SARS-CoV-2 Antiviral Activity of Zotatifin, a Host Targeting eIF4A Inhibitor

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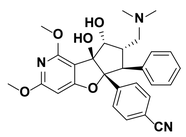
Background

- **Zotatifin (eFT226) is a potent and selective inhibitor of eukaryotic initiation factor 4A (eIF4A), a host ribonucleic acid (RNA) helicase required for SARS-CoV-2 replication**
- **Zotatifin was developed using a ligand-based design strategy[1] based on the flavagline class of natural products (e.g., silvestrol and rocaglamide A) which have broad spectrum activity against a variety of RNA viruses including coronaviruses, picornaviruses, Ebola, etc.**
- **Zotatifin selectively inhibits translation of RNA containing specific short polypurine motifs in their extended 5'- untranslated region (5'-UTR)[1]**
- **Two such highly conserved motifs are found in the SARS-CoV-2 extended 5'-UTR[2]**
- **Zotatifin is currently being evaluated in a Phase 1b dose escalation study in 36 patients with mild to moderate COVID disease (CROI poster #545)**

Figure 1. Zotatifin Is a Sequence-Specific Translational Repressor

A.



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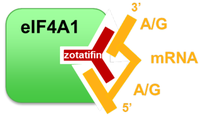

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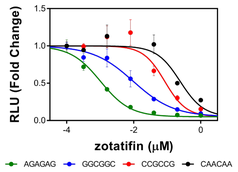
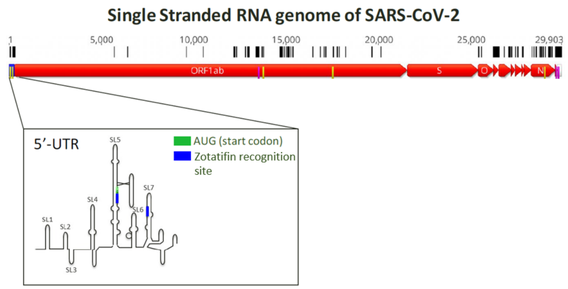


5'-UTR Sequence	Zotatifin IC_{50} (nM)
AGAGAG	1.5 ± 0.4
GGCGGC	13.8 ± 2.0
CCGCCG	92 ± 18
CAACAA	218 ± 55

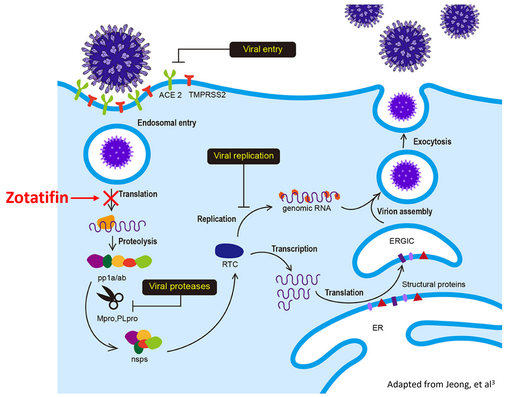
A) Chemical structure of zotatifin. **B)** Schematic of the ternary complex interactions [eIF4A-zotatifin-mRNA]. **C)** Luciferase reporter gene constructs containing 5'-UTRs with 6-mer sequence motif repeats were transiently transfected into the MDA-MB-231 cell line and treated with increasing concentrations of zotatifin for 4 hr.

Figure 2. Zotatifin Recognition Sites in the SARS-CoV-2 RNA



The extended 5'-UTR of the viral RNA is highly structured with multiple stem loops and contains two zotatifin recognition sites (blue boxes) in close proximity to the AUG start codon (green box)[2].

Figure 3. SARS-CoV-2 Life Cycle and Stage of Intervention



Adapted from Jeong, et al[3]

Objective

We evaluated the selectivity of zotatifin's inhibition of SARS-CoV-2 translation, the antiviral activity of zotatifin alone against different human coronaviruses and the antiviral activity of zotatifin in combination with other antivirals against SARS-CoV-2.

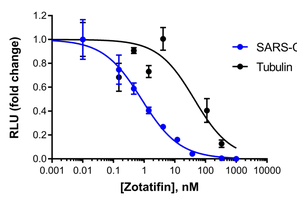
Results

Figure 4. Zotatifin Selectively Inhibits SARS-CoV-2 Translation

A.

Gene	5'-UTR Sequence
SARS-CoV-2	ATTAAAGGTTTATACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTG TTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTC ACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTT CTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTTC GTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGAAA ACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTT TGGAGACTCCGTGGAGGAGGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGCACT TGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTT CATC
Tubulin	AGTTCTCACTGAGACCTGTCACCCCGACTCAACGTGAGACGCACCGCCCGGACTCACC

B.



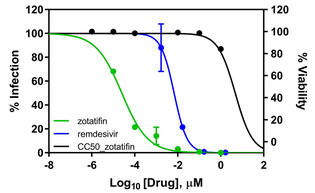
Target Gene	IC_{50}* (nM)
SARS-CoV-2	1, 5
Tubulin	43

*IC50 values from 2 independent experiments (SARS-CoV-2) or one experiment (tubulin)

A) 5'-UTR sequences in the mRNAs of SARS-CoV-2 and tubulin; cyan, zotatifin recognition sites. **B)** Relative translation was measured by luciferase expression in an in vitro luciferase reporter assay. The luciferase reporter gene constructs were transiently transfected into HEK293 cells and then treated with zotatifin for 4 hours.

Results (cont'd)

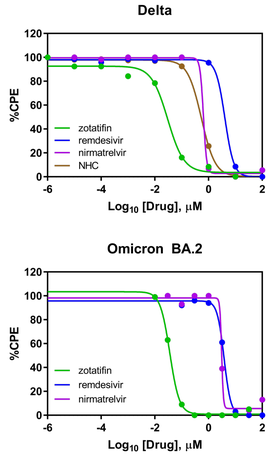
Figure 5. Zotatifin Has Potent Antiviral Activity Against SARS-CoV-2 With High Selectivity



Compound	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)
Zotatifin	0.16	1.4	>1000
Remdesivir	4.6	28	>1000

Antiviral activity of zotatifin was evaluated against SARS-CoV-2 (USA_WA1/2020) in a 3-dimensional, primary normal human bronchial cell (dNHBE) model measured by virus yield reduction assay (VYR) 4 days after infection. Cytotoxicity was measured by MTT assay.

Figure 6. Zotatifin Inhibits the Replication of Other SARS-CoV-2 Variants With Increased Potency Over Other Antivirals



Compound	EC_{50} (nM)	EC_{90} (nM)
Zotatifin	28	183
Remdesivir	3890	9266
Nirmatrelvir	639	916
NHC	645	688

Compound	EC_{50} (nM)	EC_{90} (nM)
Zotatifin	37.3	90.5
Remdesivir	3681	7226
Nirmatrelvir	3090	3819

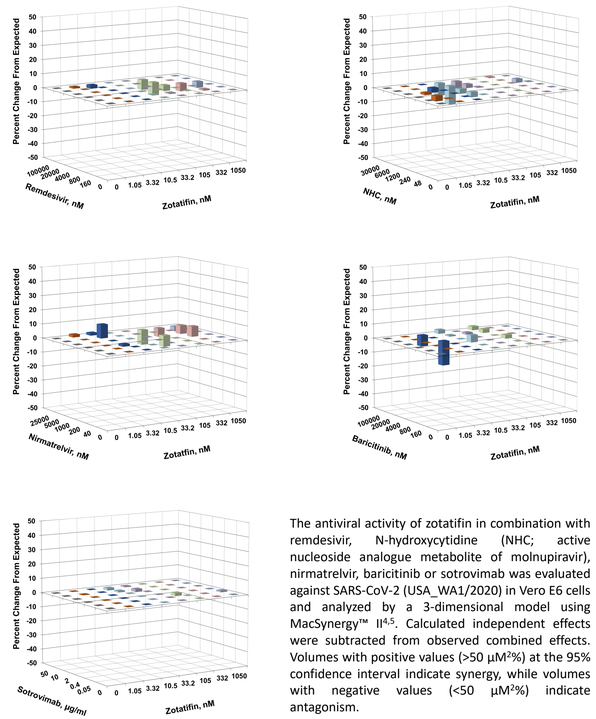
Antiviral activity of zotatifin was evaluated against SARS-CoV-2 variants in Vero E6 or 76 cells in a cytopathic effect (CPE) inhibition assay 2 days after infection.

Table 1. Zotatifin Shows Potent Broad-spectrum Antiviral Activity Against Human Coronaviruses

Virus	Cells	Endpoint	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)	SI
SARS-CoV-2 US_Wash/1/2020	dNHBE	VYR	0.06, 0.16	12.6, 1.4	2100, >1000	35,000, >6250
SARS-CoV-2 US_Wash/1/2020	Vero 76	CPE	3.58	14.0	559	156
		VYR	2.4	5.25		
SARS-CoV-2 Delta	Vero E6	CPE	28	183	>100,000	>3571
SARS-CoV-2 Omicron BA.2	Vero E6	CPE	37.3	90.5	250	7
MERS-CoV	dNHBE	VYR	0.016	42	2300	143,750
SARS-CoV-1	Vero E6	CPE	15.2	66.0	559	37
		VYR	0.6	1.26		
HCoV-229E	MRC-5	VYR	0.23	0.9	970	4217

Antiviral activity of zotatifin was evaluated against SARS-CoV-2 variants in different cells using VYR or CPE inhibition as endpoints. Cytotoxicity was measured by MTT assay.

Figure 7. Zotatifin Demonstrates Additive Interactions When Combined With Remdesivir, NHC, Nirmatrelvir, Baricitinib or Sotrovimab.



The antiviral activity of zotatifin in combination with remdesivir, N-hydroxycytidine (NHC; active nucleoside analogue metabolite of molnupiravir), nirmatrelvir, baricitinib or sotrovimab was evaluated against SARS-CoV-2 (USA_WA1/2020) in Vero E6 cells and analyzed by a 3-dimensional model using MacSynergy™ II[4,5]. Calculated independent effects were subtracted from observed combined effects. Volumes with positive values (>50 $\mu M^2\%$) at the 95% confidence interval indicate synergy, while volumes with negative values (<50 $\mu M^2\%$) indicate antagonism.

Conclusions

The potent broad-spectrum activity against a variety of human coronaviruses, and additive activity when combined with different anti-SARS-CoV-2 antivirals, highlight the advantages of the eIF4A inhibitor zotatifin as a potential treatment for COVID and warrant further evaluation in human clinical trials.

References

1. Ernst JT, Thompson PA, Nilewski C, Sprengeler PA, Sperry S, Packard G, Michels T, Xiang A, Tran C, Wegerski CJ, Eam B, Young NP, Fish S, Chen J, Howard H, Staunton J, Molter J, Clarine J, Nevarez A, Chiang GG, Appleman JR, Webster KR, Reich SH. Design of Development Candidate eFT226, a First in Class Inhibitor of Eukaryotic Initiation Factor 4A RNA Helicase. 2020. J Med Chem. 63(11):5879-5955.
2. Rangan R, Zheludev IN, Hagey RJ, Pham EA, Wayment-Steele HK, Glenn JS, Das R. RNA genome conservation and secondary structure in SARS-CoV-2 and SARS-related viruses: a first look. 2020. RNA. 26(8):937-959.
3. Jeong GU, Song H, Yoon GY, Kim D, Kwon YC. Therapeutic Strategies Against COVID-19 and Structural Characterization of SARS-CoV-2: A Review. 2020. Front Microbiol. 11:1723.
4. Prichard MN, Shipman C Jr. A three-dimensional model to analyze drug-drug interactions. 1990. Antiviral Res. 14(4-5):181-205.
5. Prichard, MN, Aseltine, KR, Shipman, C. 1992. MacSynergy II (version 1.0) user's manual. University of Michigan, Ann Arbor.