# Presentation 2: “A therapeutic neutralizing antibody targeting receptor binding domain of SARS-CoV-2 spike protein”

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# Objective of the Kim et al. Paper

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## A therapeutic neutralizing antibody targeting receptor binding domain of SARS-CoV-2 spike protein

Cheolmin Kim, Dong-Kyun Ryu, ... Soo-Young Lee + Show authors

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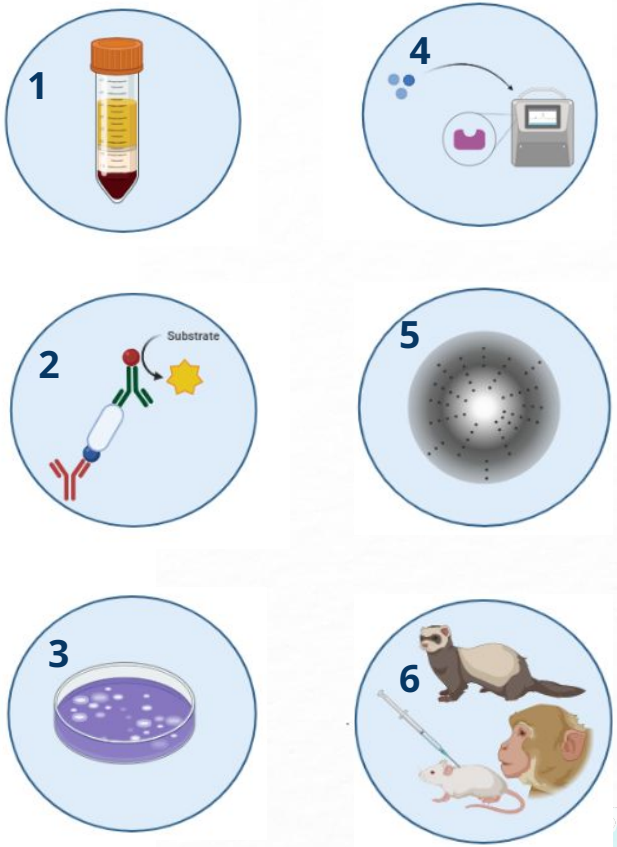
(Kim et al., 2021)

# METHODS

# Methods Overview

1. Peripheral Blood Mononuclear Cells (PBMC) Isolation
2. Phage Display & Biopanning
3. Plaque reduction neutralization test (PRNT)
4. Biolayer Interferometry (BLI)
5. X-ray Crystallography
6. Animal Studies
7. Antibody Dependent Enhancement (ADE)




(Kim et al., 2021; Sirivichayakul et al., 2014)

Image Source: BioRender

# Phage Display and Library construction

- cDNA cloned to phagemid vector for library construction.
- ER2738 competent cells transformed with scFVs cDNA library, cultured with VCSM13 helper phage cells.
- Phages displaying scFvs harvested.

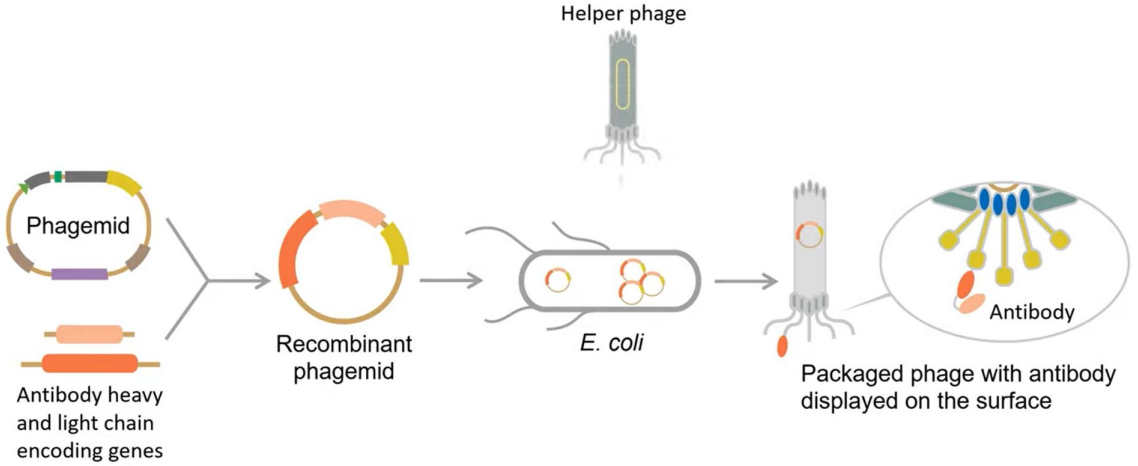



Image Source : Phage Display Technology - Creative Biolabs (Updated Version), 2020

(Kim et al., 2021; Phage Display Technology - Creative Biolabs (Updated Version), 2020; Tennant et al., 2018)

# Biopanning to find scFvs binding to SARS-CoV-2

- Screen for SARS-CoV-2 RBD-binding scFv displayed on phage.
- SARS-CoV-2 was coated with magnetic beads.
- Magnetic field helped SARS-CoV-2 RBD bound phages to be retained.
- Phage-ELISA was performed to identify scFvs binding to the RBD of SARS-CoV-2

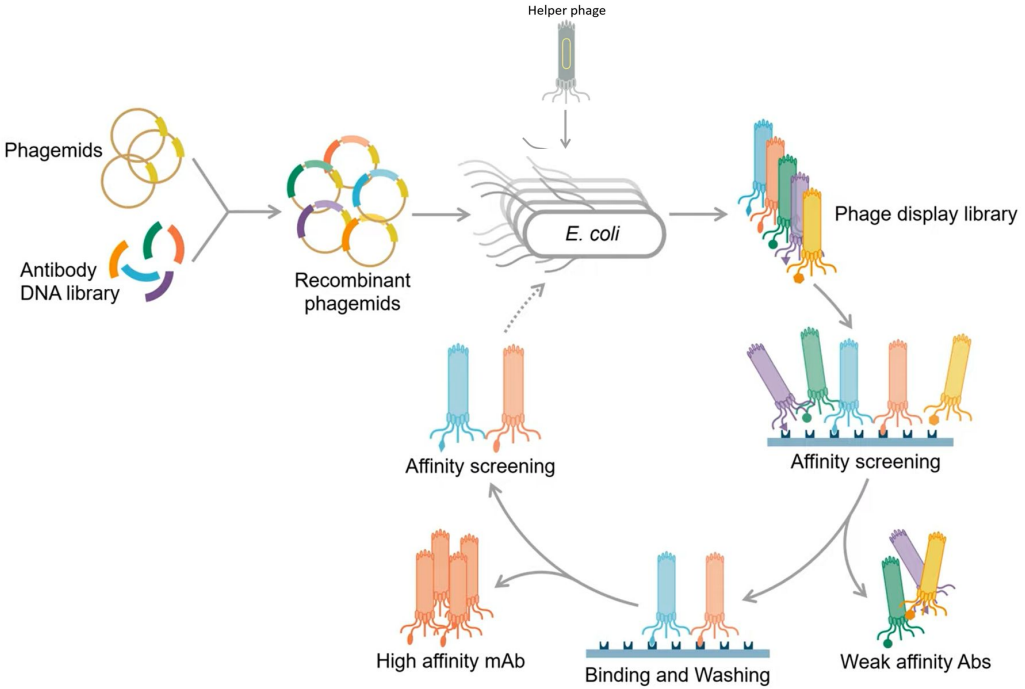
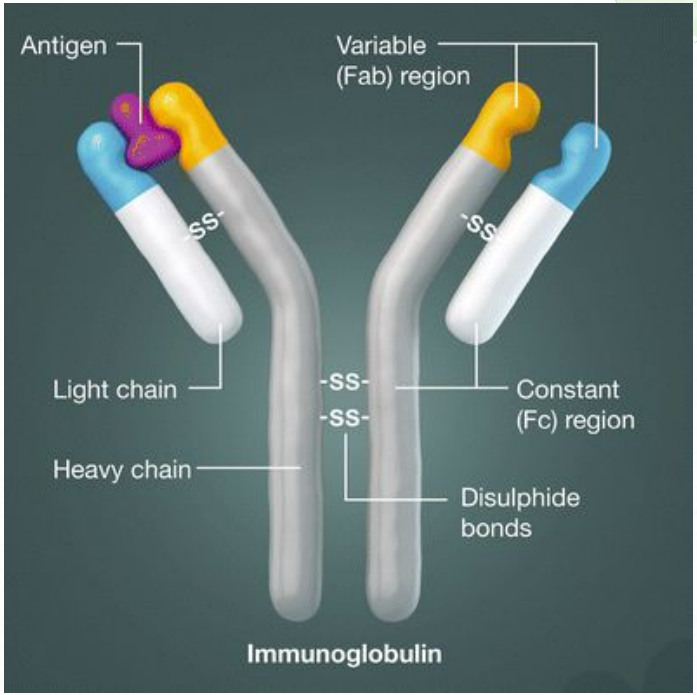



Image Source : Phage Display Technology - Creative Biolabs (Updated Version), 2020

(Ganten & Ruckpaul, 2006; Kim et al., 2021; Phage Display Technology - Creative Biolabs (Updated Version), 2020)

# Preparation of scFv-Fc and full length IgG

- scFvs cloned into constant fragment fusion vector region.
- Synthesized DNAs of heavy and light chain inserted to MarEx vectors for full length IgG expression.
- scFv-Fc and full length recombinant IgG were purified using affinity chromatography.



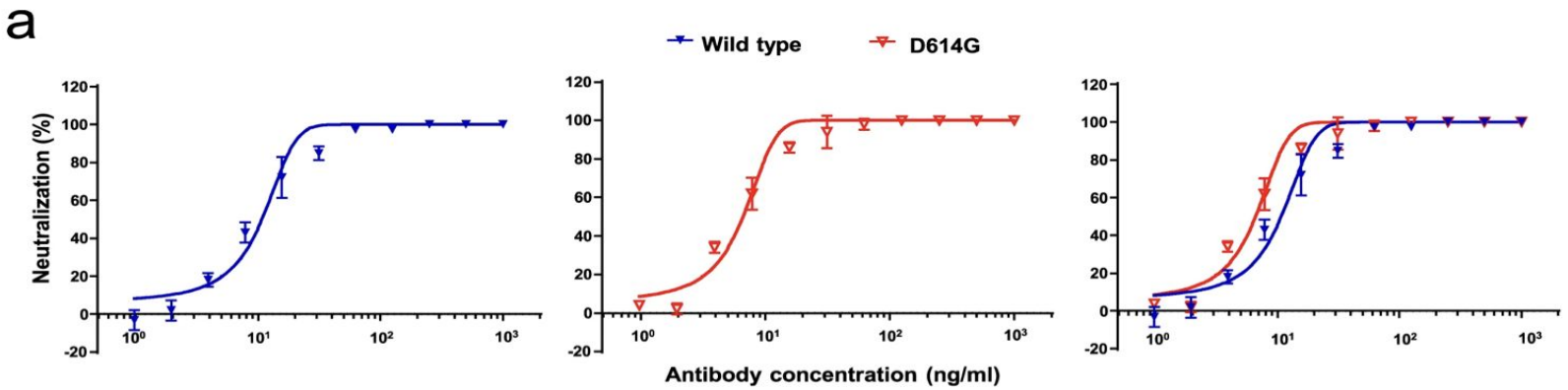

(Kim et al., 2021; Gapper et al., 2007)

# RESULTS

# Screening of CT-P59 to Evaluate Neutralization Potency

- RBD-binding single-chain variable fragments isolated via recombinant SARS-CoV-2 RBD

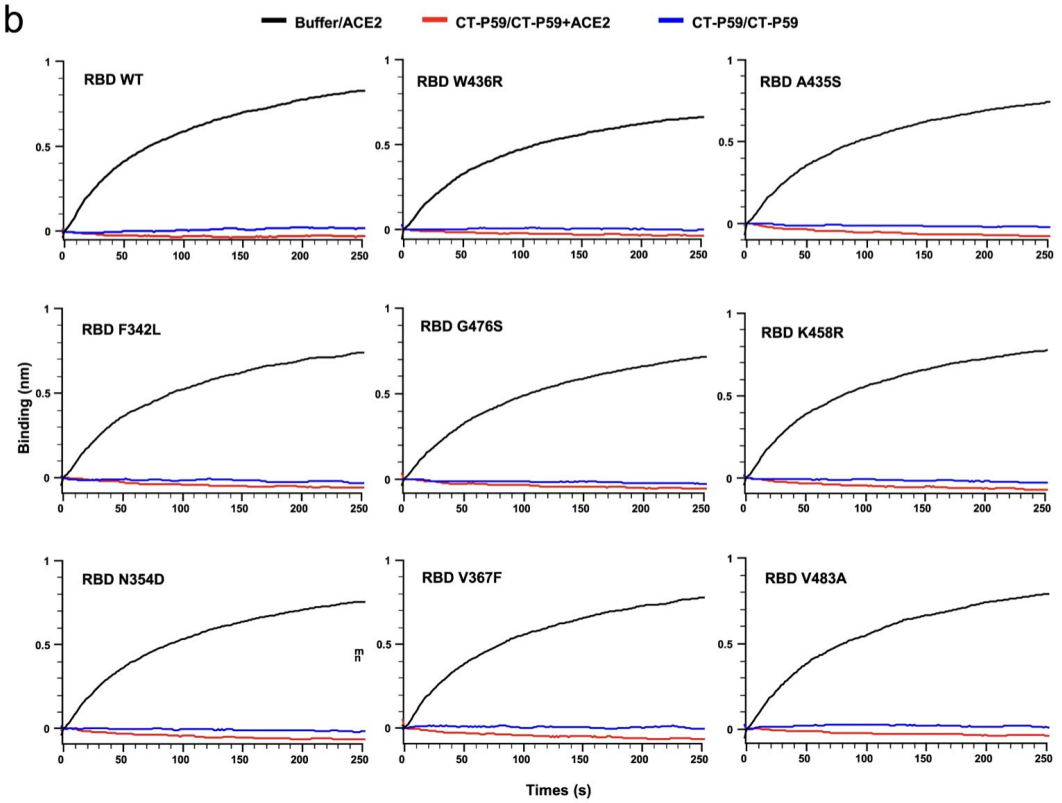
- Evaluate neutralization potency of CT-P59 against
  - SARS-CoV-2
  - SARS-CoV-2 D614G variants




(Kim et al., 2021)

# Competitive Binding of CT-P59 to RBD-ACE2

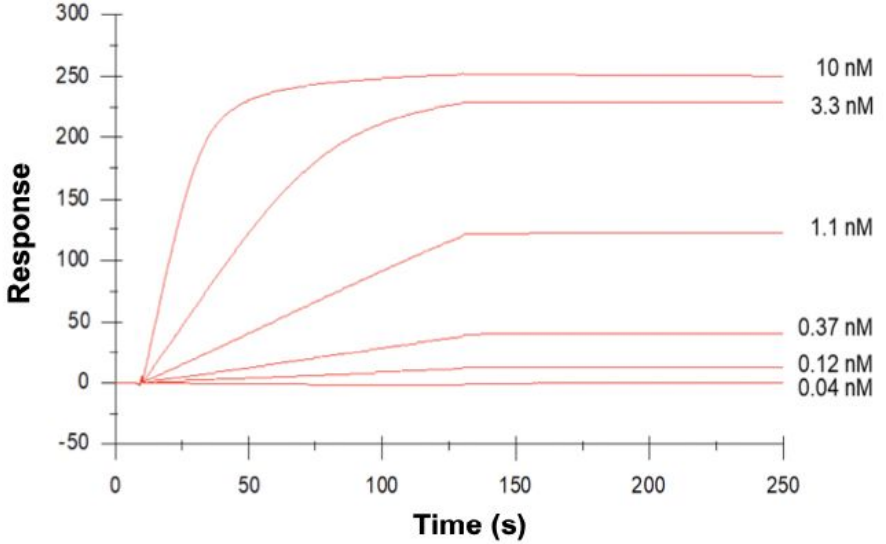
- CT-P59 significantly inhibits the viral replication of a Korean SARS-CoV-2 clinical isolate
  - IC50 = 8.4 ng/ml
- CT-P59 reduces the replication of the D614G variant
  - IC50 of 5.7 ng/ml
- CT-P59 completely inhibits the binding of RBD-ACE2.




(Kim et al., 2021)

# Evaluating CT-P59 Interactions with SARS-Co RBD

- CT-P59 specifically binds to SARS-CoV-2.
- CT-P59 has a high affinity for SARS-CoV-2 RBD.
  - KD= 27 pM
- **SARS-CoV-2 RBD mutations might alter the binding affinity of the virus for ACE2.**
  - mutations V367F, W436R, and D364Y



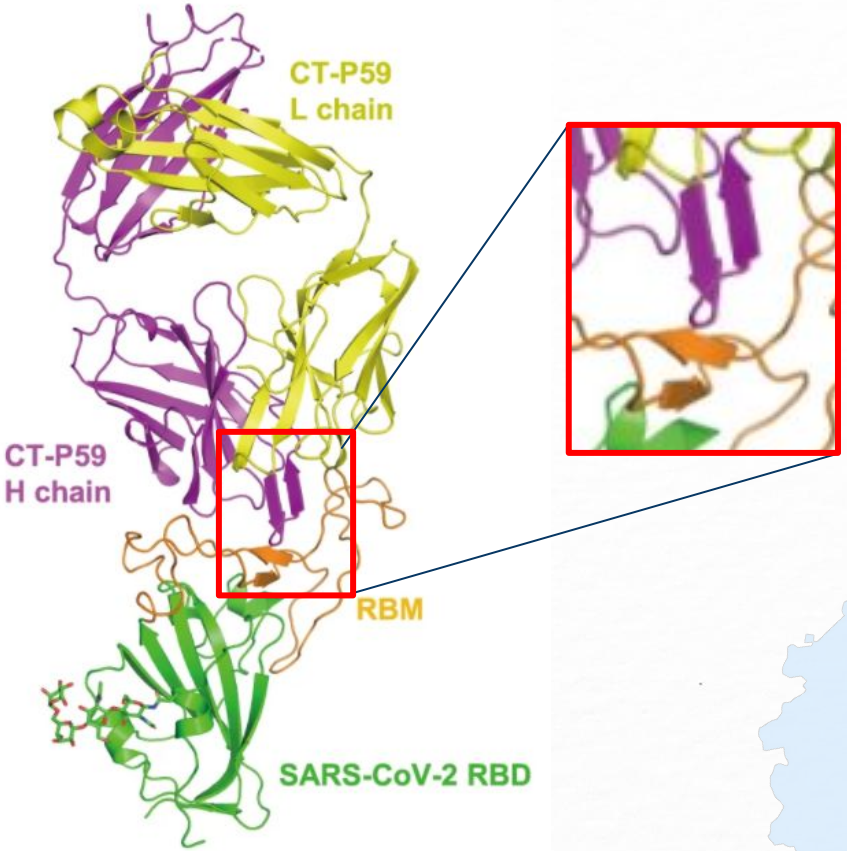

| Sample ID | $K_D$ (M) | ka (1/Ms) | kd (1/s) |
|---|---|---|---|
| CT-P59 | 2.7E-11 | 6.9E+06 | 1.9E-04 |

(Kim et al., 2021)

# Structural Analysis of CT-P59 Fab/RBD Protein Complex

- X-ray structure of CT-P59 Fab/**RBD** solved at 2.7Å

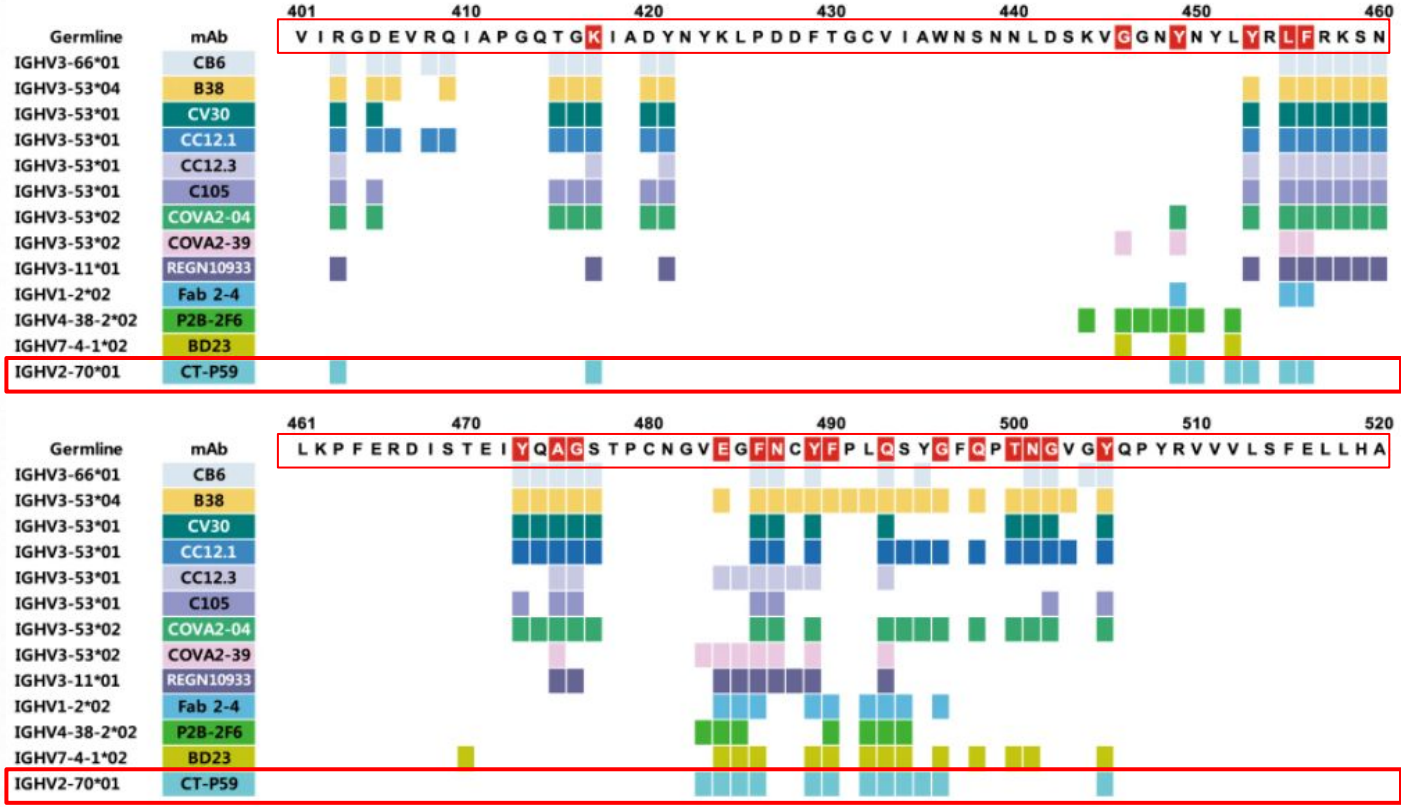
- CT-P59 Fab binds SARS-CoV-2 **RBM**




(Kim et al., 2021)

# CT-P59 Fab/RBD Complex Epitope Binding

- Compared to 12 other mAb against SARS-CoV-2
- Unique binding to RBD epitope residues
- **Red** = RBD residues that bind to ACE2

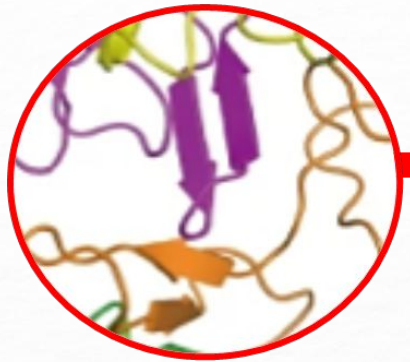


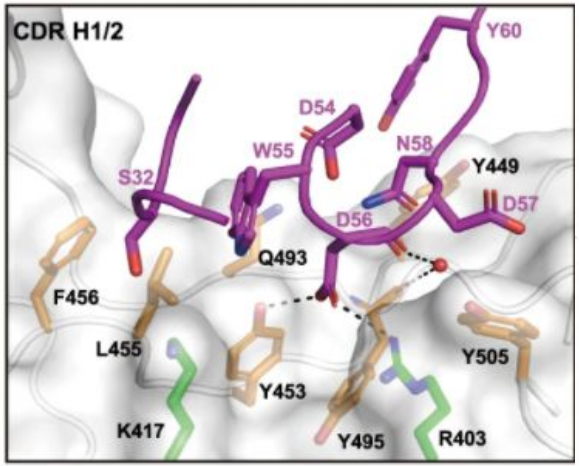

(Kim et al., 2021)

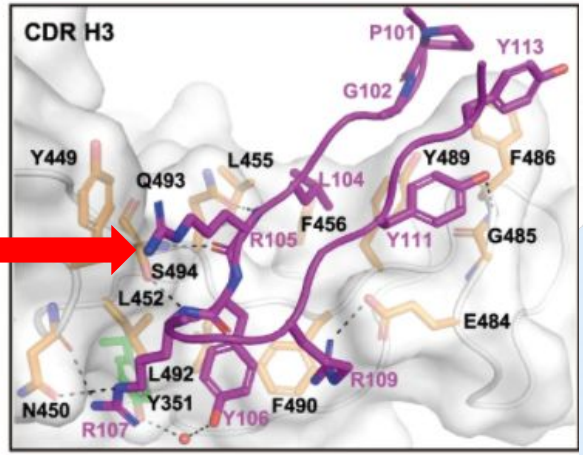
# Complementarity-Determining Regions (CDR)

- 16 residues from the **heavy chain** binds to 19 residues on the **RBD**

- **CDR H3** forms:
  - Beta hairpin
  - **8 H-bonds**

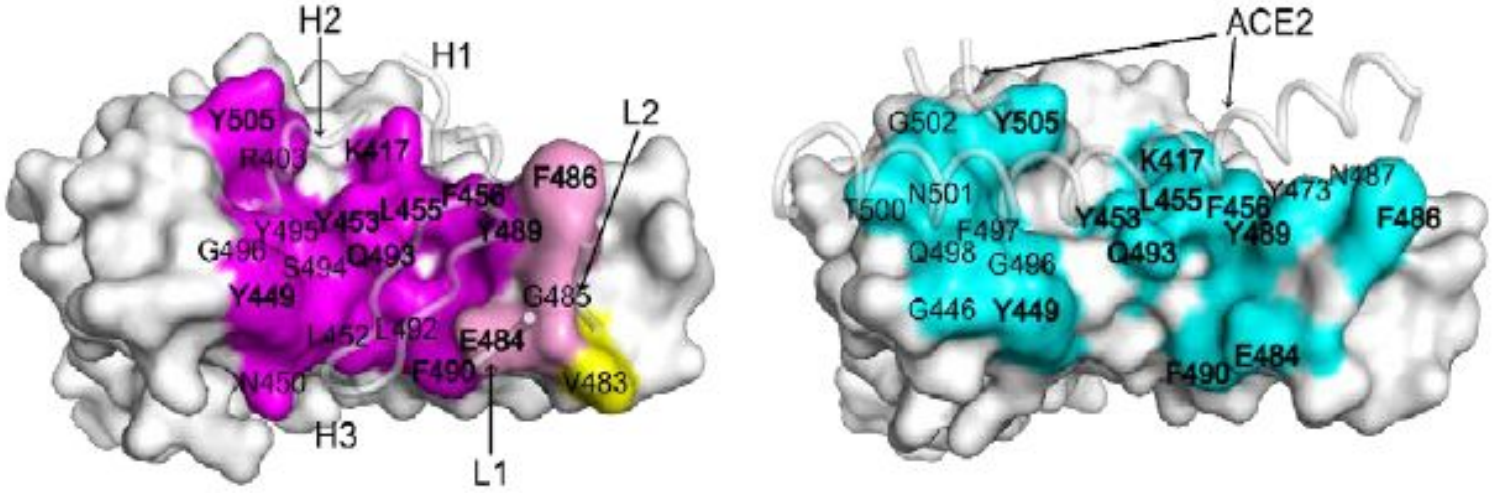





(Kim et al., 2021)

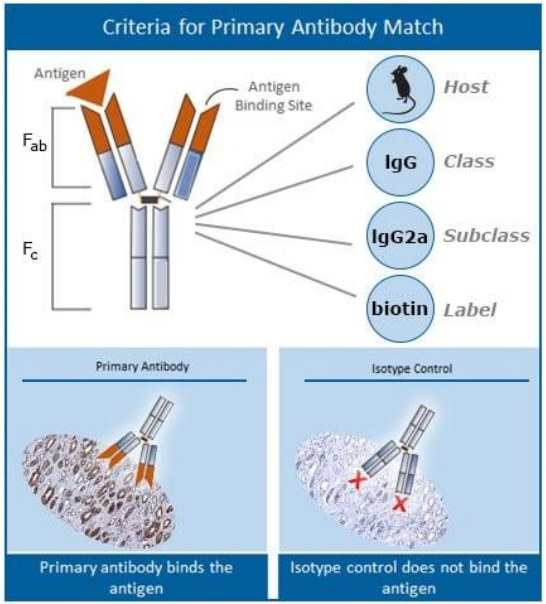
# Structural Comparison: CT-P59 Fab/RBD vs. ACE2/RBD




- Only binds to the “up” conformation
- CT-P59 binds to 12/21 ACE2 binding residues on the RBD
- Avoids binding to RBD mutants at 3 positions, notably D364Y

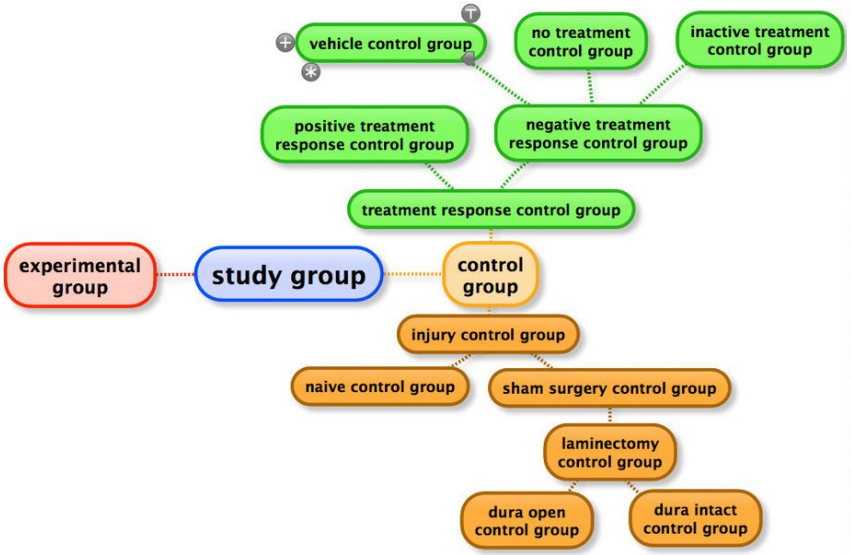
(Kim et al., 2021)

# Type of Negative Controls




## Isotype Control

- Very similar properties
- Determines extent of non-specific binding




## Vehicle-treated Control

- Administers only the vehicle
- Determines effect of vehicle

("Isotype control," n.d.; Johnson et al., 2002)

# Dosage-dependent Viral Replication Inhibition

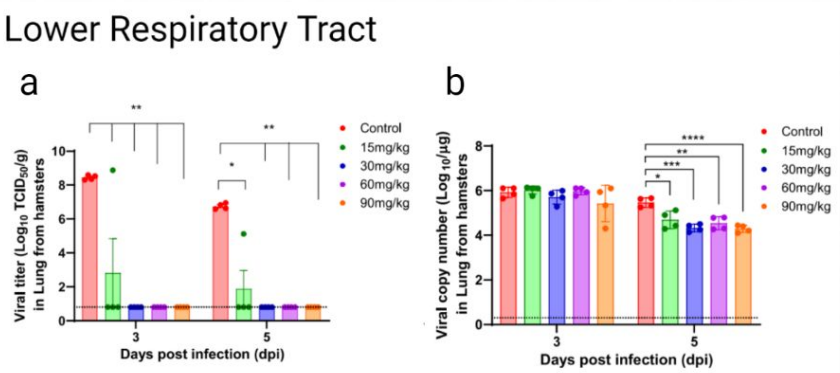
**Ferret Model**

- Cough, rhinorrhea, activity level
- Control
  - Human IgG isotype control
- Experimental
  - 18 mg/kg Remdesivir daily
  - 3 and 30 mg/kg CT-P59
- Complete inhibition of viral replication
- Similar or better therapeutic efficacy than Remdesivir

(Kim et al., 2021)

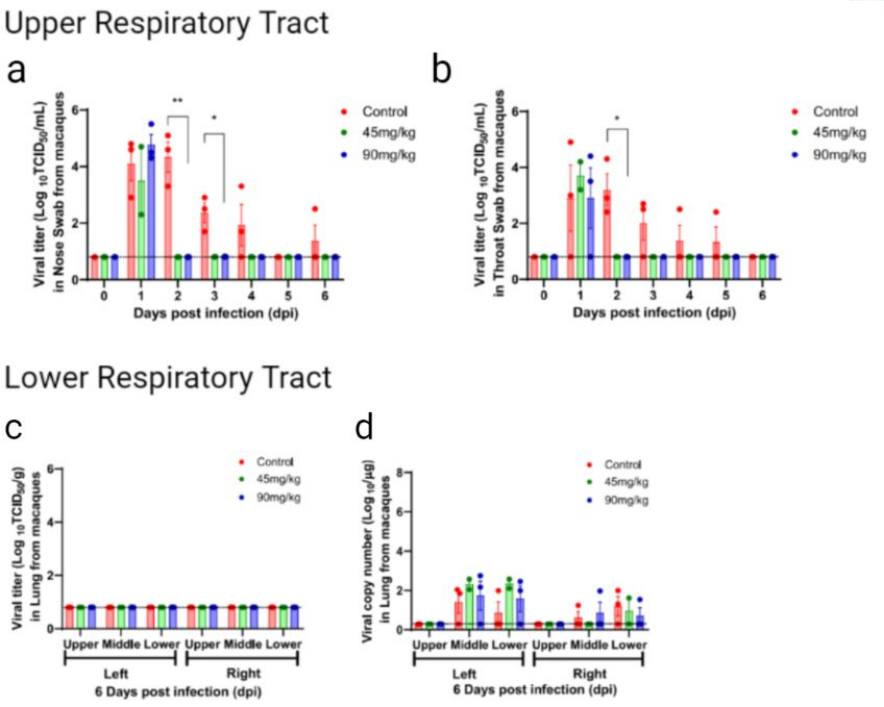
# Significant Reduction in Viral Titer

## Golden Syrian hamster model




Significant reduction or elimination of viral titer depending on dosage

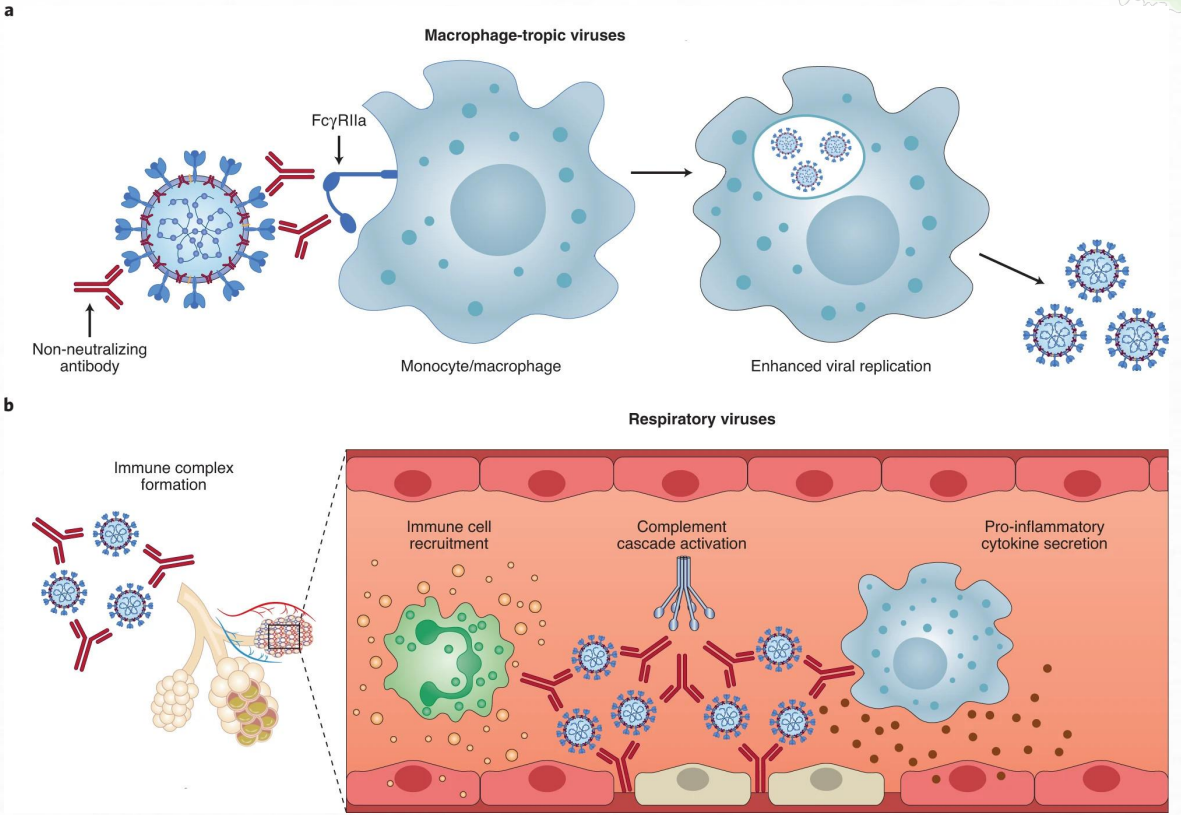
## Rhesus monkey model




(Kim et al., 2021)

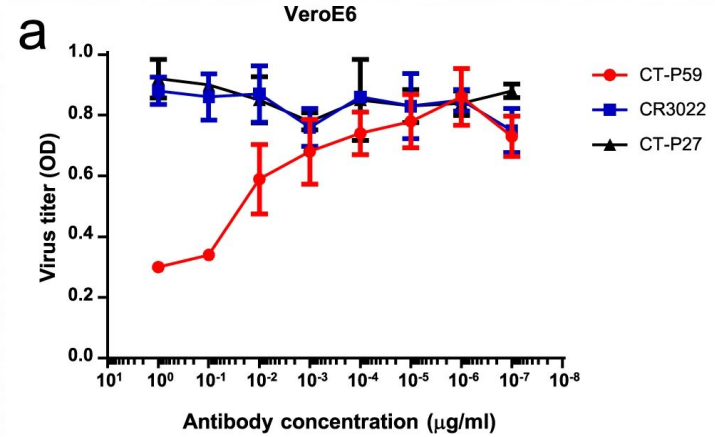
# Antibody-Dependent Enhancement (ADE)

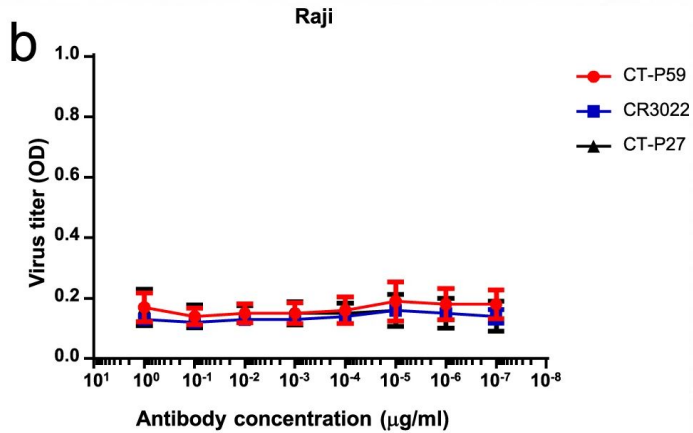
- Enhanced infection
  - Measured in vitro
- Enhanced immune activation
  - Respiratory viruses
  - Measured in vivo
- FcR-independent ADE

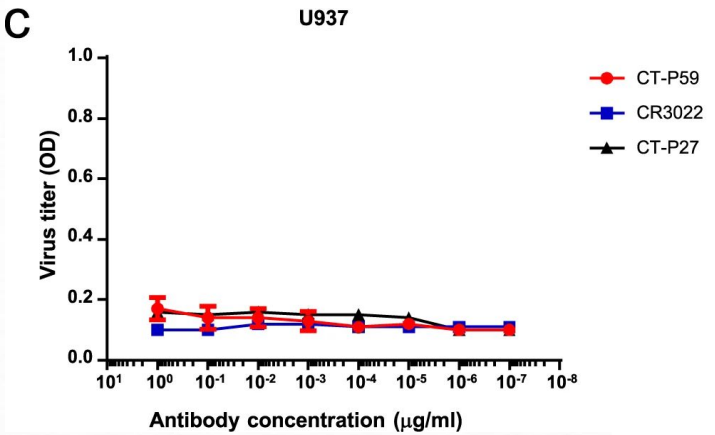



(Lee et al., 2020)

# In vitro ADE assay




- Virus-antibody complexes
  - Used to infect: FcR-bearing cells and permissive cells
- No CT-P59-mediated increase in viral infection

(Kim et al., 2021)

# CRITICAL APPRAISAL, NEXT STEPS, AND CONCLUSION

# Critical Appraisal of the Kim et al. Paper

| Pros | Cons |
| --- | --- |
| • In vivo and in vitro testing<br>• Illustrated the effectiveness of CT-P59 | • CT-P59 does not recognize the “down” conformation of the RBD<br>• Antibodies used aren’t explained in ADE<br>• In vivo animal models did not reflect clinical symptoms of severe COVID-19 in patients |

(Kim et al., 2021)

# CT-P59 is Currently Being Tested in Clinical Trials and its Broad Neutralizing Capabilities of SARS-CoV2 can be Researched

1

**Study Description** Go to

Brief Summary:

This is a Phase 2/3 study to assess the efficacy about therapeutic effect of CT-P59 to the mild to moderate SARS-CoV-2 infected patients and safety during after study drug injection.

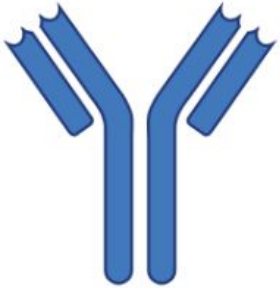
| Condition or disease | Intervention/treatment | Phase |
|---|---|---|
| SARS-CoV-2 Infection | Biological: CT-P59/Placebo | Phase 2<br>Phase 3 |

Detailed Description:

CT-P59 is a monoclonal antibody targeted against SARS-CoV-2 spike RBD as a treatment for SARS CoV 2 infection. CT-P59 is currently being developed by the Sponsor as a potential treatment for SARS-CoV-2 infection. In this study, safety, tolerability and virology of CT-P59 will be evaluated in patient with mild symptoms of SARS-CoV-2 Infection.

**Phase II and III Clinical Trials**

2



**Broadly Neutralizing Antibody for SARS-CoV2**

(Kim et al., 2021), (NIH, n.d.)

# Conclusion

Main Takeaways from the Kim et al. Paper:

- CT-P59 is able to competitively bind and inhibit RBD-ACE2 interactions
- CT-P59 Fab blocked areas of the ACE2 binding sites within the SARS-CoV-2 RBD.
- Virus titration and quantification showed CT-P59 completely inhibits viral replication, reducing viral RNA copy number and viral titer faster than remdesivir.
- ADE assay showed no evidence of CT-P59-mediated increase in viral infections via ADE

(Kim et al., 2021)

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# Thank You!