DYADIC®

MAKING HEALTHCARE ACCESSIBLE & AFFORDABLE TO PATIENTS GLOBALLY

Dyadic Thermophilic Filamentous Fungus (C1-cells) Recombinant Glycoprotein Antigen Vaccines, Antibodies & Other Therapeutic Protein Product Production Platform

Safe Harbor Regarding Forward-looking Statements

Certain statements contained in this presentation are forward-looking statements within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934, including those regarding Dyadic's expectations, intentions, strategies and beliefs pertaining to future events or future financial performance. Actual events or results may differ materially from those in the forward-looking statements as a result of various important factors, including those described in Dyadic's most recent filings with the SEC. Undue reliance should not be placed on the forward-looking statements in this presentation, which are based on information available to us on the date hereof. Dyadic assumes no obligation to update publicly any such forward-looking statements, whether as a result of new information, future events or otherwise. For a more complete description of the risks that could cause our actual results to differ from our current expectations, please see the section entitled "Risk Factors" in Dyadic's annual reports on Form 10-K and quarterly reports on Form 10-Q filed with the SEC, as such factors may be updated from time to time in Dyadic's periodic filings with the SEC, which are accessible on the SEC's website and at www.dyadic.com

Company highlights

Next Generation Protein Expression Biotech with Well-established Global Partners

Proprietary & Patented C1 gene expression platform technology	Designed to bring biologic vaccines, drugs and other biologic products to market faster, in greater quantities, at lower cost, using microbial fermenters
Competitive advantages	Robust & rapidly expanding scientific data that demonstrates high productivity, stability, and purity for a growing number of disease and drug related protein classes and types
Validating partnerships	Well-established, global biological R&D organizations, top-tier animal and human health pharmaceutical companies, as well as governmental agencies
Opportunistic business development	Emphasis on large and growing addressable human and animal health markets, many shots on goal including vaccines and antibodies for infectious diseases and therapeutic proteins for diabetes, oncology and arthritis
Experienced management	Highly experienced and energized management team and board of directors driving process and execution excellence

C1 gene Expression Platform

Filamentous Fungus C1-Cell High Level Protein Production

High Purities & Exceptional Yields/Stabilities with Scalable Benefits for Protein Based Product Manufacturing

C1-cells are thermophilic filamentous fungi with highly desirable growth and manufacturing properties

C1 initially developed to produce large quantities of low-cost enzymes for textiles, biofuels, pulp and paper, food cellulases, etc. at very large industrial scales, up to 500,000 liters

Multiple genetically engineered C1-cell lines with differentiating highly desirable properties, including reduced protease activity and desirable glycan structures

Proven low cost, high-yield and purity, scalable, robust system with improved manufacturing and downstream benefits

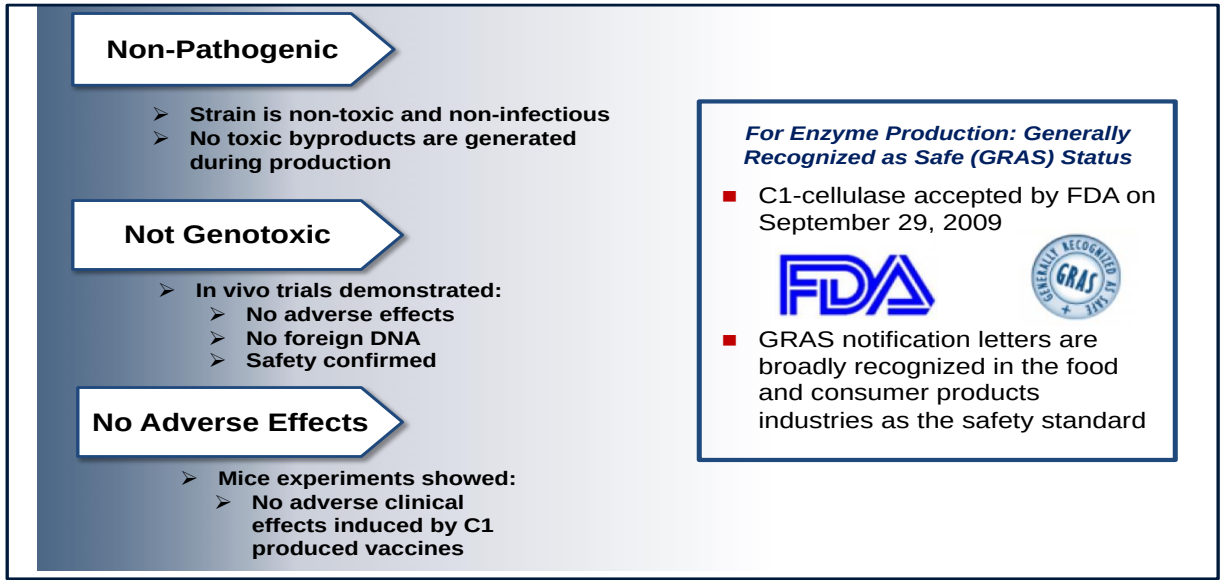
C1-cells received Generally Recognized As Safe (GRAS) certification from US FDA in 2009. Regulatory friendly, low-cost completely defined synthetic media

C1-cell chromosomal genome sequenced, annotated & full sets of genetic tools for gene engineering C1-cell strains

C1 manufactured SARS-CoV-2 antigen vaccine candidate in late pre-clinical development including toxicology & cGMP production for anticipated use in a Phase 1 clinical trial

C1 is a safe production cell line

***Dyadic's GRAS Notice* – C1 was acknowledged as a safe microorganism for the production of A Dyadic cellulase enzyme for food and feed application by the FDA on September 29, 2009.**



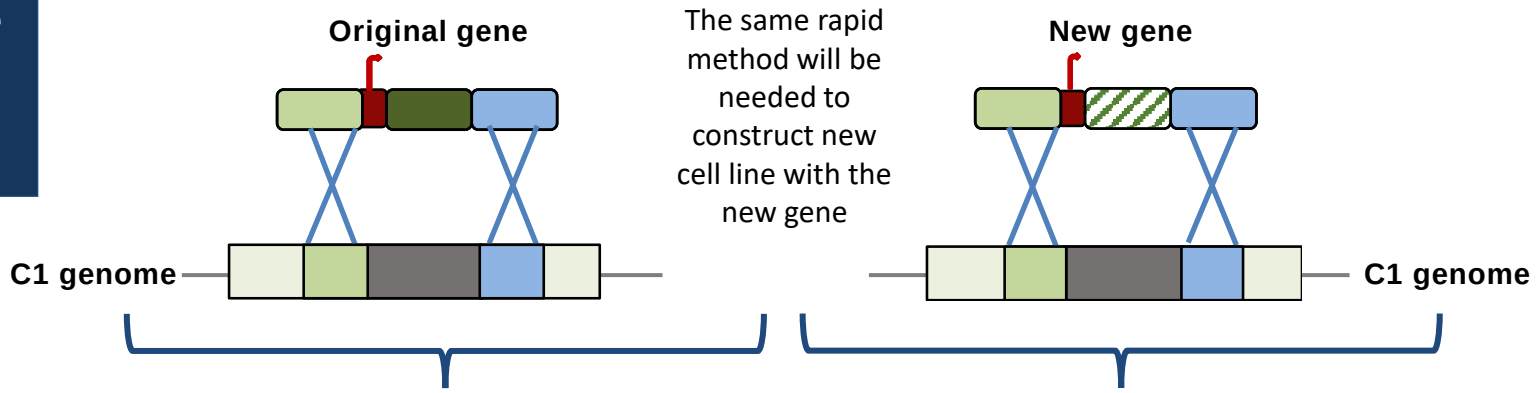
"The results of the study demonstrate that Dyadic's C1 *M. thermophila* is non-infective, non-pathogenic, and non-toxigenic, and supports the safety of Dyadic's C1 *M. thermophila* fungal strain for use in the production of a cellulase product for use in the food processing industry. Further, the study shows that, in mammals, conditions within the abdominal cavity do not favor the growth of the fungus. Intraperitoneal injection into mice elicited defense mechanisms (i.e., inflammation and abscess formation) common to the isolation and clearance of foreign proteins from host tissues".

Parexel report to Dyadic (June, 2020) - The development of C1 as a source cell system would require the same level of CMC analysis as would be required for Pichia and as mentioned earlier C1 has a glycan structure more similar to the human glycan structure than Pichia which if anything should be an advantage.

Company highlights

C1 Site Directed Transformation Method leads to quick generation of stable C1 cell lines

Rapid Response
High Doses
Scalability
Affordability



- **Set of strong promoters native and synthetic**
- **No need for induction**
- **Stable single-copy integration**
- **No need for transient stage**

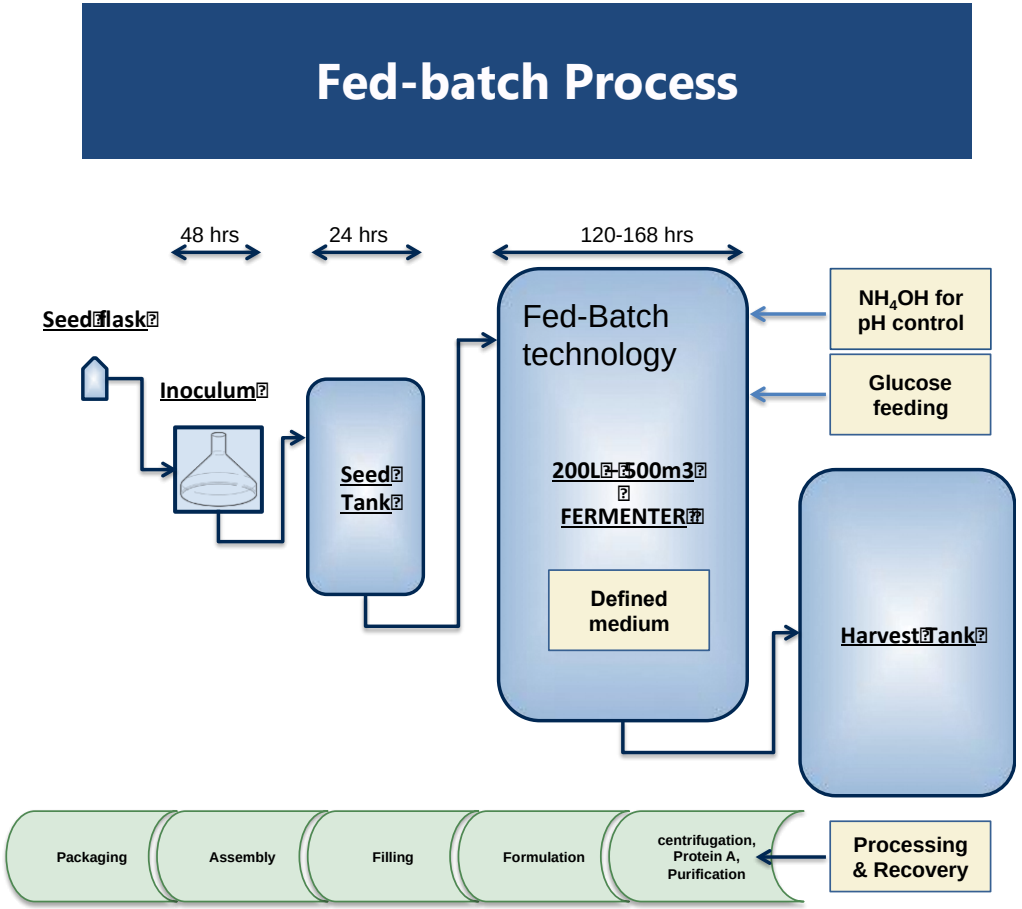
1 week	3 weeks	1 week	2 weeks
Gene synthesis	Plasmid construction	Strain construction and re-isolation for monoclonality	MTP ferm., DSP and analytics & RCB generation	1-30 l scale fermentation from RCB, DSP and analytics

Sending samples for evaluation, animal studies

cGMP grade strain and process characterization, MCB

- **Rapid Development Timelines**
- **High Productivity – large quantities**
- **Purity**
- **Stability**
- **Robust Manufacturing Process**
- **Flexible Commercial Scales**
- **Low Cost**

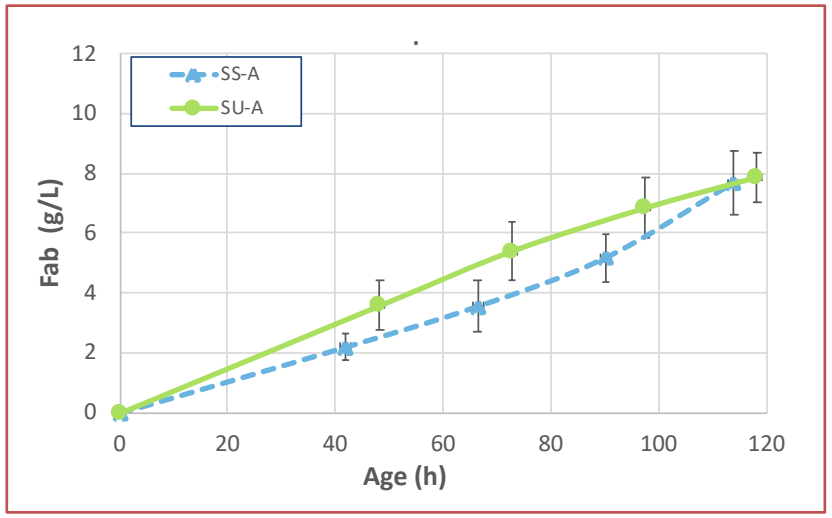
C1 Fermentation Technology

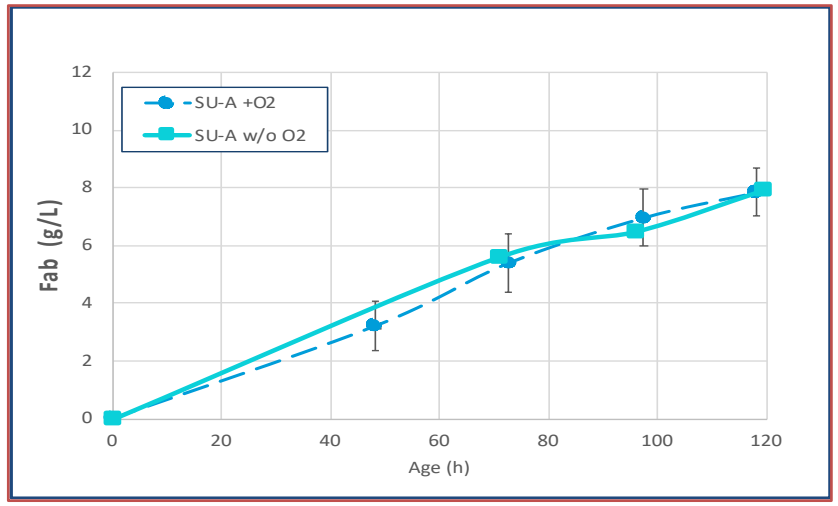


- Fully defined synthetic medium
- Fed-batch technology with glucose feeding
- Wide range of conditions available pH: 5-8, Temp: 20 - 45°C
- Low viscosity culture due to unique morphology in the fermenter
- 4-7 day process
- 1L to 500,000L fermentation scale, stainless steel or single use stirred tank fermenters
- At the end 30-40% biomass, 60-70 % supernatant (titers refer to the supernatant); g/L/day
- Protein production requires no inducer
- Protein typically secreted to the media

Certolizumab production with C1 in Single use Bioreactor (SUB)

Conditions A

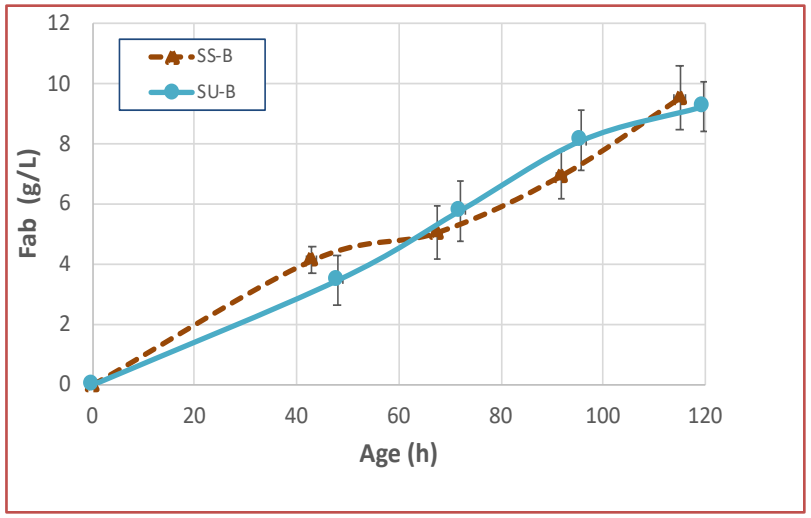


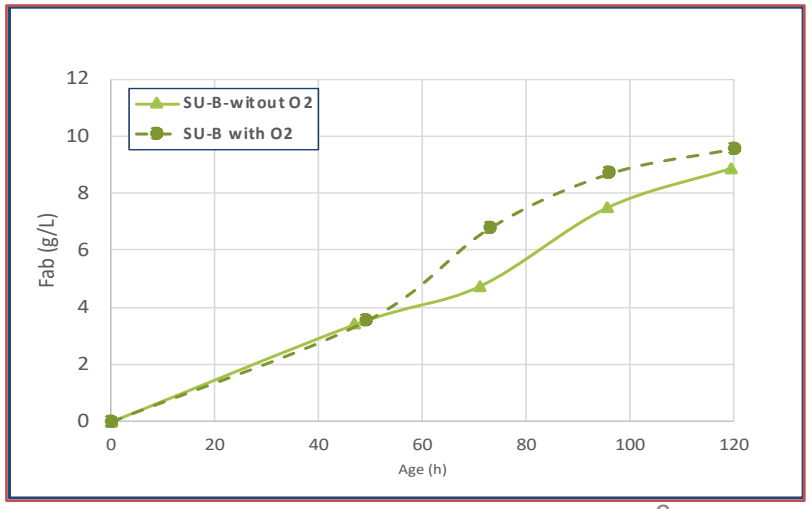


GE's Xcellerex™ XDR-50 MO

- Six batches were tested in 2 different conditions with or without O_2 supplementation.
- Conditions B have been shown to be more productive than A in both SSB and USB.
- Supplementation of O_2 slightly improve Certolizumab productivity

Conditions B





Generic Process Flow Chart for C1 (12 – 14 days) (*)

(*) Generic Process Flow Chart for CHO is 41 – 54 days

1 – 1,5 days

0,75 -2,5 days

5 days

Pre-inoculum: Mycelium activation

Seed train: inoculum expansion in flak

Inoculum expansion - bioreactors

Production bioreactor

Passage 1 plate

Passage 2 flask

N-3 ~1.6L scale

N-2 ~40L scale

N-1 ~1000L scale

N ~12,000L scale

Summary - Titers Achievable in the C1 Host

Fc-Fusion
15.3 g/l
168 Hours
2.58 g/l/day

mAbs
up to 24.5 g/l
168 Hours
up to 3.5 g/l/day

Fab (Certolizumab)
up to 14.5 g/l
164 Hours
up to 2.1 g/l/day

Trispecific
up to 6.12 g/l
144 Hours
up to 1.02 g/l/day

Bispecific
up to 6.17 g/l
72 Hours
up to 2.05 g/l/day

Therapeutic Proteins

2015

Influenza HA
up to 413 mg/l
In 137 h
(72 mg/l/day)

Virus antigen
2-3 g/L
In 96 h
(500-750 mg/l/day)

Virus-like Particles (Secreted)
up to 2400 mg/L
In 110 h
(500 mg/l/day)

Vaccines

(note: not all results are from the current best strain!)

C1-Cell Recombinant Protein Products With Competitive Advantages

Robust Recombinant Protein Production Platform Offers Competitive Advantages Over Existing Techs

Purities

- High retention of target secreted protein(s) or other C1-cell bioproduct(s) through downstream processing
- No viral or endotoxins

Productivity

- Robust & versatile C1-cell growth conditions
- High yields of C1-cell secreted protein and other products
- Low viscosity [unique morphologies]

Robustness

- Scales ranging from laboratory microtiter plates, shaker flasks, single use and/or stainless-steel bioreactors

Speeds

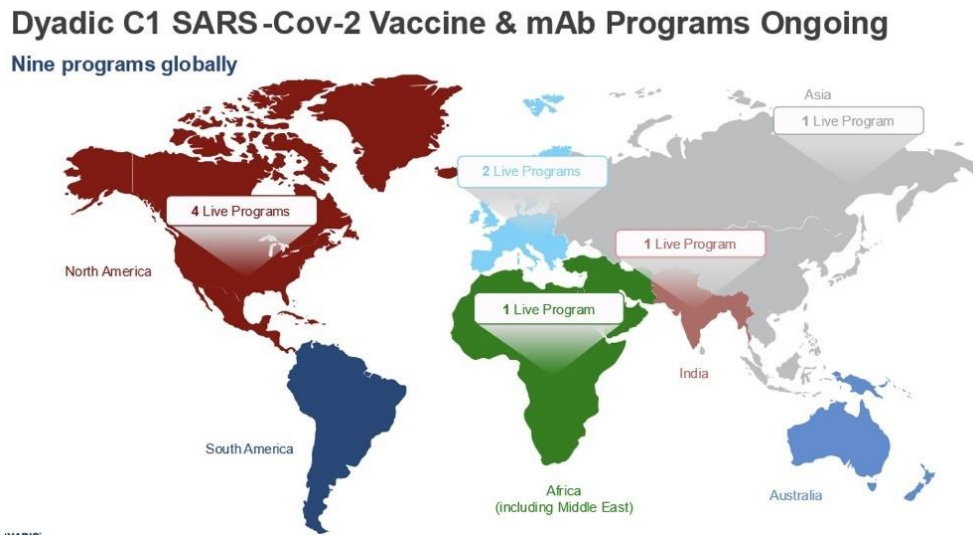
- Develop stable C1-cell lines in ~7 weeks producing recombinant proteins at grams/liter
- C1-cell production savings of ~30 days over CHO-cell production costs with very expensive media
- Manufacturing ~ 3-4 batches of mAbs at the same time it takes to make 1 batch using CHO-cells

Costs

- High yields and rapid manufacturing cycle times reduce C1 production costs and significantly reduce manufacturing footprint

Vaccines

C1 is ideally suited to help meet the global demand for Health Equity including multivalent vaccines & multi antibody cocktails



Building a more efficient biomanufacturing platform to serve a global population more affordably

Opportunistic participation in product development of vaccines, biotherapeutics for infectious, oncology, & autoimmune diseases

Novel synthetic biology tools for developing diagnostic, prophylactic, and therapeutic products

Biopharma industry-leading global commercial, government & academic strategic collaborations

European Union Zoonosis Anticipation Preparedness Initiative ZAPI Stakeholders Final Conference

Dyadic's C1 Gene Expression Platform Played a Key Role In The Success Of The Five-Year EU € 20 Million ZAPI Program

- Many infectious diseases, including influenza and Ebola, can be transmitted to humans from animals (and vice-versa). Known as zoonoses, these diseases represent a serious threat to both human and animal health.
- ZAPI brought together experts in human and animal health to create new platforms and technologies that facilitate a fast, coordinated, and practical response to new infectious diseases as soon as they emerge.

"Within the collaboration, we helped to validate a prolific fungus, developed by one of our project partners, Dyadic, who demonstrated that their C1 technology can be used to churn out vaccines and antibodies in unprecedented amounts which has the potential to further accelerate biologic manufacturing processes," said project coordinator ***Dr. Jean-Christophe Audonnet. Dr. Audonnet*** continued, "Dyadic and its C1 cell line far exceeded our initial expectations at the start of the program, turning in record antigen productivity for both the SBV and RVFV antigens."

Info about ZAPI Stakeholders Final Conference are available from the IABS website, and recording are available on YouTube:

https://zapi-stakeholders-final-conference.iabs.org/conference-information.php?parag=slides

- Day 1 – Morning, February 4, 2021
- Day 2 – Morning, February 5, 2021
- Day 1 – Afternoon, February 4, 2021
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European Union Zoonosis Anticipation Preparedness Initiative ZAPI Stakeholders Final Conference. Evidence for C1 Approach

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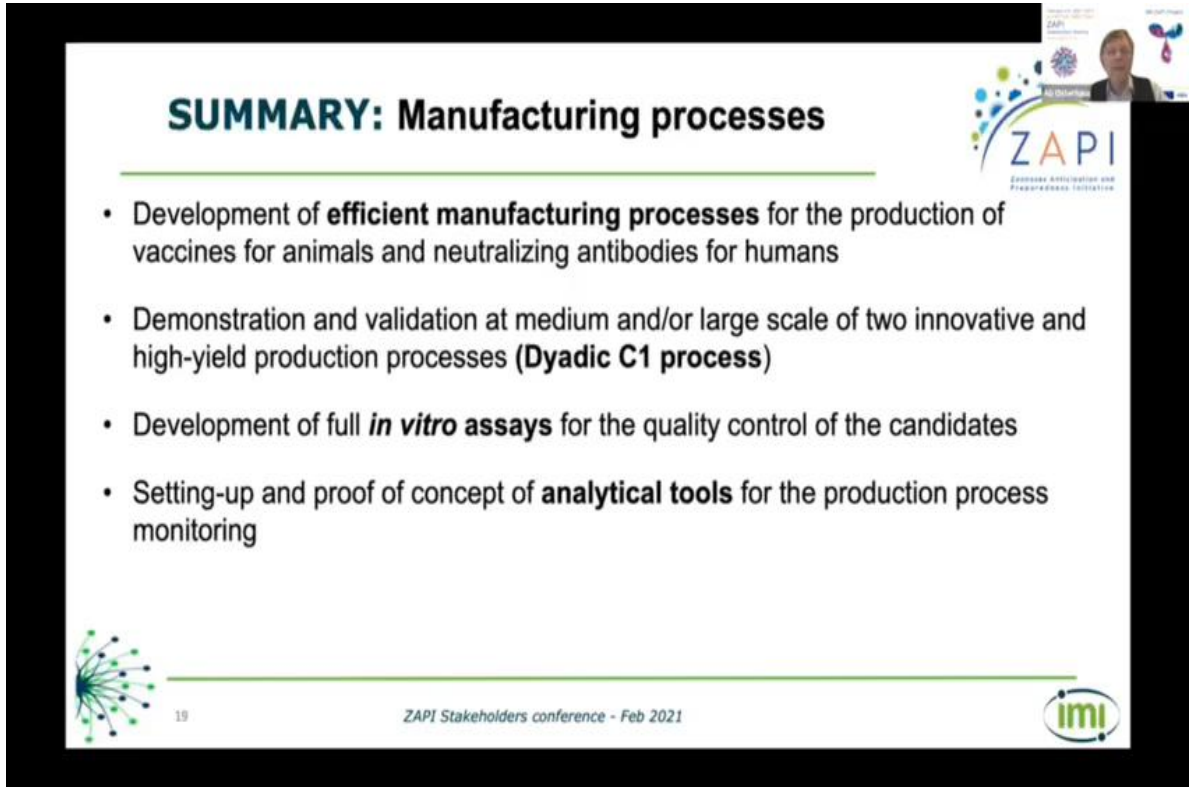
ZAPI Stakeholders Final Conference C1-cells selected for antigen manufacturing

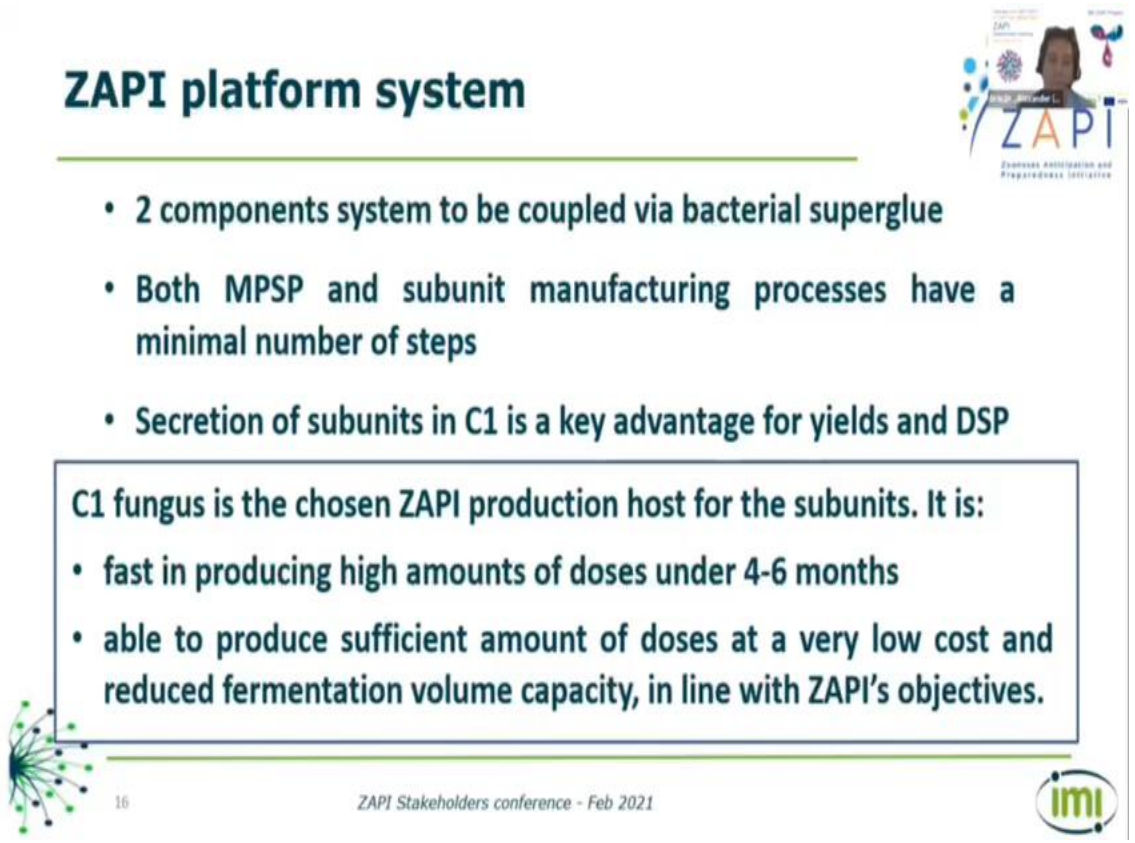


"Efficient manufacturing processes are being developed, I Specifically would like to mention Dyadic, the C1 the fungal process there which gives enormous, enormous amounts of antigens and I think especially in the face of the world's situation with the pandemic a system where you can produce much more, much more vaccine would be an ideal one to look into so if that were or to be the only spin off that would be fantastic." [1] –

Dr. Albert Osterhaus, Erasmus Medical Centre

1. ZAPI Stakeholders Final Conference Day 1 – Afternoon, February 4, 2021

ZAPI Stakeholders Final Conference



"However, If you look at the subunit production that was just also described by Ronen, It is pretty clear that the C1 expression, or using the C1 platform is the key advantage here for as is described for yields but also DSP purification, we don't have to compare those numbers again, but as Ronen described as grams per liter-based yields and IN TERMS OF SURGE CAPACITY THAT'S WHAT WE NEED, therefore for further development C1 now or has been chosen as the production host for those sub units, fast development, AND I REPEAT AGAIN, sufficient amounts and what's also is very important then from an industry perspective is the reduced fermentation volumes we don't need 4, 5, 6 thousand liters we maybe need 10, 50, 100 and then we can use smaller systems and more flexible systems obviously and I think as written here THAT IS EXACTLY WHAT WE WANT TO HAVE AND THAT'S EXACTLY IN LINE WITH THE ZAPI OBJECTIVES" [1]

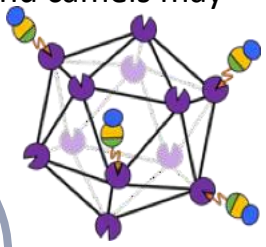
Dr. Alexander Brix, *Boehringer Ingelheim*

[1] **ZAPI Stakeholders Final Conference Day 1 – Afternoon, February 4, 2021**

Success in Expressing High Level of SBV & RVFV Antigens

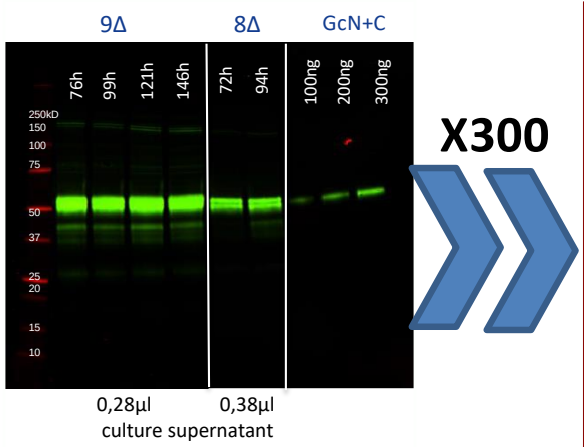
ZAPI, is a research and development program sponsored by the EU with the goal of developing a platform suitable for the rapid development and production of vaccines and protocols to fast-track registration of developed products to combat epidemic Zoonotic diseases that have the potential to effect the human population.

- SBV (Schmallenberg Virus) causes congenital malformations and stillbirths in cattle, sheep, goats, and alpaca. An antigen against SB that was developed by ZAPI group, was expressed by C1. Production level reached **1.8 g/L in 7 days fermentation – 300 fold higher than in Baculovirus**
- RVF (Rift Valley fever) is a viral disease of humans and livestock that can cause mild to severe symptoms. Animals such as cows, sheep, goats, and camels may be affected. An antigen against Rift Valley Fever Virus (RVFV) was expressed by C1.



SBV

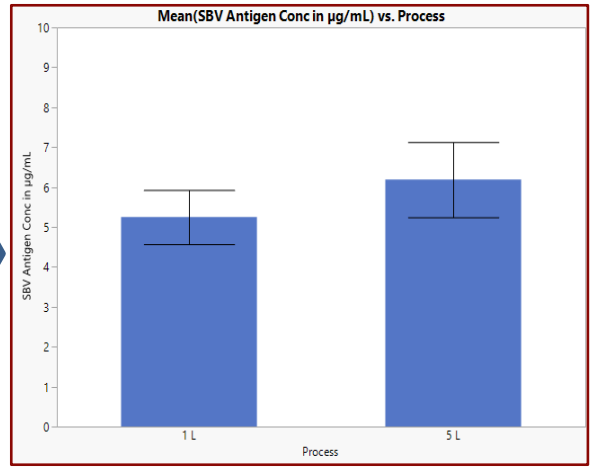
C1 Fermentation



SBV yields: **1, 800 mg/L**
(time point 121h)

X300

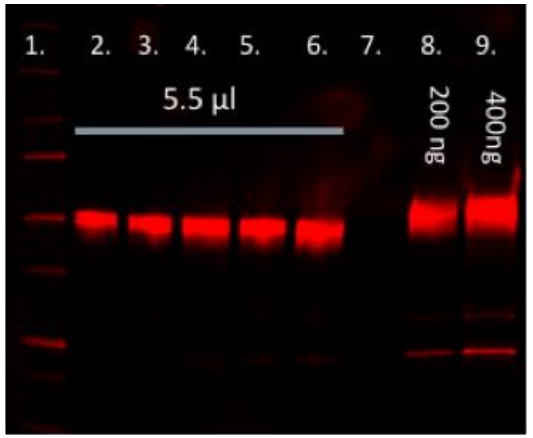
Baculovirus Fermentation



SBV yields: **6 mg/L**
(time point 192h)

RVFV

RVFV Gn_DVII-SpyCatcher-C-tag

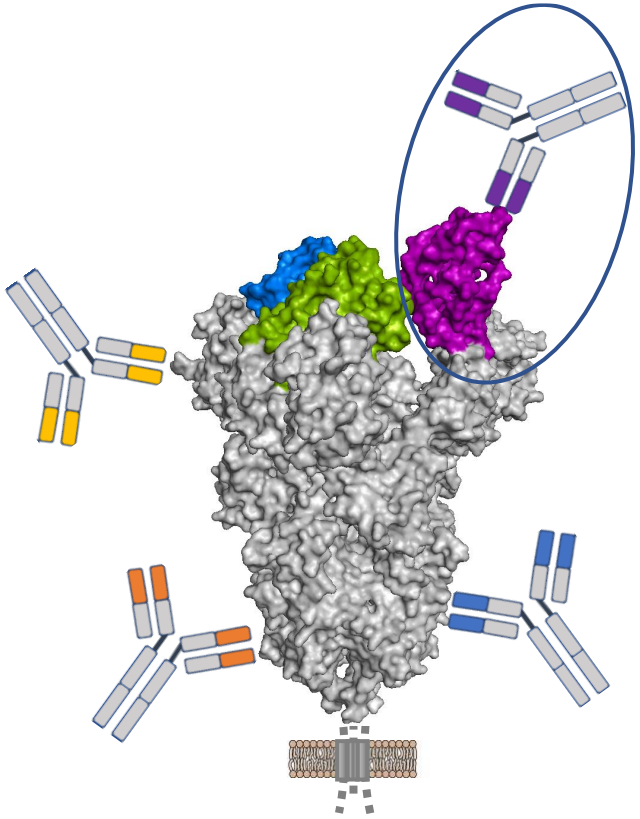


1. MW
2. Day 3
3. Day 4
4. Day 5
5. Day 6
6. Day 7
7.
8. SpyC RVFV
9. SpyC RVFV

RVFV yield: **1,24 g / L**

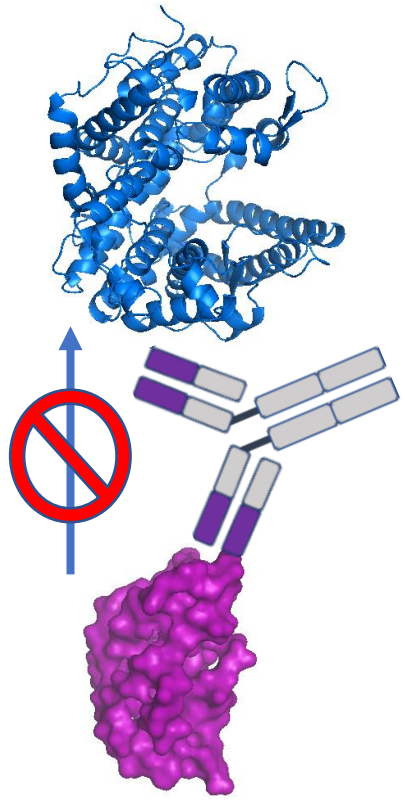
The RBD: 'Achilles heel' of the spike protein

Analyses of the antibody repertoire of infected patients show that the vast majority of (potent) neutralizing antibodies target the **receptor binding domain**.

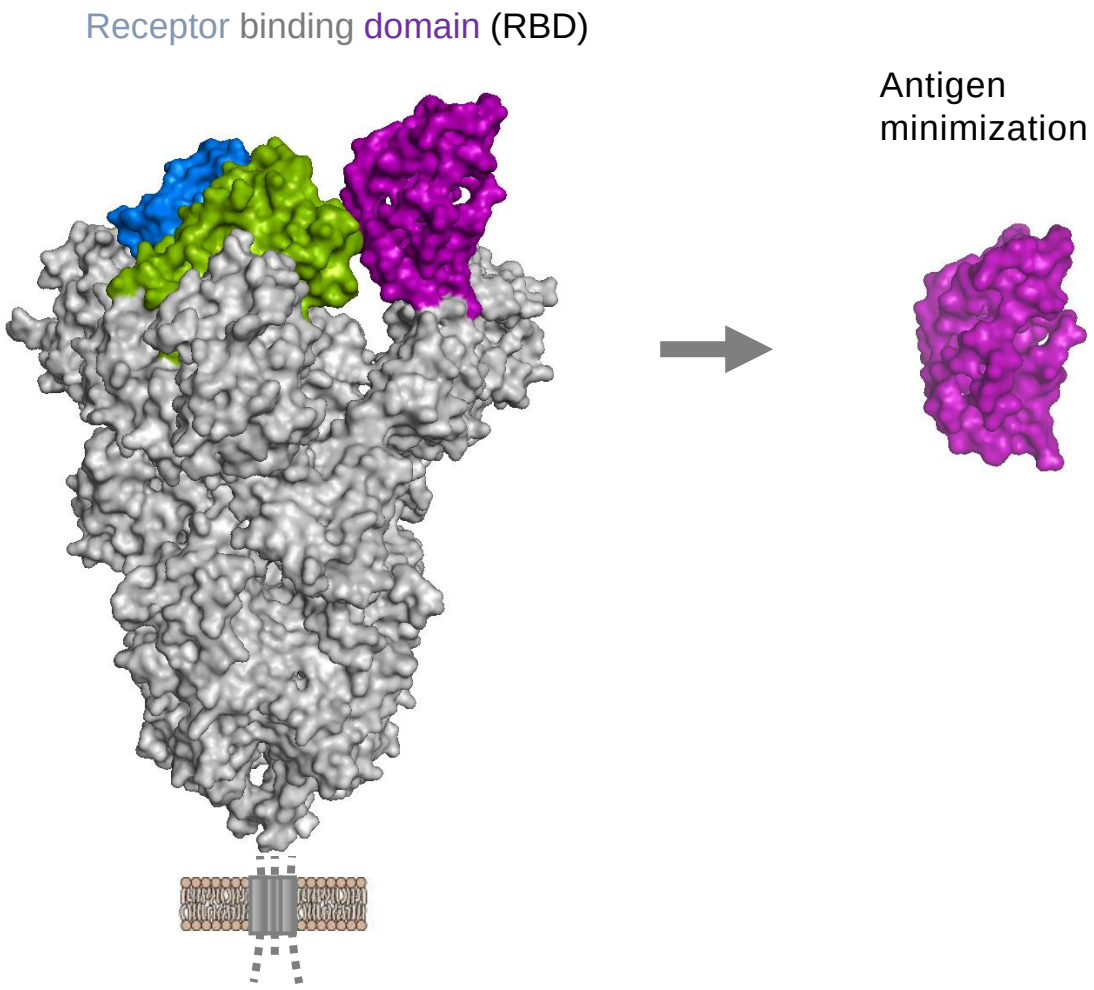


RBD-targeting antibodies are so potent, as their binding prevents virus interaction with the host cell, thereby blocking virus infection.

ACE2 receptor



RBD has key advantages as a vaccine antigen



Advantages of minimization of spike protein antigen to the RBD:

- Exposure of key neutralizing epitopes on the RBD to the immune system that are hidden in the closed spike conformation
- Efficient induction of neutralizing antibodies by focusing the immune response to primary neutralizing epitopes
- RBD is much easier to produce (18x smaller than the spike trimer, much higher yields) compared to full size S
- Immune response to RBD is sufficient to protect from disease
- Recombinant protein vaccine: use as ‘booster’ vaccine, no interference by ‘vector immunity’
- Stand-alone vaccine and potential universal boost strategy
- Reduced probability of Antibody Dependent Enhancement (ADE) / Enhanced Respiratory Disease (ERD)

Successful Execution of Government Sponsored Collaborations

Positions Dyadic to Enter Clinical Trials

Israel Institute for Biological Research (IIBR)

- Entered into initial collaboration January 2018
- Focused on advancing C1 expression platform for the development and manufacture of recombinant vaccines and neutralizing agents comprising targeted antigens and monoclonal antibodies, to combat emerging diseases and threats
- A proprietary IIBR Fc-fusion enzyme has been expressed using C1 technology provides certain countermeasures against nerve agents such as sarin and VX gas
- February 25, 2020 expanded collaboration with the IIBR to combat emerging diseases including collaborating on a potential rVaccine candidate to combat COVID-19 outbreak
- Dyadic provided one of its C1 RBD SARS-CoV-2 vaccine strains, along with samples of the C1 expressed RBD vaccine candidate, to IIBR for use in developing a potential COVID-19 vaccine
- Mice study conduced by IIBR showed that C1 expressed SARS-CoV-2 RBD has the potential to generate excellent immunogenicity responses with very high titers and neutralizing antibodies
- IIBR is conducting challenge study: transgenic mice expressing the Human Ace2 will be infected with SARS-CoV-2 virus.

SARS-CoV-2 Spike RBD Is A Key Target For Potent Neutralizing mAbs

- **In ~2 months, we developed a C1 cell line expressing the Receptor Binding Domain (23kDa) of SARS-CoV-2 spike protein**
- **C1 stable cell line was developed that expressed the RBD originally at a level of ~ 1 g/L– no need for transient stage**
  - **Fermentation optimization 2-3 g/l in 5 days in 22L fermenter**
- **C1 fermentation is based on Fed-batch technology with glucose feeding and cGMP synthetic media**
- **The RBD antigen was secreted to the media – no need for induction**
- **Transgenic mice challenge test demonstrated full protection**

Receptor binding domain:

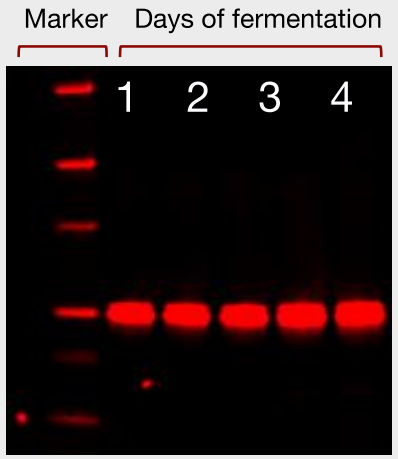
Single folded polypeptide chain

All potent neutralizing Ab target the RBD

Ag minimization -> focused immune response

Advancing Towards Phase I clinical study Q3-Q4 2021

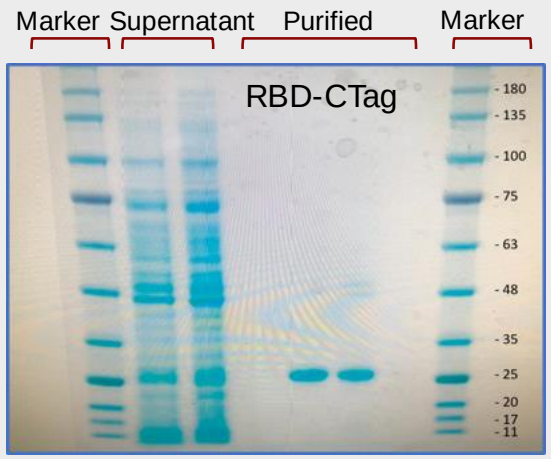
C1 cell line in Ambr250 fermenter system



C1 RBD strain was run in Ambr250 for 5 days

WB analysis off fermentation broth

C1 cell line in 1L fermenter system

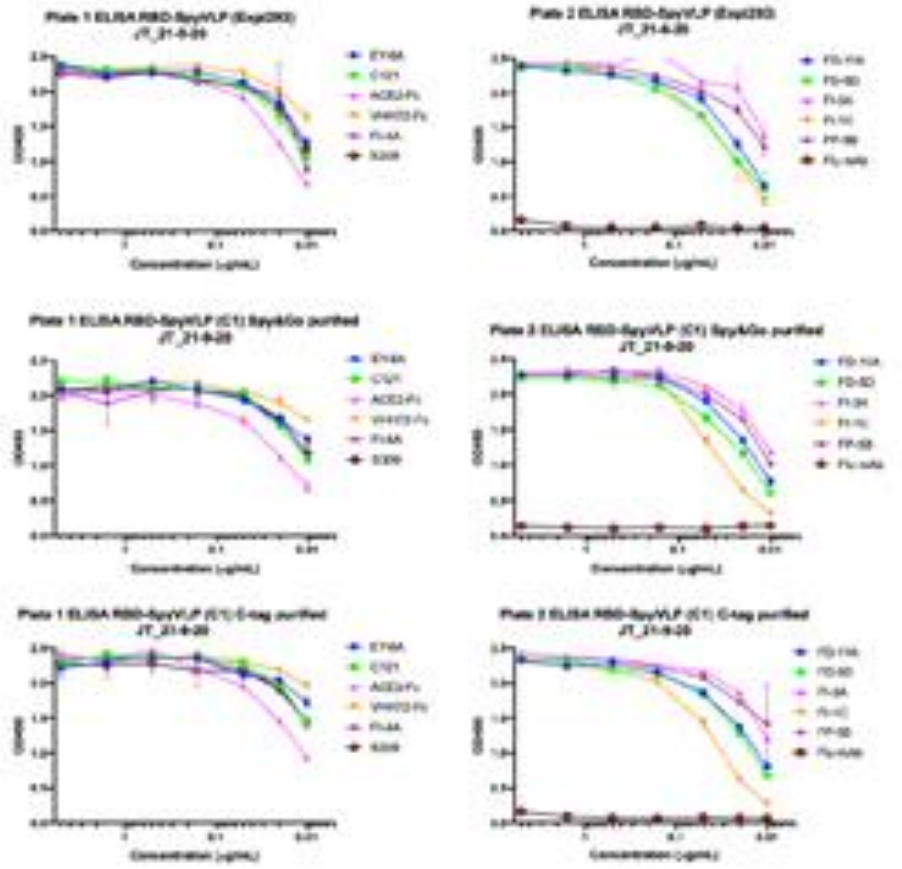


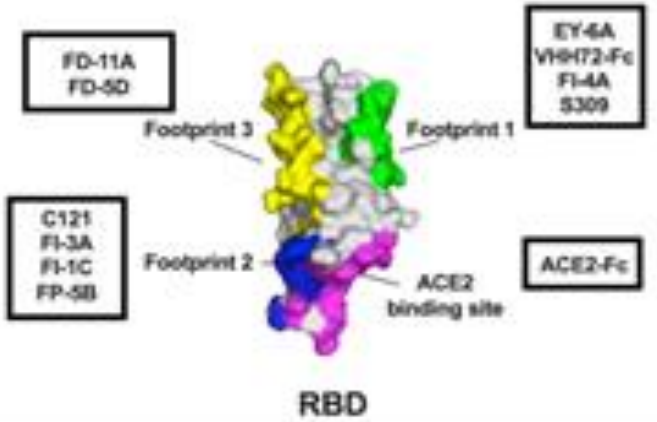
C1 RBD strain was run in 5L scale fermentation
The RBD was purified twice with CaptureSelect™ C-tag 10ml column.

98% purity
70% recovery

C1 RBD Binds to all Epitopes

ELISA 21-9-20





Source of mAb:

- C121 (Robbiani et al., 2020)
- VHH72-Fc (Wrapp et.al., 2020)
- The rest (In house)

Evaluation Of RBD Produced By C1

01

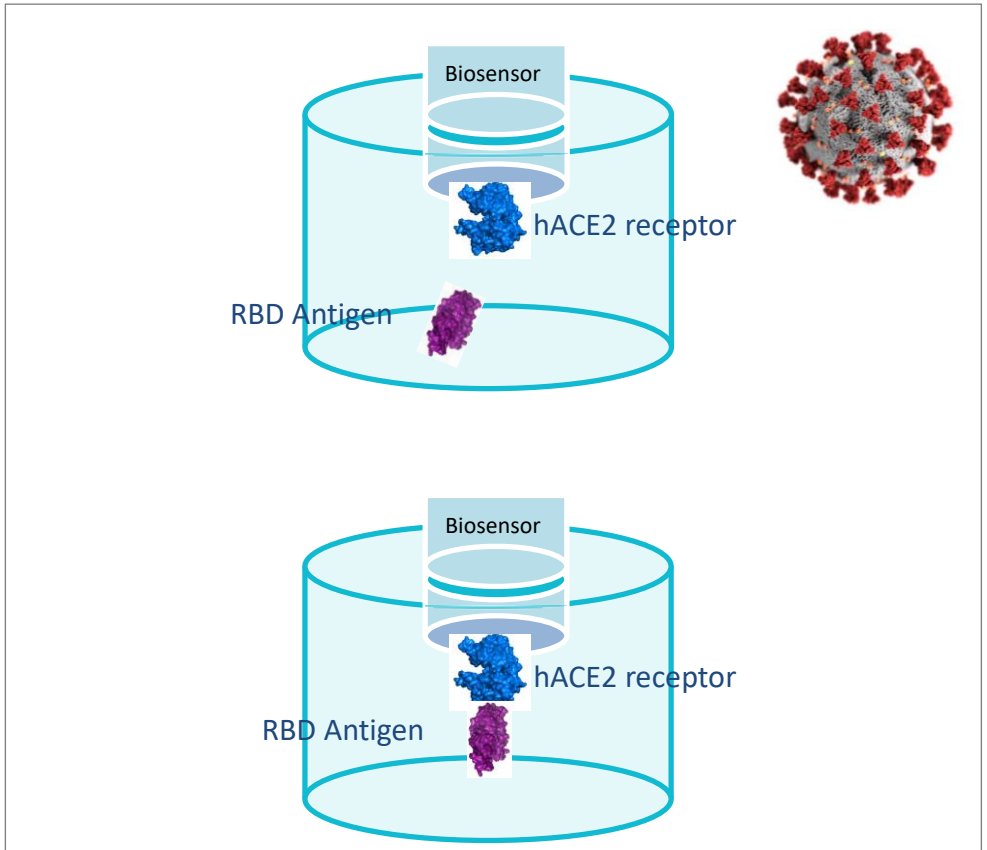
Biomolecular Binding Kinetics Assays: The equilibrium dissociation constant (KD) of C1 SARS-CoV-2-RBD-Ctag binding to recombinant hACE2 was calculated to be 4.9 Nm, which is comparable to that of the CHO SARS-CoV-2-RBD: 5.11 Nm.

02

In addition, all RBD neutralizing mAbs (that bind to different RBD epitopes) that were identified in patients infected by SARS-CoV-2 were efficiently bounded to C1 RBD-Ctag antigen. This binding clearly demonstrates that C1-RBD antigen was properly folded and has high potential to generate immune response and protection against the SARS-CoV-2 virus.

03

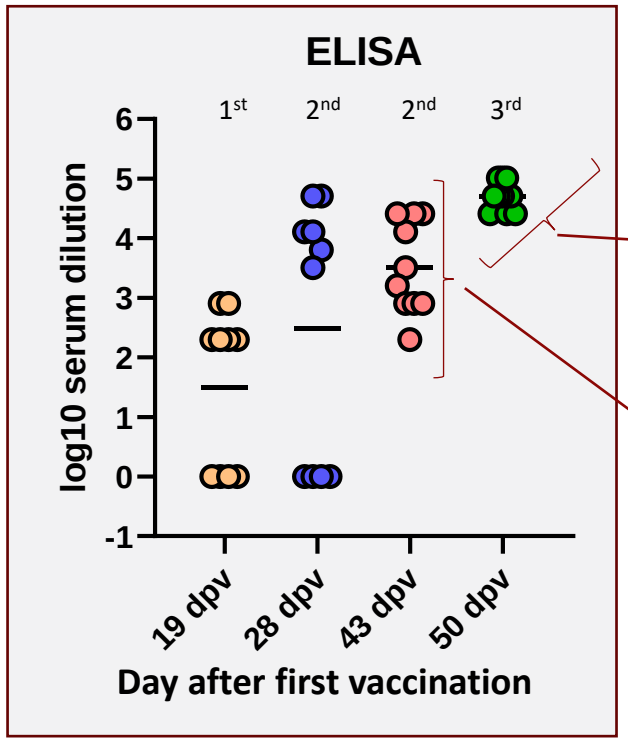
Recently – mice study confirmed that RBD antigen produced by C1 induced the production of neutralizing antibodies against the SARS-CoV-2 in mice. Additional animal studies are underway to assess the immunogenicity and protective efficacy upon challenge in hamsters and transgenic mice expressing the Human Ace2 will be infected with the SARS-CoV-2 virus.

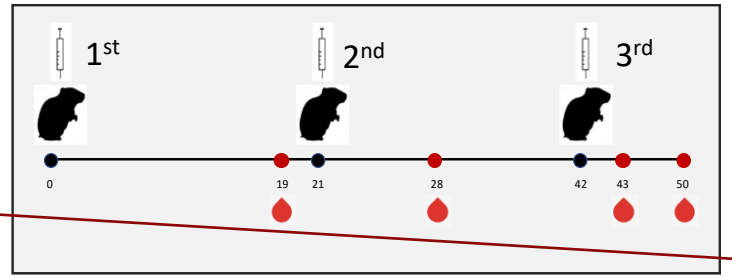


OCTET assay: RBD antigen binding assay. A ACE2 receptor is immobilized on the biosensor, followed by the binding of the RBD antigen. The binding coefficient is measured by the Biosensor

Mice study with C1 expressed SARS-CoV-2 RBD vaccine candidate - Results

Mice study demonstrated that the C1-RBD induced neutralizing antibodies at high level.





Plaque reduction neutralization test (PRNT)

SARS-CoV-2 and Vero E6 cells

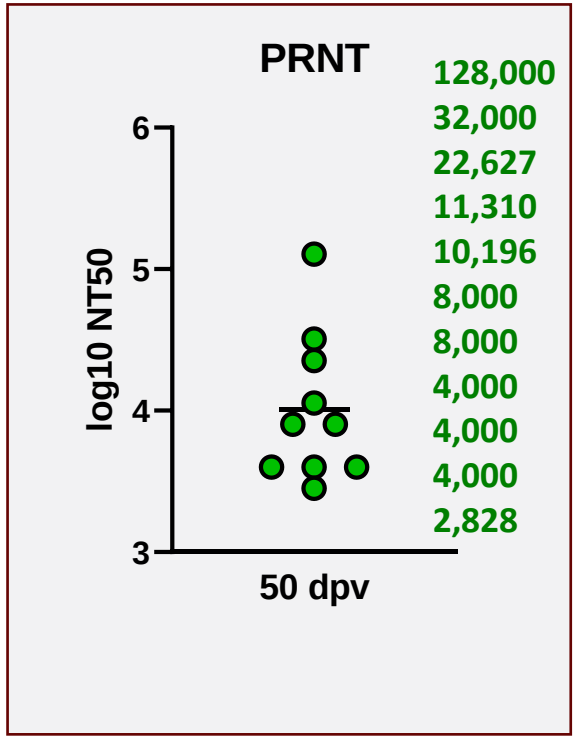
NT_{50} **Dilution** that neutralizes 50% of the virions

Conducted on pooled sera According to Titer (GEOMEAN OF THE titers):

Range 1:1,600 -3200 (Low) - **1,280**

Range 2: 6,400 -12,800 (Mid) - **5,120**

Range 3: 25,600 - 51,200 (High) - **20,400**



Direct RBD ELISA.

Serum samples obtained at 19, 28, 43 and 50 dpv were tested in a direct ELISA assay. For each mouse (n = 10) serum dilutions scoring positive in the ELISA are plotted, bars represent geometric means for each sampling time-point.

PRNT

Serum samples obtained at 50 dpv were tested in a PRNT against SARS-CoV2 on Vero E6 cells. NT50 values are plotted for each mouse (n=10) and the geometric mean value is indicated.

Challenge Test with Transgenic Mice study with C1 expressed SARS-CoV-2 RBD vaccine candidate

Challenge Mice study demonstrated that the C1-S-RBD induced full protection

A. **Vaccination of K18-hACE2 transgenic mice:**
B. **2 groups of transgenic mice were vaccinated with 20 µg of RBD-C formulated with Alhydrogel.**

Grope I of 8 mice were vaccinated: Prime = Day 1 and Boost at Day – 21. There were 3. Placebo Control Mice. At day 42- Challenge with 2000 PFU of SARS-CoV-2.

1. **Bleedings – At Day 20 and Day – 35.**
2. **Antibodies against RBD were determined by ELISA**
3. **After 2 days All Control Mice were dead. 7 out of 8 Mice survived with almost no weight loss.**

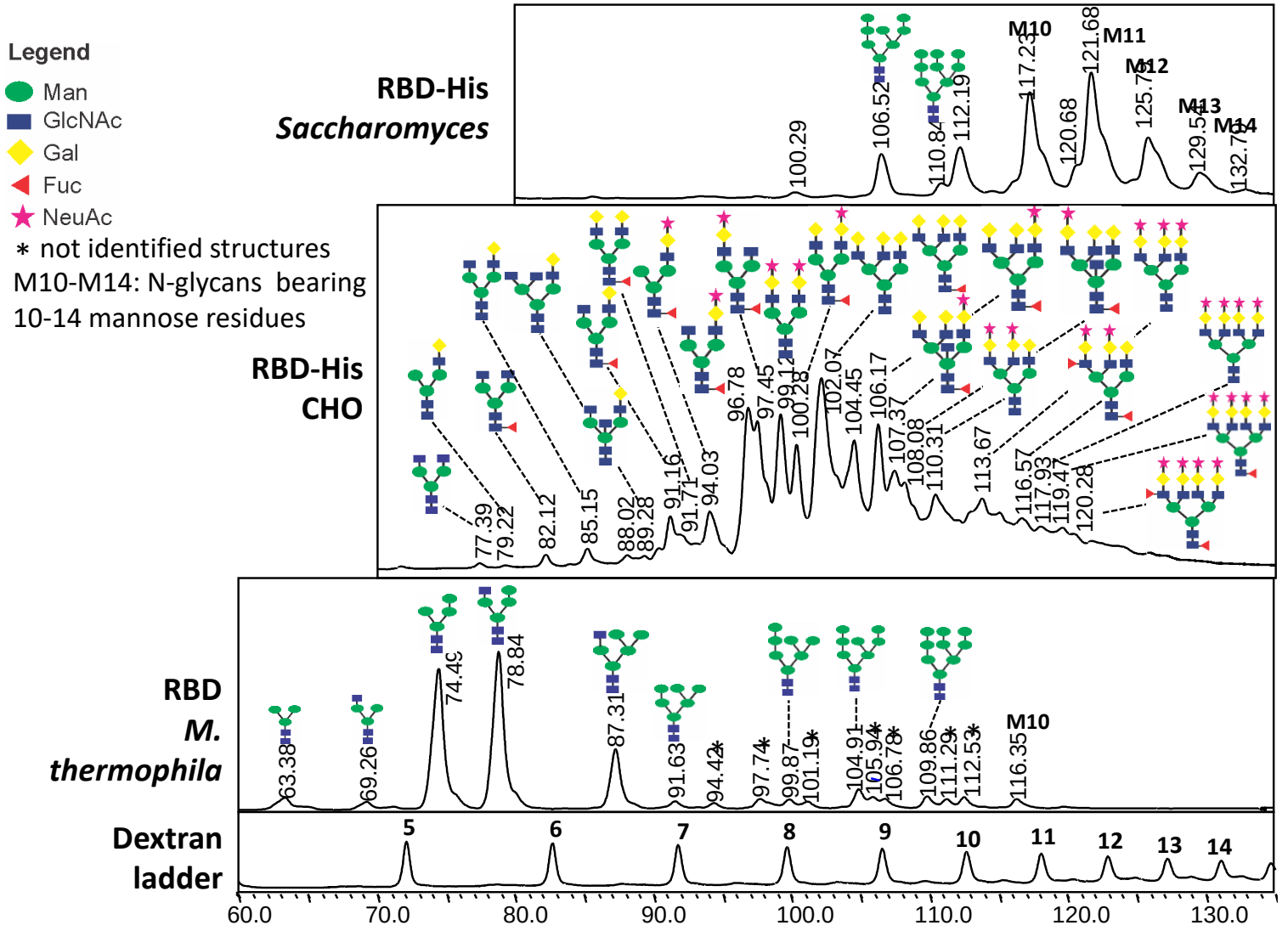
Mouse	DAY 35 Anti RBD	Day 35 Neutralizing	Challenge
1	102,400	11,314	Live
2	25,600	2828	Live
3	3,200	453	Live
4	51,200	8000	Live
5	102,400	32,000	Live
6	6,400	905	Live
7	51,200	32,000	Live
8	400	40	Dead
Cont-1	0		Dead
Cont-2	0		Dead
Cont-3	0		Dead

Grope II of 8 mice were vaccinated: Prime = Day 1 and Boost at Day – 21. And Boost at Day-42 There were 2. Placebo Control Mice. At day 57- 4 mice were Challenged with 2000 PFU of SARS-CoV-2.

i. **Bleedings – At Day 20 and Day – 41 and 56**
ii. **After 2 days All Control Mice were dead. 4 out of 4 Mice survived with no weight loss.**

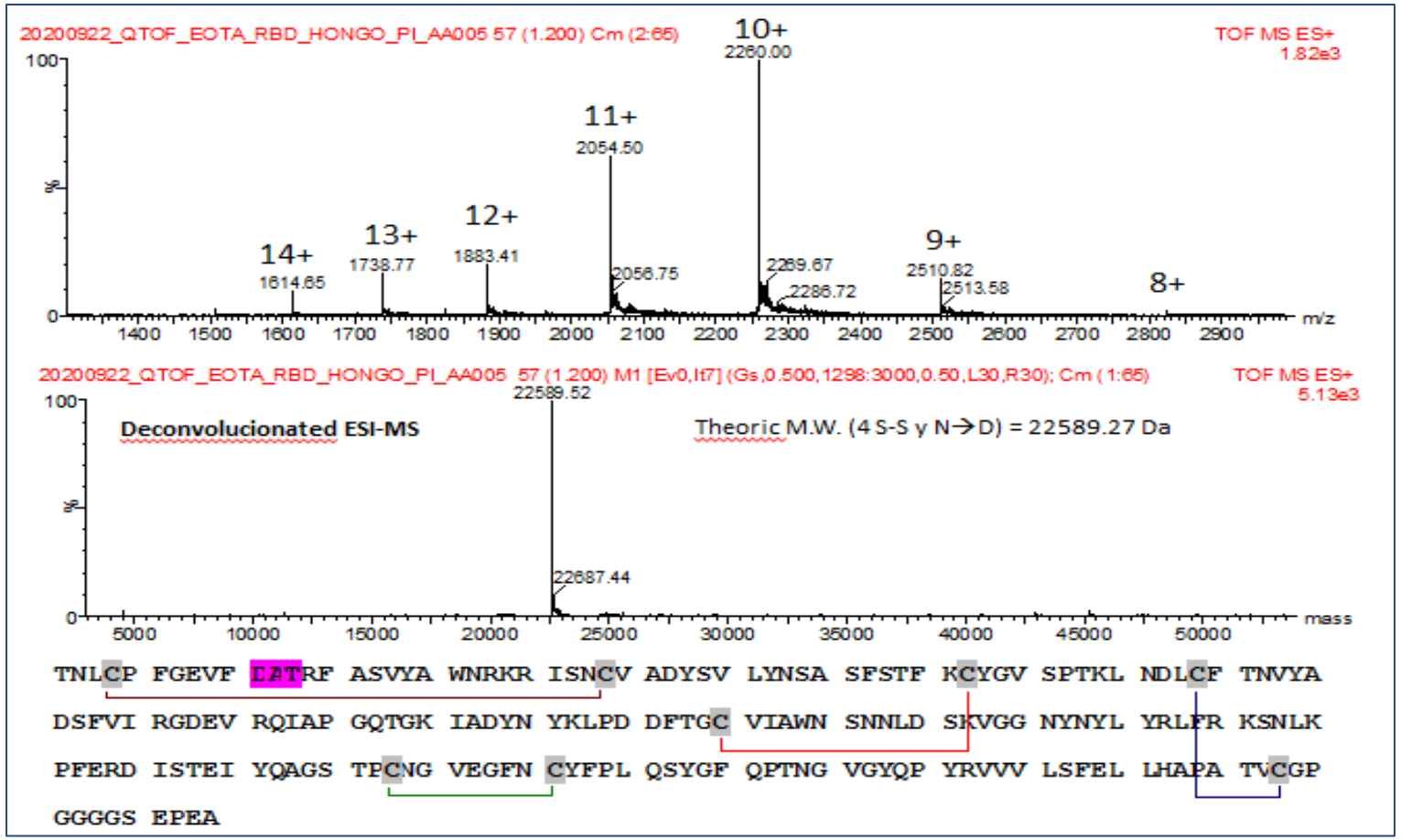
Mouse	DAY 41 Anti RBD	Challenge
1	102,400	Live
2	204,800	Live
3	204,800	Live
4	204,800	Live
5	204,800	Didn't Challenge
6	204,800	Didn't Challenge
7	25,600	Didn't Challenge
8	409,600	Didn't Challenge
Cont-1	0	Dead
Cont-2	0	Dead
Cont-3	0	ND

N-glycosylation pattern. NP-HPLC chromatography analysis



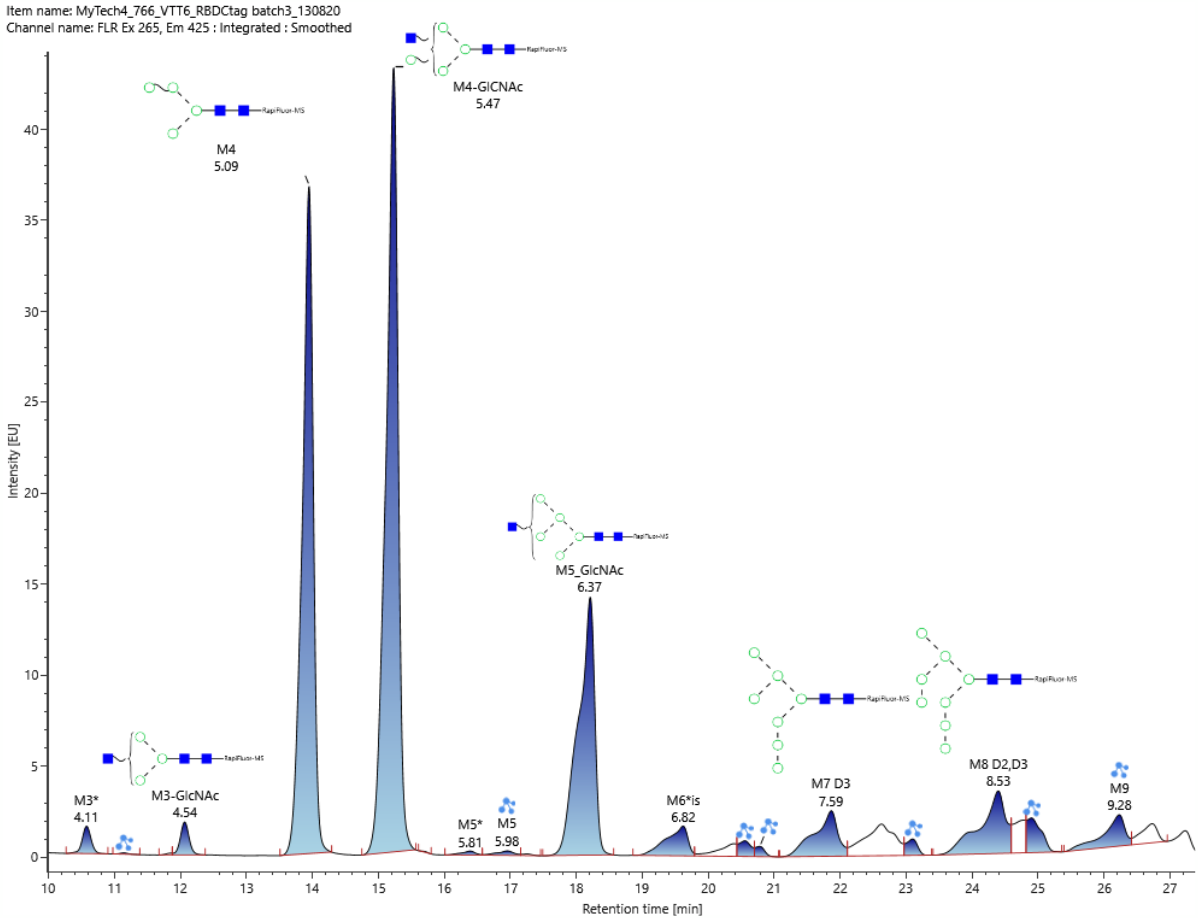
N-glycosylation pattern. NP-HPLC chromatography analysis

The protein sequence was verified and the correct formation of the four disulfide bonds was confirmed. Additionally, the molecular weight of the intact protein was confirmed since the theoretical and experimental values, considering the N-glycosylation pattern were similar



Analysis of released N-glycans by UPLC-FLR-QTOF-MS

Results for RBD-C-tag



Component name	Observed RT (min)	Amount (%)	Mass error (ppm)
M3*	10,58	0,91	1,3
M3	11,15	0,04	1
M3-HexNAc	12,06	1,09	-0,1
M4	13,95	**27,47**	1,6
M4-HexNAc	15,23	**35,52**	1,6
M5*	16,39	0,21	0,4
M5	16,95	0,3	0,8
M5_HexNAc	18,21	**15,84**	1,1
M6*is	19,62	2,43	0,8
M5A1G(4)1	20,56	0,74	0,8
A2F(3)1G(4)1	20,78	0,37	-24,5
M7	21,87	4	0,9
Hex 7	23,1	0,7	0,3
M8	24,4	5,92	0,3
M5A1G(4)1Ga(3)1	24,9	1,9	-1,3
M9	26,23	2,56	1
SUM		100	

The main glycan forms are Man4, Man4-HexNAc and Man5-HexNAc, constituting about 79% of all glycans

Phase I with C1-cell SARS-CoV-2 RBD Recombinant Vaccine in 2021

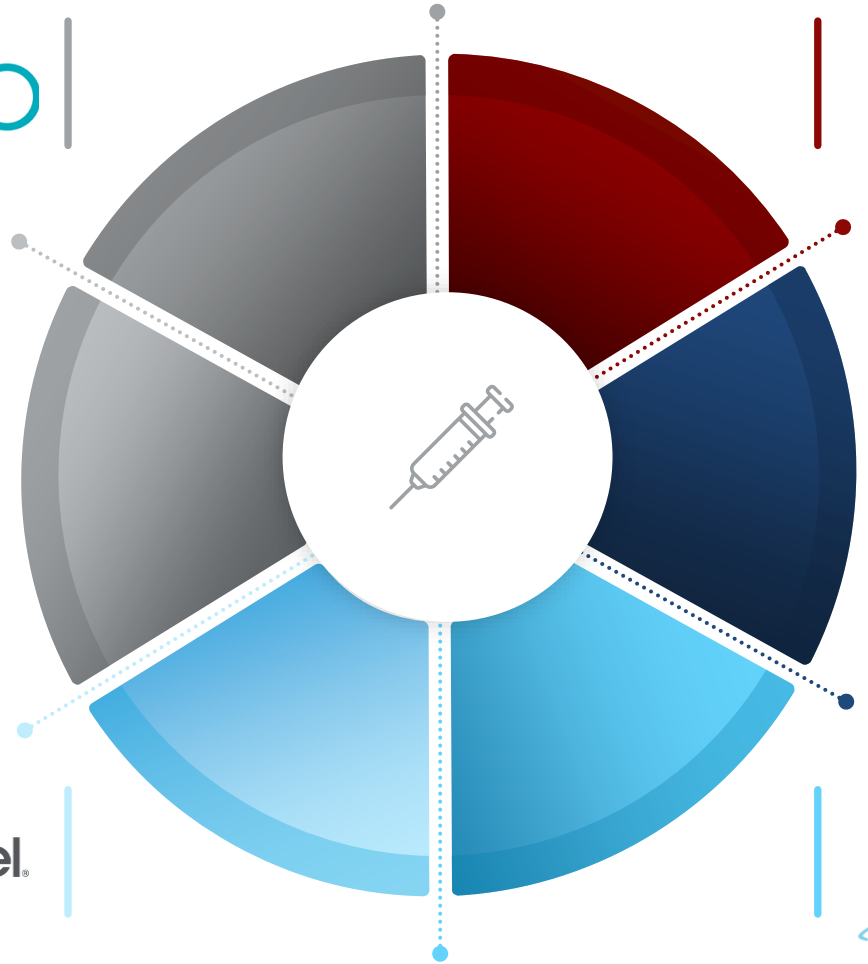
cGMP manufacturing, Fill/Finish, Toxicology, initiating and completion of an anticipated Human Phase 1 Clinical Trial Of a C1 produced SARS-CoV-2 vaccine candidate. Prove Safety & Efficacy In Humans

Coordination of clinical trials for anti-viral vaccines

cGMP manufacturing of Toxicology & Clinical Trial Material

Toxicology Study

Support with regulatory & CMC consultation towards accelerated licensure



Developed the C1 –SARS-CoV-2 RBD protein based rVaccine

Development of MonoValent RBD characterization / evaluation

Collaboration on preclinical evaluation of the vaccine candidates in small animal

DYAI-100 / SARS-CoV-2 RBD Vaccine Candidate (cGMP, Phase I)

- Meeting with Paul Ehrlich Institute (PIE) was held on April 26th and Final Minutes were received on June 2nd.
- Toxicology study
  - All animals have been vaccinated 4 times and were sacrificed. The dissections will continue till Monday – June 14. So far the rabbits don't show any adverse effects. Additional blood for serological and cytokine determination was also taken.
  - The interim/draft tox report is expected second half of July / Final tox report will be ready in September which will also include the histopathology results (organs will be analyzed in the US).
- Drug Substance - cGMP batches
  - Two engineering batches and a cGMP fermentation batch of the drug product have been completed at Biotechnology General Israel
- Drug Product - Fill & Finish
  - The F&F will be done at Eurofins - Total of - 1600-1700 vials per concentration. 400-500 vials are needed for stability, retaining and back up, so 600-700 will be kept.
- Phase I study
  - The Investigator's Brochure (IB) and the Investigational Medicinal Product Dossier (IMPD) is expected to be ready by September 2021.
  - Phase I is currently schedule to start in Germany October/November.
  - Two cohorts having 2 doses of 15µg and 30µg of RBD with alum and 2 injections (prime and boost) for each.
  - Different phase I units have been contacted to check interest in participation in the C1-RBD study as an alternative for Germany.
- In addition to India, three other Less Developed Countries Are Interested In Gaining Access to Dyadic's DYAI-100 Drug Substance and/or Drug Product Manufactured From C1-Cells
- Two of these countries are in discussion with Dyadic to help them establish in country vaccine manufacturing facilities based on the C1 vaccine manufacturing platform
- Other interested parties and governmental agencies are interested in the C1 vaccine manufacturing platform

Commercial Scale Production of C1-RBD With and Without MPSP

Predicted C1-RBD fermentation capacities for different dose requirements based on 5 days fermentation at various scales

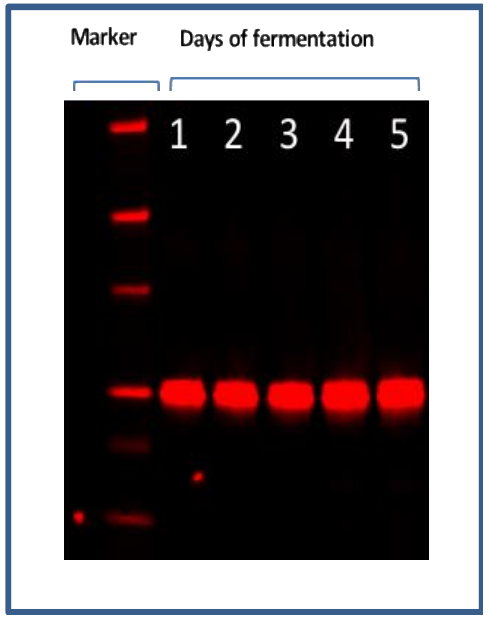
C1 productivity (2.0 g/L)	Doses (30µg and 30µg)			Doses (15µg+15µg)		
	10M	100M	1000M	10M	100M	1000M
Total volume (g)	600	6 000	60 000	300	3 000	30 000
Productivity (g/L)	2.0	2.0	2.0	2.0	2.0	2.0
RBD purification Recovery (%)	60	60	60	60	60	60
Total fermentation volume (%)	80	80	80	80	80	80
Calculated fermentation volume C1 (L)	625	6 250	62 500	313	3 125	31 250

C1-expressed SARS-CoV-2 RBD has the potential to be an effective low-cost vaccine candidate that can be rapidly manufactured at flexible commercial scales

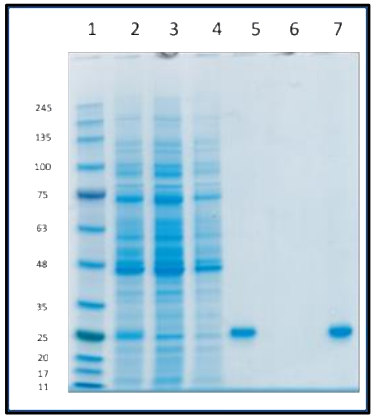
C1-Cell Expressing antigens and neutralizing mAbs for SARS-CoV-2 vaccines

- *SARS-CoV-2 proteins and neutralizing mAb successfully expressed by C1*
  - *S-RBD*
    - *Wuhan, South African, UK & Brazilian*
  - *S-RBD / Spy Tag (Nanoparticle)*
  - *Full Spike / Spy Tag (Nanoparticle)*
  - *Full Spike*
  - *FC RBD*
  - *MAb*
- *COVID-19 Variant candidates under development*
- *Infectious disease antigen targets expressed in C1 with preclinical PoC:*
  - *Influenza HA*
  - *MERS RBD*
  - *SBV antigen (Schmallenberg)*
  - *RVFV (Rift Valley Fever)*
  - *IBVD-VLPs*

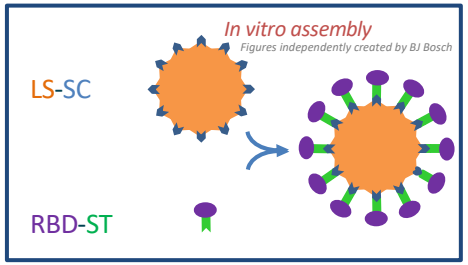
C1 / RBD Ctag



C1 / RBD-SpyTag no CTag

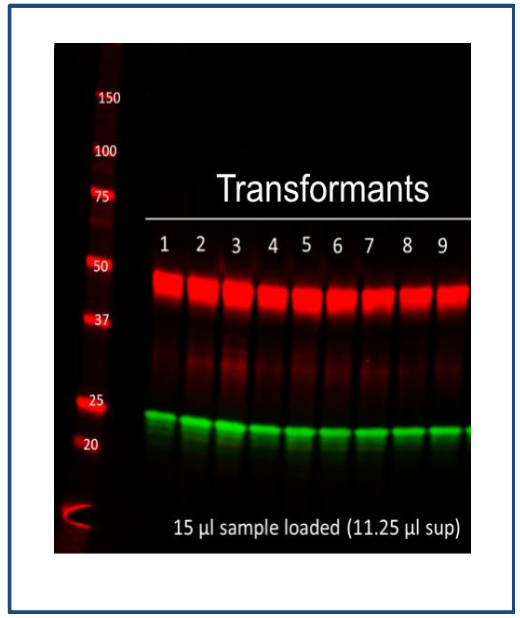


HPLC analysis RBD SpyTag - Purity 99.29%



Expression of secreted Nanoparticles at several g/L

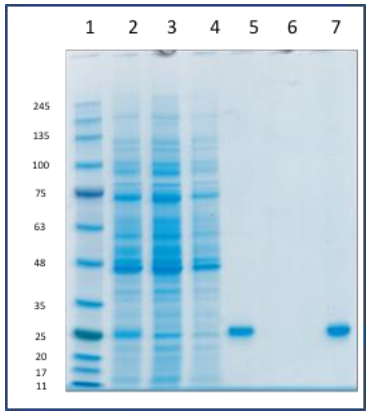
Neutralizing mAb



Stable C1-RBD-SpyTag Production Without C-Tag

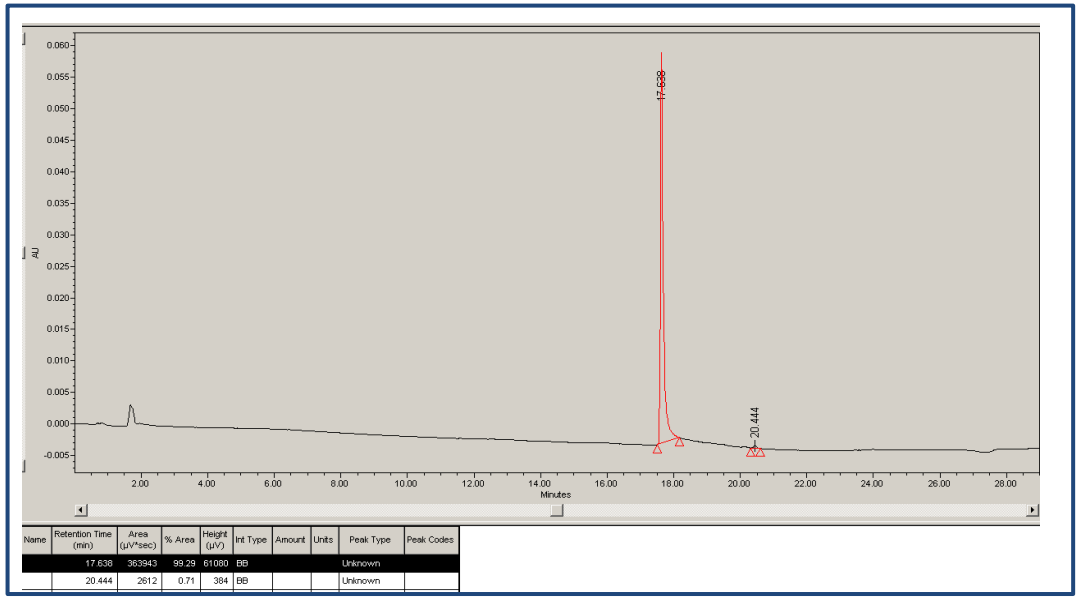
1L scale fermentation

- **Developed stable C1 cell lines expressing RBD-SpyTag without C-Tag.**
- Production level in 1L fermentor reached 0.7-0.8 g/L in 55hrs.
- Efficient and simple purification steps with Spike Protein Resin was developed.
- Purity reached a level of 99.29% with minor losses
- The RBD-SpyTag performance very well

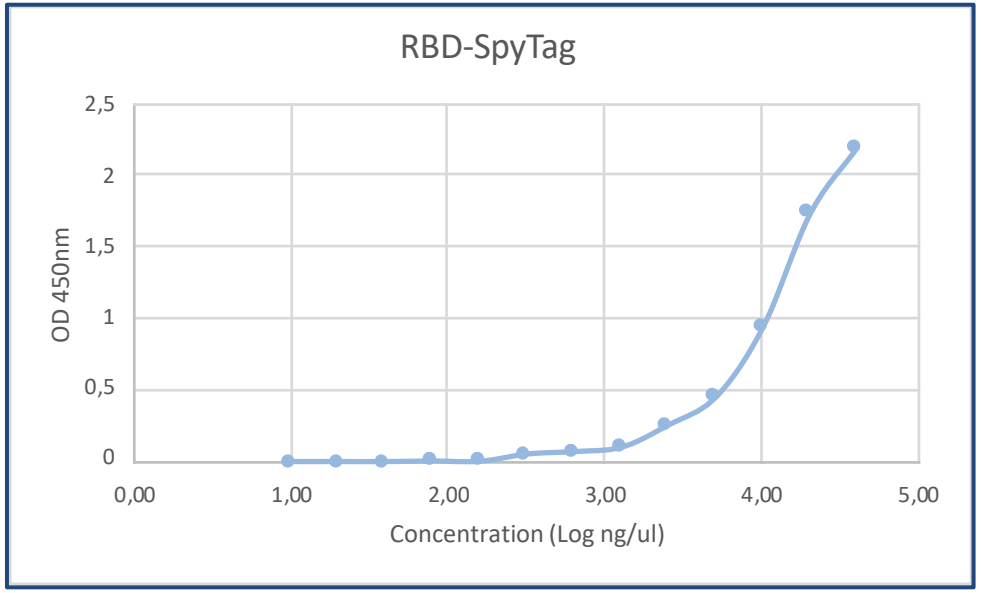


1 - Mw marker
2 – EFT 55h 8µl
3 – F.T. (15ml) 20µl
4 – Wash I 30µl
5– Elutions 1+2+3 in conc. 15µl
6 – Flow through 30µl
7 – RBD std. (2µg total) 10µl

HPLC analysis RBD SpyTag - Purity 99.29%



ELISA-ACE2 Binding Assay



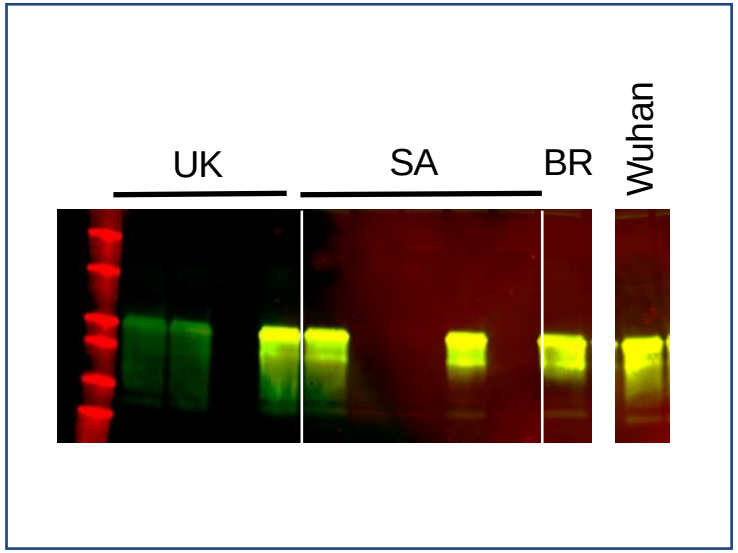
Production of SARS-CoV-2 vaccine Protein Variants

VTT is expressing the following proteins in C1:

1. Variants of Concern
   - C1 strains have been engineered to express high levels of Receptor binding domains (RBDs) of the UK, BR and SA variants
     - Delta (Indian) Variant(s) are in progress
   - Initial fermentations of Alpha (UK) , Beta (SA) and Gamma (BR) RBD have been successfully completed
2. Spike proteins of the Beta (SA) and Gamma (Indian) Variants of Concern are Being Developed
3. A growing number of proprietary and third party C1 Variants of Concern C1 strains are being engineered

Fermentations of variant RBD producing clones

- Expression constructs of the Alpha (UK) , Beta (SA) and Gamma (BR) variant RBDs were transformed into a C1 strain with 14 deletions of protease genes and grown in fermenters.
- RBD production was analyzed by Western blotting with anti-RBD antibody. RBD from Wuhan variant produced earlier is shown as control.



Alternative Adjuvant to Initiate Cells Response

Do we need alternative Adjuvants?

AS04
Beginning in 2009, monophosphoryl lipid A (MPL) was used in one U.S. vaccine (Cervarix®); however, the vaccine is no longer available in the United States due to low market demand. This immune-boosting substance was isolated from the surface of bacteria.

MF59
MF59 is the adjuvant contained in Fluad (an influenza vaccine licensed for adults aged 65 or older). MF59 is an oil-in-water emulsion composed of squalene, which is a naturally occurring oil found in many plant and animal cells, as well as in humans. MF59, used in flu vaccines in Europe since 1997 and in the United States since 2016, has been given to millions of people and has an excellent safety record.

$AS01_B$
$AS01_B$ is an adjuvant suspension used with the antigen component of Shingrix vaccine. Shingrix is the recombinant zoster vaccine recommended for persons aged 50 years or older. $AS01_B$ is made of up of monophosphoryl lipid A (MPL), an immune-boosting substance isolated from the surface of bacteria, and QS-21, a natural compound extracted from the Chilean soapbark tree (*Quillaja saponaria* Molina). In pre-licensure clinical trials, $AS01_B$ was associated with local and systemic reactions, but the overall safety profile was reassuring.
$AS01_B$ is also a component of vaccines currently being tested in clinical trials, including malaria and HIV vaccines. To date, these trials have included over 15,000 people.

CpG 1018
CpG 1018 is a recently developed adjuvant used in Heplisav-B vaccine. It is made up of cytosine phosphoguanine (CpG) motifs, which is a synthetic form of DNA that mimics bacterial and viral genetic material. When CpG 1018is included in a vaccine, it increases the body's immune response.
In pre-licensure clinical trials, adverse events after Heplisav-B were comparable to those observed after another U.S.-licensed, non-adjuvanted hepatitis B vaccine.

Liposome DNA Complex - LDC
LDC is stated to enhance both Innate immunity (Interferon, Natural Killer cell; antigen-independent immunity) and Adaptive Immunity (Humoral-Antibody-mediated and T-cell-mediated; antigen-dependent immunity) immune responses
Currently was proved safe and effective in Phase 1 and 2.

TQL1055 –is a rationally designed, semi-synthetic analogue of the saponin adjuvant QS-21. The molecule structure and synthesis structure and synthesis are designed for manufacturing efficiency. The natural source material in TQL-1055 that provides the triterpene core is sustainability harvested from Quillaja Saponaria tree. Preclinical evidence indicates that TQL-1055 will have immunostimulatory properties similar to QS-21, with improved tolerability.

Establishing Global Presence with leading organizations

Co-developing C1 enabled COVID-19 (plus variant) vaccines and/or boosters (i.e., tetravalent or quadrivalent COVID-19 vaccines)

Dyadic announces development of COVID-19 Vaccine in India

Syngene

A Biocon company

Mahesh Bhalgat, COO, Syngene International stated, "We look forward to our collaboration with Dyadic to initially explore the development of a COVID-19 vaccine, and to further evaluate the potential of developing a differentiated vaccine platform based on Dyadic's proprietary C1- cell line."

Dyadic and Medytox To Develop Vaccines Against COVID-19 Variants

(South Korea & SE Asian Countries)

Medytox

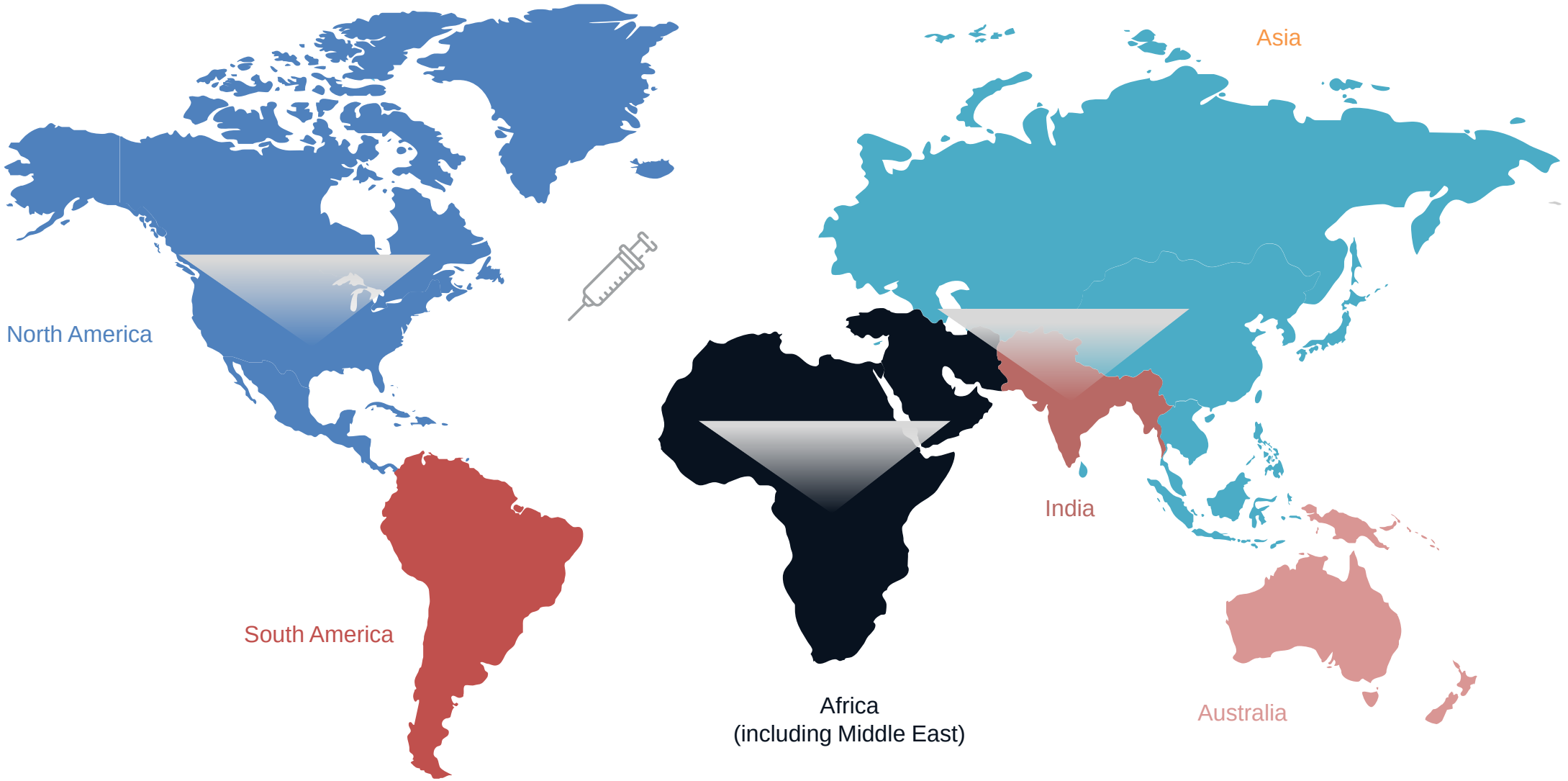
"Dr Yang, We have been working closely with Dyadic since July 2020, when we obtained access to their C1 expression platform and experienced the remarkable versatility and high productivity of the C1 platform. We believe that the fungi-derived C1 expression system is the most realistic technology to develop and manufacture multi-valent (i.e., tri-valent, and tetra-valent) vaccines, rapidly and affordably, against COVID-19 mutant viruses without the need for a large-scale bioreactor facility.

Establishing Global Presence with leading organizations

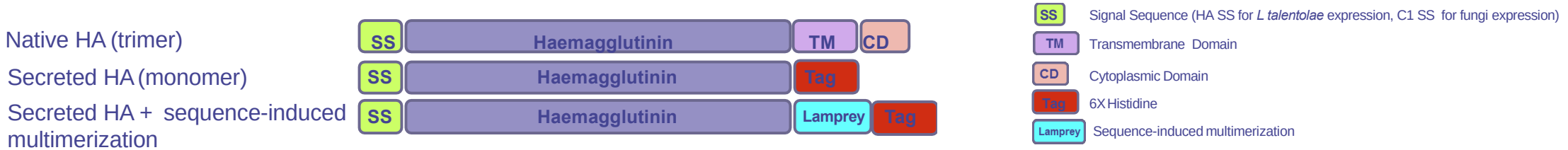
- Purified protein and/or supernatants and/or production strains of the C1 expressed SARS-CoV-2 RBD & Full Spike proteins have been sent in more than **30 shipments to more than 20 different collaborators**
- New strains have been and are continuing to be constructed to address existing and emerging variants of concern
  - RBD Variant of Concern Vaccines
    - With & Without Nanoparticles
  - Full Spike Variant of Concern Vaccines
    - With & Without Nanoparticles
- New adjuvants are being considered and evaluated

- Various nanoparticles are being considered, evaluated and developed
- Samples of Variants of Concern are being prepared for shipments globally

Establishing Additional Global Presence with leading organizations



Expression of Haemagglutinin (HA) in C1



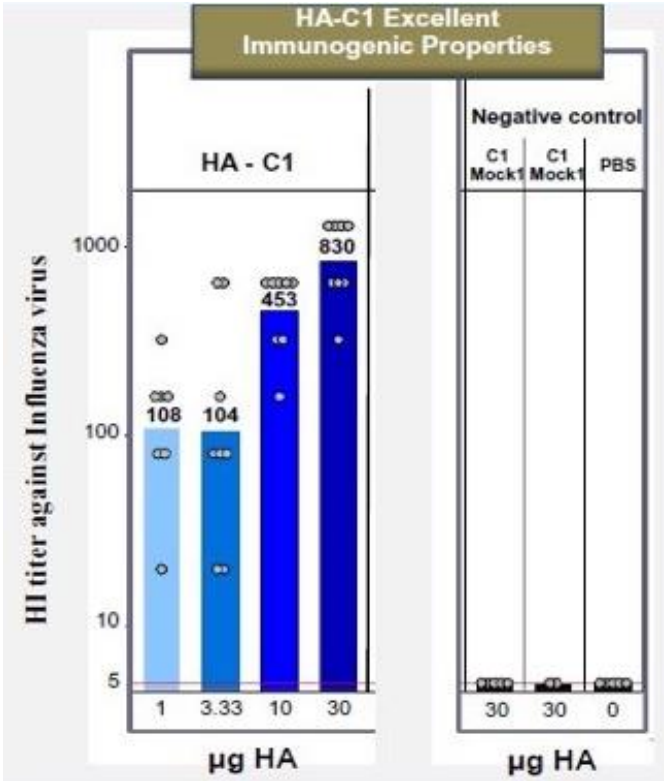
Ability to Express Biologically active HA´s

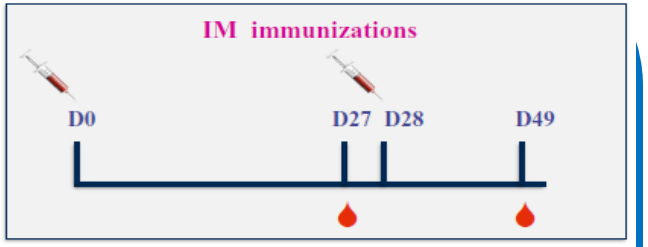
- 5 Recombinant HA´s have been expressed by C1:

Influenza Strain	Expression	Bioactive HA
New Caledonia, A (H1N1)	yes	yes
Texas, A (H1N1)	yes	yes
Puerto Rico, A (H1N1)	yes	yes
California, A (H1N1)	yes	yes
Florida, B	yes	yes

Agglutination Test:

Mice Study was conducted by Sanofi-Pasteur:





The full lengh rHA from A/New Caledonia/20/99 (H1N1) strain showed **excellent immunogenicity** properties in mice without adjuvant

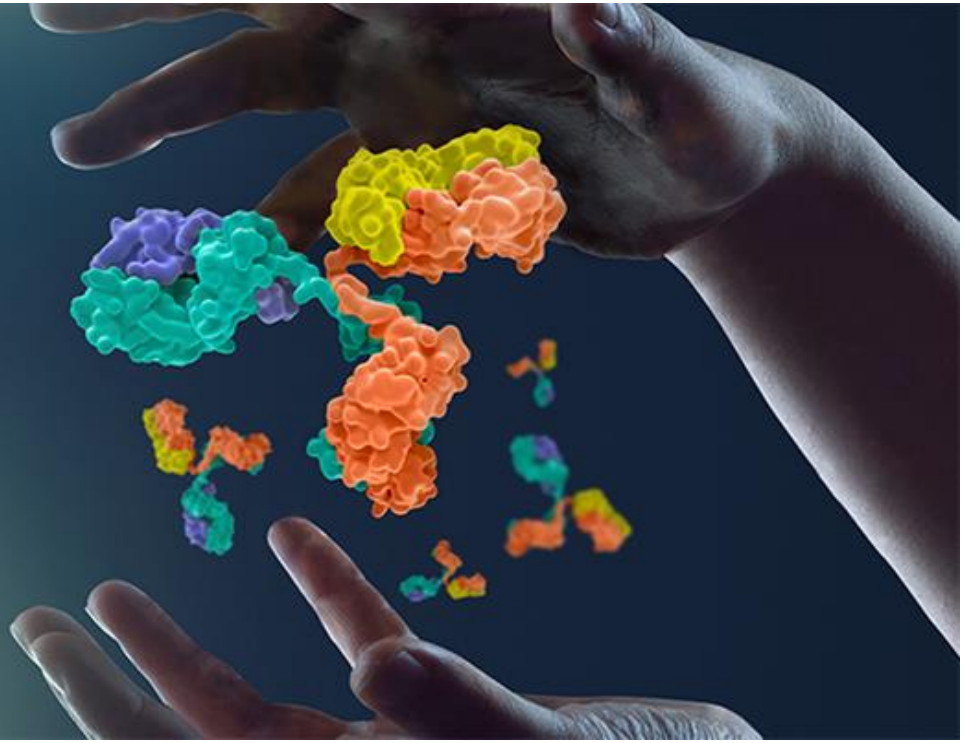
MAb overview

Biomanufacturing Capacity:

New blockbuster mAbs could leave shortage

C1-Cells Disrupting Monoclonal Antibody Development & Manufacturing

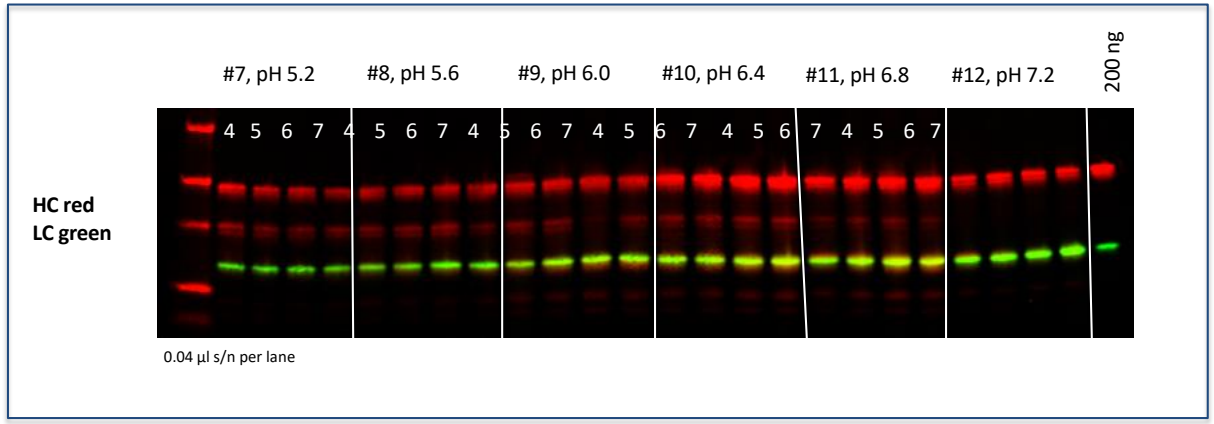
- **Three to four batches of C1 mAbs in same time as one batch of CHO mAbs**
- **Produce greater quantities from each batch more affordably**
- **Same binding and neutralizing properties**



Expression of mAbX in 2 different cell lines and different pHs

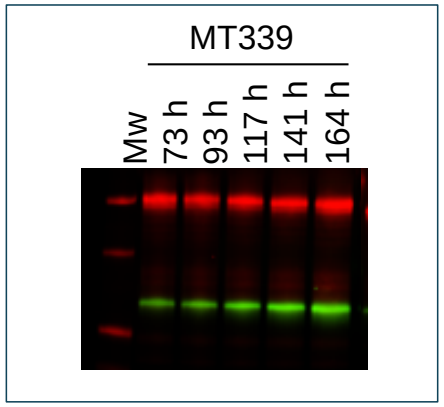
Production in (ΔXX)

Comparison of six different pH-conditions

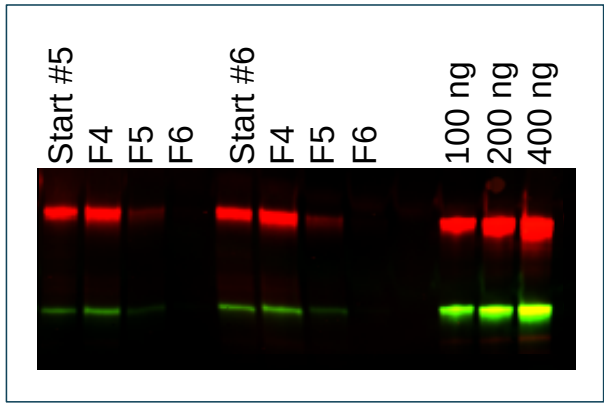


Production in (ΔXX)

Fermentation



Purification

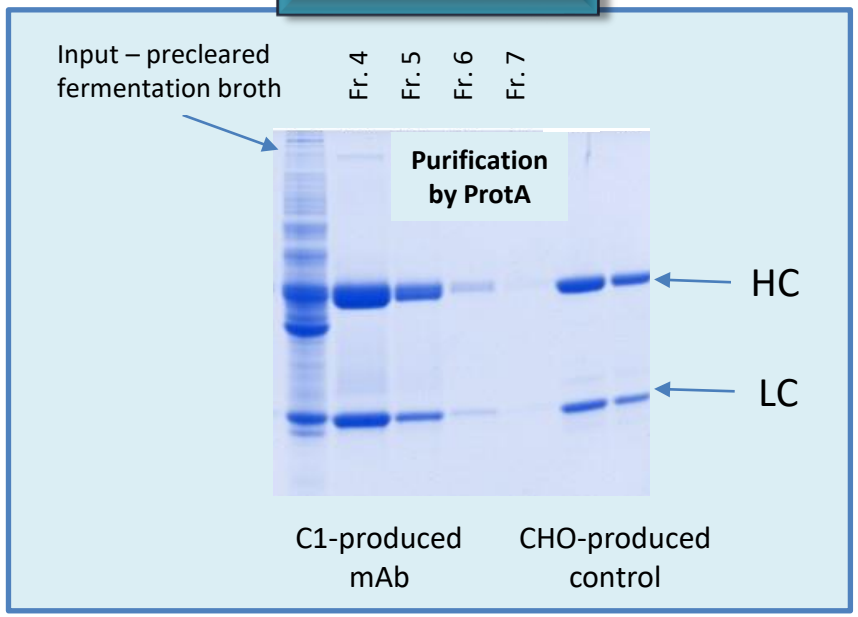


Fermentation of the mAX that was expressed in the ΔXX proteases cell line reached 16.1 g/l and 17.5 g/L in 122hrs and 145hrs respectively (2.9 g/l/day)

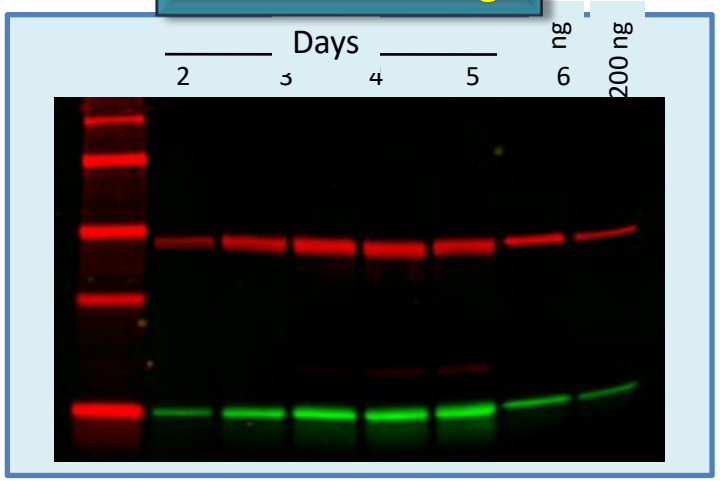
Monoclonal Antibodies are Stable and Correctly Folded

- **Typically stable mAbs production levels are between 5 – 10 g/L in 5-7 days**
- **Best productivity at Amber system:**
  - **24.5 g/L in 7 days (3.5 g/l/day)**
- **Best productivity so far at 30L scale:**
  - **20.6 g/L at 157 h (3.1 g/L/day)**

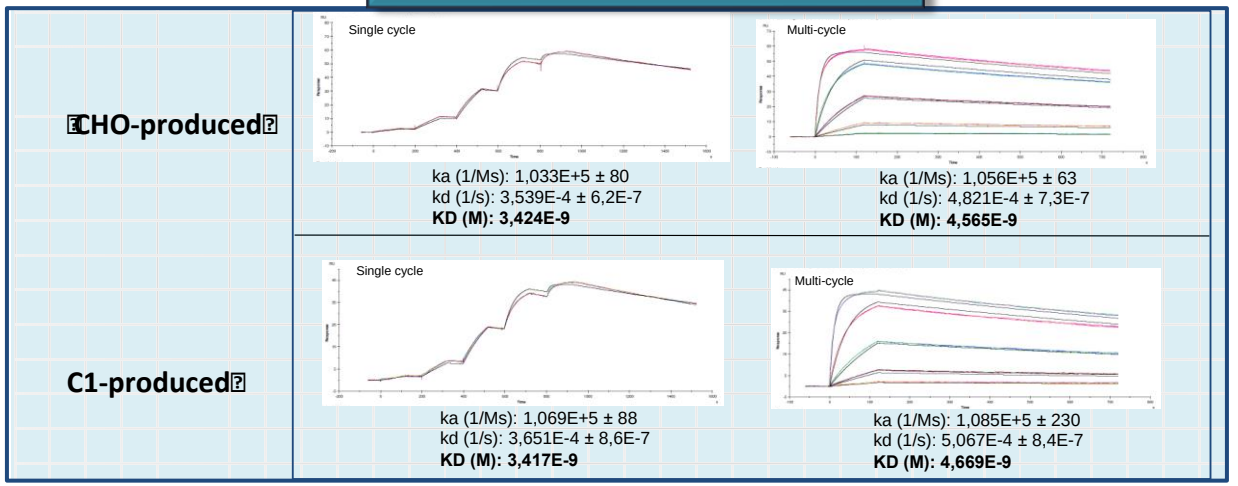
Purification



Western Blotting

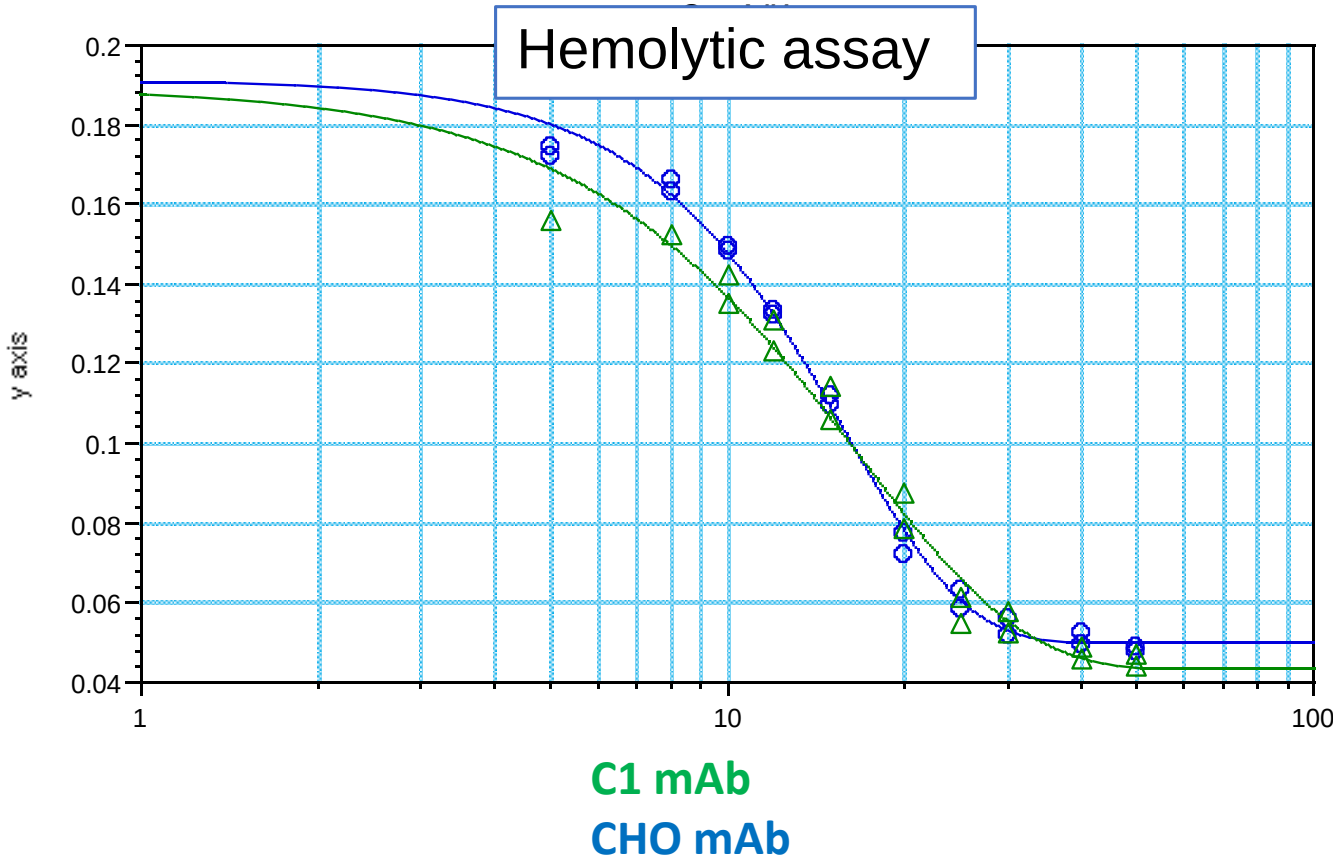


Surface Plasmon Resonance

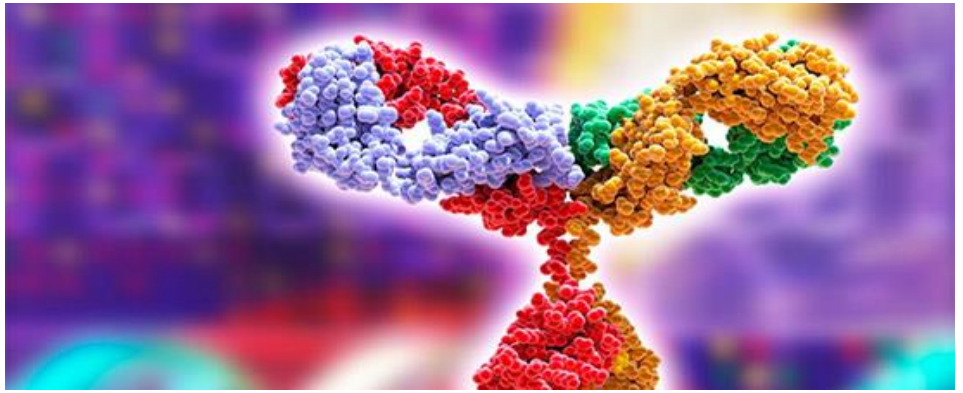


Neutralizing activity of mAb produced in fungus or CHO cells

Hemolytic assay in rabbit RBC with alpha toxin of Staphylococcus aureus



The neutralizing activity assay demonstrated great similarity between C1 produced mAb and CHO produced mAb



Hemoglobin was measured at OD450 in supernatants

GLOBAL HEALTH ACCESS, AFFORDABILITY & EQUITY