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The efficacy and safety of inhaled peptide YKYY017 for COVID-19 patients with mild illness: a phase 2 randomized controlled trial

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YKYY017 is a SARS-CoV-2 membrane fusion inhibitor. We report efficacy and safety of inhaled YKYY017 for COVID-19 patients with mild to moderate illness from a phase 2 trial (ChiCTR2300075467). 239 patients aged 18-75 years with mostly mild COVID-19 were randomly allocated to receive aerosol inhalation of 10 or 20 mg YKYY017 or placebo once daily. The primary endpoint is the change in SARS-CoV-2 viral load from baseline to Day 4. The mean (±SE) differences in viral load change from baseline were −0.48 ± 0.27 $\log_{10}$ copies/mL (95% CI, −1.01 to 0.06) for the 20 mg group and −0.27 ± 0.27 $\log_{10}$ copies/mL (95% CI, −0.79 to 0.26) for the 10 mg group, compared to the placebo group. Viral load changes at visits other than Day 4 did not differ significantly from placebo in either the 10 or 20 mg YKYY017 groups. The time to sustained symptom recovery was shorter in the 20 mg YKYY017 group (median 117.53, 95%CI 95.33 to 141.45 hours) than in the placebo group (median 143.00, 95%CI 139.17 to 186.87 hours; HR 1.552, 95%CI 1.089 to 2.214, $p = 0.0151$), whereas the 10 mg YKYY017 group showed a similar but not statistically significant trend compared to placebo ($p = 0.0833$). The time to sustained symptom alleviation was shorter in both the 20 and 10 mg YKYY017 groups than in the placebo group. The adverse events were mostly mild to moderate. The primary outcome was not met. Following a supplementary phase 1b trial, we are planning another phase 2/3 trial using a twice-daily 20 mg YKYY017 regimen to further assess efficacy and safety.

Despite World Health Organization (WHO)'s declaration of ending the COVID-19 public health emergency in May 2023[1], SARS-CoV-2 continues to cause several waves of infections annually across various geographic regions. The summer of 2024 witnessed a notable spike in COVID-19 cases, underscoring the persistent threat of COVID-19 and the need for effective treatment strategies[2].

While several small-molecule oral antivirals have gained regulatory approval, concerns about systemic adverse effects have driven the development of alternative drug delivery methods[3]. Moreover, some oral antivirals require co-administration with ritonavir, presenting clinical management challenges for patients with underlying health conditions[4]. Inhaled antiviral provides the potential for rapid and

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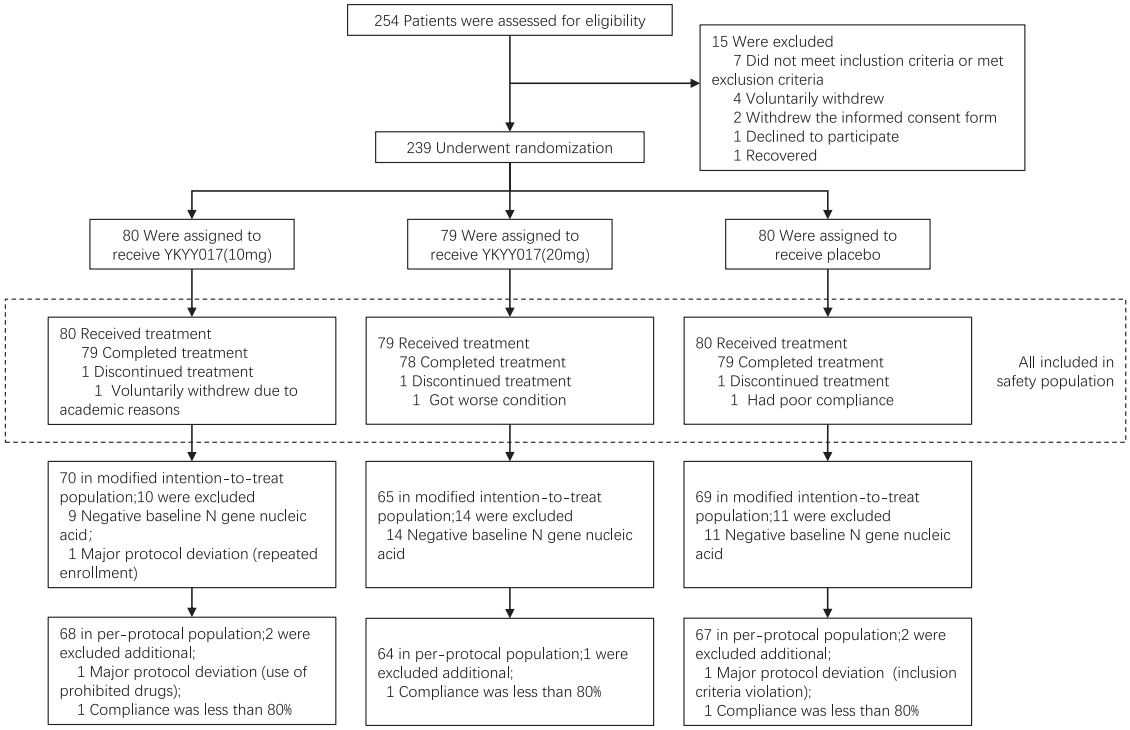

Fig. 1 | Flow chart of the trial. The modified intention-to-treat (mITT) population included randomized participants who had a positive baseline SARS-CoV-2 PCR test and received at least two doses of YKYY017 or placebo. The per-protocol (PP) population was a subset of the mITT population, comprising participants who had a positive baseline SARS-CoV-2 PCR test, no major protocol deviations, and good adherence to the study regimen. The safety population included all participants who received at least one dose of YKYY017 or placebo and had at least one post-baseline safety assessment.

localized viral suppression in the respiratory tract, the primary site of SARS-CoV-2 replication, while minimizing systemic exposure.

YKYY017, a novel lipopeptide-based SARS-CoV-2 fusion inhibitor, exerts its antiviral activity by interacting with the heptad repeat 1 (HR1) region of the S2 subunit of the SARS-CoV-2 spike protein[5–7]. This interaction prevents the formation of the homologous six-helix bundle (6-HB) between the viral HR1 and heptad repeat 2 (HR2) domains, thus inhibiting viral fusion with host cells[8]. In vitro and in vivo studies have shown YKYY017's inhibitory effects against the ancestral SARS-CoV-2 strain and several variants of concern, including emerging Omicron sublineages (EG.5.1, JN.1), suggesting a high effect barrier to viral resistance[9,10]. Toxicology studies indicate a favorable safety profile, with no significant adverse effects observed[9]. Pharmacokinetic results from single- and multiple-dose studies in healthy human participants demonstrated that YKYY017 had low systemic exposure and favorable pharmacokinetic characteristics.

Given these promising results, we conducted a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of inhaled YKYY017 in adults with mild to moderate COVID-19.

Results

Population

From September 2023 through February 2024, we enrolled 239 patients from 24 research sites (Supplementary Table 1), with 80 assigned to the YKYY017 (10 mg) group, 79 to the YKYY017 (20 mg) group, and 80 to the placebo group (Fig. 1). The modified intention-to-treat (mITT) population included 204 patients who had a positive baseline SARS-CoV-2 PCR test and received at least two doses of YKYY017 or placebo (Fig. 1). The study population was young (median age 30 years) and had >95% COVID-19 vaccine coverage (Table 1). The 20 mg group had a higher median age and lower proportion of male than the other 2 groups (Table 1). Most patients (90.2%) had mild COVID-19 disease by the National Institute of Health (NIH) COVID-19 treatment guideline definition. The 20 mg group had a higher proportion of moderately ill patients (15.4%) than the placebo group (8.7%) and the 10 mg group (5.7%). 85 patients (41.7%) had at least 1 risk factor for severe COVID-19. Overweight or obesity (28.4%) was the most common risk factor. The baseline viral load was comparable across the groups, approximately 5 $\log_{10}$ copies/mL (Table 1). Among patients with available SARS-CoV-2 genomic sequencing data, the predominant variants were HK.3.2 ($n = 54$), followed by JN.1 ($n = 46$) and HK.3 ($n = 42$) (Supplementary Table 2).

Primary endpoint

In mITT population, patients in the three groups had similar baseline viral load (Table 1). One patient in the 20 mg group progressed to severe disease on Day 4. Two patients in 10 mg group received forbidden medications on or before Day 4. For patients who experienced these events, the worst observed value between baseline and Day 4 was used as the analysis value. Missing data were reported for 3 participants in the 10 mg group, 1 participant in the 20 mg group, and none in the placebo group (Fig. 2). The missing data were imputed using multiple imputations for statistical comparison in the main analysis for the primary outcome. No significant difference for viral load change between the treatment groups and the placebo group was identified. Compared with the placebo group, the mean (±SE) differences in viral load change from baseline were -0.48 ± 0.27 $\log_{10}$ copies/mL (95% CI, −1.01 to 0.06) for the 20 mg group and -0.27 ± 0.27 $\log_{10}$ copies/mL (95% CI, −0.79 to 0.26) for the 10 mg group (Fig. 2). The results were similar for the sensitivity analysis as well as among full analysis set (FAS) population and per-protocol (PP) population (Supplementary Figs. 1-5).

Secondary endpoints

In mITT population, viral load changes at visits other than Day 4 did not differ significantly from placebo in either the 10 mg or 20 mg

Table 1 | Demographic and Clinical Characteristics (mITT)

Characteristics	10 mg YKYY017 (*N* = 70)	20 mg YKYY017 (*N* = 65)	Placebo (*N* = 69)	Total (*N* = 204)
Age, median (Q1, Q3) — yr.	30.0 (24.0,41.0)	32.0 (26.0,42.0)	28.0 (25.0,39.0)	30.0 (25.0,40.5)
Male sex — no. (%)	31 (44.3)	19 (29.2)	28 (40.6)	78 (38.2)
Risk factors for severe illness from COVID-19 — no. (%)				
At least one of the following risk factors	27 (38.6)	29 (44.6)	29 (42.0)	85 (41.7)
Overweight or obesity				
Overweight (25 ≤ BMI <28 kg/m^2)	10 (14.3)	19 (29.2)	12 (17.4)	41 (20.1)
Obesity (BMI ≥ 28 kg/m^2)	5 (7.1)	3 (4.6)	9 (13.0)	17 (8.3)
Smoking	3 (4.3)	2 (3.1)	6 (8.7)	11 (5.4)
Cardiovascular disease, including hypertension	9 (12.9)	8 (12.3)	9 (13.0)	26 (12.7)
Chronic liver disease	1 (1.4)	0	0	1 (0.5)
Age ≥ 60 yr.	6 (8.6)	5 (7.7)	2 (2.9)	13 (6.4)
Diabetes mellitus	2 (2.9)	2 (3.1)	3 (4.3)	7 (3.4)
Chronic lung disease	0	0	0	0
Stroke or cerebrovascular disease	0	0	0	0
COVID-19 severity (NIH guideline criteria)— no. (%)				
Mild	66 (94.3)	55 (84.6)	63 (91.3)	184 (90.2)
Moderate	4 (5.7)	10 (15.4)	6 (8.7)	20 (9.8)
WHO clinical progression scale— no. (%)				
Mild	69 (98.6)	64 (98.5)	69 (100.0)	202 (99.0)
Moderate	1 (1.4)	1(1.5)	0	2 (1.0)
Total score for 11 targeted symptoms at baseline				
Median (Q1, Q3)	7.5 (5.0, 11.0)	9.0 (6.0, 11.0)	8.0 (6.0, 11.0)	8.0 (6.0, 11.0)
No. of symptoms at baseline (among 14 symptoms), Median (Q1, Q3)	7 (5, 9)	8 (6, 10)	8 (6, 10)	8 (6, 9)
Time from symptom onset to initiation of the trial regimen				
≤2day — no. of patients (%)	33 (47.1)	33 (50.8)	28 (40.6)	94 (46.1)
>2day — no. of patients (%)	37 (52.9)	32 (49.2)	41 (59.4)	110 (53.9)
Median (Q1, Q3) — day	3.0 (2.0,3.0)	2.0 (2.0,3.0)	3.0 (2.0,3.0)	3.0 (2.0,3.0)
Vaccination status — no. (%)				
Unvaccinated	2 (2.9)	2 (3.1)	0	4 (2.0)
Once	2 (2.9)	2 (3.1)	0	4 (2.0)
Twice	18 (25.7)	16 (24.6)	15 (21.7)	49 (24.0)
Three times	48 (68.6)	41 (63.1)	52 (75.4)	141 (69.1)
Four or more times	0	2 (3.1)	1 (1.4)	3 (1.5)
Missing	0	2 (3.1)	1 (1.4)	3 (1.5)
Mean viral load (Q1, Q3) — log10 copies per milliliter	5.04 (4.03,5.91)	5.40 (4.20,6.45)	5.20 (4.14,6.28)	5.21 (4.06,6.28)

Median (Q1, Q3) values are reported as the median with first (25th percentile) and third (75th percentile) quartiles in parentheses. The modified intention-to-treat (mITT) population included randomized participants who had a positive baseline SARS-CoV-2 PCR test and received at least two doses of YKYY017 or placebo. COVID-19 denotes coronavirus disease 2019, IQR interquartile range, BMI body mass index.

YKYY017 groups. For example, on Day 15, the 20 mg group showed a mean difference of −0.49 ± 0.73 $\log_{10}$ copies/mL (95% CI −1.98 to 1.00), and the 10 mg group showed a mean difference of 0.26 ± 0.77 $\log_{10}$ copies/mL (95% CI −1.31 to 1.82) (Fig. 2).

In mITT population, the symptom score and symptom number in the three groups all gradually declined (Supplementary Figs. 6 and 7). The time to sustained symptom recovery was shorter in the 20 mg YKYY017 group (median 117.53, 95%CI 95.33 to 141.45 hours) than in the placebo group (median 143.00, 95%CI 139.17 to 186.87 hours; HR 1.552, 95%CI 1.089 to 2.214, $p = 0.0151$) (Fig. 3). Compared to the placebo group, the 10 mg YKYY017 group showed a similar but not statistically significant trend for time to sustained recovery (median 118.75, 95%CI 95.68 to 143.32 hours; HR 1.361, 95%CI 0.960 to 1.930, $p = 0.0833$). The time to sustained symptom alleviation was shorter in both the 20 mg (median 96.23, 95%CI 94.82 to 119.63 hours; HR 1.494, 95%CI 1.053 to 2.121, $p = 0.0246$) and 10 mg (median 115.97, 95%CI 95.20 to 119.70 hours; HR 1.545, 95%CI 1.093 to 2.184, $p = 0.0137$) YKYY017 groups than in the placebo group (median 119.63, 95%CI 117.28 to 160.15 hours) (Fig. 4). For FAS and PP population, both the 20 mg and 10 mg YKYY017 groups demonstrated shorter time to sustained symptom recovery (Supplementary Figs. 8 and 9).

Safety

239 patients who received at least one dose of YKYY017 or placebo and had at least one post-baseline safety assessment were included in the safety population (Table 2, Supplementary Table 3). Through Day 29, the 20 mg YKYY017 (27.8%, 95%CI 18.4–39.1%) and 10 mg YKYY017 (23.8%, 95%CI 15.0–34.6%) groups had higher incidence of adverse events than the placebo group (17.5%, 95%CI 9.9–27.6%). Most of the adverse events were grade 1 or grade 2. One serious adverse event (SAE) happened in both the 20 mg and 10 mg YKYY017 groups. In the 10 mg group, one patient experienced impaired consciousness, which the investigator attributed to the patient's underlying medical conditions. The patient achieved full recovery. In the 20 mg group, one patient developed meniscal injury as an SAE, which the investigator determined was caused by the patient's pre-existing

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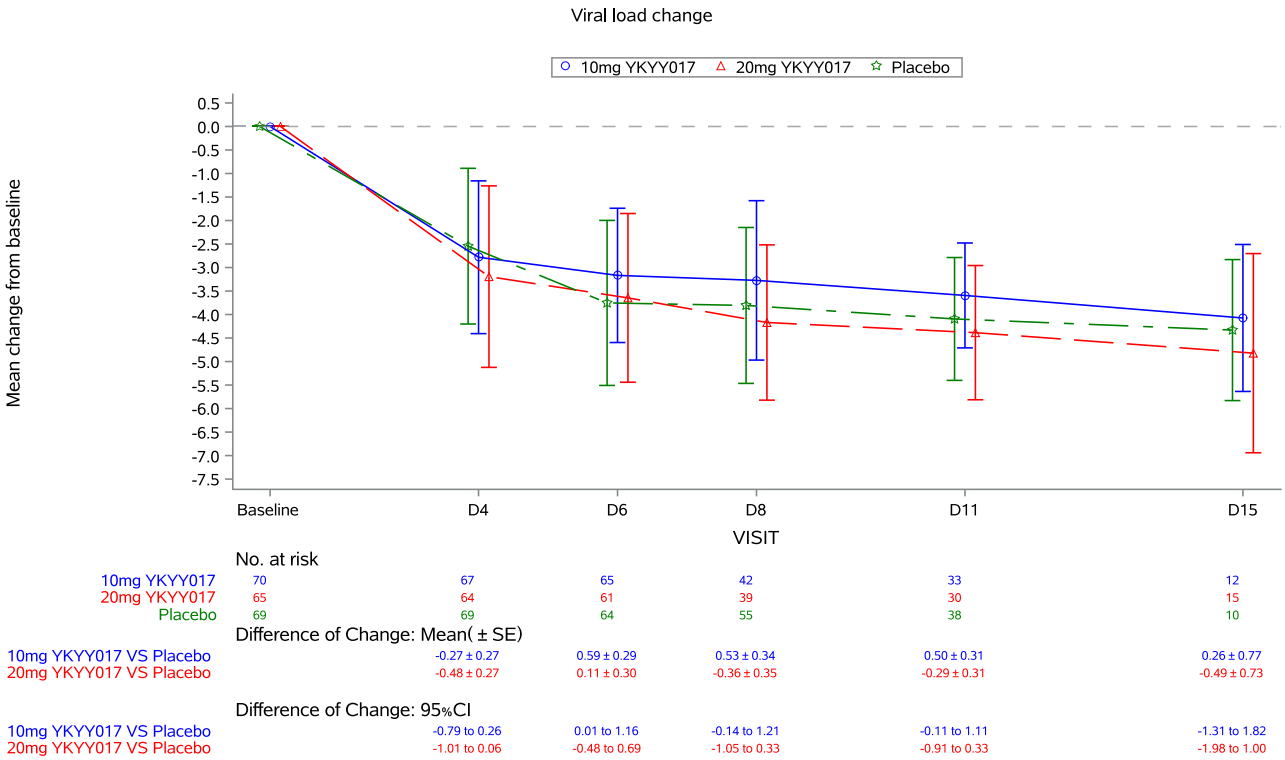

Fig. 2 | Change of viral load (mITT population). Mean changes are shown for patients with available viral load results in mITT population; I bars indicate standard deviation (upper panel). For the primary outcome (Day 4 viral load reduction), multiple imputations and ANCOVA were used (D4 in lower panel). The number of participants with available viral load results on D4 was 67, 64 and 69 in 10 mg YKYY017 group, 20 mg YKYY017 group and placebo group, respectively. For the secondary viral load outcomes (viral load reduction in other visits), the data were not imputed and ANOVA was used (D6, D8, D11, D15 in lower panel). Two-side 95% confidence intervals (CI) were calculated.

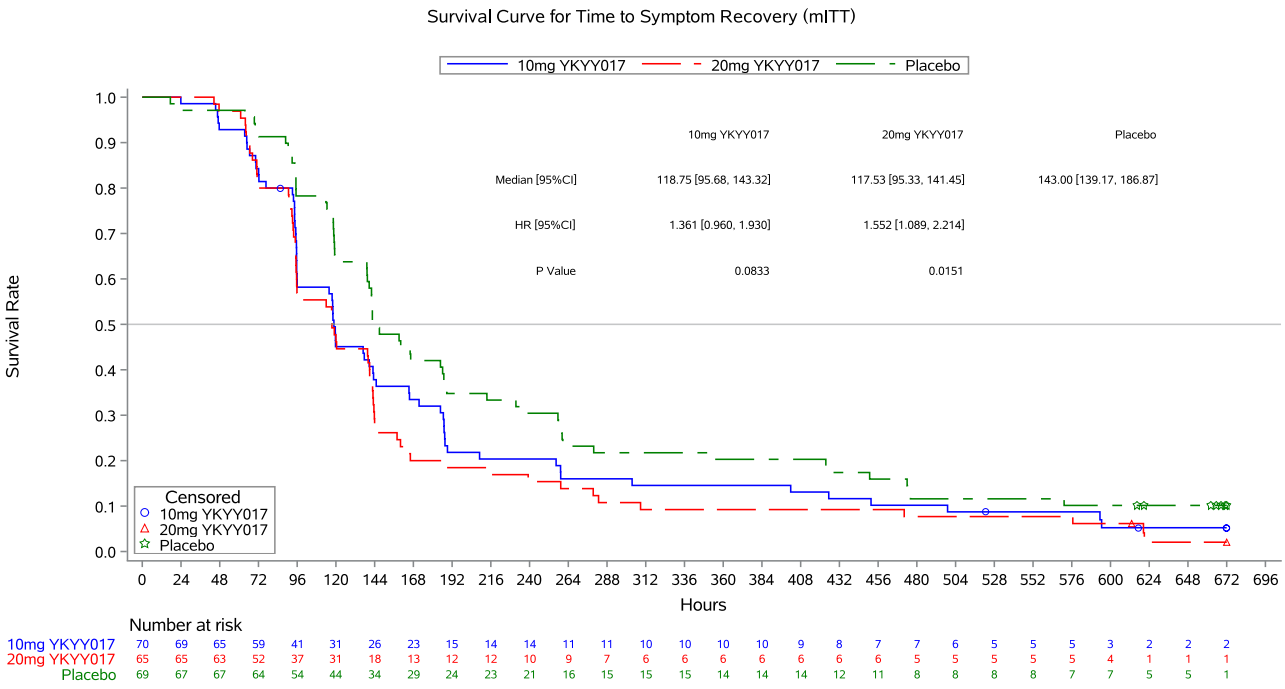

Fig. 3 | Survival curve for time to symptom recovery (mITT population). Kaplan–Meier curve for the time to symptom recovery in mITT population. The time to sustained symptom recovery was shorter in the 20 mg YKYY017 group (median 117.53, 95%CI 95.33 to 141.45 hours) than in the placebo group (median 143.00, 95%CI 139.17 to 186.87 hours; HR 1.552, 95%CI 1.089 to 2.214, $p = 0.0151$). Compared to the placebo group, the 10 mg YKYY017 group showed a similar but not statistically significant trend (median 118.75, 95%CI 95.68 to 143.32 hours; HR 1.361, 95%CI 0.960 to 1.930, $p = 0.0833$).

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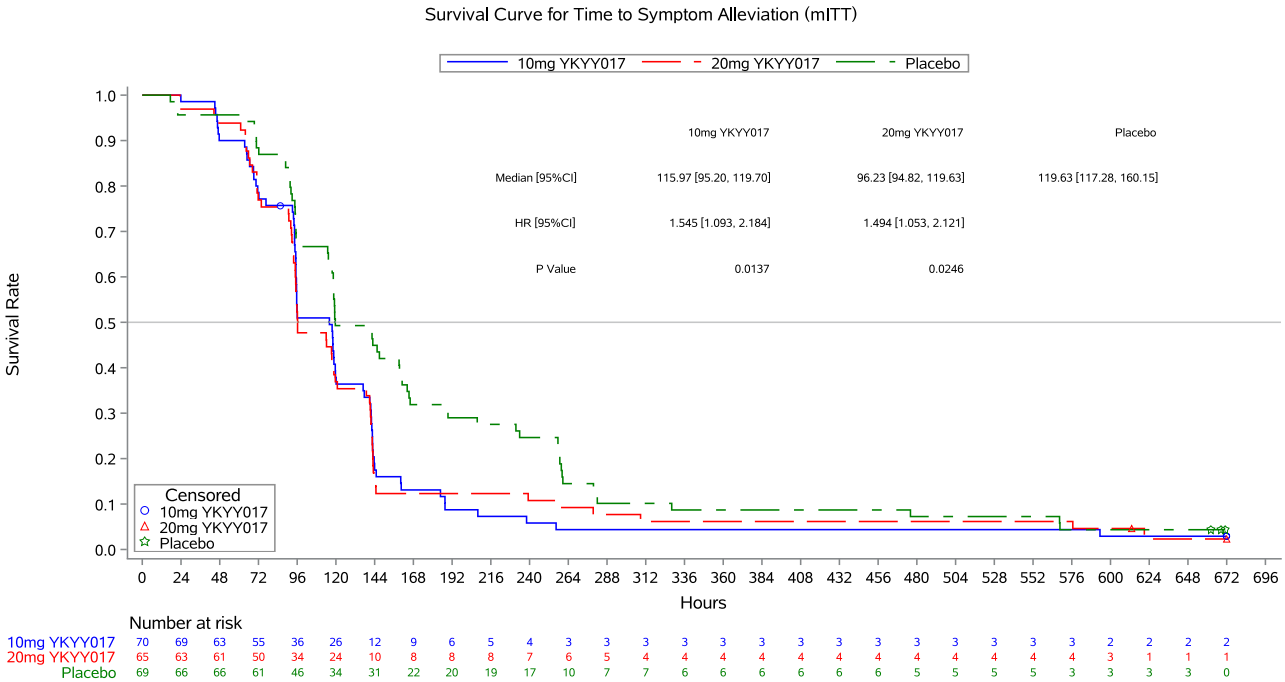

Fig. 4 | Survival curve for time to symptom alleviation (mITT population). Kaplan–Meier curve for the time to symptom alleviation in the mITT population. The time to sustained symptom alleviation was shorter in both the 20 mg (median 96.23, 95%CI 94.82 to 119.63 hours; HR 1.494, 95%CI 1.053 to 2.121, $p = 0.0246$) and 10 mg (median 115.97, 95%CI 95.20 to 119.70 hours; HR 1.545, 95%CI 1.093 to 2.184, $p = 0.0137$) YKYY017 groups than in the placebo group (median 119.63, 95%CI 117.28 to 160.15 hours).

Table 2 | Adverse Events That Emerged during the Treatment or Follow-up Period (Safety Population)

Adverse Event	10 mg YKYY017 (*N* = 80)	20 mg YKYY017 (*N* = 79)	Placebo (*N* = 80)
Events that emerged during treatment or follow-up			
No. of events	26	27	22
Grade 3	1	1	0
Grade 4	1	0	0
Grade 5	0	0	0
Patients with an event — no. (%)			
Any adverse event	19 (23.8) [15.0, 34.6]	22 (27.8) [18.4, 39.1]	14 (17.5) [9.9, 27.6]
Serious adverse event	1 (1.3) [0.0, 6.8]	1 (1.3) [0.0, 6.9]	0 [0.0, 4.5]
Grade 3 adverse event	1 (1.3) [0.0, 6.8]	1 (1.3) [0.0, 6.9]	0 [0.0, 4.5]
Grade 4 or 5 adverse event	1 (1.3) [0.0, 6.8]	0 [0.0, 4.6]	0 [0.0, 4.5]
Discontinuation trial drug or placebo because of adverse event	0 [0.0, 4.5]	0 [0.0, 4.6]	0 [0.0, 4.5]
Had temporary discontinuation owing to adverse event	0 [0.0, 4.5]	0 [0.0, 4.6]	0 [0.0, 4.5]
Events considered to be related to trial drug or placebo			
No. of events	7	10	2
Grade 3	1	0	0
Grade 4	0	0	0
Grade 5	0	0	0
Patients with an event — no. (%)			
Any adverse event	5 (6.3) [2.1, 14.0]	8 (10.1) [4.5, 19.0]	2 (2.5) [0.3, 8.7]
Serious adverse event	0 [0.0, 4.5]	0 [0.0, 4.6]	0 [0.0, 4.5]
Grade 3 adverse event	1 (1.3) [0.0, 6.8]	0 [0.0, 4.6]	0 [0.0, 4.5]
Grade 4 or 5 adverse event	0 [0.0, 4.5]	0 [0.0, 4.6]	0 [0.0, 4.5]
Discontinuation trial drug or placebo because of adverse event	0 [0.0, 4.5]	0 [0.0, 4.6]	0 [0.0, 4.5]
Temporary discontinuation owing to adverse event	0 [0.0, 4.5]	0 [0.0, 4.6]	0 [0.0, 4.5]

The safety population included all participants who received at least one dose of YKYY017 or placebo and had at least one post-baseline safety assessment.

knee condition and was not related to the study drug. No participant discontinued or temporarily interrupted the trial drug or placebo due to adverse events. Adverse events considered to be related to trial drug or placebo, as assessed by site investigators, occurred with higher frequency in both the 20 mg YKYY017 (10.1%, 95%CI 4.5–19.0%) and the 10 mg YKYY017 (6.3%, 95%CI 2.1–14.0%) groups compared to the placebo group (2.5%, 95%CI 0.3–8.7%). Most frequently reported adverse events were elevated blood bilirubin (3.8% in both YKYY017 groups vs 0 in placebo group) and diarrhea (3.8% in both YKYY017 groups vs 1.3% in placebo group) (Supplementary Table 3). No patient died.

Discussion

This randomized, placebo-controlled phase 2 trial assessed the efficacy and safety of YKYY017, an inhaled aerosolized membrane fusion inhibitor, for the treatment of COVID-19. Most enrolled patients had mild COVID-19 disease. The primary outcome of the phase 2 trial was not met. Although inhaled 20 mg YKYY017 demonstrated a signal for higher viral load reduction on Day 4, neither 20 mg or 10 mg YKYY017 showed significant difference from placebo. 20 mg YKYY017 group might have approximately 1-day shorter time to sustained symptom recovery and time to sustained symptom alleviation. The adverse events were mostly mild to moderate.

Although YKYY017 did not achieve significant differences from placebo at any measured time point versus placebo for the viral load reduction outcome, the potential antiviral effect of 20 mg YKYY017 observed on day 4 (-0.48 ± 0.27 $\log_{10}$ copies/mL; 95% CI −1.01 to 0.06) provides encouraging signals for its potential efficacy against COVID-19. Even though the 95 % CIs of the effect size contained the null value, the differences between 10 mg and 20 mg group showed a potential dose-response pattern. Therefore, we are planning to proceed with the clinical development of a higher dose regimen of 20 mg YKYY017 twice daily for 5 days. A supplementary phase 1b trial using this intensified regimen has been completed with favorable tolerability profiles, supporting our plans to continue with phase 2/3 trial to further evaluate its efficacy and safety. Besides, approximately 15% of participants were excluded from the mITT population because their PCR tests were negative for the N gene. This exclusion rate is higher than the proportion assumed in our sample size calculations and may therefore reduce the statistical power of the analysis. Effect estimates beyond Day 4 were drawn from markedly smaller datasets due to missingness and therefore fluctuate more widely. Another factor that may affect the efficacy of the treatment is the participants' severity of disease. In our trial, ~90% of the recruited participants had mild disease by NIH guideline criteria, with 20 mg group had the highest proportion of moderate disease participants (~15%). Besides, the coverage of risk factors and elder population is not optimal in this trial. Given the rapid decline of viral load among young and healthy people with mild diseases[11,12], it may be difficult for the antiviral treatment to show its impact on the disease course, and the medication might be more beneficial for the elderly population and people with more risk factors. In the planned new phase 2/3 trial, we will try to recruit a broader spectrum of patients to better reflect the antiviral effects of YKYY017.

Compared with oral and intravenous antiviral regimens, the inhaled nature of YKYY017 improves the efficiency of drug delivery to the lung and minimize systematic exposure, reducing the risk of potential drug-drug effects and systematic side effects[13,14]. YKYY017 binds to the HR1 domain, a highly conserved region of the SARS-CoV-2 spike protein[8,15]. Targeting on HR1 domain provides more stable antiviral effects against emerging SARS-COV-2 variants with immune-escaping mutations, a challenge commonly faced by monoclonal antibody therapies that interact with the receptor binding domain[6,7,16,17]. The sequencing data in a subset of patients confirmed that the efficacy of YKYY017 was consistent among emerging SARS-CoV-2 Omicron subvariants such as HK.3.2, JN.1 and HK.3. Other inhaled antiviral therapies have been investigated in clinical trials for mild-to-moderate COVID-19 patients[18,19]. In a phase 1/2 trial, inhaled monoclonal antibody cocktail therapy IBIO123 did not achieve the primary endpoint, showing a numerical decline in viral load change on Day 5 compared with placebo (−0.29 $\log_{10}$ copies/mL, 95% CI: −1.32 to 0.75; $p = 0.45$)[18]. Similar to our trial, IBIO123 showed a nominal significant benefits in symptom-based secondary outcome[18]. Inhaled interferon beta-1a SNG001 did not show acceleration for viral load reduction and time to symptom improvement in a phase 2 trial[19]. The between-group difference of Day 3 viral load reduction from Day 0 was 0.27 $\log_{10}$ copies/mL (95% CI: −0.28 to 0.82). Potential differences in time from symptom onset, sampling schedules, and patient characteristics indicate a cautious interpretation of cross-study comparisons.

Another notable finding of this study is that 20 mg YKYY017 might accelerate clinical recovery. For patients with mild to moderate COVID-19, persistent symptoms like cough remain a primary concern. Our study shows that inhaled 20 mg YKYY017 might accelerate symptom recovery by approximately one day, similar to some small-molecule oral antivirals[20,21]. As an inhaled therapy, YKYY017 provides an alternative option with comparable clinical efficacy for patients, particularly those who may prefer non-oral medications or have difficulty with oral administration. However, it is worthwhile to note that, the primary outcome of our trial was not met, and no multiplicity adjustments were applied for the secondary outcomes. Therefore, the nominally significant results should be interpreted with caution and need to be validated in our planned new phase 2/3 trial.

The safety profile of inhaled YKYY017 was acceptable. The most common adverse events were hyperbilirubinemia and diarrhea, neither causing treatment discontinuation. Importantly, hepatic and renal dysfunction, frequently observed with oral or intravenous antiviral therapies, showed similar incidence between YKYY017 and placebo groups. No dose-response relationship in adverse events was observed between the 20 mg and 10 mg YKYY017 groups.

The trial has several limitations. The exclusion of people over 75 years of age and low recruitment numbers in the 60-75 age group limited the generalizability of the results to the elderly population. Besides, the representation of risk factors in the study was limited, with overweight or obesity being the predominant one. Additionally, most of the recruited patients had mild diseases. Our planned phase 2/3 trial, using the 5-day 20 mg YKYY017 twice daily regimen, will address these limitations by including a broader spectrum of patients, thereby allowing a more comprehensive assessment of the therapeutic potential across diverse populations and disease severities.

In summary, this phase 2 trial identified potential antiviral activity and clinical efficacy, as well as acceptable safety profile of inhaled 20 mg YKYY017. The relatively modest antiviral activity may reflect the insufficient dose, and we have now initiated a new phase 2/3 trial using a higher-frequency 20 mg YKYY017 regimen to further assess its efficacy.

Methods

Trial design

This trial was a phase 2 randomized double-blind clinical trial to evaluate the efficacy and safety of YKYY017 aerosol inhalation in the treatment of patients with mild to moderate COVID-19 (Trial registration: ChiCTR2300075467). We enrolled patients aged 18 to 75 years with mild to moderate COVID-19. We conducted the trial in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. This trial was approved by the ethics committee in China-Japan Friendship Hospital (approval number: YM2023-043-02) and the ethics committee at each participating center. Written informed consent was obtained from all participants or their legal representatives.

Population

Eligible patients had positive SARS-CoV-2 PCR or rapid antigen test during participant screening; had mild-to-moderate disease; were aged 18 to 75 years; had at least one of the designated COVID-19 related symptoms/signs (fever, cough, stuffy or runny nose, sore throat, shortness of breath or difficulty breathing, chills or shivering), and the total symptom score is at least 2 points (COVID-19 related symptom score scale); and had the time of symptom onset (the time when the first symptom of 14 COVID-19 related symptoms appeared) within 72 hours of the first administration of the investigational drug. The key exclusion criteria were anticipated disease progression into severe or critical COVID-19 before randomization; the pulse blood oxygen saturation (SpO_2) in the ambient air is less than or equal to 93%, or the arterial blood oxygen partial pressure (PaO_2)/oxygen uptake concentration (FiO_2) is less than or equal to 300, or the respiratory rate is more than or equal to 30 per minute; urgently need or anticipated need of nasal high flow oxygen therapy, non-invasive ventilation, invasive mechanical ventilation, or ECMO. The evaluation of COVID-19 disease severity was based on the NIH COVID-19 treatment guideline (Supplementary Table 4)[22]. The COVID-19 related symptom score scale was based on guidance issued by the U.S. Food and Drug Administration (Supplementary Table 5)[23]. Full eligibility criteria were provided in the protocol.

Procedures

We used stratified block randomization with two stratification factors: age (18–49 years vs 50–75 years) and presence of risk factors for COVID-19 disease progression (yes vs no). This design resulted in four strata, each containing 40 blocks of six assignments. Competitive enrollment was implemented across study centers. Central randomization was performed using an interactive web response system (IWRS) in accordance with the study protocol, through which neither participants nor study personnel could access upcoming assignment allocations. An independent statistician, not involved in the trial, generated the randomization sequence using SAS (version 9.4) and uploaded it to the IWRS. The clinical, laboratory, and statistical teams remained blind to group assignments until after the database lock. The placebo and investigational drug were identical in appearance and texture, ensuring indistinguishability. The concomitant use of COVID-19 antiviral therapies other than the investigational drug was prohibited, unless investigators determined that participants met criteria for early treatment discontinuation. Ibuprofen, acetaminophen, or other single-ingredient nonsteroidal anti-inflammatory drugs (NSAIDs) could be administered when clinically indicated.

The trial had a screening period, a treatment and observation period and a follow-up period. In the treatment (Day 1 to Day X, $3 \le X \le 7$) and observation (Day X + 1) period, Enrolled patients were randomly allocated in a 1:1:1 ratio to receive aerosol inhalation of 10 mg YKYY017, 20 mg YKYY017 or placebo once daily (interval between doses: 24 ± 2 h) for consecutive 7 days or until the second day after sustained clinical recovery (whichever happened first) and observed until the following day of the last treatment dose. The follow-up period lasted until 28 days after the first treatment dose, which included visits on Day X + 4 (±1 day), Day X + 7 (±1 day), Day 15 (±2 day), Day 22 (±2 day) and Day 29 (±2 day). The investigational drug or placebo was administered via the VP-M1A nebulizer (VAPO Healthcare Co. Ltd., Jiangsu, China). Authorized physicians performed standardized assessments on COVID-19 related symptom score scale (Supplementary Table 5) and WHO clinical progression scale during the visits (Supplementary Table 6). SARS-CoV-2 rapid antigen tests and viral load quantification were performed on oropharyngeal swabs daily in treatment/observation period and Day X + 4 (±1 day), Day X + 7 (±1 day), Day 15 (±2 day) in follow-up period. Certified nurses collected oropharyngeal swab specimens during the visits, with centralized batch transportation to the central laboratory for PCR analysis. More details about procedures for investigational drugs and PCR were provided in Supplementary Table 7.

Endpoints

The primary endpoint of the phase 2 trial is the change in SARS-CoV-2 viral load from baseline to Day 4 after initiation of study treatment. Secondary endpoints include change in SARS-CoV-2 viral load from baseline at each visit (except Day 4) through Day 15, time to sustained symptom recovery, and time to sustained symptom alleviation. Sustained symptom recovery was defined as a score of 0 (no symptom or return to pre-COVID-19 status) on the 11 target COVID-19–related symptoms for two consecutive days. Sustained symptom alleviation was defined as a score of 1 (mild symptom) or 0 on all the 14 COVID-19–related symptoms for two consecutive days. The safety outcomes included adverse events and serious adverse events coded by MedDRA 26.1.

Statistical analysis

Assuming an intergroup difference of -1.5 ± 2.6 $\log_{10}$ copies/mL (treatment relative to placebo) for change of viral load, 55 participants per group would provide at least 85% power with a one-sided significance level of 0.025. Accounting for a potential dropout rate of approximately 20% and a baseline PCR negativity rate not exceeding 10% in the enrolled population, we planned to enroll 240 participants for the phase 2 trial, with 80 per group. The full statistical analysis plan (SAP) was attached in the supplementary information. The main analysis was performed among the modified intention-to-treat (mITT) population, which included randomized participants who had a positive baseline SARS-CoV-2 PCR test and received at least two doses of YKYY017 or placebo. The per-protocol (PP) population was a subset of the mITT population, comprising participants who had a positive baseline SARS-CoV-2 PCR test, no major protocol deviations, and good adherence to the study regimen. The full analysis set (FAS) included randomized participants who had a positive result on either the baseline SARS-CoV-2 antigen or PCR test and received at least one dose of YKYY017 or placebo. The safety population included all participants who received at least one dose of YKYY017 or placebo and had at least one post-baseline safety assessment.

For the primary outcome (Day 4 viral load change differences), we calculated the change in SARS-CoV-2 viral load from baseline for each participant. An analysis of covariance (ANCOVA) was performed on the $\log_{10}$-transformed changes in viral load, with treatment group as a fixed effect and baseline viral load and randomization stratification factors as covariates. Least-squares means for each group, between-group differences in least-squares means, and corresponding 95% confidence intervals (CIs) were calculated. Missing data were imputed using multiple imputation under the Missing at Random (MAR) assumption. A sensitivity analysis was conducted where missing data were imputed using placebo group data. For viral load change differences in other visits, we calculated the differences of $\log_{10}$-transformed changes between groups using analysis of variance (ANOVA), with no data imputation. For time-to-event endpoints, the Kaplan-Meier method was used to estimate the median time from treatment initiation to event occurrence for each group, and the 95% CIs were calculated using the Brookmeyer-Crowley method after log-log transformation. The Cox proportional hazards model with treatment group and randomization stratification as independent variables was employed to estimate the hazard ratio (HR). Details about strategies for intercurrent events were in Supplementary Table 7 and the SAP in the supplementary information. All statistical analyses were performed with SAS version 9.4.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data supporting the findings of this study are not openly available due to confidentiality. Data will be made available for request after product approval in China. Based on a reasonable request, a detailed proposal, and agreement with the requirements of the ethics committee, data application could be considered. A research proposal with anticipated hypothesis and hypothesis-driven statistical analysis plan will be reviewed by researchers, statisticians, the ethics committee and sponsors. Please contact the corresponding author. After approval of the research proposal and signing of a data-sharing agreement, the proposed analyses and results will be shared.

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Author contributions

Y.W., L.S., C.W. and B.C. contributed to the study design. An independent third-party statistician performed the statistical analysis. Y.W., L.S., L.W., X.W., B.D., K.H., Y.H. (Yingli He), G.L., J.Z., J.H., Y.T., J.W., L.Y., B.L., G.S., Y.H. (Yuxian He), C.W. and B.C. contributed to conducting the clinical trial. Y.W. and L.S. wrote the original manuscript. B.C., C.W., Y.H. and G.S. were the supervisors for the whole study. All authors interpreted the data. All authors contributed to revising the manuscript and approved its final version for submission.

Competing interests

Bin Cao discloses his role as PI in China for the baloxavir transmission trial sponsored by Roche and as PI of GP681 Phase II and Phase III trials sponsored by Qingfeng Pharmaceutical Group Co., Ltd. Yeming Wang serves as the sub-PI. Yeming Wang also discloses receiving compensation as rapporteur for "Optimizing the Clinical Management of Patients" in the 2024 update of the WHO Public Health Research Agenda for Influenza, led by WHO. Gengshen Song and Xia Wang are employees of Youcare Pharmaceutical Co., Ltd. Jie Zhai is an employee of Beijing Yolax Pharmaceutical Technology Co., Ltd. The remaining authors declare no competing interests.

Additional information

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