Highly potent pan-sarbecovirus neutralizing antibodies induced by serial cross-clade immunization

Linfa WANG
Programme in Emerging Infectious Diseases
• **The assay platform**: multiplex surrogate virus neutralization test (sVNT)

• **The cohort**: SARS survivors in Singapore who received the BNT162b2 vaccine

• **The finding**: strong pan-sarbecovirus NAbs against all ACE2-binding sarbecoviruses tested

• **The mechanism**: synergy of multiple neutralizing epitopes vs immunodominant cross-clade neutralizing epitopes

• **The implication**: serial cross-clade immunization is a promising/powerful approach to induce pan-sarbecovirus NAbs
The principle of sVNT

**a** Virus Neutralization Test (VNT)

Neutralizing antibody

Spike protein

ACE2 receptor

RBD of spike protein binds to ACE2 receptor

**b** surrogate Virus Neutralization Test (sVNT)

Neutralizing antibody

HRP-conjugated RBD

Signal

TMB + H₂O₂

ELISA plate

---

Tan et al. Nat Biotech (2020)
Multiplex sVNT

- Reversing the liquid-solid phase configuration: RBD on beads and PE-ACE2 in liquid
- Use of biotinylated RBD to achieve uniform coating in a multiplex system
- Presence of equimolar RBDs creates “in-tube competition”
ACE2-binding sarbecovirus RBDs (16 plex sVNT)
The cohort

• N = 8

• Recovered from SARS-CoV-1 in 2003

• Some still have good NAbs against SARS-CoV-1, but no cross-NAbs against SARS-CoV-2

• Received 1 or 2 doses of BNT162b2 mRNA vaccine

• Blood samples were taken 21-62 days after the first dose vaccination
NAbs against 10 sarbecoviruses

Tan et al. NEJM (2021)
Proposed mechanism

SARS-specific NAb epitopes

COVID-specific NAb epitopes

Cross-virus NAb epitopes

SARS

COVID

1. SARS exposure or vaccine
2. COVID exposure or vaccine
3. SARS exposure → COVID vaccine
Comparison of pan-sarbecovirus NAbs (mAb)

#1

mAb1

#2

mAb2
Highly potent against Omicron

**mAb1**

% Inhibition

Log concentration (ng/ml)

---

**mAb2**

% Inhibition

Log concentration (ng/ml)
A pan-sarbecovirus booster vaccine candidate

- Designed a “consensus” Spike protein from all known Clade-1 SARS-like CoVs (mainly from bats)
- Produced in-house trimeric Spike with PP mutations
- Immunized mice with approved human vaccines in Singapore (BNT162b2, mRNA-1273, CoronaVac) using pre-determined doses by the vaccine producers
- Boosted with two versions of Spile proteins (in experimental adjuvant): C1.25 = the consensus; WT = the human SARS-CoV-1 Spike
- All groups produced pan-sarbecovirus NAbs
Acknowledgments

Duke-NUS Team

NCID Team

DxD Hub Team

NIBSC

GenScript