Zelicapavir demonstrates high clinical and functional barrier against antiviral resistance: results from a randomized, double-blind, placebo-controlled pediatric RSV antiviral clinical trial

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BACKGROUND

- Respiratory syncytial virus (RSV) vaccines and monoclonal antibodies leave >75% of RSV medically attended childhood visits unprevented, even if all indicated infants receive them[1]
- There is a large unmet medical need for RSV antiviral treatments[2]
- Antiviral resistance plagues the treatment of RNA viruses including Influenza, SARS-CoV-2, and RSV (e.g., monoclonal antibodies and fusion inhibitor small molecule antivirals)
- Zelicapavir is an oral, once-daily, nonnucleoside RSV antiviral targeting the highly conserved RSV N-protein, with potent activity against RSV-A and RSV-B strains
- Zelicapavir has a high barrier to resistance in vitro[3]
- Zelicapavir showed antiviral activity and improvements on clinical endpoints in recently completed clinical trials of adults and children[4,5]
- We therefore sought to characterize the genotypic and phenotypic resistance of RSV in these studied children treated with zelicapavir or placebo.

METHODS

Study Design

- A phase 2, randomized, double-blind, placebo-controlled, multinational clinical trial (NCT04816721)
- Study population: hospitalized and nonhospitalized pediatric patients with RSV infection, 28 days to 36 months of age
- Treatment
  - 2:1 or 4:1 (depending on stage of study) randomization to zelicapavir oral suspension or placebo
  - Dose of 5 mg/kg or 7.5 mg/kg zelicapavir oral suspension once daily for 5 days
- Sample collection of mid-turbinate nasal swabs at baseline and days 3, 5, 9, and 14

Treatment-Emergent Sequence Assessment

- Next-generation sequencing (Illumina) of the full RSV-N gene on longitudinally collected respiratory secretions
- Samples from all 96 patients receiving zelicapavir (n=69) or placebo (n=27) for 5 days
- All samples from individual patients who had ≥3 log_{10} copies/mL (or culture-positive) for ≥3 timepoints were submitted for sequencing
- Sequences from placebo and zelicapavir recipients were compared against their patient-specific baseline to identify treatment-emergent nucleotide variations
- Amino-acid-level variations were reported
- Criterion for reporting successful sequencing: >1% of >500 nucleotide position reads

RESULTS

Baseline Characteristics of Study Population

- Demographic and baseline characteristics were generally well balanced between the treatment groups
- Most (80.2%) patients were hospitalized at baseline
- Mean (SD) duration of RSV symptoms prior to randomization was 4.0 (1.6) days and 4.1 (1.8) days in the zelicapavir and placebo groups, respectively
- High baseline viral loads were observed (≈6.5 log_{10} copies/mL)

Table 1: Baseline Characteristics	Zelicapavir (n=70)	Placebo (n=26)*
Age, months, mean (SD)	10.4 (9.1)	10.7 (9.0)
Sex, female, n (%)	35 (50.0)	14 (53.8)
Race, white, n (%)	51 (72.9)	11 (42.3)
RSV viral load by RT-qPCR, log_{10} copies/mL		
n	63	23
Mean (SD)	6.60 (1.52)	6.19 (1.44)
Duration of symptoms prior to randomization, days, mean (SD)	4.0 (1.6)	4.1 (1.8)
Participants hospitalized at enrollment, n (%)	57 (81.4)	2 (7.7)

Abbreviations: RSV, respiratory syncytial virus; RT-qPCR, reverse-transcription quantitative polymerase chain reaction.

Next-Generation Sequencing Results

- Sequencing was successful in 160/166 (96.4%) of specimens
- This represented 45 patients (n=34 zelicapavir, n=11 placebo; n=26 RSV-A, n=19 RSV-B)
- No patients developed resistance-associated mutations during treatment
- No patients had consensus sequences representing resistance-associated mutations through follow-up (**Tables 2 and 3**)

Table 2. Frequency of variant detection in study population sequenced and treated with zelicapavir (sample level)

Zelicapavir clinical trial type	Samples evaluated for resistance from zelicapavir-treated patients	Samples with consensus sequence resistance from zelicapavir-treated patients	Samples with resistant subpopulations† from successfully sequenced‡ zelicapavir-treated patients
Pediatric RSV treatment trial (NCT04816721)	124	0/124 (0%)	2/120 (1.7%)

†Treatment was daily for 5 days. Two subjects had minority subpopulations of sequences containing N: L139I which first occurred at day 9 (4 days after last dose of zelicapavir).
‡96.8% of samples were successfully sequenced. Abbreviations: NGS, next-generation sequencing; RSV, respiratory syncytial virus.

RESULTS (cont.)

Next-Generation Sequencing Results (cont.)

Table 3. Frequency of variant detection in study population sequenced and treated with zelicapavir (patient level)

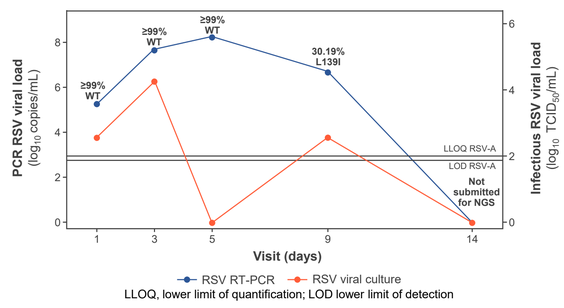
Zelicapavir clinical trial type	RSV-infected patients randomized to zelicapavir†	Patients developing minority subpopulation resistance during treatment‡	Patients developing consensus sequence resistance at any time during study	Patients developing minority subpopulation resistance at any time during study‡
Pediatric RSV treatment trial (NCT04816721)	35	0 (0%)	0 (0%)	2/35 (5.7%)

*†Samples from 35 subjects were submitted for NGS sequencing; 34 subjects were successfully sequenced.
‡Treatment was daily for 5 days. Two subjects had minority subpopulations of sequences containing N: L139I, first occurring at day 9 (4 days after last dose of zelicapavir).

- Two patients treated with zelicapavir had day 9 (4 days after end of treatment) single specimens containing treatment-emergent sequence variant minority viral subpopulations representing 8.28% and 30.19% of the viral populations at that timepoint, respectively
- Both patients had N: L139I variants, both of which subsequently cleared polymerase chain reaction (PCR)-detectable RSV at the next timepoint (day 14) (**Figure 1**)

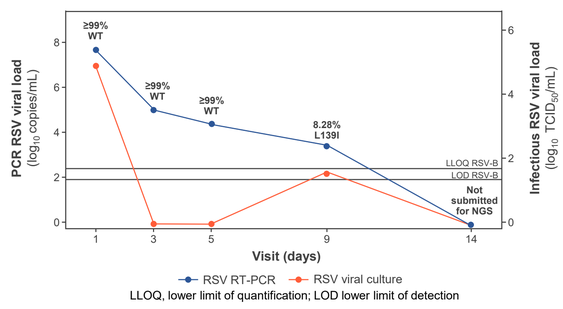
Fig 1: Viral and Sequence Variant Dynamics in Individual Patients

Patient 1062: Viral kinetics (qPCR and viral culture) and N-gene variant frequency



LLOQ, lower limit of quantification; LOD lower limit of detection

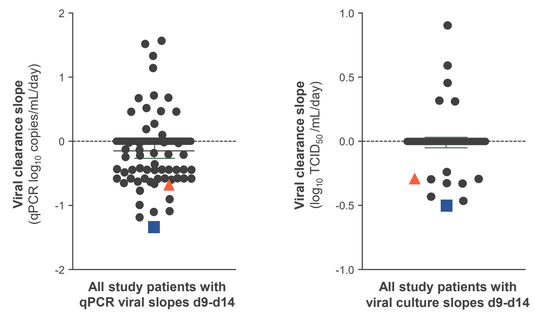
Patient 1037: Viral kinetics (qPCR and viral culture) and N-gene variant frequency



LLOQ, lower limit of quantification; LOD lower limit of detection

Fig 2: Phenotypic Behavior of N: L139I Variant RSV in Patients and in vitro

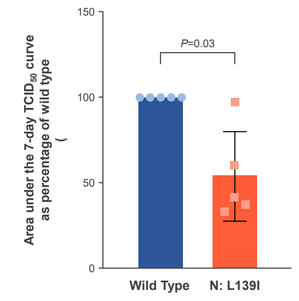
A. Patient's clinical viral clearance rates are faster if containing subpopulations with N: L139I variants



■ **Patient 1062:** A single timepoint detection of subpopulation N: L139I occurred on day 9 at 30.19% sequence frequency, 4 days after last zelicapavir dose (Fig 1)
▲ **Patient 1037:** A single timepoint detection of subpopulation N: L139I occurred on day 9 at 8.28% sequence frequency, 4 days after last zelicapavir dose (Fig 1)

Individual patient's RSV viral clearance slopes measured from nasal swabs by quantitative PCR (left panel) and by quantitative viral culture ($TCID_{50}$) (right panel) are shown. Scatterplots show all patients with quantitative viral assessments, including placebo and zelicapavir recipients. Placebo recipients are included in these scatterplots because viral clearance rates are determined 4-11 days after last study drug dose. The more negative the viral clearance slopes, the faster the clearance. Error bars are mean ± 95% CI.

B. In vitro study of RSV N: L139I shows lower functional fitness



HEp-2 cells were infected at a multiplicity of infection of 0.1 with the indicated recombinant strain of RSV-A containing either wild-type N gene (WT) or containing N: L139I variant. Cells and supernatant were harvested and subject to $TCID_{50}$ analysis. The AUC was normalized to wild type and expressed as the percent AUC relative to Wild Type. Data are expressed as the mean ± standard deviation AUC. Statistical analysis was by 1-way repeated measured ANOVA followed by Dunnett multiple comparison test. Abbreviations: AUC, area under the curve; N, nucleoprotein; $TCID_{50}$, 50% tissue culture infectious dose. Error bars are mean ± SD.

- RSV viral load clearance rates (PCR and viral culture quantification) in these 2 patients after N: L139I detection was faster than wild-type viral clearance from other study patients (**Fig 2A**), suggesting reduced clinical fitness of this viral variant. In vitro, the RSV N: L139I variant showed an ≈10x increase in zelicapavir EC_{90} (90% effective concentration) but with reduced viral fitness (50% infectious virus production vs wild-type)[3] (**Fig 2B**)

In Silico Search for N: L139I Variant RSV in Global Sequence Databases

- In nature, no N: L139I sequences were found during searches of global RSV protein or nucleotide sequence databases representing >10,000 sequences of RSV-A and B (BV-BRC database, 2020-2025). This analysis evaluated the in silico alignment of the past 5 years of RSV N-protein sequences within BV-BRC assessed resistance-associated natural variants.

CONCLUSIONS

- RSV immune-naive young children with high viral loads and high numbers of RSV replication cycles associated with zelicapavir exposure represent a scenario in which viral resistance, if occurring, would likely be detectable
- No resistant variants were detected during zelicapavir treatment
- A single resistance-associated minority variant was detected 4 days after drug discontinuation in 2 patients, showed reduced fitness, and was not found in nature
- Zelicapavir demonstrates a high clinical and functional barrier against antiviral resistance, thus supporting ongoing clinical development

DISCLOSURES, ACKNOWLEDGEMENTS, REFERENCES

- All authors are employees and stockholders of Enanta Pharmaceuticals, Inc.
- We extend our thanks to the parents who gave their physicians their trust by participating in this study
- References

1. Hall CB, et al. *N Engl J Med* 2009;360(6):588-598. DOI: 10.1056/NEJMoa0804877.
2. Anderson LJ, Walsh E. N Engl J Med 2022 N Engl J Med 2022;386:696-697. DOI: 10.1056/NEJMe2118465
3. Levene RE, et al. *J Infect Dis.* 2025;231(2):e290-e298. DOI: 10.1093/infdis/jiae471.
4. Harris C, et al. ESCMID Global 2026; April 17-21, 2026; Munich, Germany.
5. Harris C, et al. ID Week 2025; October 19-22, 2025; Atlanta, Georgia, USA.