oseltamivir PLUS Baloxavir in hospitalised patients is underway pending results (COMBO Trial 1 2020-2023) (5), although plans to recruit only 60 with the imprecise measure of time to viral clearance.

16.8.1.2. Drug-to-Drug interactions

There are no known drug-to-drug interactions. Safety data for Oseltamivir-Baloxavir combination therapy used in FLAGSTONE does not delineate between each of the neuraminidase inhibitor-Baloxavir combinations, however there was no meaningful difference in the rate of adverse events between arms. In addition, no meaningful drug to drug interactions were observed between Oseltamivir and Baloxavir in a phase I open label DDI study of 18 healthy subjects (6), and it is not expected that there would be any interactions based on pharmacokinetic properties.

16.8.1.3. Pharmacokinetic characteristics

Pharmacokinetic sampling schedule (only if there is evidence of accelerated viral clearance):

All patients will need to be admitted for 12 hours for intense sampling

The timings are:

- Pre-dose D0H0, then
- 1,2,3,4,6,9,12 and 24h post dose

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentrations

16.8.1.4. Toxicity

Baloxavir plus Neuraminidase Inhibitors were well-tolerated in FLAGSTONE and no new safety signals were observed; serious adverse events occurred in 29 (12%) of 239 patients in the Baloxavir group versus 19 (15%) of 124 patients in the control group, of which one was considered related to treatment (orthostatic hypotension in a patient in the control group). Overall, four deaths (2%) occurred in the Baloxavir group and seven (6%) in the control group; none were considered related to treatment (4).

16.8.2. Oseltamivir PLUS Favipiravir

16.8.2.1. Rationale

In-vitro studies have demonstrated combination effectiveness against neuraminidase inhibitor resistant influenza strains of this combination in-vitro (1, 7) and superiority against severe influenza in immunosuppressed mice, but did not prevent emergence of oseltamivir resistant strains. (8)

Data from 2 separate prospective studies compared combination oseltamivir and favipiravir (n=40) to oseltamivir only (n=138) in critically ill adults with A(H1N1)pdm09 and A(H3N2) influenza. The combination arm was part of a phase 2a combination therapy trial comparing combinations 2018-2019 but with two dose regimens of favipiravir. The control arm was made up of patients enrolled to a different study 2016-19. showed better cumulative clinical improvement for combination, with lower proportion of patients with severe outcomes on day 7 (60.0% combination vs 63.3% P=0.0257) and day 14 (30% combination vs 48.5%, P=0.0069). (9) 62.5% had improved by day 14 in the combination group vs 42.2% in monotherapy, P=0.025, but no differences were found on day 7 or day 28, or in mortality. Clinical improvement was defined as a decline in 2 categories on the 7-category ordinal scale. The proportion of patients with negative RT-PCRs was significantly lower in the combination group on days 2 (10% vs 0.8%), 5 (30% vs 5.5%), 7 (45.0% vs 15.6%) and 10 (67.5% vs 21.9%, P<0.01 for all) (9). Resistance development wasn't monitored across both groups. 53% of the control group received corticosteroids, whilst the PLUS Favipiravir group did not.

16.8.2.2. Drug to Drug interactions

No theoretical interactions, no known overlap in metabolism pathways.

16.8.2.3. Pharmacokinetic characteristics

Pharmacokinetic sampling schedule (only if there is evidence of accelerated viral clearance):

All patients will need to be admitted for 12 hours for intense sampling

The timings are:

- Pre-dose D0H0, then
- 1,2,3,4,6,9,12 and 24h post dose

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentrations

Favipiravir is mainly metabolised by aldehyde oxidase, leading to the inactive metabolite T705M1, and marginally by xanthine oxidase. Most metabolites are excreted under hydroxylated forms via the kidney.[6]

16.8.2.4. Toxicity

Combination well-tolerated in human study (n=128 received combination therapy for severe influenza), no severe adverse events occurred, 3 reversible increases in ALT occurred.(9)

16.8.3. Favipiravir PLUS Baloxavir

16.8.3.1. Rationale

Synergy with CIwt's of 0.54 A(H1N1) 0.16 A(H3N2) have been shown in-vitro. (2) To our knowledge, the combination of favipiravir plus Baloxavir has only been tested in vitro.

16.8.3.2. Drug Interactions

There are no theoretical DDIs between Oseltamivir and Baloxavir.

16.8.3.4. Pharmacokinetic characteristics

Pharmacokinetic sampling schedule (only if there is evidence of accelerated viral clearance):

All patients will need to be admitted for 12 hours for intense sampling

The timings are:

- Pre-dose D0H0, then
- 1,2,3,4,6,9,12 and 24h post dose

Note that blood will be taken on D3, and D7 for routine haematology and biochemistry. The EDTA sample will be used to measure the drug concentrations

Favipiravir is mainly metabolised by aldehyde oxidase, leading to the inactive metabolite T705M1, and marginally by xanthine oxidase. Most metabolites are excreted under hydroxylated forms via the kidney.[6]

16.8.3.5. Toxicity

No human data are available

16.8.4. References for combination therapies

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17. Appendix 3: Sample Size Simulations

Planned interim analyses

Figure 1 describes the decision flow for interim analyses. At the start of the study we planned to have an unblinded interim analysis at the point we have PCR data for the first 50 participants enrolled. The aim of this is analysis is to assess the methodology as relates to the influenza virus and inform the statistical analysis plan, and not to stop arms. The interim analysis for an intervention arm begins with a minimum sample size of 20 participants (relative to 20 concurrent negative control arm). If the arm meets the futility criteria, it is removed from the platform. If interim results are ambiguous, the arm continues with an additional 10 participants in both the intervention and concurrent negative control arms until the next analysis.

Once an arm meets success criteria, the analysis shifts to non-inferiority, comparing antiviral efficacies to the positive control arm. The non-inferiority interim analysis begins with a minimum sample size of 40 participants in the intervention arm (with 40 concurrent positive control arm). If the antiviral efficacy is inferior to the positive control arm, recruitment stops, and the arm is removed. If it's superior, the arm becomes the new positive control. Ambiguous results prompt continuation with an additional 10 participants in both arms until the next analysis. If antiviral efficacy falls within the non-inferiority margin, decisions depend on context, such as drug tolerability.

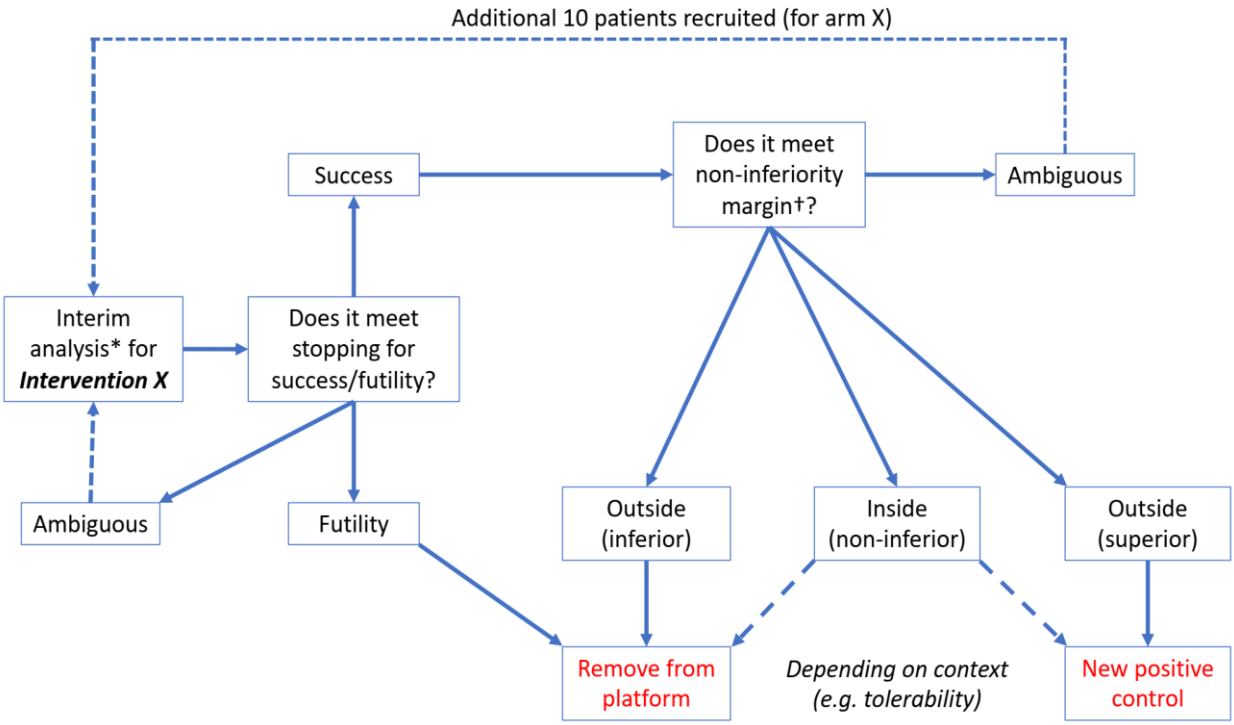

Figure 1 A flowchart describes planned interim analyses and decision rules for removing an arm from the platform or making an arm the new positive control.

During interim analyses the study will not be stopped. The pre-defined stopping rules are based in-vivo antiviral efficacy and not safety (which is reviewed on a continuous basis- see section 9. Safety Reporting). It is not possible to infer individual benefit, or harm, to a participant based on a relative change in viral clearance, so continued recruitment during analysis does not pose any disadvantage, and allows for further precision on the clearance estimate. This is in line with other adaptive studies (e.g. RECOVERY).

The determinations of the futility/success margin λ_1 and the non-inferiority margin λ_2 were informed by insights gained from simulations using data from the first 500 participants enrolled in the PLATCOV trial, which has a similar study design for assessing the efficacies of SARS-CoV-2 antivirals. The simulations assessed thresholds $\lambda_1 \in \{1.125, 1.15, 1.2\}$ (i.e. 12.5%, 15% or 20%) for the futility/success assessments relative to the no study drug

arm; and thresholds $\lambda_2 \in \{0.905, 0.875, 0.85\}$ (i.e. -9.5%, -12.5%, or -15%) for the non-inferiority assessments relative to the positive control arm. 100 iterations were simulated for each permutation of λ_1, λ_2 and for three hypothesised effect sizes (0%, 40% and 60% increase relative to no study drug), i.e. 2700 simulations in total. The first interim analysis is triggered at 20 participants per arm, with subsequent analyses for each additional 10 per arm. For the non-inferiority comparisons, they start when there are at least 40 participants per arm, and only if the success criteria have been met.

- **Choosing futility/success margin λ_1** The results of the simulations for the success and futility stopping rules are shown in Figures 2-3.

  For λ_1 = 1.125 (12.5%) and an intervention that had no effect, the median sample size was 40 participants per arm with 4% false positive results and 28% of inconclusive trials at 120 participants per arm. Increasing λ_1 to 1.2 (20%) would reduce the median sample size to 30 participants per arm and result in approximately 1% false positive results and 18% of inconclusive trials at 120 participants per arm.

  For an intervention with an effect of 40%, the median sample size was 30 participants per arm for a threshold of 12.5% and 50 participants per arm for a threshold of 20%. In all cases, false negative results were less than 3% of simulations, and inconclusive results increased from 14% for a threshold of 12.5% to 34% for a threshold of 20%.

  For an intervention with an effect of 60%, the median sample size was 20 participants per arm, regardless of the λ_1 threshold. False negative results were less than 1% of simulations, and inconclusive results increased from 1% for a threshold of 12.5% to 9% for a threshold of 20%.

  Following discussion with the trial steering committee for the PLATCOV trial, it was decided to increase the λ_1 threshold to 20% in order to stop poorly performing arms earlier (this reflects how the therapeutic priorities of the trial have changed as the pandemic has progressed).

- **Choosing non-inferiority margin λ_2** The results of the simulations for the non-inferiority stopping rules are shown in Figures 4-5.

  In simulations which met the stopping rule for success, stopping for non-inferiority or inferiority was then assessed for each of the three threshold values. Only in 1 simulation with an effect of 0% was the non-inferiority threshold met (<0.3% false positive result).

  An effect of 40% relative to no study drug arm is very close to/on the boundaries defined by the λ_2 values, assuming that the positive control has an effect of 60% relative to the no study drug. For all three boundary values, around half the simulations met the non-inferiority criterion, 10% were inconclusive and 40% met the inferiority criterion.

  For an effect of 60% relative to the no study drug arm (i.e. same as the positive control), 70% to 78% of simulations met the non-inferiority criterion for the -9.5% (i.e. most stringent) to the -15% (least stringent) thresholds, respectively. Around 6% of simulations were inconclusive at 120 participants, and 24 to 17% resulted in a false inferiority result for the -9.5% (i.e. most stringent) to the -15% (least stringent) thresholds, respectively.

  Following discussion with the trial steering committee, it was decided to initially set the λ_2 threshold at -10%. This results in earlier stopping of interventions which are clearly less effective than the positive control and later stopping for interventions with an effect close to the positive control.

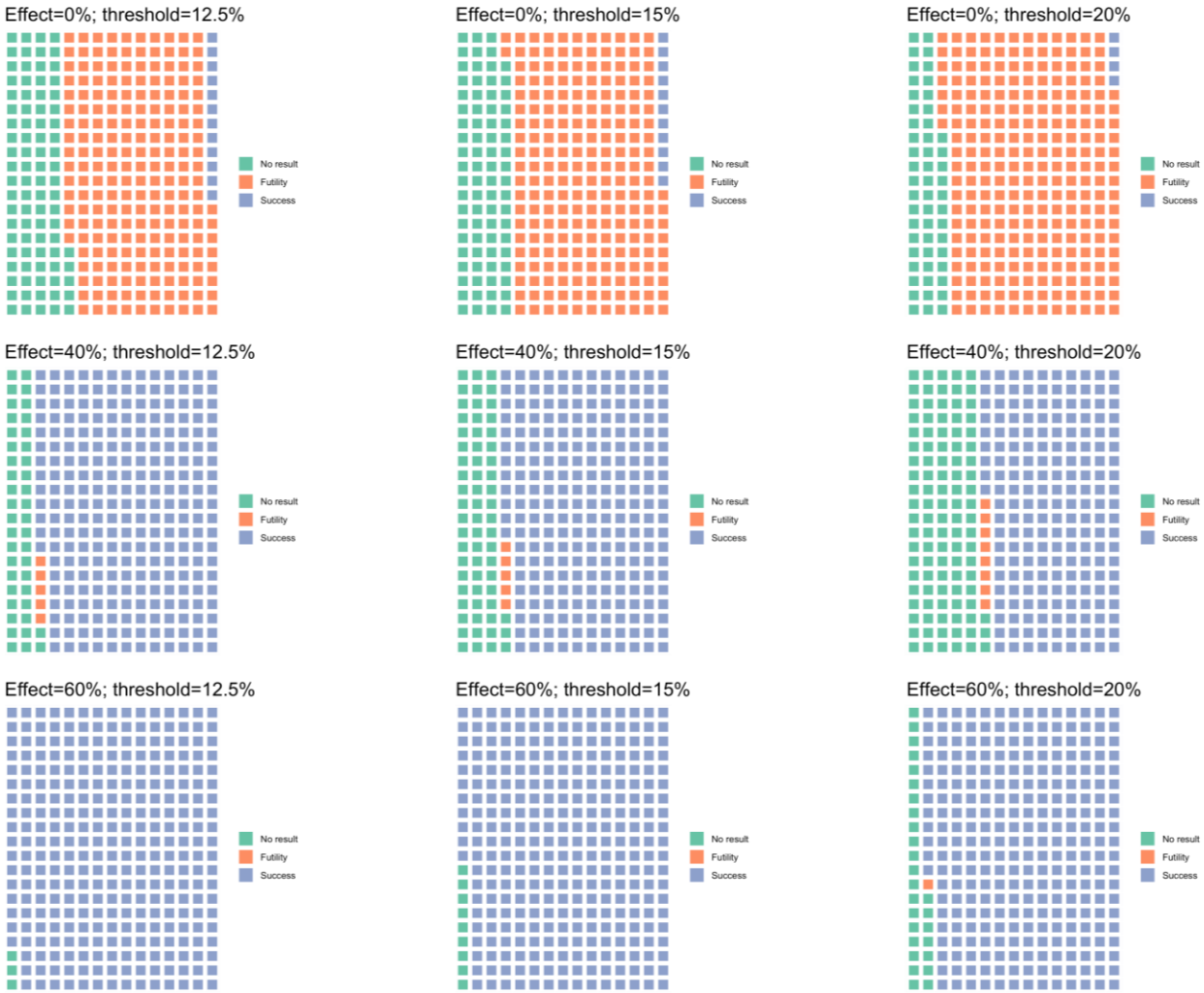

Figure 2 Waffle plots showing the proportion of each outcome (success: blue; futility: orange; no result by 120 participants: green) for the success versus futility stopping rule. 100 simulations were run for each permutation of the effect size and λ_1 threshold.

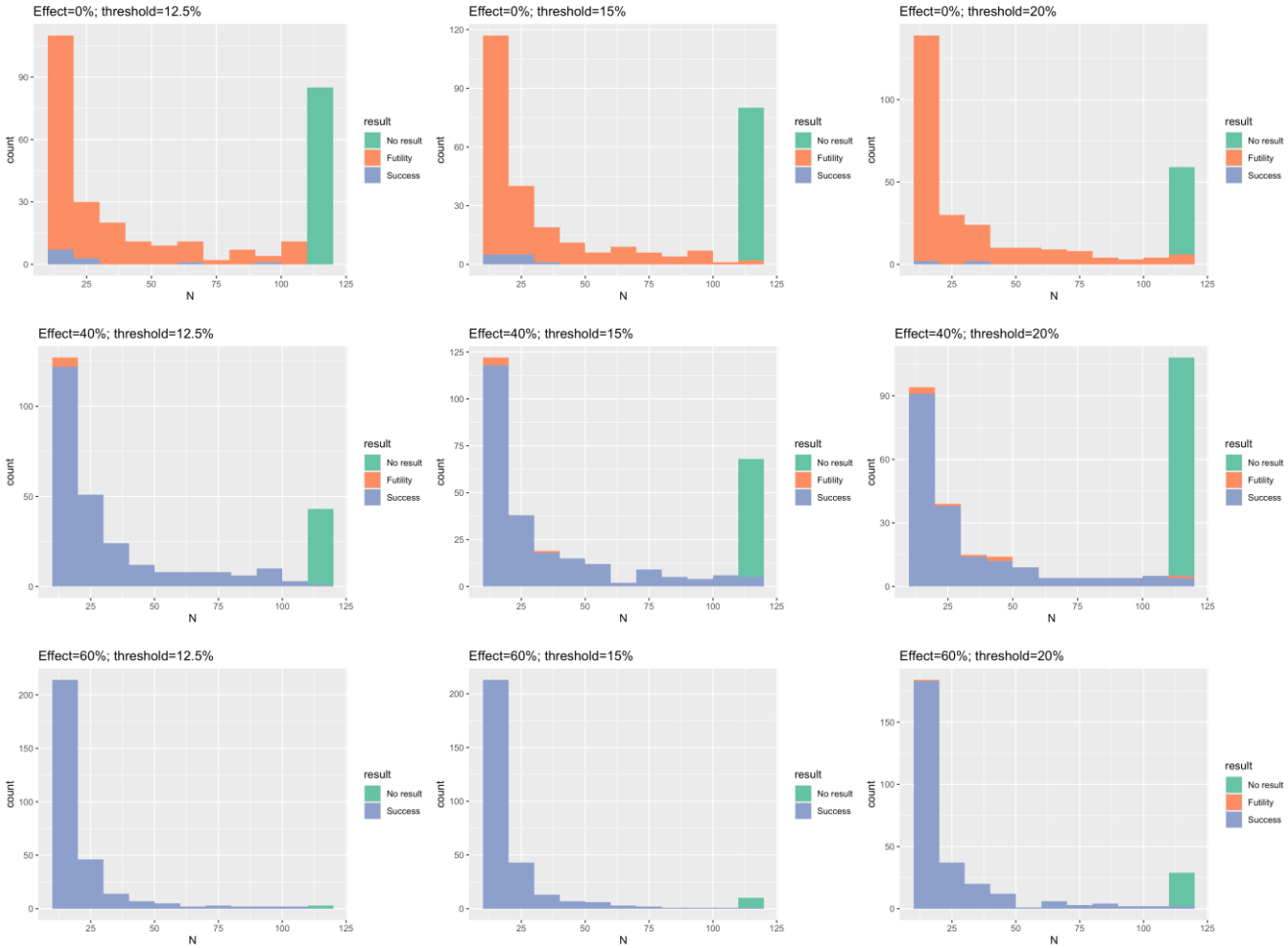

Figure 3 Histogram of sample size until stopping rule is met (success: blue; futility: orange; no result by 120 participants: green).

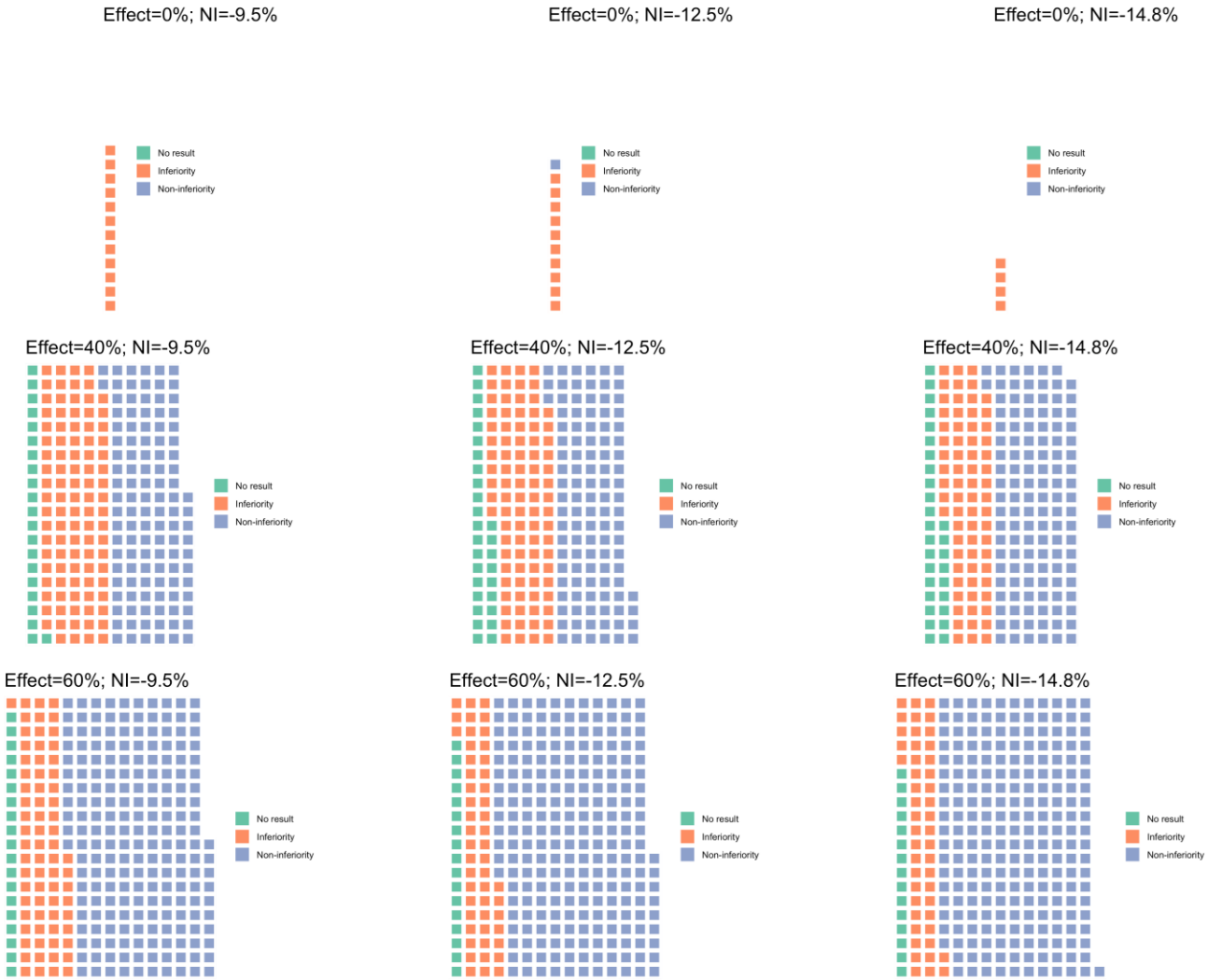

Figure 4 Waffle plots showing the proportion of each outcome (non-inferiority: blue; inferiority: orange; no result by 120 participants: green) for the non-inferiority stopping rule. 100 simulations were run for each permutation of the effect size and λ_2 threshold.

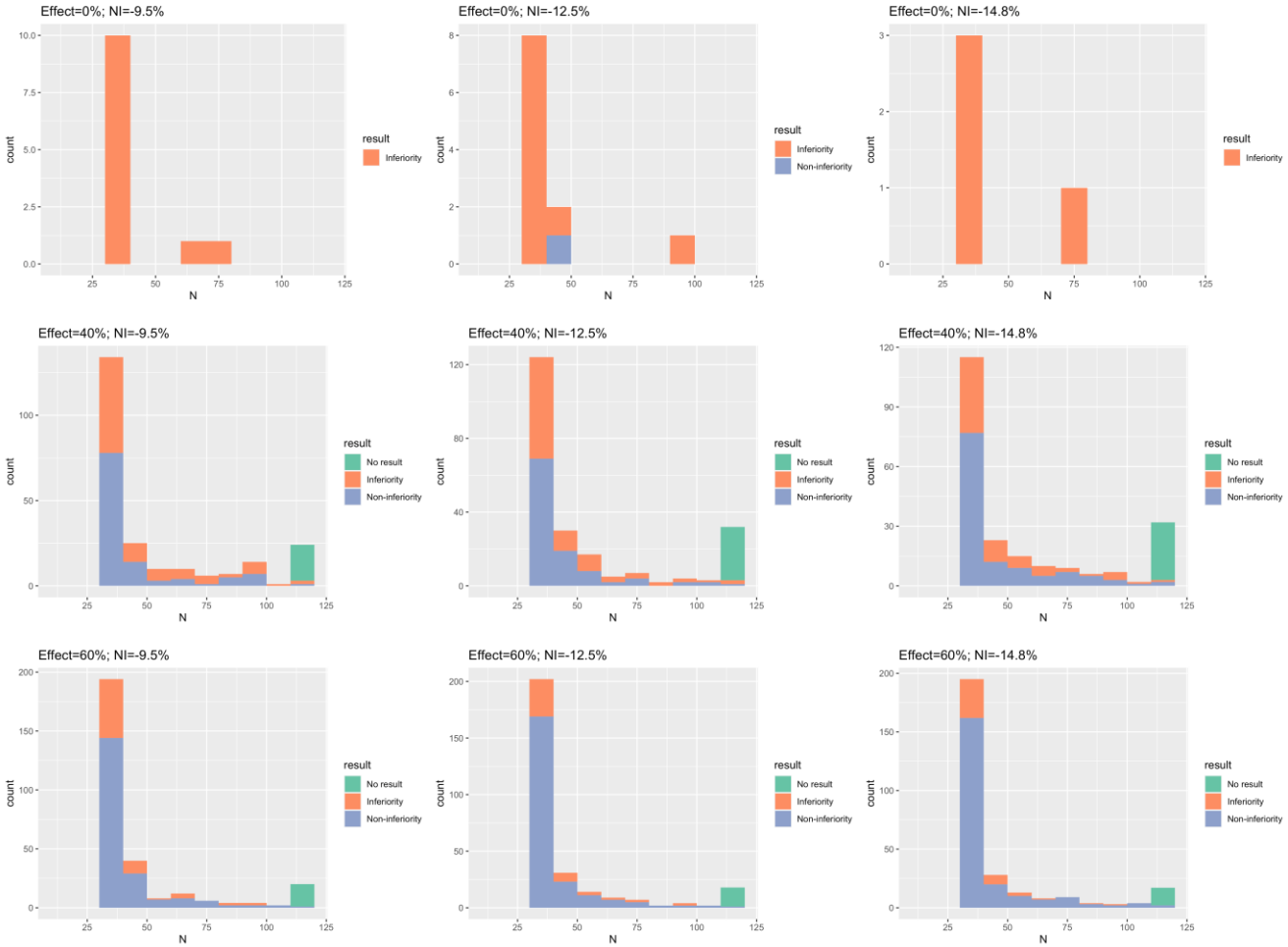

Figure 5 Histogram of sample size until stopping rule is met (non-inferiority: blue; inferiority: orange; no result by 120 participants: green).

18. Appendix 4: Exclusion Criteria

The exclusion criteria in the Master Protocol are expanded upon here. The exclusion criteria in **bold** below have a corresponding section in this appendix where more details are given.

The patient may not enter the study if ANY of the following apply:

- **Taking any concomitant medications or drugs which could interact with the study medications or have antiviral activity**
- **Presence of any chronic illness/condition requiring long term treatment or other significant comorbidity**
- BMI ≥35 Kg/m^2
- Clinically relevant laboratory abnormalities discovered at screening
  - Haemoglobin <10g/dL
  - Platelet count <100,000/uL
  - ALT > 2x ULN
  - Total bilirubin >1.5 x ULN
  - eGFR <70mls/min/1.73m^2
- For females: pregnancy, actively trying to become pregnant or lactation (women on OCP are eligible to join)
- **Contraindication to taking, or known hypersensitivity reaction to any of the proposed therapeutics**
- Currently participating in another interventional influenza or COVID-19 therapeutic trial
- Clinical evidence of pneumonia- e.g. shortness of breath, hypoxaemia, crepitations (imaging not required)
- Known to be currently co-infected with SARS-CoV-2 (i.e. confirmed with positive ATK or RT-PCR)
- Received live attenuated influenza virus vaccine within 3 weeks prior to study entry

1. Taking any concomitant medications or drugs which could interact with the study medications or have antiviral activity

The study will exclude all patients taking **regular drugs** including herbal drugs, but some patients may take over the counter drugs or other vitamin and herbal supplements if considered safe in the judgement of the site investigator upon screening assessment. Patients should be counselled to end and avoid all non-essential medications during study participation. In certain circumstances where a patient is on regular medication, which does not reflect an excluded underlying illness and in the opinion of the study PI is not likely to interfere with the study outcomes, or predispose that patient to any increased risk, a decision can be made to enrol. In all cases, the risks/benefits of this decision should be documented, as well as the person(s) making the decision.

If any doubt about drug interactions, please refer to some of the websites below:

https://crediblemeds.org/healthcare-providers

https://compendium.ch/fr/account/logon?returnUrl=%2Ffr%2FPatient – this is a Swiss website dealing with drug interactions

https://www.bnf.org/products/bnf-online/ - available only in the UK

https://www.uptodate.com/content-not-available#di-druglist

https://www.covid19-druginteractions.org/checker - This can be used for checking drug interactions for Favipiravir and Molnupiravir

Contraindication to taking, or known hypersensitivity reaction to any of the proposed therapeutics

Oseltamivir
No additional contraindications

Zanamivir
- Milk protein allergy

Peramivir
No additional contraindications

Laninamivir
No additional contraindications

Baloxavir
No additional contraindications

Favipiravir
- Gout or history of gout
- For females of childbearing age, they should use effective contraception during the study and for 7 days after the last dose. For males, contraception should be used during the study and for 7 days post last dose and they should abstain from sex with females of child bearing potential

Molnupiravir
- As per US FDA advice, those on molnupiravir need to be aware of avoiding pregnancy. Females of child bearing potential need to use a reliable method of contraception for the duration of the treatment, and also for 4 days after the final dose of molnupiravir. Males of reproductive potential, if they are sexually active with females of child bearing potential, should use a reliable form of contraception during the treatment and at least 3 months after the final dose

Oseltamivir PLUS Favipiravir
- Gout or history of gout
- For females of childbearing age, they should use effective contraception during the study and for 7 days after the last dose. For males, contraception should be used during the study and for 7 days post last dose and they should abstain from sex with females of child bearing potential

Oseltamivir PLUS Baloxavir
No additional contraindications

Favipiravir PLUS Baloxavir
- Gout or history of gout
- For females of childbearing age, they should use effective contraception during the study and for 7 days after the last dose. For males, contraception should be used during the study and for 7 days post last dose and they should abstain from sex with females of child bearing potential

2. Prescence of any chronic illness/condition requiring long term treatment, or other significant comorbidity

The protocol excludes almost all individuals with a chronic illness of any severity and those with an illness that requires long term treatment.
In addition, the following are considered significant comorbidities associated with severe illness or complications according to US centers for Disease Control and Prevention and WHO:
- Age 65 years of age or older
- Asthma
- Neurological and neuro-developmental conditions (including disorders of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy, epilepsy [seizure disorders], stroke, moderate to severe developmental delay, muscular dystrophy or spinal cord injury)
- Chronic lung disease (including COPD and asthma)

- Chronic heart disease (such as coronary heart disease, congenital heart disease, congestive heart failure)
- Haematological disorders (such as sickle cell disease)
- Endocrine disorders (such as diabetes mellitus)
- Chronic renal disease including known end stage renal function or reduced kidney function - creatinine clearance <60ml/min/1.73m^2
- Liver disorders
- Metabolic disorders (such as inherited metabolic disorders and mitochondrial disorders)
- Individuals with immunosuppression due to disease or medication (such as people with HIV/AIDS, or cancer, chronic steroids or other medications causing immune suppression)
- Pregnant or 2 weeks postpartum
- BMI ≥40 kg/m^2

*eGFR for males is calculated based on the Chronic Kidney Disease Epidemiology Collaboration (Andrew S Levey, Lesley A Stevens et al. 2009)
https://qxmd.com/calculate/calculator_251/egfr-using-ckd-epi

19. Appendix 5: Potential Participating Countries

Thailand
Laos
Brazil
Nepal

20. Appendix 6: Amendment History

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	V4.0	24 Feb 23	Dr William Schilling	• Include host genetic test on day 3, 7 and 14. • Include serology test on day 3 and 7.
2	V4.2	06 Nov 23	Dr William Schilling, Dr Ellen Beer	1. Updated co-investigators. 2. Upper limit inclusion age extended from 50 to 60 years old. 3. Viral clearance analysis changed from day 0-7 to day 0-5 4. Primary objective to compare antiviral efficacy amended to clarify that a superiority comparison will be done comparing study drug to no study drug arm and the current best available treatment i.e. positive control. 5. Secondary objective 'time to symptom resolution' clarified to indicate comparison between study drug arm and no treatment arm 6. Secondary objective endpoint amended to specify each endpoint e.g. Area Under the Curve of recorded temperature 7. Objective to determine viral mutant development by looking at number of mutations in detectable virus added. 8. Tertiary objective to characterise the relationship between viral clearance, study arms, and the development of persistent post-acute symptoms added. 9. Tertiary endpoint recording the occurrence of persistent symptoms of post-acute influenza using the Modified Influenza Yorkshire Rehabilitation Scale (Flu-YRSm), adapted from the Modified COVID-19 Yorkshire Rehabilitation Scale 10. The follow up period of the participant has extended from 28 days to 120 days 11. Sample size increased to 1500 participants. 12. Addition option to ask some patients to have one extra set bilateral swabs taken on any day between day 0 and day 5 by study personnel or by self-swab. 13. Clarified saliva collection can be done between day 0 and day 5 14. Updated intervention arms to include combination therapies. 15. Formatting to insert section hyperlinks, minor grammatical corrections where identified, clarity added to order of asterisk typography in appendix 1. 16. Study duration extended by one year.

3	V6.0	15 Sep 25	Dr William Schilling, Dr Stije J Leopold	1. Updated list of co-investigators: some co-investigators have been removed and two new co-investigators have been added, Dr Stije J Leopold and Dr Abhilasha Karkey. 2. Removed a tertiary objective regarding monitoring symptoms associated with post-acute symptoms. 3. Removed all activities on day 6, day 14, day 28, and day 120, thereby shortening individual participants' involvement to 7 days and the total blood volume was decreased. 4. Removed blood collection for cytokine analysis (TNF/IL-6/IL-1) on day 0 (baseline), day 3, day 7, and day 14 5. Removed blood collection for host genetic test on day 3, day 7, and day 14. 6. Removed blood collection for serology analysis and stored blood samples on day 3 and day 14. 7. Added oropharyngeal swabs at hour 1 (H1) time point on day 0 8. Changed D0 pre-dose swab requirement from two sets (4 swabs) to one required set, with the second set be optional. 9. Updated the procedure for observation of multiple doses of drug administration from 'observe' to 'confirm' 10. Administrative changes, including updated grant reference (funder), revised wording from observed to confirmed, changed the brand of VTM, formatting adjustments, and correction of typographical errors, updated web site for data sharing etc.
4	V7.0	18 Nov 25	Dr William Schilling, Dr Stije J Leopold	1. Updated section 10: statistics and analysis, including the sample size estimation, determinations of the futility/success margin (from 12.5% to 20%) and the non-inferiority margin, a link to access AD ASTRA statistical analysis plan (SAP) on GitHub. 2. Revised the interim analysis plan and sample size simulation on appendix3. 3. Updated the wording and structure in Section 7 regarding the oropharyngeal swab schedule to improve clarity and understanding.