REPURPOSING ERAVACYCLINE FOR THE TREATMENT OF SARS-CoV-2 INFECTIONS

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BACKGROUND:

- Pathogenic coronaviruses are a major threat to global public health, as exemplified by Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), Middle East respiratory Syndrome coronavirus (MERS-CoV) and the newly emerged SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19).
- Coronavirus 3CLpro (or Main protease, Mpro) is a cysteine protease that is essential for the replication and life cycle of coronaviruses, making it an attractive target for antiviral development.
- We have identified a new 3CLpro inhibitor through a drug repurposing strategy. Eravacycline dihydrochloride is a tetracycline class antibacterial indicated for the treatment of complicated intra-abdominal infections (cIAI) caused by Gram positive and negative bacteria. It is a safe drug that has been in the US market since 2018 and that can be repositioned for the treatment of coronavirus infections.

OBJECTIVE:

- Our objective was to **identify approved and safe drugs that could be repositioned for the treatment of COVID-19** and that could quickly initiate clinical trials in COVID-19 patients.

METHODS:

SOM^{AI}PRO
- Artificial intelligence screening technology based on molecular fields
- Identification of 3CLpro inhibitors in a database of clinically safe compounds
- Top 300 hits according to potential functional similarity to N3 (PDB 6LU7; Jin et al 2020: https://doi.org/10.1038/s41586-020-2223-y)

Docking
- Docking at the catalytic domain of SARS-CoV-2 3CLpro, and covalent docking on a subset of hits (Cys145)
- Top 30 compounds for *in vitro* testing

3CL protease
- SARS-Co-V2 3CL protease inhibition assay to identify potential hits (3 hits identified, Eravacycline was the most potent)
- SARS-CoV and MERS-CoV 3CL protease inhibition assays

VeroE6 infection
- Positive hits were tested for inhibition of SARS-CoV-2 infection in VeroE6 cells

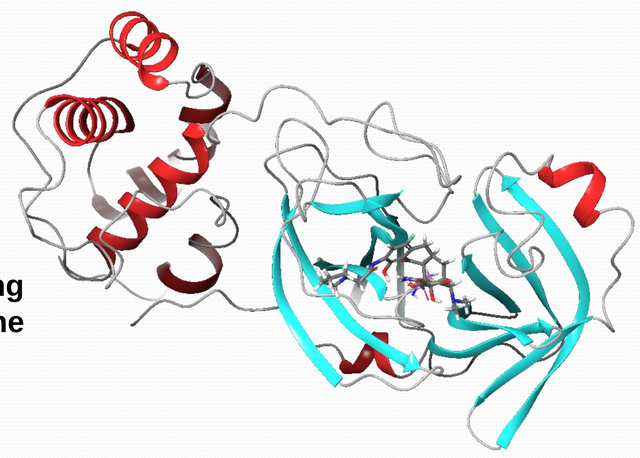


Fig.1: Covalent docking of eravacycline with the catalytic domain of SARS-CoV-2 3CL protease

RESULTS:

- **Eravacycline potently inhibits SARS-CoV-2 3CL protease activity**
- SARS-CoV and MERS-CoV 3CL proteases are also inhibited by Eravacycline

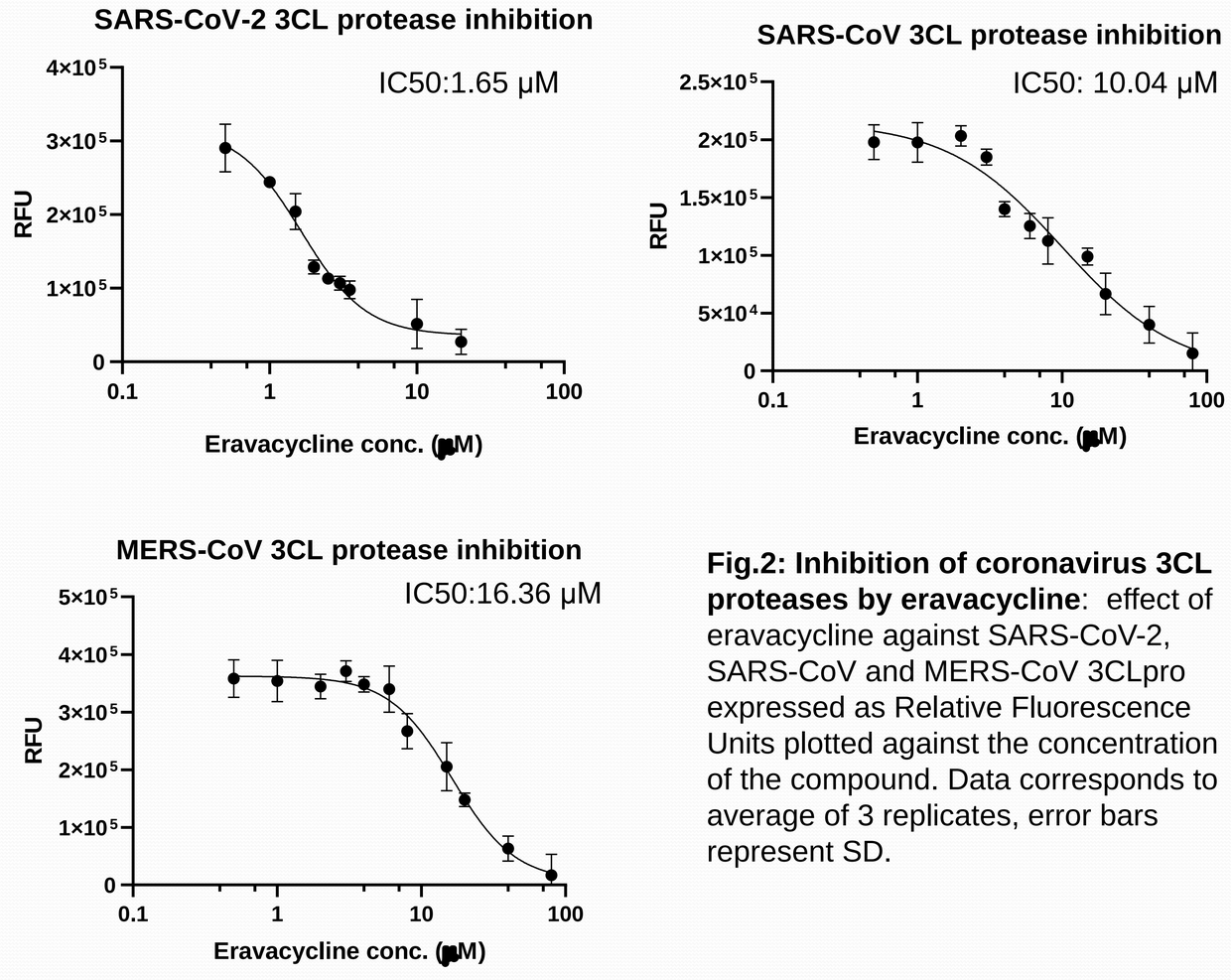
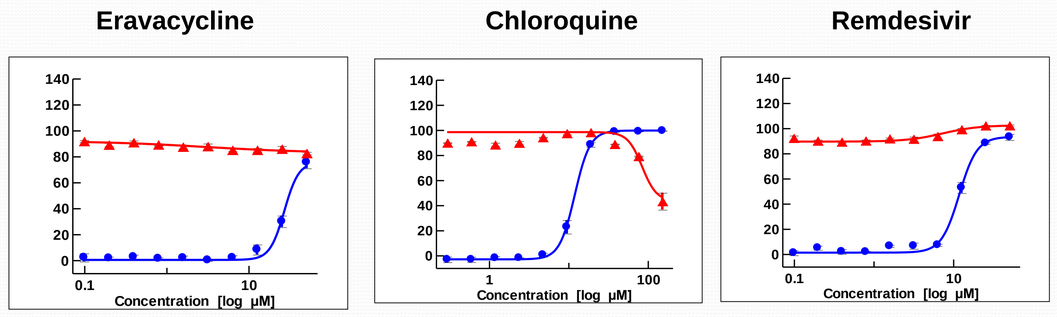


Fig.2: Inhibition of coronavirus 3CL proteases by eravacycline: effect of eravacycline against SARS-CoV-2, SARS-CoV and MERS-CoV 3CLpro expressed as Relative Fluorescence Units plotted against the concentration of the compound. Data corresponds to average of 3 replicates, error bars represent SD.

- **Eravacycline inhibits SARS-CoV-2 infection in VeroE6 cells**



	Eravacycline	Chloroquine	Remdesivir
IC50 infection	30,61 µM	12 µM	12,08 µM
CC50 tox	> 50 µM	127,81 µM	> 50 µM

Fig.3: Inhibition of VeroE6 cells infection: VeroE6 cells were pretreated 1h with different concentrations of compounds and infected with SARS-CoV-2 at a MOI=0,0125. 24h after infection, cells were fixed, permeabilized and stained with SARS-CoV-2 nucleocapsid N antibody. Infection was calculated as the number of cells expressing nucleocapsid protein / total number of cells per well. Red: % cell viability; Blue: % inhibition of infection

CONCLUSIONS:

- **Eravacycline has the expected properties for a drug to target respiratory illness caused by an intracellular pathogen like a virus.**
- **The drug has an IC50 in the low µM range at the 3CLpro and accumulates in the lung reaching the intracellular space.**
- **Eravacycline is safe and additionally has anti-inflammatory properties.**
- **The antibacterial activity of eravacycline is also suitable to prevent secondary infections in COVID-19 patients**
- **A Ph2a clinical trial is underway to prove the effectiveness of eravacycline in hospitalized COVID-19 patients**

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