EP-023938, A NOVEL NON-FUSION REPLICATION INHIBITOR OF RESPIRATORY SYNCYTIAL VIRUS (RSV)

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ABSTRACT

◆ **BACKGROUND:** RSV is one of the most prevalent community-acquired respiratory viruses. Currently there are no approved therapies for adult RSV and limited therapy options for infants. There is a high unmet need for better treatment or prophylaxis of RSV infections.

◆ **METHODS:** Cyto-protection and qRT-PCR assays were run utilizing multiple clinical isolates, RSV-A and -B using HEp-2 or HBEC (RSV-A), or A549 (RSV-B) cells infected at 0.1 or 0.5 MOI respectively. Protein binding effect was assessed by adding 40mg/mL Human Serum Albumin (HSA). Cytotoxicity was determined in HEp-2, A549, CHO, and HEK-293 cells. In time-of-addition assays, cells were infected at 0.1 MOI and drugs were added at 0, 2, 6, and 24 hours post infection (hpi). Combination studies for EP-023938 were conducted with fusion inhibitor GS-5806, L-inhibitor AZ-27, or nucleoside ALS-8112. Resistant virus was selected through serial passage of RSV-A with increasing concentrations of compounds.

◆ **RESULTS:** Lead optimization efforts resulted in the identification of EP-023938 as a novel non-fusion inhibitor of RSV. EP-023938 inhibited RSV-A, RSV-B and clinical isolates with an EC_{50} < 200nM, with only a 3-fold shift in the presence of HSA. No significant cytotoxicity was observed (CC_{50} > 25µM). In time-of-addition studies, compound activity was observed 24 hpi. Combinations with GS-5806, AZ-27, or ALS-8112 were additive to moderately synergistic. EP-023938 displayed a high barrier to resistance, while maintaining activity against GS-5806-resistant virus.

◆ **INTERPRETATION:** EP-023938 is a potent inhibitor of RSV, maintaining antiviral activity post infection while presenting a high barrier to resistance. With a minimum effect from protein binding and demonstrated synergy with inhibitors of other mechanisms, EP-023938 is a promising therapeutic in the fight against RSV.

RESULTS

TABLE 1. *In vitro* antiviral activity: EC_{50} (nM)

	Virus	Cell Type	Readout	Compound: EP-023938 Non-Fusion	RSV-604 N-inhibitor	GS-5806 Fusion inhibitor	AZ-27 L-inhibitor (Non-nuc)	ALS-8112 L-inhibitor (Nuc)
Laboratory RSV Strains	RSV-A Long	HBEC	(qRT-PCR)	3.4 ± 2.9	390 ± 120	1.1 ± 0.7	2.3 ± 1.8	284 ± 93
		HEp-2	(qRT-PCR)	94 ± 10	1,906 ± 377	3 ± 1	32 ± 0	28,554 ± 846
			(ATPlite)	53 ± 12	1,451 ± 685	0.72 ± 0.18	8.8 ± 1.2	4,298 ± 1,569
	RSV-A2	HEp-2	(ATPlite)	50 ± 0.6	973 ± 51	< 1	5.8 ± 0.08	674 ± 93
	RSV-B Washington	A549	(ATPlite)	72 ± 7	1,164 ± 681	0.49 ± 0.22	1,297 ± 45	25,080 ± 3,259
Clinical RSV Isolates	RSV-A 629-Q0284	HEp-2	(qRT-PCR)	85 ± 4	2,072 ± 52	3 ± 1	39 ± 10	20,969 ± 1,119
	RSV-A 629-8-2			88 ± 5	2,028 ± 132	2 ± 0.5	44 ± 7	16,530 ± 9,809
	RSV-A 629-2/0607	HEp-2	(ATPlite)	48 ± 2	1,092 ± 186	< 1	14 ± 0.8	790 ± 31
	RSV-A 629-9-2			53 ± 4	3,702 ± 237	< 1	40 ± 1	1,486 ± 49
	RSV-A 121301018			185 ± 95	9,221 ± 5,022	3 ± 0.5	14 ± 2	8,803 ± 1,887
	RSV-A2 Tracy			73 ± 9	1,507 ± 137	1 ± 0.2	11 ± 0	4,312 ± 1,281
	RSV-A 79223			43 ± 9	676 ± 15	0.3 ± 0	3.8 ± 0.1	328 ± 7
	RSV-A 79309			51 ± 5	1,139 ± 32	1.1 ± 0.2	8.5 ± 0.9	12,093 ± 932
	RSV-A 80189			88 ± 16	1,425 ± 112	1.2 ± 0.3	10 ± 2	5,051 ± 664
	RSV-A 37425			63 ± 4	955 ± 37	0.6 ± 0.1	24 ± 0.2	2,190 ± 1,050
	RSV-A 61245			98 ± 9	2,236 ± 45	1.5 ± 0.4	5.3 ± 1.1	3,408 ± 387
	RSV-A 79365			47 ± 10	812 ± 120	0.46 ± 0.08	6.8 ± 0.7	6,014 ± 355
	RSV-A 79303			85 ± 24	1,791 ± 473	1.4 ± 0.3	11 ± 2	5,484 ± 1,791
	RSV-A 121301343			53 ± 7	1,033 ± 18	1 ± 0.3	3.9 ± 1	1,628 ± 133
	RSV-B 629-24/2007	A549	(qRT-PCR)	81 ± 5	502 ± 34	0.79 ± 0.06	> 128	239 ± 28
	RSV-B 57097	HEp-2	(ATPlite)	83 ± 22	968 ± 38	0.41 ± 0.04	>250	1,067 ± 278
	RSV-B 61138			163 ± 10	1,694 ± 30	0.85 ± 0.1	>250	2,885 ± 312
	RSV-B 60567			66 ± 16	637 ± 124	0.14 ± 0	>250	645 ± 120
	RSV-B 65859			138 ± 11	1,756 ± 40	1.0 ± 0.2	>250	1,860 ± 265
	RSV-B 60188			152 ± 13	1,444 ± 105	1.1 ± 0.3	>250	2,701 ± 545
	RSV-B 61736			82 ± 7	978 ± 83	0.46 ± 0.06	>250	600 ± 53
	RSV-B 65848			190 ± 21	1,727 ± 221	6 ± 9	>300	2,111 ± 121
	RSV-B 79222			98 ± 10	1,030 ± 60	0.3 ± 0.06	235 ± 38	1,493 ± 261
	RSV-B 121301314			96 ± 12	1,074 ± 86	0.79 ± 0.09	>250	1,287 ± 54
	RSV-B 80145			179 ± 9	2,091 ± 1	0.86 ± 0.2	> 250	6,894 ± 845

- Single digit nM EC_{50} against RSV-A in primary Human Bronchial Epithelial Cells (HBEC)
- Strong antiviral activity below 200 nM across all strains of RSV tested; interrogated with both ATPlite (cell viability) and qRT-PCR (viral load)
- ***Acknowledgements***: Dr. Pedro Piedra at Baylor University and Dr. Kelly Henrickson at Medical College of Wisconsin for supplying the RSV-A and –B clinical isolates

TABLE 2. *In vitro* activity of EP-023938 not significantly affected by protein-binding

	Virus	Readout	Compound: EP-023938 Non-Fusion	RSV-604 N-inhibitor	GS-5806 Fusion inhibitor	AZ-27 L-inhibitor (Non-nuc)	ALS-8112 L- inhibitor (Nuc)
EC_{50} fold increase in the presence of 40mg/mL HSA	RSV-A Long	(ATPlite)	2.4	3.5	3	4	1.7
	RSV-B Wash.		3	3.4	12	-	> 2.0
	RSV-A2	(ATPlite)	3.5	7.3	-	-	-
		(qRT-PCR)	3	7.5	-	-	-

- Physiological level of Human Serum Albumin (HSA) ranges from 30-50 mg/mL
- EP-023938 EC_{50} shifts are comparable to or less than other RSV inhibitors due to protein binding

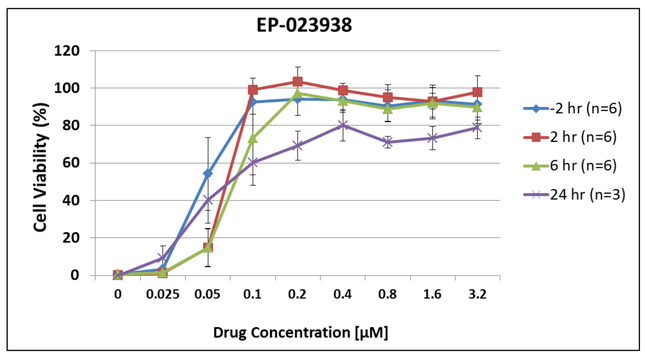
TABLE 3. *In vitro* cytotoxicity:

Cell Line	Cell Type	EP-023938 CC_{50} (µM)
HEp-2	Human Epithelial (HeLa contaminated)	> 50
A549	Human lung	> 50
CHO	Chinese Hamster Ovary	26.9
HEK-293	Human Embryonic Kidney	> 50

- Minimum cytotoxicity observed, yielding an *in vitro* selectivity index (SI, CC_{50}/EC_{50}) of >1000

RESULTS CONTINUED

FIGURE 1. *In vitro* time-of-addition study



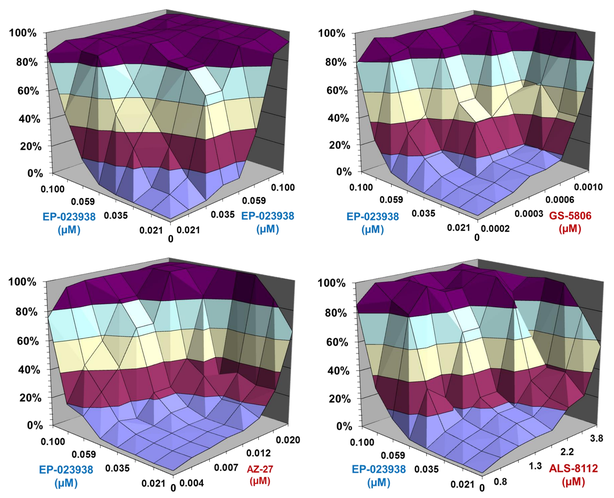
- **EP-023938 demonstrates significant antiviral potency even when dosed 24 hours following infection with virus**
- *Method Notes*:
  - Hep-2 cells were seeded at -24 hours
  - 0.1 MOI RSV-A Long used to infect for 1 hour prior to removal of virus
  - Assay ran 5 days post infection with ATPlite used to measure cell viability

TABLE 4. EC_{50} (nM) and fold shift values against drug resistant RSV-A strains

	Type	DMSO (WT RSV-A)	Drug Resistant (^{R}) RSV-A Virus Used: EP-023938^R 16X EC_{50}	Fold Change	AZ-27^R 312X EC_{50}	Fold Change	GS-5806^R 14,236X EC_{50}	Fold Change
EP-023938	Non-Fusion	53 ± 5	250 ± 53	5	68 ± 8	1	< 100	< 2
ALS-8112	L	28,978 ± 9,487	19,123 ± 3,790	1	> 50,000	> 2	698 ± 13	0.02
AZ-27		19 ± 2	29 ± 5	2	> 20,000	> 1,060	5 ± 1	0.3
GS-5806	F	5 ± 0.4	2 ± 0.6	0.4	6 ± 0.3	1	> 200,000	> 40,000
MDT-637		4.9 ± 2	-	-	-	-	> 50,000	> 10,203
BMS-433771		40 ± 11	-	-	-	-	> 50,000	> 1,250
AZD-4316		1.0 ± 0	-	-	-	-	> 50,000	> 50,000

- **Viral resistance to the fusion inhibitor GS-5806 results in loss of efficacy for all other fusion inhibitors tested**
- **EP-023938 shifts only 5-fold against the highest drug resistance developed while maintaining activity against other drug resistant viral strains**
- Resistance mutations are currently being sequenced and confirmed
- *Method Notes*:
  - Drug resistance to EP-023938 was only achievable when selection was started at 1X EC_{50} followed by multiple passages, increasing drug concentration 2-fold each time.
  - Multiple attempts were unsuccessful at passaging EP-023938-resistant virus above 16X EC_{50}
  - Virus was repeatedly lost when primary selection utilized EP-023938 at 4X EC_{50} or above
  - Drug resistance to AZ-27 and GS-5806 were commenced with multiple passages at 10X EC_{50} and rapidly progressed to the EC_{50} multiples listed above
  - 0.1 MOI used for all viruses
  - DMSO, EP-023938^R, AZ-27^R used qRT-PCR readout; GS-5806^R used ATPlite readout

FIGURE 2. Combination analysis: % viral inhibition curves



- Viral inhibition quantified using ATPlite to measure cell viability
- EP-023938 and other companies' drugs were tested in combination with each other or by themselves
- Curves show good signal resolution across the concentrations tested allowing for accurate combination index calculation

TABLE 5. Combination Analysis: Loewe additivity model

Compounds	Avg. Combination Index (CI) at EC_{50}	EC_{75}	EC_{90}	EC_{95}	Avg.
EP-023938 + EP-023938	0.8	0.8	0.9	0.9	**0.9**
EP-023938 + ALS-8112	0.7	0.6	0.5	0.4	**0.6**
EP-023938 + AZ-27	0.8	0.6	0.5	0.4	**0.6**
EP-023938 + GS-5806	0.9	0.7	0.6	0.5	**0.7**

CI <0.9 = synergy
CI >1.1 = antagonism
CI 0.9 - 1.1 = additivity

- The control of EP-023938 dosed with itself gives an additive effect, as expected
- EP-023938 dosed with other companies' drugs resulted in observed mild synergy, especially in the higher end of the EC curve

CONCLUSIONS

- **EP-023938** is a potent inhibitor of both RSV-A and RSV-B, maintaining antiviral potency across all clinical isolates tested
- Displays single digit nM potency in the highly relevant primary Human Bronchial Epithelial Cell (HBEC) line
- Inhibits viral growth even when administered post infection
- Low cytotoxicity, with a selectivity index of >1000
- A high barrier to resistance while maintaining full potency against viruses resistant to other companies' compounds
- Synergy is observed when EP-023938 is dosed in combination with inhibitors utilizing alternative mechanisms of action