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Analysis of Presatovir (GS-5806) Resistance Emergence in Human Healthy Adult Subjects Experimentally Infected With Respiratory Syncytial Virus

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Introduction

- Presatovir is a potent, small-molecule respiratory syncytial virus (RSV) fusion inhibitor with activity against a broad spectrum of RSV A and B clinical isolates
- The efficacy of presatovir was evaluated in a randomized, double-blind, placebo-controlled, Phase 2a viral challenge study in healthy adults (aged 18–45 years) experimentally infected with RSV Memphis 37b (NCT01756482)[1]

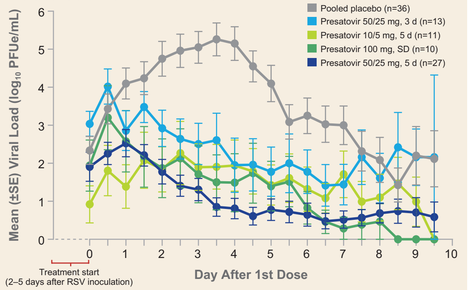
Presatovir Challenge Study Design*

Subjects/Cohort Presatovir	Subjects/Cohort Placebo	Cohort Presatovir mg/d or placebo					
39	39	1–4 50/25 mg, 5 d	50	25	25	25	25
17	5	5 50/25 mg, 3 d	50	25	25		
14	4	6 100 mg, SD	100				
17	5	7 10/5 mg, 5 d	10	5	5	5	5

*Subjects were randomized into 7 sequential treatment cohorts. Subjects with low serum RSV neutralizing antibody titers were inoculated via intranasal administration with ~4 $\log_{10}$ plaque-forming unit equivalents (PFUe) of RSV Memphis 37b. Subjects were monitored for RSV in nasal wash samples by qualitative reverse-transcription–polymerase chain reaction (RT-PCR) assay and were randomized into cohorts when RSV-positive or 5 days after inoculation. SD, single dose.

- In all cohorts, presatovir treatment reduced viral load from nasal wash samples, total mucus weight, and symptom scores compared with placebo

Posttreatment Viral Load*



*Viral load measured by quantitative RT-PCR assay was plotted from start of treatment to end of quarantine period (Day 12). SE, standard error.

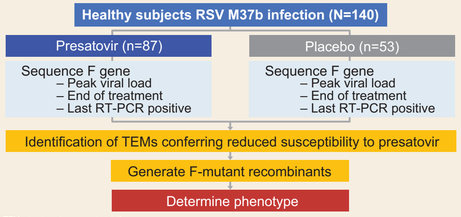
- No significant adverse events were reported
- Presatovir is currently being evaluated in several Phase 2a studies in adults with natural RSV infection (NCT02254421, NCT02254408, NCT02534350, and NCT02135614)
- Here we present the results of drug-resistance emergence analyses in subjects enrolled in the Phase 2a RSV challenge study

Objectives

- To identify and characterize treatment-emergent presatovir-resistant RSV variants

Methods

Resistance Sampling

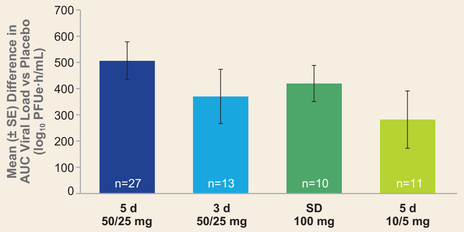


TEMs, treatment-emergent mutations.

- RSV sampling: emergence of drug resistance analyzed by population sequencing of RSV from nasal wash samples taken at viral load peak, end of dosing, and last day of RSV detection
- RSV F-gene sequencing
  - Viral RNA extracted from nasal wash samples using SV 96 Total RNA Isolation System (Promega Corporation, Madison, WI)
  - RSV F gene amplified by QIAGEN OneStep RT-PCR Kit (QIAGEN, Valencia, CA)
  - Nucleotide sequence of RSV F gene determined by Sanger sequencing
- RSV recombinant virus production
  - Point mutations representing individual TEMs introduced by site-directed mutagenesis into plasmid clones encoding wild-type (WT) RSV F protein
  - Recombinant mutant RSV strains expressing individual TEMs constructed by transferring mutant RSV F gene into RSV genome using modified RSV reverse-genetics methodology[2]
- Phenotypic analysis
  - Susceptibility of RSV recombinants containing TEMs related to RSV Memphis 37b determined in RSV cell-based antiviral enzyme-linked immunosorbent assay (ELISA)
  - HEp-2 cells ($3x10^3$ cells/well) infected with RSV 100 µL ($1x10^{4.5}$ tissue infectious doses/mL) in media in presence of serial dilutions of presatovir
  - Virus replication quantified 4 days postinfection by RSV-specific ELISA using RSV F-protein-specific murine monoclonal antibody
- Competitive fitness assay
  - Mixtures of WT and TEM-expressing RSV passaged 3 times in HEp-2 cells
  - Each virus contained a silent-marker sequence in the N gene that was recognized by unique TaqMan® probes
  - The proportions of WT and presatovir-resistant variants were determined by a multiplexed quantitative RT-PCR assay using probe pairs with minimal spectral overlap to quantify the level of WT or resistant variants

Results

Exposure-Driven Antiviral Activity of Presatovir*



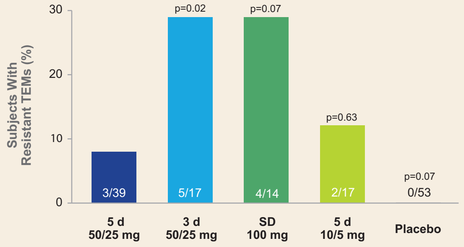
Plasma Presatovir, ng/mL	5 d 50/25 mg	3 d 50/25 mg	SD 100 mg	5 d 10/5 mg
C_{24h}	209	200	396	36.6
C_{48h}	236	216	209	40.8
C_{72h}	238	223	113	40.2
C_{96h}	246	154	68.0	43.5
C_{120h}	250	94.1	39.8	44.1

>4-fold EC_{95}† | 1–3 fold EC_{95}† | < EC_{95}†

*Area under curve (AUC) viral load analysis restricted to subjects who were RSV positive within 5 days postinoculation; †95% maximal effective concentration (EC_{95}) for RSV Memphis 37b. C, concentration.

- Exposure level of presatovir above EC_{95} for RSV Memphis 37b correlated with antiviral response

Resistance Frequency*



	5 d 50/25 mg	3 d 50/25 mg	SD 100 mg	5 d 10/5 mg	Placebo
TEMs conferring resistance (EC_{50} ≥3-fold WT value)	F140L T400I I474T	T138I T323A T400I D486N D488L	D127A, F140L L141F/T400I S398L K399N	L141W D386N/T400I	
TEMs not conferring resistance (EC_{50} <3-fold WT value)	R133G I291V V360A T423I S509I	M396I D338Y		S215T T397S G566S	N63D I153F V379L F483S

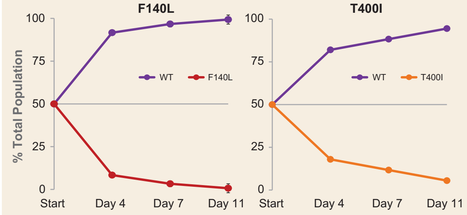
*Calculated by dividing number of subjects with resistant variants by total number of presatovir-treated subjects. p-values for each cohort are relative to 5d (50/25 mg) cohort. EC_{50}, half maximal effective concentration.

Antiviral Susceptibility of RSV Variants Expressing TEMs Associated With Presatovir Resistance*

	Presatovir	Palivizumab†	Ribavirin
WT RSV A2 EC_{50}, µM	0.0002 ± 0.0001	0.23 ± 0.27	12.41 ± 5.04
Resistant variant	EC_{50} shift relative to RSV A2		
L138I	>200	0.7	0.8
V127A, F140L‡	>200	0.1	0.7
F140I	>200	0.9	1.5
L141F	>200	0.6	0.7
L141W	>200	1.5	1.2
L141F/T400I§	>200	0.5	1.2
T323A	5.0	0.5	0.5
D338Y	2.9	0.8	0.8
S398L	>200	0.3	0.3
K399N	38	0.6	0.6
T400I	>200	1.3	1.2
T400V	>200	1.5	1.2
T400A	>200	3.3	1.8
I474T	3.2	0.7	1.2
D486N	>200	0.8	1.3

*Data represent mean of ≥3 experiments; †Values in µg/mL; ‡Mutants genetically linked; §Mutants not genetically linked.

Competitive Fitness Assay



- Competitive fitness assays demonstrated WT RSV outcompetes presatovir-resistant variants in mixed-infection assays

Clinical Response to Presatovir in Subjects With TEMs Associated With Presatovir Resistance

Clinical Parameter/ Endpoint*	Subjects Treated With Presatovir: With Resistant Mutations n=15	Subjects Treated With Presatovir: Without Resistant Mutations n=72	p-Value
Viral load, $\log_{10}$ PFUe x h/mL	784 (189)	149 (191)	<0.001
Symptom score, x h	152 (204)	181 (300)	0.72
Mucous weight, g x h	11.2 (18.8)	11.7 (15.3)	0.93

*Values in parentheses are standard deviations.

Conclusions

- Treatment-emergent mutations (TEMs) conferring reduced susceptibility to presatovir were identified in the F gene of several viral isolates from subjects experimentally challenged with RSV and treated with presatovir
- The frequency of resistance to presatovir was lowest for the 5 day 50/25-mg treatment group
- Subjects harboring presatovir-resistant variants had higher AUC viral load values than subjects with WT virus
- Clinical significance of TEMs is unknown: no significant differences were observed in clinical symptom scores or mucous weights between subjects with presatovir-resistant variants and WT virus
- RSV recombinants with reduced susceptibility to presatovir remained sensitive to inhibition by palivizumab and ribavirin
- The most frequently observed presatovir-resistant TEMs (F140L and T400I) conferred reduced in vitro replicative fitness of RSV
  - In vivo assessment of replication capacity is in progress

References

1. Devincenzo J, et al. *N Engl J Med* 2014;71:711-22; **2.** Collins PL, et al. Proc Natl Acad Sci USA 1995;92:11563-7.

Acknowledgments

We extend our thanks to the patients and their families, and to Michael Miller and Hongmei Mo for advice and critical review of the data for the RSV resistance analysis program.
This study was funded by Gilead Sciences, Inc.

Presented at IDWeek™ 2015, October 7–11, 2015, San Diego, CA