Discovery and Development of SARS-CoV 3CL Protease Inhibitors

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SARS: Overview

First known case of atypical pneumonia Nov. 2002 in Guandong Province, China; first official outbreak reported to WHO Feb. 2003

FIGURE 1. Chain of transmission among guests at Hotel M — Hong Kong, 2003

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5212a1.htm
SARS: Overview (2)

- Disease characterized by fever, malaise, dry cough, dyspnea, hypoxaemia, GI symptoms, lymphopenia, elevated aminotransferase levels.
- Death due to progressive respiratory failure.
- As of August 2003: 8422 cases; 916 deaths (11% CFR).
- Virus isolated from nasopharyngeal aspirates, BAL, sputum, kidney, urine, feces, plasma.
SARS: Coronavirus (CoV) Identified

- CPE in cell culture and EM:
- RT-PCR analysis confirms identity
- Demonstration of seroconversion
- Interstitial pneumonia in monkeys

SARS: A New CoV

CoV: Overview

- Large, enveloped (+) stranded RNA viruses
- Respiratory and enteric disease in man and animals
  - 2 human CoVs cause 15-30% of colds with seasonal incidence
  - Fatal systemic and epizootic disease in animals: FIPV, HEV, IBV, TGEV
- Narrow host range and cell/tissue specificity
CoV Life Cycle

SARS CoV 3CL Protease

- CoV known to encode critical enzyme (3CL) homologous with picornavirus 3C protease\(^1\)
  - Substrate specificity (Q/S,G)
  - Cysteine as active site nucleophile

CoV 3CL Protease

- Structural similarities also noted

Purple: Human rhinovirus (HRV-2) 3C protease¹

Green: porcine transmissible gastroenteritis coronavirus (TGEV) 3CL protease²

HRV 3C Protease Inhibitors
Project Summary

- X-Ray crystal structure of HRV 3CP solved in 1994
- Peptidomimetic irreversible protease inhibitor incorporating ester as Michael acceptor developed
- AG7088 (Ruprintrivir) selected and formulated for intranasal delivery.
- Potent in vitro antiviral activity against diverse rhinovirus and other picornaviruses (mean EC90 = 82 nM)
- Clinical studies:
  - Phase I: Safe and well tolerated
  - Phase II challenge: moderated illness severity when initiated either before or one day after rhinovirus infection
  - Phase II (natural infection): no antiviral activity or reduction in symptom severity

Homology modeling suggests Michael acceptor-containing HRV-3CP inhibitors may also interact with SARS 3CL\textsuperscript{1,2}

**Pink:** HRV-2 3C protease\textsuperscript{3}

**Cyan:** SARS-CoV 3CL protease homology model\textsuperscript{2}

SARS-CoV infection of Vero 76 cells

Cell viability assay using neutral red or MTS as endpoint
AG-7088 vs SARS?

- AG-7088 not optimized for SARS 3CL (multiple hypotheses why)


Easily accommodated by SARS 3CL

Para-F too large for SARS 3CL but H may fit
AG-7088 vs SARS?

- AG-7088 does not inhibit SARS-CoV in vitro

EC$_{50}$ >167 µg/ml
CC$_{50}$ >167 µg/ml
(Strain 200300592)

Other Michael Acceptors

- Truncated molecule (AG-7122) exhibits in vitro anti-SARS-CoV activity

EC$_{50}$ = 14.1 µg/ml
CC$_{50}$ >100 µg/ml
(Strain 200300592)

Next Steps

- Evaluate physiochemical properties of active SARS-CoV 3CL protease inhibitors
- Utilize structure-based design and combinatorial synthesis strategies to design novel peptidomimetic 3C protease inhibitors
Key Learnings

- Rapid identification of etiological agent
- Rapid access to key scientific publications
- Existence of significant knowledge base, technical expertises and compounds (3C protease inhibitors)
- Efficient external networking and deep sense of collaboration
- Antiviral assay in development
Key Learnings

- Streamline processes
- Establish infrastructure and identify single points of contacts
- Early and frequent communication
## Acknowledgements

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