values of baloxavir ranged from 1.4 to 3.1 nM (n=4) for influenza A viruses and 4.5 to 8.9 nM (n=3) for influenza B viruses in a PA endonuclease assay. Viruses with reduced susceptibility to baloxavir have amino acid substitutions in the PA protein.

Antiviral Activity

The antiviral activity of baloxavir against laboratory strains and clinical isolates of influenza A and B viruses was determined in an MDCK cell-based plaque reduction assay. The median 50% effective concentration (EC_{50}) values of baloxavir were 0.73 nM (n=31; range: 0.20–1.85 nM) for subtype A/H1N1 strains, 0.83 nM (n=33; range: 0.35–2.63 nM) for subtype A/H3N2 strains, and 5.97 nM (n=30; range: 2.67–14.23 nM) for type B strains. In an MDCK cell-based virus titer reduction assay, the 90% effective concentration (EC_{90}) values of baloxavir against avian subtypes A/H5N1 and A/H7N9 were in the range of 0.80 to 3.16 nM. The relationship between antiviral activity in cell culture and clinical response to treatment in humans has not been established.

Resistance

Cell Culture

Influenza A virus isolates with reduced susceptibility to baloxavir were selected by serial passage of virus in cell culture in the presence of increasing concentrations of baloxavir. Reduced susceptibility of influenza A virus to baloxavir was conferred by amino acid substitutions I38T (A/H1N1 and A/H3N2), E198K (A/H1N1) and E199G (A/H3N2) in the PA protein of the viral RNA polymerase complex.

Clinical Studies

Treatment-emergent substitutions were identified in influenza A and B viruses in clinical studies. Substitutions associated with a >3-fold reduction in susceptibility to baloxavir are shown in Table 7.

Table 7 Treatment-Emergent Amino Acid Substitutions in PA Associated with Reduced Susceptibility to Baloxavir Identified in Clinical Specimens

Influenza Type/Subtype	**A/H1N1**	**A/H3N2**	**B**
Amino Acid Substitution	E23G/K/R, A37T, I38F/N/S/T	E23G/K, A37T, I38M/T, E199G	T20K, I38T

Clinical studies in adult and adolescent subjects ≥ 12 years of age:

In adult and adolescent subjects who had a confirmed influenza virus infection, the overall frequencies of treatment-emergent amino acid substitutions associated with reduced susceptibility to baloxavir were 5% (6/134), 11% (53/485), and 1% (2/224) in influenza A/H1N1, A/H3N2, and B virus infections, respectively, in pooled data from Trials T0821, T0831, and T0832 *[see Clinical Studies (14)]*. In Trial T0834, of 303 subjects ≥ 12 years of age who received XOFLUZA post-exposure prophylaxis, 32 were viral RNA-positive post-baseline, including 17 subjects who were evaluated for resistance. Of these 17 subjects, influenza virus with substitutions associated with reduced susceptibility to baloxavir was identified in 4/4 subjects who developed clinical influenza (as described for the primary endpoint) and 6/13 other subjects evaluated who did not meet the primary endpoint definition for clinical influenza *[see Clinical Studies (14)]*.

Clinical studies in pediatric subjects 5 to < 12 years of age:

Selection of influenza viruses with treatment-emergent amino acid substitutions associated with reduced susceptibility to baloxavir has occurred at higher frequencies in pediatric subjects 5 to <12 years of age compared to subjects ≥ 12 years of age. Such viruses were detected with overall frequencies of 17% (2/12), 18% (17/93), and 0% (0/13) in influenza A/H1N1, A/H3N2, and B virus infections, respectively, in pooled data from 4 pediatric treatment trials in subjects 5 to < 12 years of age.

In Trial T0834, of a subgroup of 57 subjects 5 to < 12 years of age who received XOFLUZA post-exposure prophylaxis, 12 were viral-RNA positive post-baseline, including 10 subjects who were evaluated for resistance. Of these 10 subjects, influenza virus with substitutions associated with reduced susceptibility to baloxavir was identified in 2/2 subjects who developed clinical influenza (as described for the primary

population was defined as those with a positive rapid influenza diagnostic test (Trial T0821) or positive influenza reverse transcription polymerase chain reaction (RT-PCR) (Trial T0831) at trial entry.

The primary endpoint of both trials, time to alleviation of symptoms, was defined as the time when all seven symptoms (cough, sore throat, nasal congestion, headache, feverishness, myalgia, and fatigue) had been assessed by the subject as none or mild for a duration of at least 21.5 hours.

In both trials, XOFLUZA treatment at the recommended dose resulted in a statistically significant shorter time to alleviation of symptoms compared with placebo in the primary efficacy population (Tables 9 and 10).

Table 9 Time to Alleviation of Symptoms After Single Dose in Otherwise Healthy Adults with Acute Uncomplicated Influenza in Trial T0821 (Median Hours)

	XOFLUZA 40 mg (95% CI[a]) N=100	**Placebo (95% CI[a]) N=100**
Adults (20 to 64 Years of Age)	50 hours[b] (45, 64)	78 hours (68, 89)

[a]CI: Confidence interval
[b]XOFLUZA treatment resulted in a statistically significant shorter time to alleviation of symptoms compared to placebo using the Gehan-Breslow's generalized Wilcoxon test (p-value: 0.014, adjusted for multiplicity using the Bonferroni method). The primary analysis using the Cox Proportional Hazards Model did not reach statistical significance (p-value: 0.165).

Table 10 Time to Alleviation of Symptoms After Single Dose in Otherwise Healthy Subjects 12 Years of Age and Older with Acute Uncomplicated Influenza in Trial T0831 (Median Hours)

	XOFLUZA 40 mg or 80 mg (95% CI[a]) N=455	**Placebo (95% CI[a]) N=230**
Subjects (≥ 12 Years of Age)	54 hours[b] (50, 59)	80 hours (73, 87)

[a]CI: Confidence interval
[b]XOFLUZA treatment resulted in a statistically significant shorter time to alleviation of symptoms compared to placebo using the Peto-Prentice's generalized Wilcoxon test (p-value: < 0.001).

In Trial T0831, there was no difference in the time to alleviation of symptoms between subjects (age ≥ 20 years) who received XOFLUZA (54 hours) and those who received oseltamivir (54 hours). For adolescent subjects (12 to 17 years of age) in Trial T0831, the median time to alleviation of symptoms for subjects infected with influenza and who received XOFLUZA (N=63) was 54 hours (95% CI of 43, 81) compared to 93 hours (95% CI of 64, 118) in the placebo arm (N=27).

The number of subjects who received XOFLUZA at the recommended dose and who were infected with influenza type B virus was limited, including 24 subjects in Trial T0821 and 38 subjects in Trial T0831. In the influenza B subset in Trial T0821, the median time to alleviation of symptoms in subjects who received 40 mg XOFLUZA was 63 hours (95% CI of 43, 70) compared to 83 hours (95% CI of 58, 93) in subjects who received placebo. In the influenza B subset in Trial T0831, the median time to alleviation of symptoms in subjects who received 40 mg or 80 mg XOFLUZA was 93 hours (95% CI of 53, 135) compared to 77 hours (95% CI of 47, 189) in subjects who received placebo.

Pediatrics (5 to < 12 Years of Age)

Trial CP40563 (NCT03629184) was a randomized, double-blind, multicenter, active-controlled study, designed to evaluate the safety, efficacy, and pharmacokinetics of a single oral dose of XOFLUZA compared with oseltamivir in otherwise healthy pediatric subjects (including subjects aged 5 to < 12 years of age) with influenza-like symptoms. Eligible subjects had a tympanic temperature of at least 38°C and at least one

respiratory symptom of either cough or nasal congestion.

A total of 118 subjects 5 to less than 12 years of age were randomized and received a single one-time oral dose of XOFLUZA (N=79) based on body weight (2 mg/kg for subjects weighing < 20 kg or 40 mg for subjects weighing ≥ 20 kg) or oseltamivir (N=39) for 5 days (dose based on body weight). The primary objective was to compare the safety of a single one-time dose of XOFLUZA with 5 days of oseltamivir administered twice daily. The secondary efficacy endpoint included time to alleviation of influenza signs and symptoms, which was defined as the time when all of the following were met for at least 21.5 hours: cough and nasal symptoms were assessed by the caregiver as no problem or minor problem, subject was able to return to normal daily activity, and subject was afebrile (temperature ≤ 37.2°C). However, the trial was not powered to detect statistically significant differences in this secondary endpoint.

Of the 118 randomized subjects 5 to less than 12 years of age in Trial CP40563, 94 subjects had influenza confirmed by RT-PCR at baseline or during the trial; 89% percent of subjects were White, 3% Black or African American and 8% Other/unknown/multiple races. The mean age was 8 years [SD=1.97]; 56% of subjects were female and 44% male. The predominant influenza virus strain in this study was the A/H3N2 subtype (67%), followed by A/H1N1 (20%) and type B (9%).

The median time to alleviation of influenza signs and symptoms was 138 hours in the XOFLUZA arm (95% CI of 117, 163) and 126 hours in the oseltamivir arm (95% CI of 96, 166).

14.2 Treatment of Acute Uncomplicated Influenza—High Risk Subjects (12 Years of Age and Older)

Trial T0832 (NCT02949011) was a randomized, double-blind, placebo- and active-controlled trial to evaluate the efficacy and safety of a single oral dose of XOFLUZA compared with placebo or oseltamivir in adult and adolescent subjects 12 years of age or older with influenza who were at high risk of developing influenza-related complications.

A total of 2,182 subjects with signs and symptoms of influenza were randomized to receive a single oral dose of 40 mg or 80 mg of XOFLUZA according to body weight (subjects who weighed 40 to less than 80 kg received 40 mg and subjects who weighed 80 kg and above received 80 mg) (N=729), oseltamivir 75 mg twice daily for 5 days (N=725), or placebo (N=728). Twenty-eight percent of subjects were Asian, 59% were White, and 10% were Black or African American. The mean age was 52 years, and 3% of subjects were less than 18 years of age; 43% of subjects were male and 57% female.

High risk factors were based on the Centers for Disease Control and Prevention definition[1] of health factors known to increase the risk of developing serious complications from influenza. The majority of subjects had underlying asthma or chronic lung disease, diabetes, heart disease, morbid obesity, or were 65 years of age or older.

In Trial T0832, 1,158 of the 2,182 enrolled subjects had influenza confirmed by RT-PCR and were included in the efficacy analysis (XOFLUZA N=385, placebo N=385, or oseltamivir N=388). Among subjects in whom only one type/subtype of influenza virus was identified, 50% were infected with subtype A/H3N2, 43% were infected with type B, and 7% were infected with subtype A/H1N1.

Eligible subjects had an axillary temperature of at least 38˚C, at least one moderate or severe respiratory symptom (cough, nasal congestion, or sore throat), and at least one moderate or severe systemic symptom (headache, feverishness or chills, muscle or joint pain, or fatigue), and all were treated within 48 hours of symptom onset. Subjects participating in the trial were required to self-assess their influenza symptoms as “none,” “mild,” “moderate,” or “severe” twice daily. A total of 215 subjects (19%) had preexisting symptoms (cough, muscle or joint pain, or fatigue) associated with their underlying high risk condition that were worsened due to influenza infection. The primary efficacy endpoint was time to improvement of influenza symptoms (cough, sore throat, headache, nasal congestion, feverishness or chills, muscle or joint pain, and fatigue). This endpoint included alleviation of new symptoms and improvement of any preexisting symptoms that had worsened due to influenza. A statistically significant improvement in the primary endpoint was observed for XOFLUZA when compared with placebo (see Table 11).