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EDP-938 Has a High Barrier to Resistance in Healthy Adults Experimentally Infected with Respiratory Syncytial Virus

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Background. EDP-938 is an oral once-daily RSV nucleoprotein (N) inhibitor with potent antiviral activity. In a human RSV challenge trial, EDP-938 significantly reduced viral load and symptom severity. During antiviral development, it is critical to understand the propensity for resistance to develop. In vitro studies of EDP-938 suggest a higher barrier to resistance as compared to RSV fusion inhibitors. We evaluated the development of viral resistance to EDP-938 in a human challenge trial.

Methods. A subset of the 124 participants with RSV infection were chosen for genetic analysis; 159 nasal wash samples from 48 participants were analyzed using next-generation sequencing of the N gene of RSV. Of the 48 participant sampled, 37 were from EDP-938–treated and 11 were placebo-treated participants, representing 45% and 26% of the participants, respectively. The effects of treatment-emergent mutations on viral load, EDP-938 efficacy, and viral fitness were evaluated.

Results. Two of the 37 EDP-938–treated participants with samples sequenced had treatment-emergent mutations: N:L139I and N:E112G. From in vitro analysis, N:L139I reduced sensitivity to EDP-938 by approximately 10-fold, while N:E112G had no effect. However, N:L139I was associated with a reduction in viral fitness, suggesting clinical resistance is associated with fitness costs. Neither of these variants were associated with reduced viral clearance.

Conclusions. In human RSV infections treated with EDP-938, emergence of RSV variants with reduced sensitivity to EDP-938 occurred in only 1 participant and was associated with reduced viral fitness. EDP-938's high barrier to resistance highlights its robust mechanism of action.

Clinical Trials Registration. NCT03691623.

Keywords. RSV; antiviral; antiviral resistance; clinical trials; challenge trial; N inhibitor; EDP-938.

Respiratory syncytial virus (RSV) is a ubiquitous pathogen that infects the respiratory tract. For young children, the elderly, and those with underlying medical conditions, RSV can result in substantial morbidity and mortality [1–4]. While there are prophylactic monoclonal antibodies and vaccines approved for the prevention of RSV [5–7], there is no specific treatment for RSV infection. Aerosolized ribavirin has been used to treat severe RSV infection, but it is not recommended by the American Academy of Pediatrics due to its unclear therapeutic benefits and concerns about teratogenicity and toxicity [8].

EDP-938 is an orally bioavailable RSV nucleoprotein (N) inhibitor currently being studied in 2 phase 2b clinical trials (NCT04816721, NCT05568706). EDP-938 displays potent anti-RSV activity in vitro and was efficacious in a nonhuman primate model of RSV infection [9]. In phase 1 clinical trials, EDP-938 was safe and generally well tolerated [10], prompting its entry into a phase 2a human challenge trial (NCT03691623). Healthy volunteers were inoculated with RSV-A M37b and randomly assigned to an EDP-938 dosing group or placebo cohort. The study was conducted in 2 independent parts. In the first, 115 participants received either EDP-938 at 600 mg once daily, 300 mg twice daily after a 500-mg loading dose, or placebo. In the second part, 63 participants received 300 mg once daily after a 600-mg loading dose, 200 mg twice daily after a 400-mg loading dose, or placebo. In all dosing groups, EDP-938 significantly reduced viral load and symptom score as compared to placebo [11].

A major concern with direct acting antivirals (DAAs) is the development of resistance. Indeed, experimental generation of in vitro resistance to RSV antivirals targeting the fusion protein resulted in generation of fusion protein mutations upon initial

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exposure of 10-fold the 50% maximum effective concentration (EC_{50}) that mediated >1000-fold decreases in potency with minimal effect on viral fitness [12–17]. Resistance to one fusion inhibitor has, to date, mediated cross-resistance to other fusion inhibitors, which is not unexpected because many of them bind in the same region of the fusion protein [14, 18, 19]. The rapid emergence of fusion inhibitor resistance has also been observed in both a human challenge trial and naturally occurring infection [15, 17]. In contrast, the barrier to in vitro resistance for EDP-938, a replication inhibitor, is high, as demonstrated by extensive passaging (approximately 10 generations) below 10-fold of the EC_{50} needed to generate resistant mutants [9]. Much smaller potency shifts (≤70-fold) were observed for EDP-938–resistant mutants with an inverse relationship between resistance and viral fitness [9]. These data suggest that the barrier to clinical resistance is higher for EDP-938 as compared to fusion inhibitors.

To determine the propensity for EDP-938–resistant viruses to emerge in a clinical setting, we performed next-generation sequencing (NGS) of the RSV N gene on nasal swabs from participants enrolled in a human challenge trial. We found only 1 EDP-938–treated participant with a mutation that confers resistance. Thus, EDP-938's barrier to resistance in a clinical setting is relatively high compared to fusion inhibitors, providing a distinct advantage of its mechanism of action.

METHODS

Clinical Study Design and Sample Collection

EDP-938 efficacy and safety was assessed in a randomized, double-blind, placebo-controlled human challenge trial (NCT03691623) [11], conducted by hVivo. Nasal wash samples were collected 1 day prior to challenge, and then twice daily from 2 days after challenge until the morning of study day 12, for a total of 21 nasal washes postchallenge. Upon positive detection of RSV by qualitative polymerase chain reaction (PCR; 3M Integrated Cycle; Focus Diagnostics) or after day 5, participants were randomized into a treatment group. All participants provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki (1996 version), the International Conference on Harmonization Good Clinical Practice guidelines.

Plasmids

The RSV reverse genetics plasmid system was obtained from Dr Marty Moore [20]. Site-directed mutagenesis was performed using NEB Q5 on a shuttle vector containing the N open reading frame (ORF) between the *Avr*II and *Bst*BI sites. Upon sequence confirmation of the mutation, the N ORF region was PCR amplified and gel purified using the Macherey Nagel NucleoSpin Gel and PCR Clean-Up Mini Kit. The pSynkRSV-I19F bacterial artificial chromosome (BAC) was digested with *Avr*II and *Bst*BI and gel purified using the Macherey Nagel NucleoSpin Gel and PCR Clean-Up Mini Kit. The PCR amplified regions were then inserted into the *Avr*II and *Bst*BI site of pSynkRSV-F:I19F using HiFi assembly mix (NEB). All primers are listed in Supplementary Table 1.

Cell Lines

HEp-2 cells (CCL-23) and Vero cells (CCL-81) were obtained from American Type Culture Collection. HEp-2 cells were cultured and infected in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% fetal bovine serum (HyClone), 1% penicillin/streptomycin (Life Technologies), and 1% minimal essential media nonessential amino acids (Life Technologies). Vero cells were cultured in and infected in minimum essential medium (MEM; Life Technologies) supplemented with 10% fetal bovine serum (HyClone). All cell lines were maintained in and all infections were performed at 37°C in 5% CO_2 atmosphere.

Chemical Inhibitors

AK0529 (Ziresovir) and RV521 (Sisunatovir) were purchased from MedChemExpress. All other compounds were synthesized by Enanta Pharmaceuticals, Inc, Watertown, MA.

Viruses

Preparation and recovery of recombinant wild-type and mutant RSV was performed using the BAC-based system described in Hotard et al [20] with the following modifications. The wild-type (WT) or mutant versions of pSynkRSV-I19F along with the 4 helper plasmids and pCDNA3.1-T7 were transfected into Vero cells using Lipofectamine 2000CD (Life Technologies) following the manufacturer's instructions. Cells were expanded and the media was replaced every 2–3 days until the observation of multiple mKATE fluorescent foci. Upon florescence detection in greater than 70% of the monolayer, the virus was harvested. Mutations were confirmed by Sanger sequencing.

Viral stocks were titered by 50% tissue culture infectious dose ($TCID_{50}$) in HEp-2 cells. The presence or absence of mKATE signal was assessed at 7 days postinfection. The Marco-Binder calculator was used to calculate $TCID_{50}$/mL (Marco Binder, University of Heidelberg).

RNA Purification, cDNA Preparation, and PCR

Viral RNA was extracted from viral stocks and purified using the Quick-RNA Viral 96 Kit (Zymo Research). cDNA of the N ORF region of the RSV genome was created with SuperScript IV One-Step RT-PCR (Invitrogen) and Sanger sequenced using the primers listed in Supplementary Table 1.

In Vitro Compound Screen

HEp-2 cells were infected at a multiplicity of infection (MOI) of 0.1 and treated with the indicated compound or dimethyl sulfoxide (DMSO) at the time of infection, without removing the

inoculum. mKATE fluorescent intensity was measured 5 days postinfection using a Molecular Device SpectraMax i3x. Percent viral inhibition was calculated by normalizing mKATE values to DMSO-treated infected cells. EC_{50}/EC_{90} values were calculated using XLFit Dose-Response Model 200.

Viral Fitness Assays

HEp-2 cells were infected at an MOI of 0.1. mKATE fluorescent intensity was measured or cells and supernatant were collected for $TCID_{50}$ analysis at each day postinfection for 7 days, as described above.

Statistical Analysis

All statistical analysis was performed using GraphPad Prism version 9.0 using analysis of variants (ANOVA) followed by a post hoc comparison test. All graphs show mean ± standard deviation. A minimum of 3 biological replicates were conducted for all dose-response and fitness experiments.

Next-Generation Sequencing

NGS methods are in Supplementary Methods.

RESULTS

Two Treatment-Emergent Mutations Were Observed in Samples from the Human Challenge Trial

To assess the emergence of resistance, viral loads from approximately 2480 nasal wash samples collected over 12 study days from the 124 subjects with RSV infection in the human challenge trial were evaluated by reverse transcription quantitative PCR (RT-qPCR) [11]. To maximize the likelihood of sequencing samples with EDP-938 resistance mutations, we employed a selective strategy for determining which participants and which participants' samples to sequence. Samples were sequenced from patients treated with EDP-938 or placebo that had transient increases in viral load or a slow viral decline. The first category included EDP-938–treated participants with additional peaks of viral shedding ≥1 log from their previous nadir on or off therapy. These additional peaks of viral shedding could suggest the emergence of a variant with reduced sensitivity to EDP-938. This group was labeled group A and included 24 participants and 80 samples. The second group were EDP-938–treated participants that did not show a rapid or typical reduction in viral shedding compared to placebo-treated participants. The slower response could be indicative of the presence of a variant(s) with decreased sensitivity to EDP-938. This group was labeled group B and included 11 participants and 34 samples. The last group were placebo-treated participants, of whom some had an additional peak of viral shedding while most were randomly selected. These participants were included as controls to understand viral genotypic variance in the absence of EDP-938 selective pressure. These comprised group C and included 40 samples collected from 11 participants. Participants who had a response to EDP-938 with a consistent decline in viral load upon initiation of treatment followed by subsequently undetectable viral loads (group D) were judged to have the lowest probability of on-treatment detection of EDP-938–resistant variants, and thus were not selected for sequencing. Two participants (10035 and 20002) initially classified with additional peaks of shedding (group A) were reclassified into group D as there was a rapid decline in viral load following initiation of treatment. Nonetheless, 5 samples from these 2 participants underwent sequencing. The viral shedding patterns of all participants selected for sequencing are in Supplemental Figures 1–4. Theoretical viral load patterns that could be observed in each group are outlined in Figure 1*B*. Based on a percentage of the intention-to-treat infected population, the number of participants sequenced represents 45% of the EDP-938–treated participants and 26% of the placebo-treated participants. Group A compromised 65% of sequenced, EDP-938–treated participants, while groups B and D compromised 30% and 5%, respectively.

Specific nasal wash time point samples were chosen to enrich the likelihood of identifying variants with decreased sensitivity to EDP-938. When viral loads supported it, 3 time points were selected for sequencing: a pretreatment baseline, immediately prior to the triggering event (nadir), and immediately after the triggering event (postnadir). This enabled identification of variants and how they changed over the study. The target viral concentration for sequencing was 3.5 $\log_{10}$ copies/mL; however, in cases where samples did not meet the threshold, samples slightly below the cutoff were chosen. In total, 159 samples from 48 participants were chosen for sequencing (Figure 1*A*). Detailed information about samples is in Supplementary Table 2.

As in vitro resistance generation identified mutations in the N protein [9], we performed NGS on amplicons from the N region of the RSV genome. Samples had on average 87 000 reads after trimming and filtering. Detailed information about sequence reads and mapping is in Supplemental Tables 3–5. The input virus also underwent NGS as part of quality assurance mechanisms. As expected, the input virus matched the RSV-A M37b reference genome [21], and no variants were detected at the 1% level (accession number, KM360090). All sequences were aligned and compared with the RSV-A M37b reference genome. Four different SNPs were detected: 3 within the RSV N gene and 1 in the phosphoprotein (P) gene. Two SNPs (N:K5K, A1128G in RSV genome, and P:A5A, T2335A) were silent and present in most samples, including placebo-treated participants, at all time points (Supplementary Figure 5 and Supplementary Table 6). The other SNPs were changes in the N protein. N:E112G (A1448G) was detected in a single post–EDP-938 time point collected prior to the additional peak and was transient as all subsequent samples were WT until RSV was undetected (Figure 1*C*). N:L139I (C1528A) was detected in nasal wash samples collected after EDP-938 treatment on study day 6, AM and study day 7, AM in more than 50% of

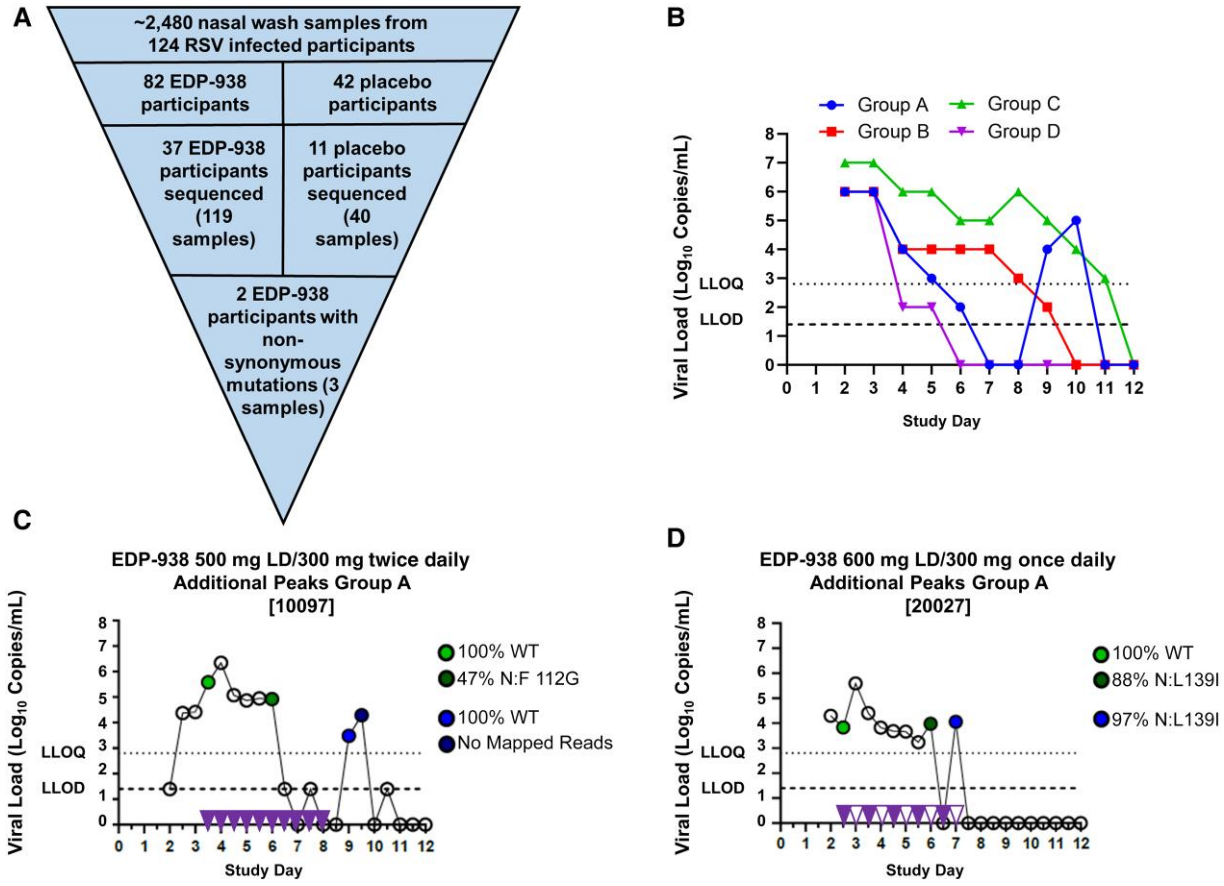


Figure 1. Variants detected in the human challenge trial. *A*, Diagram detailing selection of participants for sequencing analysis. *B*, Theoretical viral load patterns for each group. These data are not from any participants but an example of viral load patterns that could be observed in each group. *C* and *D*, Viral shedding patterns for (*C*) LLOQ and (*D*) N:L139I for each participant from the human challenge trial with treatment-emergent mutations. Shaded triangles indicate EDP-938 dosing. Participant 20027 was assigned to a once daily treatment group. As such, this participant received a placebo for 1 of the 2 daily doses (open triangles). Light dashed line indicates the LLOQ (2.8 $\log_{10}$ copies/mL). Bold dashed line indicates the LLOD (1.4 $\log_{10}$ copies/mL). No mapped reads indicate no sequencing reads were able to be aligned to the RSV M37b reference genome. Abbreviations: LD, loading dose; LLOD, lower limit of detection; LLOQ, lower limit of quantification; N, nucleoprotein; RSV, respiratory syncytial virus; WT, wild type.

reads, indicating that it became the dominant variant (Figure 1*D*). After study day 7, AM, no further RSV was detected, indicating viral clearance (Figure 1*D*). Of the approximately 2480 samples from 124 participants with RSV infection in the study only 3 samples from 2 participants had treatment-emergent nonsynonymous mutations. Neither treatment-emergent mutation impaired viral clearance. Importantly, neither N:E112G nor N:L139I were detected in any pretreatment samples.

N:L139I But Not N:E112G Reduces the Sensitivity of RSV to EDP-938

To determine how N:E112G or N:L139I affect the sensitivity of RSV to EDP-938, we created recombinant RSV strains with these mutations [20]. The $EC_{50/90}$ of EDP-938 against N:E112G was comparable to those values obtained against a recombinant WT virus (Table 1 and Figure 2*A*). However, N:L139I resulted in an approximately 10-fold decrease in both the EC_{50} and EC_{90} of EDP-938 (Table 1 and Figure 2*A*).

We tested these recombinant viruses against AK0529, RV521, and EDP-323. AK0529 is a fusion inhibitor that completed a phase 3 trial in China (AirFLO, NCT04231968), and reduced symptoms and viral loads [22]. RV521 is also a fusion inhibitor that reduced symptom scores and viral loads in a human challenge trial [16]. EDP-323 is a nonnucleoside large (L)-polymerase inhibitor with picomolar in vitro potency and in vivo efficacy in a mouse model of RSV infection [23, 24]. EDP-323 completed phase 1 clinical trials and was generally safe and well tolerated in healthy volunteers [25]. None of the variants decreased the sensitivity of the virus to any of these compounds (Table 1 and Figure 2*B*–*D*).

N:L139I Reduces In Vitro Fitness of RSV

To determine the effects of N:E112G or N:L139I on viral fitness, we analyzed in vitro growth kinetics. As the recombinant viruses express mKATE, we measured the mKATE florescent signal each day following infection for 7 days. Comparing the area

Table 1. Antiviral Activity of Anti-RSV Compounds Against Identified Variants

	WT		N:L139I		N:E112G	
Compound	EC_{50}, nM	EC_{90}, nM	EC_{50}, nM	EC_{90}, nM	EC_{50}, nM	EC_{90}, nM
EDP-938	61 ± 12	91 ± 5	590 ± 69	1075 ± 388	100 ± 33	124 ± 34
EDP-323	0.21 ± 0.04	0.37 ± 0.08	0.14 ± 0.04	0.31 ± 0.03	0.10 ± 0.04	0.14 ± 0.03
AK0529	7 ± 1	25 ± 18	9 ± 6	52 ± 41	2 ± 1	15 ± 8
RV521	0.42 ± 0.32	2.69 ± 2.84	0.37 ± 0.11	1.24 ± 0.80	0.62 ± 0.38	3.97 ± 5.02

For each biological replicate, a 4-parameter logistic regression using the 200 dose-response model in XLFit was applied to the curves in Figure 2 and the EC_{50} and EC_{90} are reported as the mean ± standard deviation from at least 3 independent biological replicates.

Abbreviations: EC_{50}, 50% maximum effective concentration; EC_{90}, 90% maximum effective concentration; WT, wild type.

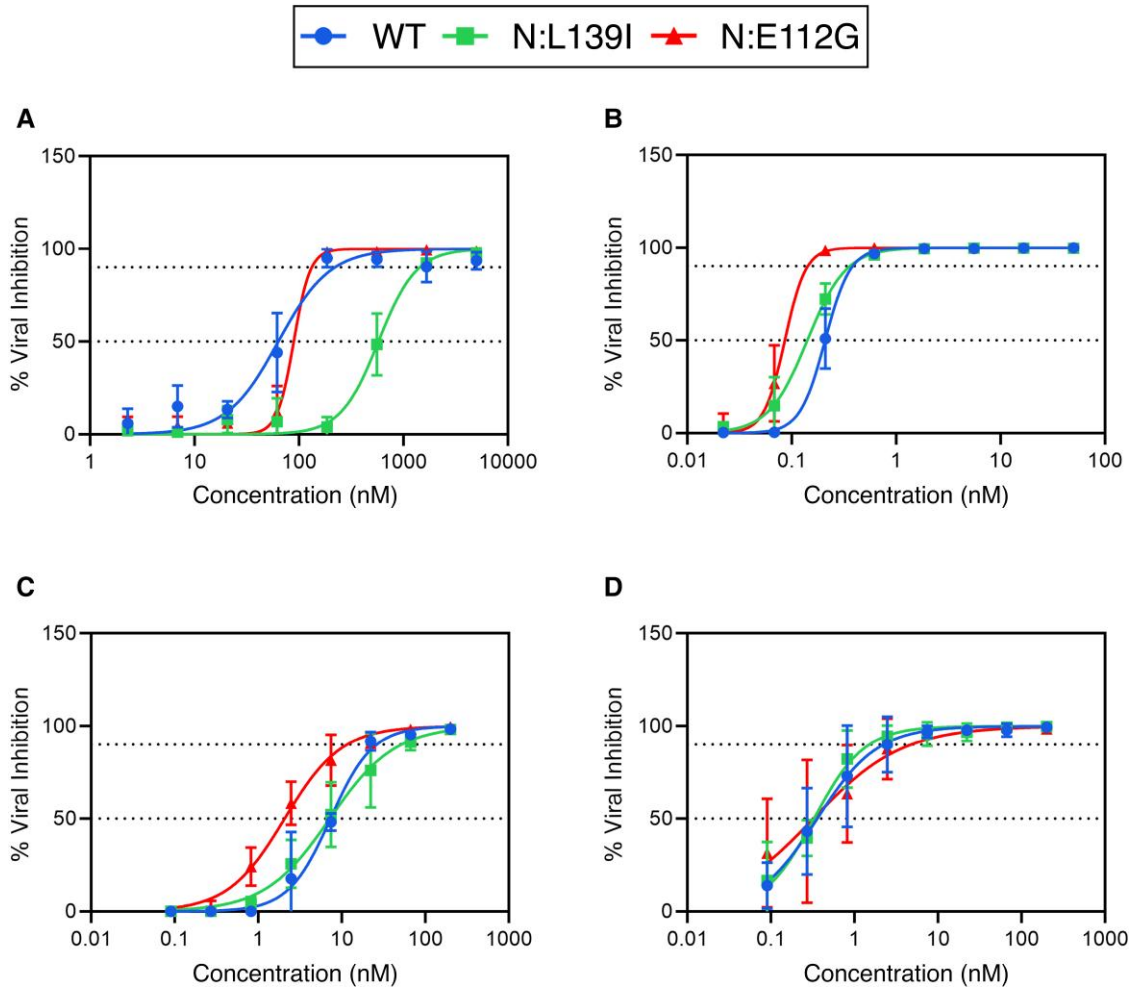


Figure 2. N:L139I mediates resistance to EDP-938. HEp-2 cells were infected with the indicated recombinant strain of RSV-A expressing an mKATE reporter at a multiplicity of infection of 0.1. At the time of infection, cells were treated with the indicated concentration of the listed compound or DMSO vehicle control: (*A*) EDP-938, (*B*) EDP-323, (*C*) AK0529, and (*D*) RV521. Five days postinfection, the mKATE fluorescence intensity was measured. Percent viral inhibition at each concentration was calculated. Data are expressed as the mean percent viral inhibition at each concentration ± standard deviation from at least 3 independent biological replicates. Dotted lines indicate 50% and 90% viral inhibition. Abbreviations: DMSO, dimethyl sulfoxide; N, nucleoprotein; RSV, respiratory syncytial virus; WT, wild type.

under the curve (AUC) of the mKATE signal over 7 days showed that signals were comparable to WT for all mutant viruses (Figure 3*A* and 3*B*). While mKATE signal measures the production of viral mRNA and its subsequent translation, it does not necessarily translate to infectious virus production. Thus, we measured infectious virion production over 7 days. N:L139I took 7 days to achieve its peak viral titer while WT achieved peak titer in 6 days, suggesting a slight in vitro growth

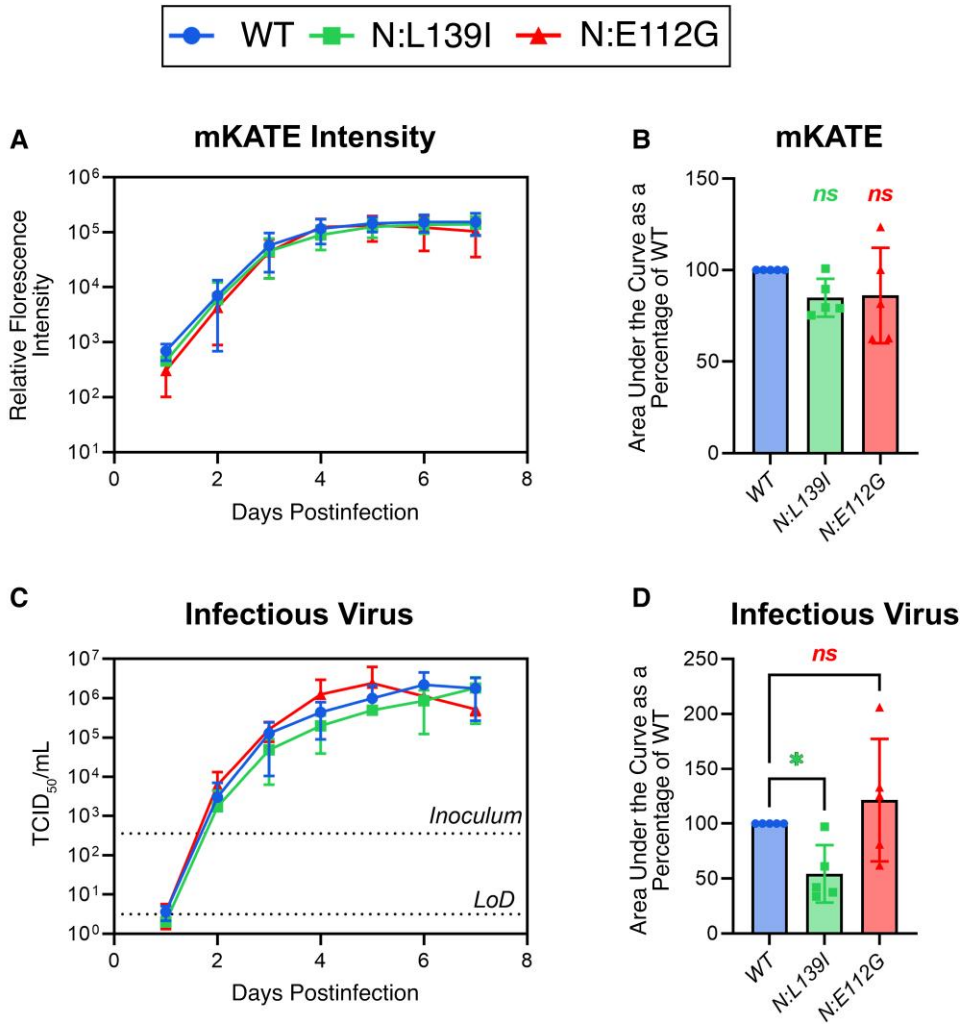


Figure 3. N:L139I has in vitro fitness defects compared to WT. *A* and *C*, HEp-2 cells were infected with the indicated recombinant strain of RSV-A expressing an mKATE reporter at a multiplicity of infection of 0.1. At each day postinfection, mKATE florescence intensity was measured (*A*) or cells and supernatant were harvested and subject to $TCID_{50}$ analysis (*C*). Data are expressed as the mean mKATE florescence intensity (*A*) or $TCID_{50}$/mL (*C*) at each day postinfection ± standard deviation from at least 3 independent biological replicates. LoD was 3.16E + 0 $TCID_{50}$/mL. Values at or below the LoD are set at 2.0E + 0 $TCID_{50}$/mL. *B* and *D*, For each replicate, the AUC was determined using a linear-linear plot. The AUC was normalized to WT and expressed as the percent AUC relative to WT. Data are expressed as the mean ± standard deviation AUC. $^{*}P < .05$ by 1-way repeated measured ANOVA followed by Dunnett multiple comparison test. Abbreviations: AUC, area under the curve; LOD, limit of detection; N, nucleoprotein; ns, not significant; RSV, respiratory syncytial virus; $TCID_{50}$, 50% tissue culture infectious dose; WT, wild type.

delay. The peak titer for N:E112G was obtained 1 day before that of WT. The AUC of the infectious virus produced over 7 days for N:L139I was only approximately 50% of that of the WT (Figure 3*C* and 3*D*), further suggesting reduced viral fitness for N:L139I. No significant changes in AUC were observed for N:E112G (Figure 3*C* and 3*D*). These data demonstrate that resistance to EDP-938 is associated with reduced viral fitness.

DISCUSSION

Like all antimicrobials, generation of antiviral resistance is a major concern. We report that only 1 EDP-938–treated participant out of the 37 sequenced (approximately 3%) had a treatment-emergent mutation associated with resistance (Figure 1, Figure 2, and Table 1). Additionally, in vitro characterization of mutant viruses suggests that resistance is associated with reduced fitness (Figure 2 and Figure 3). Moreover, clinical resistance to EDP-938 was not associated with resistance to other classes of inhibitors (Figure 2). Lastly, resistance to EDP-938 did not impair viral clearance, as all remaining virus was cleared shortly after detection of N:L139I (Figure 1).

We identified a single treatment-emergent resistant mutant, N:L139I, which was also identified in preclinical resistance studies of RSV-604 [26], another RSV N inhibitor, whose clinical development was discontinued [27]. N:L139I resulted in an approximately 7-fold decrease in in vitro RSV-604 potency

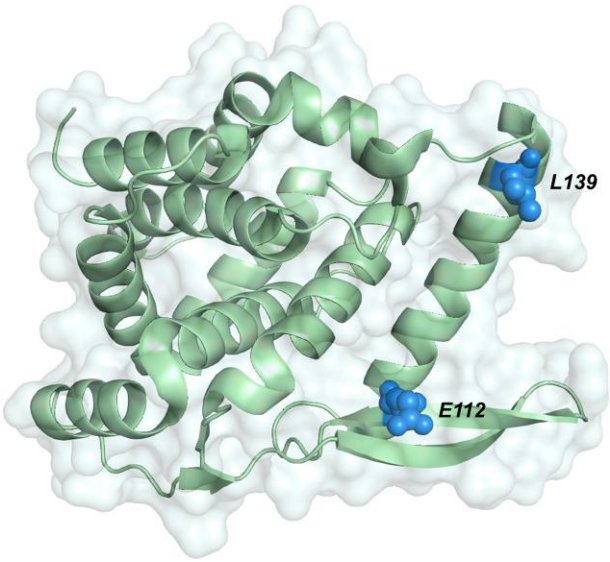


Figure 4. Mapping of observed mutations on the nucleoprotein (N) crystal structure. N crystal structure [9] (Protein Date Bank file No. 4UC6) of amino acids 32–251 containing all observed mutations in this region. Observed mutated residues are shown as spheres.

[26]. Similar results were obtained for EDP-938 (Table 1). N:L139I was not detected in EDP-938 preclinical resistance generation. Nonetheless, N:L139I confers reduced viral fitness as it reduced both the AUC of infectious virus and increased the time to peak titer (Figure 3). While there was no significant change in mKATE intensity (Figure 3), this metric is not an indication of infectious virus. In contrast, this variant was rapidly cleared in the clinical setting (Figure 1) in an otherwise healthy participant that can mount an effective immune response, further suggesting reduced viral fitness. Nonetheless, the isolation of N:L139I in 2 independent N-inhibitor studies suggests the need for continued vigilance in clinical development.

There appeared to be a complete loss of viral shedding in participant 20027 between the 2 detections of N:L139I (Figure 1). The lack of detection could be due to sampling variation, the dilution of the sample associated with nasal washing, and/or remnants of noninfectious viral RNA released from dead or dying infected cells. However, it is also possible that infectious virus below the limit of detection at study day 6, PM was rapidly replicated to near baseline levels by study day 7, AM. The possibility of a multi-log increase is unlikely, especially given N:L139I's reduced viral fitness (Figure 3). The percentage of reads that were N:L139I only changed approximately 10% from study day 6, AM compared study day 7, AM (Figure 1) with less than a log change in viral load, suggesting there was not infectious virus below the limit of detection on study day 6, PM. Most of the groups A and D show similar patterns to participant 20027, that is a time point with lack of detection sandwiched between 2 time points with similar viral loads (Supplemental Figures 1–4). The additional peak could be the result of sampling variation, especially when viral loads are just barely above the limit of detection/quantification and/or release of noninfectious viral RNA from dead or dying cells. Given that the goal with participant selection for sequencing was to maximize the detection of resistance, we likely overclassified participants into groups associated with additional peaks of shedding or a reduced response to not miss variants. Nonetheless, this overclassification also emphasizes the relatively low occurrence of treatment-emergent mutations when compared to Presatovir, where more than 20% of participants had treatment-emergent mutations in a human challenge trial [15].

The 2 observed nonsynonymous mutations are located near where the P proteins are hypothesized to interact with N [9, 28, 29] (Figure 4, right). In EDP-938 in vitro resistance generation studies, most of the identified mutations were also in this area, although many did not mediate meaningful changes in antiviral potency [9]. It is unlikely that EDP-938 disrupts N-P interactions, as EDP-938 has no effect on either mRNA transcription or genome replication in a minigenome system [9] and the similar RSV inhibitor, RSV-604, had minimal effect in a replicon assay [30]. It is currently hypothesized that EDP-938 exerts its antiviral activity either directly on the N protein or through a cellular protein [9]. Further elucidation of the mechanism of action of EDP-938 will enable determination of the role of these residues, especially with respect to N:L139I.

Our results highlight EDP-938's higher barrier to resistance relative to fusion inhibitors, differentiating EDP-938 from other small-molecule RSV therapies in clinical development. In a clinical trial setting, development of resistance to fusion inhibitors is well documented. In a human challenge trial, approximately 28% of Presatovir-treated participants had treatment-emergent mutations, with 17% of Presatovir-treated participants having resistance-associated amino acids [15]. We did not sequence every participant and are unable to determine the exact percentage of participants with treatment-emergent mutations. However, in a population preselected to have higher likelihood of resistance mutations, only 2 of 37 or 5% of EDP-938–treated participants had treatment-emergent mutations and only 1 (approximately 3%) reduced the sensitivity of the virus to EDP-938, which is less than one-fifth the number of Presatovir-treated participants. Another key difference is that Presatovir-resistant variants were associated with higher viral load AUCs versus nonresistant variants [15]. These data are in direct contrast to EDP-938, where the resistant variant was rapidly cleared (Figure 1). Together these data highlight a key advantage of EDP-938 over fusion inhibitors.

EDP-938's improved barrier to resistance relative to that of fusion inhibitors may be explained by its mechanism of action. EDP-938 targets the nucleoprotein, the most conserved viral protein [31]. Mutations that drastically decrease the susceptibility of the virus to EDP-938 may not be tolerated as they could disrupt

an essential function(s) of N. The low mutability of N may account for EDP-938's observed high barrier to resistance, which is in direct contrast to fusion protein targeted therapies. The fusion protein is mutable and associated with sequence variability within the antigenic sites [32], suggesting an ability to adapt to antibody responses, including palivizumab. Palivizumab-resistant mutants have been recovered from 5%–10% of children hospitalized with RSV breakthrough infections [33–37]. The same adaptability likely applies to DAAs that target the F protein, including Presatovir.

Collectively, our results demonstrate EDP-938's high barrier to resistance in a clinical setting, differentiating EDP-938 from other RSV DAAs. It will be critical to evaluate resistance development in the background of pediatric and immunocompromised patients, who have longer durations of viral shedding and may not be able to adequately mount of a complete immune response [38].

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. R. E. L., J. D., A. L. C., A. A., Y. S. O., and M. H. J. R. contributed study conception. R. E. L. and hVIVO collected data. R. E. L., J. D., A. L. C., A. A., and M. H. J. R. analyzed data. J. D., A. L. C., A. A., Y. S. O., and M. H. J. R. contributed supervision. R. E. L. wrote the manuscript. All authors reviewed the manuscript.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Data availability. Data available upon request.

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