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# *AvATher Group Meeting with Guangdong Raynovent Biotech Co., Ltd (China) on Onradivir (ZSP1273)*
# *13 March 2025*

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**ANRS-MIE**: Meena MURMU

**Invited Participants (The First Affiliated Hospital of Guangzhou Medical University，Guangzhou Institute of Respiratory Health):** Prof. Nanshan ZHONG, Prof. Zifeng YANG, Dr. Yangqing ZHAN

**Invited Participants (Guangdong Raynovent Biotech Co., Ltd):** Dr. Xiaoxiokn CHEN, Dr. Haijun LI

**Meeting Objectives:** On behalf of AvATher, the ANRS-MIE invited Guangdong Raynovent Biotech to present Onradivir, an antiviral currently in development for influenza.

**Presentation Topic: "Onradivir: Outpacing Antiviral Resistance & New Treatments For Influenza A Virus Infection"**.

## 1. Background: Onradivir (ZSP1273)

- **Onradivir (ZSP1273)** is an antiviral agent, an analogue of VX-787 (pimodivir), currently in late-stage clinical development by Guangdong Raynovent Biotech Co., Ltd. (China).
- **Mechanism of Action**: Onradivir (ZSP1273) blocks viral RNA synthesis by inhibiting the cap-snatching process of the PB2 subunit of influenza virus RNA polymerase.

## 2. Preclinical Assessment of ZSP1273 Efficacy[1]:

- **In vitro activity**: ZSP1273 has demonstrated potent inhibitory activity against circulating influenza A virus strains (H1N1 and H3N2) with $EC_{50}$ values ranging from 0.012nM to 0.063nM, surpassing the efficacy of the currently approved antiviral oseltamivir. No activity was observed against influenza B virus (B/Lee/40 strain).
- ZSP1273 also demonstrated strong antiviral activity:
    - Against oseltamivir-resistant influenza A virus strains, specifically A/Weiss/43 (H1N1), with $EC_{50}$ values of 0.014nM and 0.017nM, comparable to its activity against wild-type influenza A/PR/8/34 (H1N1) ($EC_{50}$=0.042nM),
    - Against baloxavir-resistant influenza A/PR/8/34 (H1N1), ZSP1273 maintained potent inhibitory activity, with an $EC_{50}$ value of 0.028nM, similar to its efficacy against the wild-type strain.

[1] Chen X, Ma Q, Zhao M, Yao Y, Zhang Q, Liu M, Yang Z, Deng W. Preclinical Study of ZSP1273, a Potent Antiviral Inhibitor of Cap Binding to the PB2 Subunit of Influenza A Polymerase. Pharmaceuticals (Basel). 2023 Feb 27;16(3):365. doi: 10.3390/ph16030365.

- Against avian influenza viruses A/Anhui/01/2013 (H7N9), A/Qingyuan/GIRD01/2017 (H7N9), and A/Guangzhou/39715/2014 (H5N6), ZSP1273 showed inhibitory activity with IC50 values of 0.627 ± 0.312 nM, 0.777 ± 0.427 nM, and 0.245 ± 0.03 nM, respectively.

These results suggest that ZSP1273 retains high antiviral potency against oseltamivir- and baloxavir-resistant strains, highlighting its potential as an antiviral for drug-resistant influenza viruses.

## 3. Clinical Development Program:

- ZSP1273 has undergone Phase I–III clinical trials, evaluating its efficacy and safety across diverse patient populations **(Table 1**):

### Table 1: Clinical Trial Overview

| Clinical Trial Title (ID), Phase | Primary Purpose | Criteria Evaluated | Population Tested | Publications |
|---|---|---|---|---|
| The Safety, Tolerability and Pharmacokinetic Study of ZSP1273 in Healthy Volunteers (NCT03679143), Phase I | Treatment | **Primary Outcome Measures**: Number and severity of treatment-emergent adverse events (TEAEs) and Serious Adverse Events (SAE). **Secondary Outcome Measures**: Pharmacokinetic parameters, including Tmax (time to maximum concentration), Cmax (maximum plasma concentration), $t_{1/2}$ (elimination half-life), and AUC (area under the concentration-time curve), among others. | Adults (18 - 50 years) | Hu Y et al (2021), Expert Opin Investig Drugs. 30(11):1159-1167. doi: 10.1080/13543784.2021[2]. |
| A Study of ZSP1273 Tablets in Patients With Acute Uncomplicated Influenza A (NCT04683406), Phase III | Treatment | **Primary Outcome Measures**: Time to symptom alleviation in participants randomized to ZSP1273, placebo, or Oseltamivir, assessed up to 14 days post-initial dose. **Secondary Outcome Measures**: Proportion of participants with positive influenza virus titer and changes from baseline in viral titer, assessed via RT-PCR from | Adults (18 - 64 years) | Yang Z et al (2025), Lancet Respir Med. 2025 Jun 6:S2213-2600(25)00046-3. doi: 10.1016/S2213-2600(25)00046-3[3]. |

[2] Hu Y, Li H, Wu M, Zhang H, Ding Y, Peng Y, Li X, Yu Z. Single and multiple dose pharmacokinetics and safety of ZSP1273, an RNA polymerase PB2 protein inhibitor of the influenza A virus: a phase 1 double-blind study in healthy subjects. Expert Opin Investig Drugs. 2021 Nov;30(11):1159-1167. doi: 10.1080/13543784.2021.

[3] Yang Z, Zhan Y, Li Z, Lin Z, Fang Z, Li H, Chen X, Ding B, Zeng H, Zhang X, Song Y, Lin Z, Liang S, Luo J, Huang J, Chen X, Zhong N; Onradivir Trial Recruitment and Medical Monitoring Group. Efficacy and safety of onradivir in adults with acute uncomplicated influenza A infection in China: a multicentre, double-blind, randomised, placebo-controlled and oseltamivir-controlled, phase 3 trial. Lancet Respir Med. 2025 Jun 6:S2213-2600(25)00046-3. doi: 10.1016/S2213-2600(25)00046-3. Epub ahead of print. PMID: 40489986.

| | | | | |
|---|---|---|---|---|
| | | nasopharyngeal swabs at days 2, 4, and 6**,** AUC (area under the curve) of viral titer and time to cessation of viral shedding**,** monitored for up to 6 days post-initial dose in participants randomized to ZSP1273, placebo, or Oseltamivir**,** and other virological and clinical outcome measures. | | |
| Study of ZSP1273 in Patients With Acute Uncomplicated Influenza A (NCT04024137), Phase II | Treatment | **Primary Outcome Measures**: Time to resolution of influenza symptoms in participants, measured from day 1 (treatment initiation) to day 15. **Secondary Outcome Measures**: Time to resolution of individual influenza symptoms, changes in viral titer and viral RNA load measured on days 2, 4, and 6, AUC of the log10 pharyngeal viral load, and other virological and clinical outcome measures. | Adults and Older Adults (18 - 65 years) | Yang Z et al (2024). Lancet Infect Dis-24(5):535-545. doi: 10.1016/S1473-3099(23)00743-0[4]. |
| Pharmacokinetic, Safety and Efficacy of ZSP1273 in Children 2-17 Years Old With Influenza A (NCT06656026), Phase II | Basic Science | **Primary Outcome Measures**: Plasma concentrations of ZSP1273. **Secondary Outcome Measures**: Number of participants experiencing treatment-related adverse events**,** time to resolution of influenza symptoms**,** and percentage of participants with detectable viral titer**.** | Children (2 - 17 years) | The study is currently ongoing. |

- A **New Drug Application (NDA)** was submitted to the **China National Medical Products Administration (NMPA)** in December 2023.
- **Approval:** Onradivir was recently approved by the China National Medical Products Administration (NMPA) in May 2025.

## 4. Clinical Trial Findings Presented at the Meeting:

### 4.1 Phase II Study: Safety and Efficacy of Onradivir in Adults with Acute Uncomplicated Influenza A Infection.

*A Multicentre, Double-Blind, Randomized, Placebo-Controlled Phase II Trial*

[4] Yang Z, Li Z, Zhan Y, Lin Z, Fang Z, Xu X, Lin L, Li H, Lin Z, Kang C, Liang J, Liang S, Li Y, Li S, Yang X, Ye F, Zhong N; Onradivir Trial Recruitment and Medical Monitoring Group. Safety and efficacy of onradivir in adults with acute uncomplicated influenza A infection: a multicentre, double-blind, randomised, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2024 May;24(5):535-545. doi: 10.1016/S1473-3099(23)00743-0.

**Study Design:**
Participants were randomly assigned (1:1:1:1) into four groups:

- Onradivir 200 mg twice daily
- Onradivir 400 mg twice daily
- Onradivir 600 mg once daily
- Placebo group (matching placebo) twice daily

**Population:**

- Adults (18–65 years) with influenza-like illness, confirmed by rapid antigen testing at the initial clinical visit, with an axillary temperature >38°C and symptom onset within ≤48 hours.

**Key Exclusion Criteria:**

- Pregnant individuals
- Allergy to onradivir
- Receipt of any influenza antiviral medication within 7 days prior to enrolment

**Primary Endpoint:**

- **Time to resolution of influenza symptoms**, defined as the duration from study treatment initiation to the point at which all seven influenza-related symptoms (headache, fever, muscle or joint pain, fatigue, cough, sore throat, nasal congestion) are reported by the participant as either absent (0) or mild (1) for a minimum of 21.5 hours.

**Secondary Endpoints:**

- **Duration of Detectable Virus** defined as the period from treatment initiation until the viral titre falls below the lower limit of quantification, as measured by viral culture and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).
- **Time to Fever Resolution** defined as the period from treatment initiation to when axillary temperature drops below 37°C and remains below this threshold for at least 12 hours.
- **Change in Viral RNA Load** defined as the baseline-adjusted change in viral RNA load, measured by viral culture and qRT-PCR on Days 2, 4, and 6.
- **Selection of PB2 resistance-associated mutations**
- **Safety**
- **Other virological, clinical, and quality-of-life outcome measures**[5]

## Results:

**Symptom Resolution:**

- All three onradivir groups showed decreased median time to alleviate influenza symptoms (**46·92hr** [IQR 24·00-81·38] in the 200 mg twice per day group, **54·87hr** [23·67-110·62] in the 400 mg twice per day group, and **40·05hr** [17·70-65·82] in the 600 mg once per day) compared with the placebo group (**62·87hr** [36·40-113·25]). The median difference between the onradivir 600mg once per day group and the placebo group was **-22·82hr** (p=0·03).

[5] https://clinicaltrials.gov/study/NCT04024137

- All three onradivir groups showed significantly shorter time to fever relief (axillary temperature <37°C).

**Adverse Events:**

- The most frequently reported treatment-emergent adverse event was diarrhoea (71 [42%] of 171), ranging from **33-65%** of the patients in the onradivir-treated groups, compared with **10%** in the placebo group. No serious adverse events were observed. The severity of diarrhoea was reported as grade 1 or 2, with the majority being grade 1, and symptoms resolved after 1–2 days.

**Virological Response:**

- Patients in the three onradivir groups had significantly shorter viral detection time than those in the placebo group. The median time to negative viral RNA detection was **41·95 hr** (95% CI 23·08–64·00; p=0·0037) in the onradivir 200 mg twice per day group, **62·26 hr** (23·80–69·78; p=0·0048) in the onradivir 400 mg twice per day group, and **41·77 hr** (23·00–70·25; p=0·0066) in the onradivir 600 mg once per day group compared with **71·85 hr** (61·42–100·08) in the placebo group.

**Mutations Identified**:

- Sequencing of paired pharyngeal samples using the Sanger method revealed **PB2 amino acid mutations** in three patients from the **onradivir** groups:
  i) **Onradivir 600 mg once daily**: **Ile66Val**
  **ii) Onradivir 400 mg twice daily**: **Ile66Thr**
  **iii) Onradivir 200 mg twice daily**: **Asn510Ile**
- Additionally, three patients in the **placebo group** had different **PB2 mutations**: **The521Ala**, **Val67Ile**, and **Arg144Ile**
- No mutations in other **influenza viral genes** were tested in this study.

**Conclusions: Phase II Study**

- Onradivir demonstrated a safety profile comparable to placebo.
- The 600 mg once-daily regimen showed higher efficacy than placebo in alleviating influenza symptoms and reducing viral load in adult patients with uncomplicated influenza infection.

### 4.2 Phase III Study: Assessment of the Efficacy and Safety of ZSP1273 Tablets in the Treatment of Acute Uncomplicated Influenza A.

*A Multicentre, Double-Blind, Randomized, Placebo-Controlled Phase III Trial*

**Study Design:**

Participants were randomly assigned (2:1:1) into three groups:

- Onradivir tablets, 600mg once daily for 5 days
- Oseltamivir phosphate capsules, 75mg twice daily for 5 days
- Placebo (matching placebo) for 5 days

**Population:**

- Adults (18–64 years) with influenza-like illness, confirmed by rapid antigen testing at the initial clinical visit, with an axillary temperature >38°C and symptom onset within ≤48 hours.

**Key Exclusion Criteria:**

- Pregnant individuals
- Allergy to oseltamivir or onradivir
- Severe influenza virus infection requiring hospitalization
- Use of any influenza antiviral medication within 7 days before enrolment

**Primary Endpoint:**

- **Time to resolution of influenza symptoms**, defined as the duration from study treatment initiation to the point at which all seven influenza-related symptoms (headache, fever, muscle or joint pain, fatigue, cough, sore throat, nasal congestion) are reported by the participant as either absent (0) or mild (1) for a minimum of 21.5 hours.

**Secondary Endpoints:**

- **Percentage of participants with a positive influenza virus titre**, quantified from nasopharyngeal swabs using RT-PCR or tissue culture, measured at three time points: Days 1, 4, and 6.
- **Pharmacokinetic parameters**: AUC of change from baseline in virus titre from Day 1 to Day 5, calculated using the trapezoidal method up to 6 days after the first dose.
- **Time to cessation of viral shedding**, determined by virus titre or viral RNA levels in participants randomized to onradivir or placebo.
- **Percentage of participants with influenza-related complications**, assessed in those randomized to onradivir or placebo, defined as the duration from study treatment initiation to the resumption of normal daily activities.
- **Other virological, clinical, and quality-of-life outcome measures**[6]

## Results:

**Symptom Resolution:**

- The median time to alleviation of influenza symptoms was significantly shorter in participants treated with onradivir (**38.83hr**; 35.31–41.18) compared to placebo (**63.35hr**; 55.48–68.48, p<0.001) but not significantly different from oseltamivir (**42.17hr**; 38.26–52.83, p=0.21, log rank test).
- Similarly, as compared with placebo, onradivir significantly shortened the time to resolution of both systemic symptoms (onradivir: **18.67hr**, Placebo: **28.07hr,** p<0.001**)** and respiratory symptoms (onradivir: **33.68hr**, Placebo: **58.38hr,** P<0.001). By contrast, no significant difference was observed when compared to oseltamivir (**20.02 hr** and **37.92 hr,** p=0.75 and p=0.35, respectively)

[6] https://clinicaltrials.gov/study/NCT04683406?cond=NCT04683406&rank=1

- The onradivir group experienced fever alleviation in **26.22 hours,** which was significantly shorter than the placebo group (**43.27hr**, p<0.001), and comparable to oseltamivir (**28.53hr**, p=0.67).

**Virological Response:**

- Viral titres
  - Onradivir showed a greater viral titre reduction (as measured in $\log_{10}$ $TCID_{50}$/mL) than placebo and oseltamivir at 24 hours (**p=0.017** and **p=0.002, respectively**), and 48 hours, (**p=0.013** and **p=0.040**, respectively).
  - At 72 hours, viral load reduction was similar across all groups, suggesting that onradivir provides an early but transient advantage in accelerating viral decline.
  - The median time to achieve a negative viral titre was **19.75** hours in the onradivir group, significantly shorter than in the placebo (**23.20hr**) and oseltamivir (**22.44hr**) groups (p<0.001 for both).
- RNA viral load:
  - Onradivir rapidly and sustainably suppressed viral load, as measured by viral RNA ($\log_{10}$ copies/mL) decline from baseline. Within 24 hours after the first dose, the viral load in the onradivir tablet group decreased by more than **1$\log_{10}$**. Additionally, from 24 to 96 hours post-dosing, onradivir led to a significantly greater reduction in viral RNA load compared to both the placebo and oseltamivir groups.
  - The time to achieve a negative viral load was **68.65** hours in the onradivir group, also significantly shorter compared to placebo (**88.62hr**) and oseltamivir (**86.03hr**) (p<0.001 for both).

**Safety:**

- No death was reported. One **serious adverse event (SAE)** occurred in both the placebo and oseltamivir groups, while no SAEs were observed in the onradivir group.
- The incidence of **adverse events (AEs) and adverse drug reactions (ADRs)** was **significantly higher** in the onradivir group compared to the placebo group. **Diarrhoea** was the most common AE, but it was generally **mild (Grade 1–2),** with most cases involving ≤3 episodes per day**.** Symptoms began on the second day after administration and lasted for approximately two days**.**

**Mutations Identified**:

- PB2 gene sequence analysis on baseline samples and last positive samples after treatment identified **28 mutation profiles** in both the **placebo** and **onradivir groups**, while **10 amino acid substitutions** were detected in the **oseltamivir phosphate group**.
- **Amino acid substitutions selected by onradivir:**
  **- N510T/K/I** (10 cases) – **5.3%**
  **- S324R** (4 cases) – **2.1%**
  Compared to **pimodivir**, mutations at the same gene sites were observed (at position **S324, K376, M431, and N510**).

**Drug Resistance:**

4 different patients (0200, 0774, 0119, and 0847) had viruses showing a fold change greater than 4

after treatment as compared to baseline. However, since the S324R mutation in other samples (0140 and 0798) was not associated with drug resistance ($IC_{50}$ ratio is 0.83 and 0.04), S324R was not suspected to be associated with drug resistance ➔ 3/189 = 1.6%.

- **N510T (H1N1):** $IC_{50}$ increased from **0.1711nM to 3.607nM**
- **M475I/I554del (H3N2):** $IC_{50}$ increased from **0.0514nM to 2.502nM**
- **I674M (H3N2):** $IC_{50}$ increased from **0.4713nM to 2.356nM**
- ***S324R (H3N2) 0.1486 to 7.607 (patient ID 0774),0.03087 to 0.2527 (patient ID 0140)*** *and* ***S324R (H3N2) 0.3994 to 0.01334 (patient ID 0798)***

Except for N510T, the time to alleviation of influenza symptoms in patients with suspected drug-resistant mutations was shorter than the median time.

**Conclusions: Phase III Study**

- Onradivir tablets significantly shorten the alleviation time of clinical symptoms in influenza A patients.
- The drug is safe and effective against influenza A subtypes H1 and H3.
- Onradivir demonstrated a better antiviral effect than oseltamivir, with greater viral concentration reduction and faster viral clearance.
- Diarrhoea was the most common adverse reaction, generally mild to moderate in severity.

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## 5. Discussion:

***Q1. You mentioned H5N1, but we did not see any data. Could you comment on the in-vitro activity of onradivir against H5N1 and, if available, its efficacy in preclinical models?***

**Answer:** Following the avian influenza outbreak in China in 2014, we evaluated the effect of onradivir against H7N9 and H5N6 using both cell cultures and a mouse model. More recently, with the emergence of H5N1 in North America, we collaborated with researchers at Hong Kong University to assess onradivir efficacy against H5N1. While we did not present detailed results today, our findings demonstrate antiviral potency at the nanomolar level. Our paper detailing these results was published in *The Lancet Respiratory Medicine* (Yang Z et al (2025), Lancet Respir Med. 2025 Jun 6:S2213-2600(25)00046-3. doi: 10.1016/S2213-2600(25)00046-3[3]).

***Q2. In your Phase III study, did you detect any known resistance-associated substitutions at baseline (i.e., before treatment)? If so, do you have data on their frequency or prevalence in circulating influenza viruses?***

**Answer:** There is significant concern about mutations, particularly those selected by PA inhibitors. To address this, we analysed over 100 isolates. While we detected the substitutions, they have not, so far, been identified as drug-resistant or clinically significant. In the Phase III clinical trial, we analyzed 574 baseline samples (184 H1N1 and 390 H3N2). For the H1N1 subtype, the frequency of amino acid mutations was lower than 10%. For the H3N2 subtype, the most frequent mutations were E60D (34.92%), I607L (34.92%), D107N (12.17%), I147T (11.38%), and V410M (11.38%). These mutations are recorded in virus databases and are considered natural genetic polymorphisms.

***Q3. Do you plan to study the synergistic effect of combining oseltamivir with your drug, given that their mechanisms of action are quite different? There may be additional effects or benefits from using both together.***

**Answer:** During severe influenza outbreaks in China, including both seasonal and avian flu, we observed that combination therapy with oseltamivir or polymerase inhibitors showed promising results. However, this approach should be considered primarily for severe pneumonia cases. In our Phase II and III studies, we focused on mild and uncomplicated influenza infections. Future studies will explore whether combining oseltamivir with our drug offers additional benefits.

We are still in the early stages of clinical studies, with a primary focus on patients with mild to moderate infections. For severe cases, combination therapy may be more beneficial, as it targets different viral components, such as PA or PB2, potentially leading to a synergistic effect.

***Q4. Did you also sequence PA and PB1 to check for any compensatory mutations that might contribute to resistance, in addition to PB2?***

**Answer:** We do not have PA and PB1 sequence data in this study. In our *in-vitro* study, we did not identify any drug-related resistance. However, in our Phase III studies, we detected four mutations, but three of them did not prolong the median symptom resolution time. Only in one subject did the median symptom resolution time exceed 38 hours, suggesting that these three mutations were not clinically significant.

I agree that this is an important concern. In one strain, symptom resolution took more than 60 hours, making this an interesting finding that warrants further investigation.

***Q5. The virological efficacy appears to be lower than that of baloxavir. How do you interpret this finding?***

**Answer:** We do not consider the virological efficacy of onradivir to be inferior to that of baloxavir.

Both baloxavir and onradivir interact with the influenza polymerase, locking it into two distinct conformations. However, the exact role of these conformations within the viral life cycle remains unclear.

Our latest data, which we were unable to present here, suggest that while baloxavir and onradivir were traditionally thought to inhibit only mRNA transcription, they actually exert a broader effect. They appear to completely lock the polymerase, disrupting not only mRNA transcription but also cRNA and vRNA synthesis. This indicates a more complex mechanism than initially believed. Understanding how these molecular interactions translate into clinical efficacy requires further investigations.

Onradivir demonstrated an EC50 value that was 50 times lower than that of baloxavir, suggesting greater potency in laboratory studies. However, this difference was not observed in clinical trials. As these clinical trials were conducted during different influenza seasons and employed different viral detection methodologies, direct comparison between the two treatments is not possible. Furthermore, no head-to-head study has been performed to directly evaluate their relative efficacy, emphasizing the need for further research into its real-world impact.

***Q6. Do you plan to conduct further studies using clinical endpoints as the primary criteria?***

**Answer:** Studies addressing clinical endpoints are an important consideration, and we acknowledge the need to conduct such research.

***Q7. Can you share your plans for onradivir in China regarding regulatory approval?***

**Answer:** Onradivir is anticipated to receive approval from the Chinese FDA in the coming months, as it has already undergone thorough evaluation.

***Q8. Are there plans to conduct an international clinical trial or collaborate with partners outside China, given the drug's promising potential?***

**Answer:** The current Phase III clinical trial has been terminated. However, there is interest in collaborating with partners outside China for a future clinical trial.

## 6. Concluding Statement by Prof. Nashan Zhong

Professor Zhong emphasized that further discussions on this topic are warranted, as there are many aspects still to be explored, both in basic research and clinical studies. This includes comparisons between baloxavir and onradivir. He inquired whether such discussions could be arranged in the future.
The ANRS-MIE responded that it will continue communication with Dr. Haijun LI, and further discussions can certainly be initiated. Additionally, the ANRS-MIE mentioned that it will follow up with Dr. Haijun Li via email, providing a brief outline of how AvATher proceeds once invited participants have presented their products at the meeting.

The discussion concluded with the presentation of additional information, particularly on drug interaction results for onradivir, including the following:

- **CYP3A4:** Induction effect observed. However, no clinically significant impact on midazolam (a CYP3A4 substrate) was found.
- **CYP2C9:** No pharmacokinetic effects on warfarin (a CYP2C9 substrate).
- **UGT** (Uridine Glucuronosyltransferase): Co-administration increased drug exposure by 40.79% and 51.87%. However, no increased safety risks were identified.
- **P-gp (P-glycoprotein):** No pharmacokinetic effects observed on its substrate, digoxin.
- **BCRP (Breast Cancer Resistance Protein):** BCRP inhibitors had no impact on **onradivir** pharmacokinetics.
- **OATP (Organic Anion Transporting Polypeptides):** No inhibitory effect observed on OATP/BCRP transporter substrates, or the risk of inhibition was minimal.
- **OAT (Organic Anion Transporters):** No drug-drug interaction effect detected with OAT1/OAT3 substrates, including **oseltamivir carboxylic acid**.

In conclusion, participants agreed to continue discussions between the two groups to address the key points raised. ANRS-MIE will circulate the meeting minutes, after which the AvATher working group will provide its consultative opinion on the drug, if necessary.

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## 7. Internal Discussion: AvATher Team Members

## 1. Inclusion Criteria

During our internal discussion, a point of clarification came up regarding patient inclusion. Were patients enrolled based solely on RAT positivity, or was PCR confirmation required for all? We were also wondering if any participants were included based on clinical signs alone (e.g., fever >38°C) in the absence of PCR confirmation. Clarifying this would help us better understand the study population.
**Answer:** All enrolled participants underwent rapid antigen test (RAT) screening, with confirmed influenza A positivity as the enrollment criterion. Post-enrollment, nasopharyngeal swabs were collected and sent to a central laboratory for testing, including polymerase chain reaction (PCR). The primary study endpoint was analyzed based on the PCR-positive cohort defined as ITTI (Intention-To-Treat Infected).
In conclusion, all RAT-positive patients were treated, but only the subset who were later confirmed PCR-positive were used for the main analysis.

Also, it is unclear whether the study population included 688 patients (as indicated on slide 11: Onradivir tablets 600 mg QD: 334 patients, Oseltamivir phosphate capsules 75 mg BID: 167 patients, Placebo: 167 patients), or 702 patients (as indicated on slide 12: Onradivir group: 349 patients, Oseltamivir: 177 patients, Placebo: 176 patients). We noticed a discrepancy in the reported numbers—would you be able to clarify the actual number of patients included in the study?
**Answer:** Slide 11 shows the Phase 3 clinical trial design. The numbers in this slide refer to the planned sample size. A total of **668 subjects** are planned to be enrolled in the ITTI population, including **334 in the ZSP1273 tablet group**, **167 in the oseltamivir phosphate capsule group**, and **167 in the placebo group**.

Considering a **10% false positive rate of Influenza Virus A RAT** (with the diagnosis of influenza virus infection established based on the result of RT-PCR of IFV), the ZSP1273 tablet group, oseltamivir phosphate capsule group, and placebo group require **372, 186, and 186 subjects**, respectively. Thus, a total of **744 subjects with Influenza Virus A infection detected by RAT** are required to be enrolled.

The patient numbers shown in Slide 12 correspond to the **ITTI population**, representing the actual number of patients confirmed to be Influenza Virus A PCR-positive. The **ITTI population** refers to patients who were initially detected as RAT-positive and subsequently confirmed to have Influenza A infection through RT-PCR.

Only **6.4%** of patients were not PCR-confirmed. Based on the analysis of all patients with clinical signs (**ITTI population**), the median time for alleviation of influenza symptoms was **38.37 hours** (95% CI: 35.317–40.900) in the Onradivir group and **61.45 hours** (95% CI: 49.600–66.050) in the placebo group, which was consistent with the results of the ITTI analysis.

## 2. Virological Responses

*Viral Titre*

We found the viral titre data interesting, especially the observed reduction in $\log_{10}$ $TCID_{50}$/mL with onradivir. However, to fully appreciate the significance of these findings, it would be helpful to understand the experimental methodology used to determine $TCID_{50}$. Could you share more details on the assay or protocol?
**Answer: Influenza A Virus TCID50 Titer Assay Protocol:**

MDCK cells were counted and diluted to a concentration of 30,000 cells/mL, then inoculated into 96-well cell culture plates and incubated for 24 hours. The samples to be examined were diluted in a 10-fold gradient series and inoculated into 96-well cell culture plates that had been set for MDCK cells. Incubate for 48 hours. Fixation, closure, incubation of primary antibody, incubation of secondary antibody, color development and finally determination of OD value were performed according to ELISA assay. The results of each well culture were judged, and the TCID50 titer of influenza A virus was calculated based on the Reed and Muench method.

"Full methodological details are provided in the annex '**Methodology**'".

*Viral Load*

The greater reduction in viral RNA load with onradivir ($log_{10}$ $TCID_{50}$/mL) is notable, though the results were somewhat heterogeneous. Are there any known factors that might explain this variability in participant response?
**Answer:** The issues contributed to the heterogeneous may be different populations, seasons, geographical settings, and even using different analytical methodologies. For example, the use of MDCK cells in drug sensitivity tests, which have a significantly lower efficiency for influenza virus isolation and culture compared to MDCK-SIAT1 cells.

## 3. Drug Resistance

The resistance data sparked some discussion. We noticed that 4 out of 16 virus isolates showed $IC_{50}$ ratios > 4. It looks thus like 5 viruses (in 5 different patients) were resistant. However, the overall resistance rate was reported as 3 out of 189 patients (1.6%). Could you clarify how resistance was defined and reconciled with those susceptibility results?
**Answer:** Some subjects (0200, 0774, 0119, and 0847) showed a $TCID_{50}$ ratio >4. The S324R mutation in more samples (0140 and 0798) indicates no development of drug resistance ($IC_{50}$ ratio is 0.83 and 0.04), thus we considered S324R is not suspected drug resistant.

Also, it remains unclear whether the resistance-associated mutations identified were pre-existing in the viral population or emerged as a result of selective pressure from onradivir treatment. For instance, for mutations like N510T, do we know whether these are naturally occurring polymorphisms or could they be treatment-emergent?
**Answer:** In the study, no N510T, M475I/I554del, or I674M mutations were detected in the baseline samples, suggesting that these substitutions emerged later, after treatment.

We understand that during the meeting, it was clarified that 100 isolates were analysed, and while a few mutations were detected, none have been identified as drug-resistant or clinically significant to date. Would it still be possible to receive further information on drug resistance?
**Answer:** A search of the GISAID (gisaid.org) database revealed that from 2022 to October 2023, there were 18 H1N1 subtype and 35 H3N2 subtype isolates from China. By comparing the PB2 gene sequences of vaccine strains with those of the prevailing influenza A virus strains of the season, it was found that the PB2 gene of H1N1 is relatively conserved, with fewer amino acid variation and a lower mutation frequency. Compared to the H3N2 vaccine strain, the prevailing H3N2 strains exhibit high-frequency of D60E, K62R, and L607I substitutions. In 60% of the prevailing strains, amino acid site 60 is E, site 607 is I, and in all prevailing strains, site 62 is R. This indicates that D60E and L607I are natural polymorphisms.

In this study, the PB2 gene sequences of RVD0304 and RVD0003 from the V1 visit period were selected as the reference sequences for H1N1 and H3N2 PB2 genes, respectively. Sequence comparison showed that the amino acid sequence of the PB2 gene in H1N1 clinical strains is consistent with that of the vaccine strain. For H3N2 clinical strains, compared to the vaccine strain, all viruses harbored PB2-D60E, PB2-K62R, and PB2-L607I. This suggests that the PB2 gene sequences of the selected clinical strains are more representative than those of the vaccine strains and are more suitable as reference sequences for this experiment.

In this study, the sequencing of the PB2 gene of influenza virus was completed for 1,176 samples. Among them, 552 samples from the V1 visit period could be accurately sequenced for genetic diversity analysis, including 174 H1N1 samples and 378 H3N2 samples. Comparative analysis with the reference amino acid sequences of the PB2 subunit revealed that the proportion of H1N1 samples exhibiting amino acid variation in the PB2 subunit is relatively low, with the frequency of different amino acid mutations all below 10%. Moreover, the proportion of samples without mutations is essentially consistent across different groups. In the ZSP1273 tablet group, only the H1N1 isolate from subject 0200 after medication showed a PB2 subunit mutation, suspected to be related to drug resistance. This mutation was N510T, which was not observed in samples from the V1 visit period or in samples from other groups after medication. Given that the IC50 value of the sample from this subject after medication was only 4.35 times higher than the reference value, and considering that this mutation occurred only once without statistical significance, further systematic in vitro experiments are required to confirm the mechanism of amino acid mutation and drug resistance.

The analysis of the polymorphism of the PB2 subunit in H3N2 samples from the V1 visit period identified E60D, I607L, D107N, I147T, and V410M as high-frequency amino acid mutations, with mutation frequencies exceeding 10%. A comparison with H3N2 samples in the GISAID database from January 1, 2022, to October 10, 2023, revealed that these five amino acid mutations are also high-frequency. When comparing the sequences of the PB2 subunit across different visit periods, we observed reverse substitutions at the same amino acid positions. These include R41K, I106T, A468T, and V546L for H1N1, and N82S, E74G, N107D, T147I, F384L, A400V, M410V, I545V, E680D, and Q753R for H3N2. This phenomenon may arise because the virus strains inherently possess two genotypes at these positions, leading to varying types observed across different visits. Alternatively, the mutations might be unstable, reverting to their original state when viral survival conditions are restricted or altered. This phenomenon was observed across the placebo group, oseltamivir phosphate capsule group, and ZSP1273 tablet group. Additionally, drug sensitivity tests did not reveal significant differences in IC50 values for subjects with reverse substitution mutations across other visit periods, such as A468T in H1N1 and E74G and Q753R in H3N2. Thus, these reverse substitutions appear to be natural mutations rather than induced ones. In the ZSP1273 tablet group, three H3N2 subjects exhibited amino acid mutations in the PB2 subunit after medication, potentially linked to drug resistance. The specific mutations were S324R; M475I, I554del, and I674M. These mutations were not observed in samples from the V1 visit period or in other groups post-medication, nor were they found in the aforementioned GISAID samples. The S324R mutation also occurred in samples from subjects 0140 and 0798. However, drug sensitivity test results indicated no resistance, and the association between this mutation and the resistant phenotype requires further investigation. Additionally, M475I, I554del, and I674M mutations occurred only once in this experiment, lacking statistical significance. Their mutation mechanisms and drug resistance require confirmation through more systematic in vitro experiments. As was shown in Table 5 (during the talk), there were 10 samples with mutations at the N510 amino acid site, nine of which were H3N2 samples. Among these

nine subjects, only sample 1089 met the analysis requirements after drug sensitivity testing. The mutations were K391E, N510K, and Q753R, and the test results showed no drug resistance.

Other samples either failed to culture live viruses or had low viral loads. This may be attributed to the use of MDCK cells in drug sensitivity tests, which have a significantly lower efficiency for influenza virus isolation and culture compared to MDCK-SIAT1 cells. Alternatively, N510 amino acid mutations might substantially reduce viral survival rates in MDCK cells. Further research is needed to determine the specific causes. It should be noted that in this experiment, drug sensitivity tests were only conducted on samples with amino acid mutations before and after ZSP1273 tablet administration. No drug sensitivity tests were performed on samples from the placebo group and oseltamivir phosphate capsule group. Additionally, the selection of gene mutations and the correlation between genotype and drug-resistant phenotype typically require extensive and systematic research experiments to establish. The number of samples with amino acid mutations potentially related to drug resistance, as identified in this experiment, is small and lacks statistical significance. Therefore, the data obtained cannot directly lead to conclusions about induced mutations and drug resistance.

In the case of patient 0774, the resistance profile appeared quite distinct. Could there be other contributing mutations elsewhere in the genome that might explain the distinct resistance profile?
**Answer:** We analyzed the viral samples of the 0774 before and after administration, except S324R, no other amino acid mutations were found.

One last curiosity—despite showing a high fold-change in susceptibility, one patient actually experienced faster symptom resolution than average. Are there any hypotheses for this apparent disconnect between resistance and clinical response?
**Answer:** We analyzed factors such as the time from symptom onset to medication administration and baseline viral load in patient 0847, who had the I674M mutation, an $IC_{50}$ ratio of 7.7, and a median TTAS of 11.4 hours; however, but no clear explanations were identified.

## 4. Safety

We noted that diarrhoea was the main adverse effect, usually resolving within two days. Since the median time to fever resolution was ~26 hours, do you think the persistence of gastrointestinal side effects beyond symptom relief might affect patient acceptability or adherence?
**Answer:** In the Onradivir tablet group, placebo group, and oseltamivir phosphate group, diarrhea onset occurred at 2.0 days, 3.5 days, and 2.6 days after the first dose, respectively, with durations of 2.2 days, 1.7 days, and 1.7 days, respectively. Stool consistency was primarily watery or loose. Most subjects either did not experience abdominal pain or reported pain that resolved after defecation.

The severity was predominantly Grade 1-2, and the majority of cases resolved spontaneously without requiring specific therapeutic interventions.

No patients withdrew from the clinical study due to diarrhea. Based on the stool characteristics and severity of diarrhea, we conclude that diarrhea did not impact medication adherence. However, some patients may have discontinued treatment voluntarily after experiencing rapid fever resolution post-dosing, though this is unrelated to adherence issues.

## 5. Clinical Trial Population

It's encouraging to see results in uncomplicated influenza cases. We understand that during the meeting it was clarified that onradivir was still in the early stages of clinical studies, with a primary focus on patients with mild to moderate infections. Given the burden of influenza in hospitalized patients, especially outside pandemic periods, are there plans to explore onradivir's use in more severe cases?
**Answer:** We are planning to initiate a clinical study evaluating Onradivir in non-hospitalized high-risk adult or adolescent populations.

For hospitalized severe patients, antiviral therapy can contribute to clinical improvement. However, these patients often present with concurrent severe complications, necessitating additional symptomatic and supportive care beyond antiviral treatment.

## 6. Combination Therapy

From a mechanistic point of view, combining a PB2 inhibitor like onradivir with a neuraminidase inhibitor like oseltamivir sounds promising. Has there been any consideration on combination therapy to enhance efficacy or reduce resistance development?
**Answer:** In vitro studies combining Onradivir with Oseltamivir yielded synergy and antagonism indices of 852.41 and -0.19, respectively, suggesting a strong synergistic interaction. These results support the potential *in vitro* application of the Onradivir and Oseltamivir combination.

Additionally, Pimodivir combined with Standard of Care (SoC) did not provide any additional clinical benefit compared to SoC alone in hospitalized patients. However, in high-risk outpatients, Pimodivir plus SoC resulted in a shorter time to resolution (TTR) of influenza symptoms compared to placebo plus SoC (Leopold et al 2024)[7]. A similar outcome was reported for the combination of baloxavir with neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir, Kumar et al 2022)[8]

Therefore, the combination of Onradivir and Oseltamivir may face considerable challenges in hospitalized patients.

We understand that during the exchange, it was clarified that such a combination may be beneficial, as it targets different viral components, potentially leading to a synergistic effect. However, this approach should be considered primarily for severe pneumonia cases. In the Phase II and III studies conducted so far, only mild and uncomplicated influenza infections were included. It remains to be explored whether combining oseltamivir with onradivir could also provide added benefit.
**Answer**: We are in agreement with this statement.

## 7. Regulatory Strategy

[7] Lorant Leopold, Johan Vingerhoets, Sofie Deleu, Catherine Nalpas, Karin Weber, Ilse van Dromme, David Lowson, Bart Michiels, Wilbert van Duijnhoven, Efficacy and Safety of Pimodivir Combined With Standard of Care in Hospitalized and Nonhospitalized High-Risk Adolescents and Adults With Influenza A Infection, *The Journal of Infectious Diseases*, Volume 231, Issue 1, 15 January 2025, Pages e132–e143, https://doi.org/10.1093/infdis/jiae408.
[8] Kumar D, Ison MG, Mira JP, Welte T, Hwan Ha J, Hui DS, Zhong N, Saito T, Katugampola L, Collinson N, Williams S, Wildum S, Ackrill A, Clinch B, Lee N. Combining baloxavir marboxil with standard-of-care neuraminidase inhibitor in patients hospitalised with severe influenza (FLAGSTONE): a randomised, parallel-group, double-blind, placebo-controlled, superiority trial. Lancet Infect Dis. 2022 May;22(5):718-730. doi: 10.1016/S1473-3099(21)00469-2.

Is there an intention to pursue EU marketing authorization for onradivir? If so, have early interactions with the EMA been initiated to align on expectations for further development?

**Answer:** We believe that for Onradivir to obtain regulatory approval in the European Union (EU), clinical data from Chinese populations alone would be insufficient, and additional clinical studies conducted within the EU would be required. Currently, establishing a strategic partner is essential to co-develop markets outside China. Should we pursue EU registration in the future, we will engage with the European Medicines Agency (EMA) to align on the clinical development pathway.

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## 8. Conclusions of the AvATher Group:

The resolution of the seven symptoms is considered a positive clinical outcome. However, interpreting the change in viral load between days 2 and 6 remains challenging, particularly regarding its implications for viral transmission.

On the positive side, onradivir seems to have a relatively low risk of resistance development. *In vitro* studies demonstrated strong antiviral activity against strains resistant to oseltamivir and baloxavir, with an EC50 value approximately 50 times lower than that of baloxavir, indicating higher potency in laboratory settings. However, this enhanced efficacy was not reflected in clinical trials, highlighting the need for further research to understand its real-world effectiveness.

There are still some uncertainties regarding onradivir's virological characteristics, particularly concerning how resistance may develop under selective pressure and whether such resistance would persist, underscoring the need for additional investigation.

The group acknowledges the molecule's potential, particularly in **combination therapies**, c**ombining a PB2 inhibitor with a neuraminidase inhibitor**. Given the lack of data, especially in vulnerable populations such as immunocompromised or severely ill patients, further investigation is warranted, with a specific focus on **evaluating combination strategies**.

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**Annex: Methodology**

**Name of Centre Laboratory**

Guangzhou KingMylab Pharmaceutical Research Co., Ltd

**Address:** 6F, Building #2, Unit 2, Luoxuan 4th Road, Guangzhou International Bio-Island,510005, Guangzhou, P.R. China

| No. | Test name | Method | Quantitative/qualitative (semi-quantitative) | Lower limit of quantification (LLOQ) |
|---|---|---|---|---|
| 1 | Quantitative nucleic acid testing for influenza A virus | Real-time PCR | Quantitative | 200copies/mL |
| 2 | Influenza A virus TCID50 titer assay | Virus isolation and culture＋ELISA | Quantitative | $10^{1.5}$ $TCID_{50}$/mL |
| 3 | Sequencing analysis of influenza A virus PB2 gene | RT-PCR＋Sanger sequencing | Qualitative (semi-quantitative) | 1.15E+04~1.56E+05 copies/mL |
| 4 | Influenza A virus polymerase activity assay | Virus culture ＋ELISA | Qualitative (semi-quantitative) | MOI≥0.01 |

**I. Quantitative nucleic acid testing for influenza A virus**

After the sample to be isolated containing the target nucleic acid was lysed in the cell by lysis solution, the magnetic beads were used to specifically recognize and efficiently bind to the DNA/RNA molecules, and the magnetic separator was used to make the magnetic beads adsorbed to the tube wall, and finally the high purity RNA was obtained through the process of washing, elution, and purification. The highly conserved sequence of HA gene of influenza A virus H1 subtype was used to design specific primers and probes, and the real-time fluorescence quantitative PCR detection technology was applied, and finally the detection of influenza A virus RNA was realized by the change of fluorescence signal.

**II. Influenza A virus TCID50 titer assay**

MDCK cells were counted and diluted to a concentration of 30,000 cells/mL, then inoculated into 96-well cell culture plates and incubated for 24 hours. The samples to be examined were diluted in a 10-fold gradient series and inoculated into 96-well cell culture plates that had been set for MDCK cells.

Incubate for 48 hours. Fixation, closure, incubation of primary antibody, incubation of secondary antibody, color development and finally determination of OD value were performed according to ELISA assay. The results of each well culture were judged and the TCID50 titer of influenza A virus was calculated based on the Reed and Muench method.

**III. Sequencing analysis of influenza A virus PB2 gene**

The RNA of the influenza A virus samples was extracted using nucleic acid extraction reagent, and then the PB2 gene fragments were cloned using influenza A virus-specific RT-PCR primers and One step RT-PCR kit. For the gene fragments that do not meet the conditions, they should be purified by agarose electrophoresis and gel cutting, and then the PB2 gene fragments should be enriched by high-fidelity PCR; for the gene fragments that meet the conditions, they should be sequenced by Sanger method, and then the gene sequences should be analyzed.

**IV. Influenza A virus polymerase activity assay**

MDCK cells were inoculated in 96-well plates and cultured overnight at 37°C, 5% CO2. On the next day, the virus was inoculated into 96-well plates with an MOI of 0.01 TCID50/well, and then adsorbed at 35°C, 5% CO2 for 1 h. The viral inoculum was aspirated, and the plate was washed once with PBS, and 8 gradient concentrations (200-0.01 nM) of Infection medium (containing 2 μg/ml TPCK) were added, and 4 replicate wells were set up for each concentration of the drug, and the simultaneous After 24 hours, the supernatants of the four wells were collected together, and the TCID50 of the viruses in the supernatants was determined by ELISA, the inhibition rate was calculated, and the drug sensitivity of each strain was determined by nonlinear regression analysis.