FULL NARRATIVE REPORT – SAMUEL LAI

PRECLINICAL DEVELOPMENT OF A POTENT MUCO-TRAPPING ANTIBODY AGAINST SARS-COV-2 FOR INHALED IMMUNOTHERAPY AND PROPHYLAXIS AGAINST COVID-19

Executive Summary

At the onset of the COVID-19 pandemic, we recognized both the power of inhaled therapy, and the challenge associated with advancing antibody therapies in light of the potential for viral escape. Indeed, Eli Lilly's Bamlanivimab is effectively evaded by the latest South African variant (100-fold lower neutralization potency). Other studies also showed that a single mutation could escape binding by *both* antibodies included in Regeneron's cocktail. As such, when making a strategic decision for how we want to approach engineering an antibody, we focused on engineering an antibody molecule that is *not at risk for viral escape*.

We were able to engineer such a molecule by replacing the part of the antibody that typically recognizes viruses (Fab domain) with the receptor targeted by SARS-CoV-2 virus (ACE2). This created a molecule that binds all ACE2-targeted viruses, including ALL SARS-CoV-2 variants. In fact, the more the virus evolves to bind the receptor more tightly to increase transmission (as is case with the latest variants), the more potent our antibody. We successfully optimized the linkage between the ACE2 domain and the rest of the antibody molecule. This linkage is key to enable our molecule to bind bivalently against the virus (i.e. both arms of the antibody molecule can engage the virus simultaneously), thus creating a molecule with much greater potency than other similar attempts. This is the major focus of the patent application filed by the university. Finally, we engineered the antibody to possess the key glycans (i.e. sugars) that enable interactions with mucins. The end result is an antibody molecule that can bind all SARS-CoV-2 variants with picomolar affinity and neutralization potency, and can trap the virus in mucus upon inhalation and immediately block the infection from spreading the progressing. The molecule was effective in treating SARS-CoV-2 infections in a pilot study in hamsters. By delivering the molecule directly to the airways, we expect to enable efficacious and cost-effective treatment against SARS-CoV-2, with little risk of adverse side effects.

Below is a synopsis of our key scientific findings. The molecule has been licensed to Inhalon Biopharma, and is tentatively named IN-007.

Scientific Accomplishments

Below is a highlight of the scientific accomplishments that arose from extensive work in engineering and development the antibody. We did not include describing work that are considered "mundane" but is nonetheless essential for ensuring scientific rigor and the developability, including ensuring consistent transient expression of the molecule, formulation optimization to ensure stability, and development of cGMP-compliant cell lines for producing clinical trial materials.

1. Enabling bivalent binding and muco-trapping. The S protein spikes that SARS-CoV-2 use to bind ACE2 are comprised of (3) S proteins assembled in the form of trimers, with individual S-protein spikes located on average ~15 nm apart on SARS-CoV [62]. The typical wingspan of the two Fabs on typical IgG_1 is roughly ~10-12 nm. Thus, a typical mAb molecule against any particular S-protein epitope is unlikely to bind bivalently to the virus (**Figure 1**). Due to the orientation of the (3) S proteins on an individual S-protein spike, simply linking an ACE2 molecule to Fc would also not allow the same ACE2-Fc mAb to bind bivalently to any 2 S-proteins on the trimeric S spike (**Figure 1**). The same problem is well described for HIV, which explains, in part, why it is so difficult for the body to generate potent neutralizing Abs against HIV [63, 64] and why fairly high doses of broadly neutralizing mAb are needed to neutralize the virus *in vivo*. To overcome this problem, we incorporated a flexible 30 AA GS linker between the ACE2 domain and Fc, such that when assembled, the overall reach of the two ACE2 domains on IN-007 would span ~15-18 nm. This greatly increases IN-007 binding SARS-CoV-2 bivalently both inter-spike and intra-spike (see C.4). Finally, IN-007 possesses the G0/G0F-dominant Fc glycosylation for muco-trapping, enabling IN-007 to effectively trap SARS-CoV-2 in human AM (see C.4).

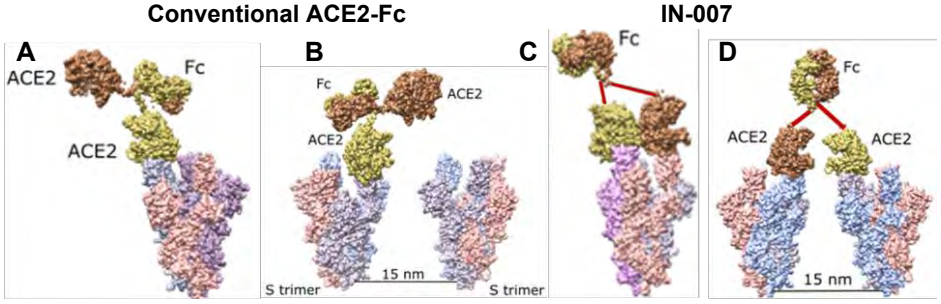

Figure 1. Schematic of conventional **(A-B)** ACE2-Fc vs. **(C-D)** IN-007. The key difference is our incorporation of (i) a flexible linker (shown in red) that enables bivalent binding both intra-spike (C) and inter-spike (D), as well as (ii) muco-trapping Fc. Without the linkers, ACE2-Fc alone is unable to bind bivalently effectively.

2. Binding and neutralization potencies of IN-007. We measured the binding affinity of different ACE2 constructs to S-protein by ELISA. Using 4-parameter fit of absorbance data (**Figure 2**), we found the K_D of IN-007 to be ~40 ng/ml), ~10-fold greater affinity than conventional ACE2-Fc constructs with the dimerizing CD domain (K_D ~600 ng/ml) and >40-fold greater affinity than ACE2-Fc constructs without CD domain and without optimized linker (K_D ~2.5 μg/ml). This much greater binding affinity also translates to greater neutralization potencies (IC_{50} ~35 ng/mL), which is markedly more potent than other ACE2-derived constructs reported in the literature, including a ~54-fold improvement over the same ACE2-Fc construct without linkers, and a ~5-fold improvement over affinity-matured ACE2 recently reported in Science [67]. Notably, IN-007's binding affinities and neutralization potencies are comparable to those for IN-002 against RSV that we observed exceptional potency. In our lamb study, nebulizing a target inhaled dose of 0.8 mg/kg resulted in ~1.6 μg/ml in the BALF and ~1 μg/ml in the blood 24 hours later. This suggests that nebulizing the same dose of IN-007 will result in local and systemic IN-007 levels that are 40- and 25-fold greater than IN-007's IC_{50} even at the trough. While several other mAbs possess greater neutralization potencies, we believe the advantages associated with IN-007 (no risk of viral escape and the ability to bind and neutralize other ACE2-targeted viruses, present and future) make IN-007 a promising molecule to advance into the clinic despite the large number of mAbs currently in clinical development.

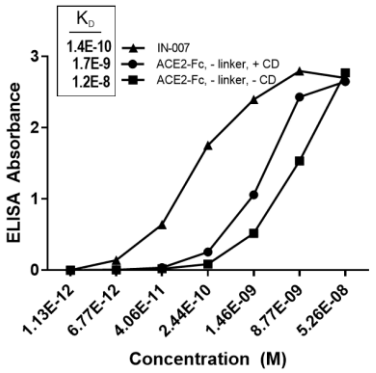

Figure 2. (A) IN-007 binds SARS-2 VLP with much greater avidity than ACE2-Fc.

3. IN-007 traps SARS-CoV-2 VLPs in AM. We prepared fluorescent SARS-2 VLP by co-expressing S protein with GAG-mCherry fusion construct, and visualized its mobility in fresh human AM isolated from extubated endotracheal tubes. IN-007 effectively trapped SARS-2 VLP in AM, reducing the fast moving viral populations (defined as possessing suffiicent diffusivity to diffuse across ~50 um layer in ~1 hr) by ~14 fold vs. saline control even at just 1 ug/mL conc in AM (**Figure 3**). In contrast, CR3022, a high affinity mAb against S protein from JNJ/Crucell, was not able to reduce viral mobility to the same extent at 10x higher concentration.

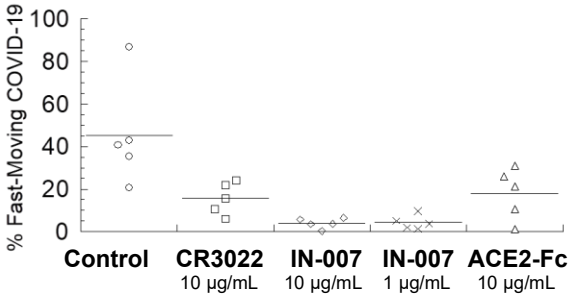

Figure 3. IN-007 effectively traps SARS-2 VLP in human AM with much greater potency than ACE2-Fc or CR3022, a control anti-SARS-CoV-2 mAb.

4. IN-007 reduces SARS-CoV-2 viral load in hamsters even when dosed as late as 48 h post-infection. As an *in vivo* proof-of-concept, we conducted a pilot study at University of Hong Kong assessing the efficacy of IN-007 in Golden Syrian Hamsters infected with live SARS-CoV-2 (the work was performed by Prof KY Yuen's group, the lab that pioneered the hamster SARS-CoV-2 model [95]). Hamsters presents clinical signs of weight loss, and histopathological changes with high viral loads in the lungs, making it a suitable model for testing mAb-based approaches despite differences in anatomy of the respiratory tract). Rather than evaluate dosing mAb within 2-6 hrs following infection (as reported in most prior mAb studies in hamsters [96-98]), we started treatment much later, giving IN-007 once daily intransally, beginning only 48 hrs after infection. We found that IN-007 treatment, even when delayed until 48 hrs, provided a 10-fold reduction in viral load in the nasal turbinate tissues by 96 hrs (i.e. 2 single doses; **Figure 4A**). PCR-based quantitation of viral load yielded similar reduction (data not shown). This translated to substantial reduction in weight loss over just the 2 day period (p=.03, **Figure 4B**). Notably, waiting until 48h to initiate treatment presents an exceptionally challenging model; other preclinical efficacy studies usually assess efficacy of mAb dosed 2-12 hr [96-98] post-infection, or up to 24hr in larger animals with REGN-COV2 [99]. Encouraged by the protection seen in this proof-of-concept experiment, we are now repeating this work and testing a dosing regimen beginning at 24h, which would more conservatively represent dosing human patients in the early stages of disease soon after diagnosis.

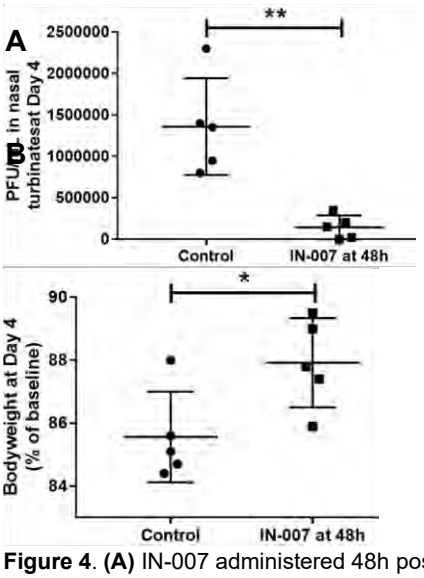

Figure 4. **(A)** IN-007 administered 48h post-infection significantly reduces SARS-CoV-2 PFU/mL in nasal turbinates of hamsters at 96h post-infection (p=.002). **(B)** IN-007 reduces loss of body weight by 96h post-infection compared to control (p=0.03).

5. IN-007 can be stably nebulized using the Philips InnospireGo vibrating mesh nebulizer. To demonstrate we can stably nebulize IN-007, we collaborated with Tony Hickey at RTI to perform initial characterization of nebulization with Philip's Innospire Go. Using the Copley Next Generation Impactor (NGI) (**Figure 5A**) for inertial impaction (i.e. distribution of antibody mass with respect to impaction stage cutoff diameter), we can determine the aerodynamic particle size distribution. We measured a Mass Median Aerodynamic Diameter (MMAD) of 4.96 ± 0.01 µm (GSD = 2.16 ± 0.01), with a fine particle fraction of 53.0% ± 0.10% (indicative of the percentage of mass deposited on stage 3 of the NGI and below which, at 15 L/min, has a cutoff diameter of 5.39 µm) using an anti-spike mAb. These results confirmed our ability to generate aerosol droplets suitable for lung airway deposition. To validate that IN-007 could be stably nebulized, we performed ELISA assays coated with pre-fusion-stabilized trimeric SARS-CoV-2 spike protein to assessing IN-007 binding pre- and post-nebulization, with nebulized IN-007 collected via a glass impinger sampling system [100]. We did not observe any loss in binding affinity following nebulization (**Figure 5B**). Finally, we assessed whether nebulization induced formation of aggregates by analyzing the size distribution of nebulized mAb via dynamic light scattering (DLS is a much more sensitive technique to detect aggregates than conventional size-exclusion HPLC, since the intensity of scattering scales to the 6th power of MW). We saw no detectible aggregates post-nebulization, with size distribution virtually identical to pre-nebulization (**Figure 5C**). Together, these results confirmed we can stably nebulize IN-007. In Year 1 of this project, we will further optimize the IN-007 formulation to increase the concentration of IN-007 that can be nebulized.

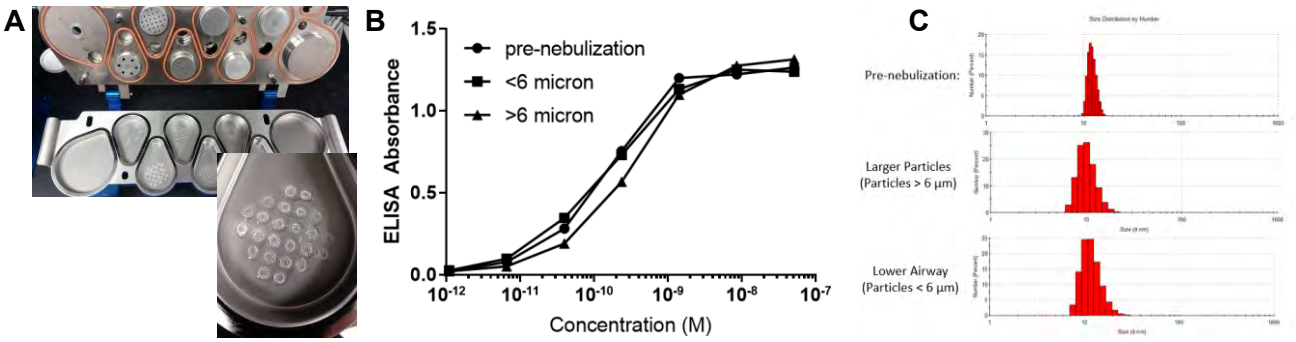

Figure 5. (A) Photograph of NGI. Inset: zoomed-in image of stage three. **(B)** ELISA binding curves of IN-007 pre- and post-nebulization, recovered from glass impinger collection setup allowing separation of large (>6 µm) and small/respirable (<6 µm) particles. **(C)** Dynamic light scattering of pre-nebulization and post-nebulization IN-007 shows lack of aggregates following nebulization.

Brief Summary of Expenses

The primary expenses are (1) personnel; (2) a variety of materials and supplies; (3) a contract with Bionova to develop cGMP-compliant manufacturing cell line. During project execution, it also became necessary to acquire a multi-angle light scattering instrument for characterizing the actual size of our molecule – a key readout to confirm stability. Unfortunately due to the unique size of our molecule, no such capability was available, and we had to purchase this instrument which allowed us to assess our molecule across a large number of conditions as part of formulation optimization. There was no expenditure for the hamster study as the lab conducting the study was very intrigued by our molecule and offered to conduct the study free-of-charge.

BUDGET OVERVIEW

			NON-PERSONNEL EXPENSES							
ORIGINAL BUDGET	**REVISED BUDGET**	**PERSONNEL EXPENSES** (Payroll and benefits cost for employee that are dedicated to COVID-19)	**Contracted Labor Expenses**	**Other Service Expenses** (e.g. utilities, telephone, data, lease related expenses)	**Subcontract Expenses** (e.g. construction, maintenance)	**Goods Expenses (e.g. supplies, PPE)**	**Equipment Expenses**	**TOTAL NON-PERSONNEL EXPENSES**	**TOTAL EXPENDITURES**	**BALANCE**
$798,352	**$788,352**	**$158,626.71**	0	$316,848.87	0	$195,527.45	$70,632	**$583,008.32**	**$741,635.03**	**46,716.97**

Personnel Supported by this Award

1. Sam Lai, Associate Professor, UNC Eshelman School of Pharmacy, with joint appointment in Immunology & Microbiology and Biomedical Engineering
2. Karthik Tiruthani, Postdoctoral Fellow, UNC Eshelman School of Pharmacy
3. Carlos Cruz-Teran, Postdoctoral Fellow, UNC Eshelman School of Pharmacy
4. Whitney Wolf, Research Specialist, UNC Eshelman School of Pharmacy
5. Jasmine Edelstein, Graduate Student, Biomedical Engineering, UNC

NC Policy Collaboratory Project-related Grants

Awarded

1. **Sponsor**: **Mucommune**
   SBIR Title: *Aerosol immunotherapy for treatment of human metapneumovirus infection*
   Total award: $262,000; UNC portion $74,250

2. **Sponsor**: **DoD** (with contract to MTEC) - 9-month lab service agreement from Nov 19, 2020 – Aug 31/2021
   Title: *Measurement of muco-trapping potencies of monoclonal antibodies against SARS-CoV-2*
   Award: $50,615 (through MTEC)

Pending

1. **Sponsor**: **Inhalon**
   SBIR Title*: Inhaled muco-trapping immunotherapy for COVID19*
   Amount: $2M; subcontract to UNC $106,539

2. **Sponsor**: **Mucommune (SBIR from NIAID)**
   SBIR Title: *In vivo validation and IND-enabling development of MM004, a bispecific inhaled immunotherapy for RSV and MPV*
   Amount: $2.4M; UNC portion $97,000
   Note: this award is now being finalized with grants management at NIH

3. **Sponsor: NIH**
   Title: *Engineered muco-trapping antibodies for inhaled therapy of parainfluenza and human metapneumovirus infections*
   Amount: $2,990,128

4. **Sponsor: NIH**
   Title: *Quantitative assessment of magnitude and temporal dynamics of anti-PEG immunity in vaccines receiving COVID mRNA vaccines*
   Amount: $2,170,954