Next-Gen vaccines without the right platform limit pandemic effectiveness

C1, Protective, Rapid, High Yield, Low-Cost Global Solution

C1-cell Protein Production Platform

US FDA Workshop on Recombinant Protein-based COVID-19 Vaccines

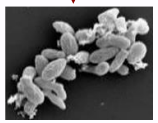
April 27, 2023

Safe Harbor Regarding Forward-looking Statements

This press release contains 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 International's expectations, intentions, strategies, and beliefs pertaining to future events or future financial performance, such as the success of our clinical trial, our research projects and third-party collaborations, as well as the availability of necessary funding. Actual events or results may differ materially from those in the forward-looking statements because of various important factors, including those described in the Company's most recent filings with the SEC. Dyadic assumes no obligation to update publicly any such forward-looking statements, whether because 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

Industrial heritage provides knowledge for rapid scalability and high productivity





INDUSTRIAL — **30 Years and Over $100MM Invested in C1 Platform Development** — **PHARMACUETICAL**

1990's

C1 strain discovered

Commercial launch of industrial enzymes (150K L scale)

C1 morphology change leading to low viscosity, high yield, and non sporulating cell line.

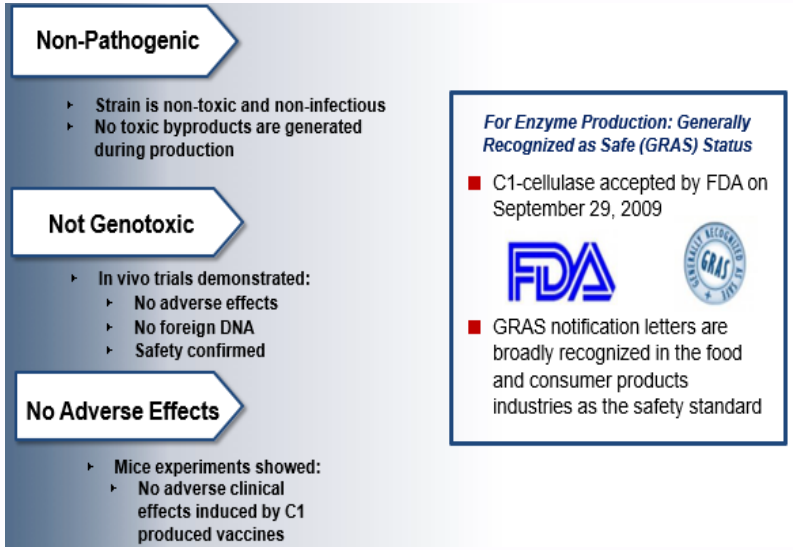
Genome sequenced and fully annotate

2000's

Enzyme sales in 35 countries

C1 licensed in biofuels & other industries Abengoa, BASF, Shell Oil enzymes **(500K L scale)**

Generally Recognized as Safe (GRAS) designation by FDA



2015+

Launch of human biologics development for vaccines and biotherapeutics

Proof-of-Concept collaborations with pharma industry accelerate

Global partnerships and R&D projects with academia, government agencies, top pharma and leading animal health companies

Today



Regulatory Status: DYAI-100 COVID Vaccine Phase 1 Clinical Trial in South Africa underway & planned in India

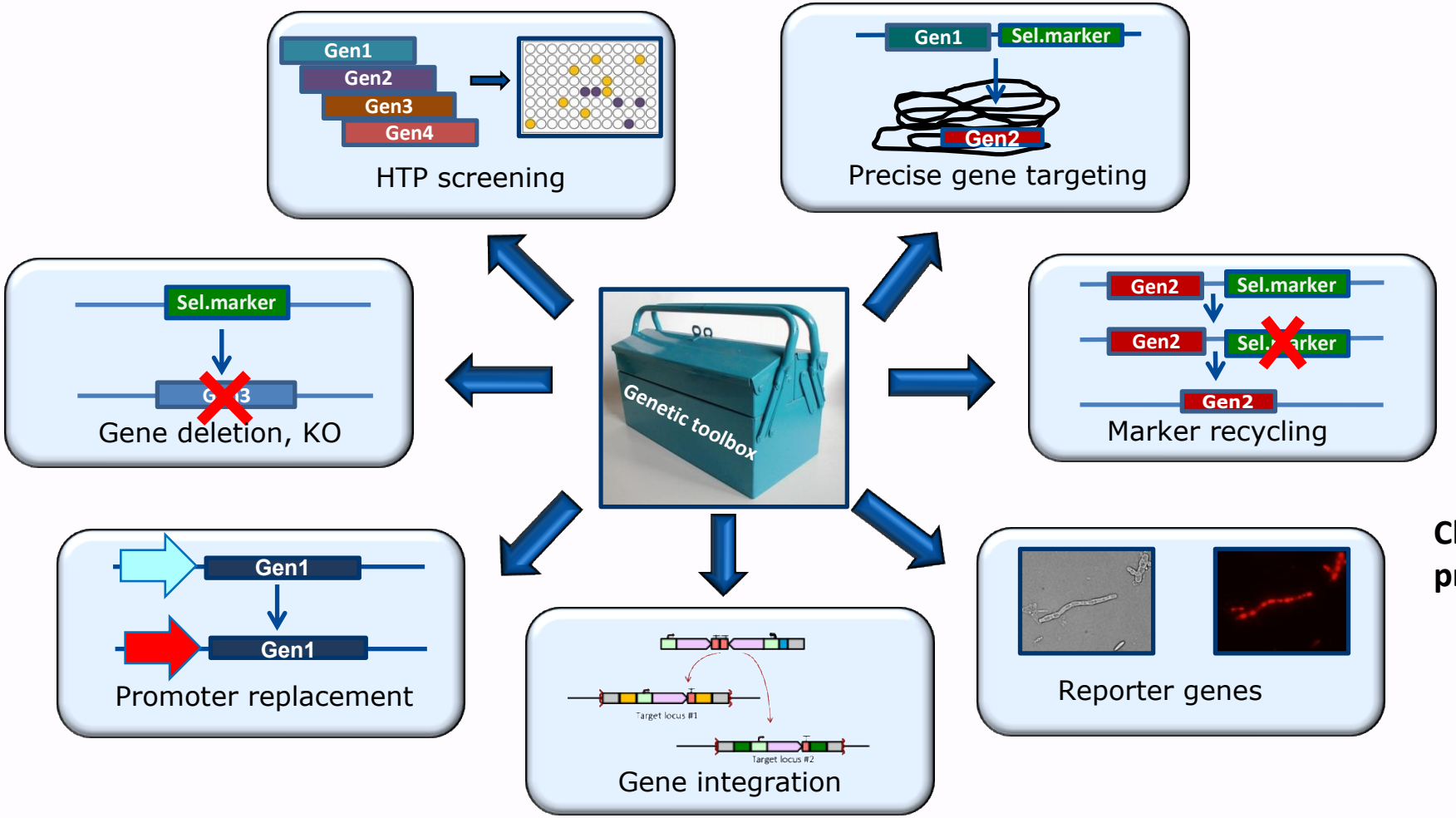
C1 produced COVID-19 mAb shows safety and efficacy in hamsters and nonhuman primate studies

Strategic partnerships and collaborations:

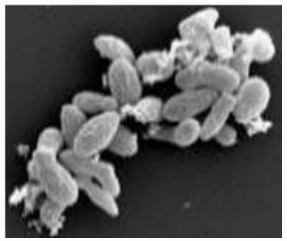
- Janssen
- NIIMBL
- Rubic
- Epygen
- Syngene

Genetic toolbox for rapid, stable, high yield C1 strain development

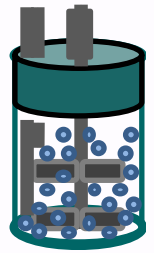
The advanced genetic toolkit for synthetic biology







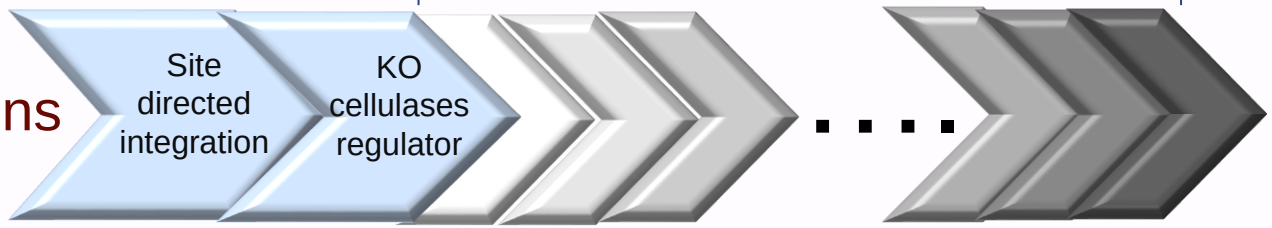
Change in morphology led to hyper-productivity and low viscosity.



Systematic deletion of active proteases in C1 host to improve stability

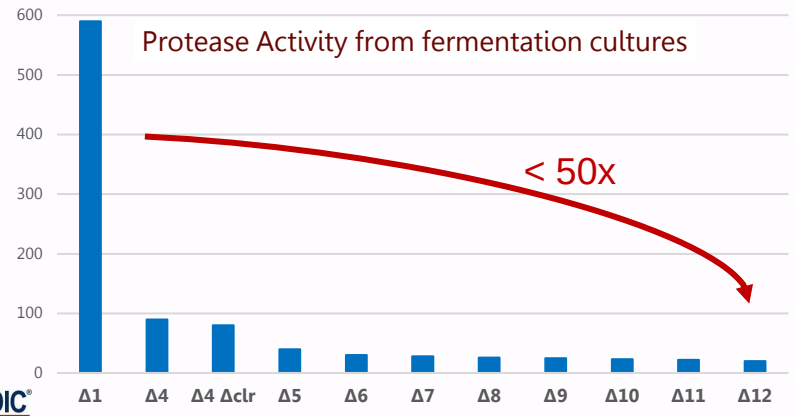
KO proteases and main secreted proteins (15 genes knocked out of C1 cell)

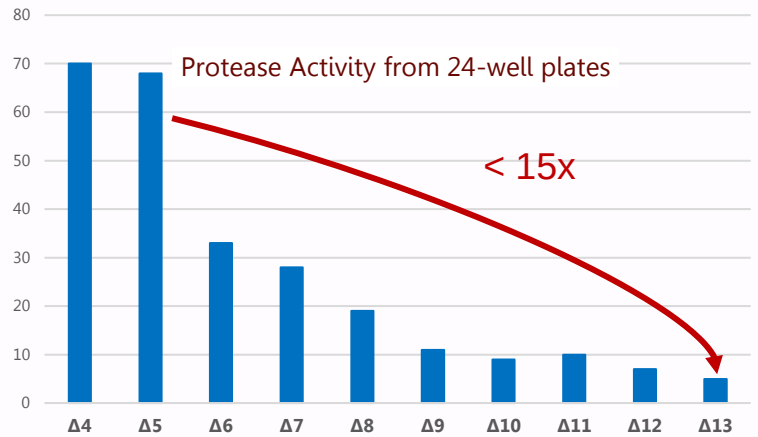
Pharma C1 strains



Systematic deletion of proteases genes based on:

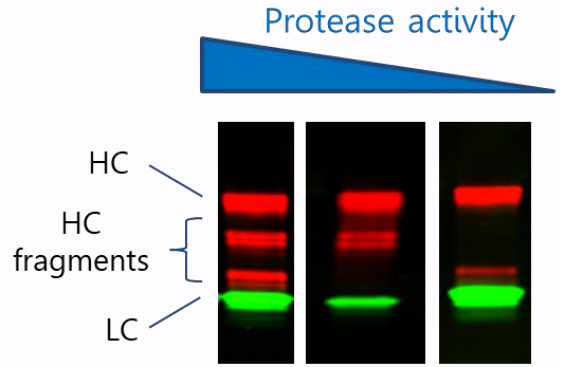
- Isolation and identification of extracellular proteases
- C1 protease library in Pichia pastoris that allows to test more than 50 proteases
- Effect of different protease inhibitors on protease activity
- mRNA sequencing data
- Protease gene annotation



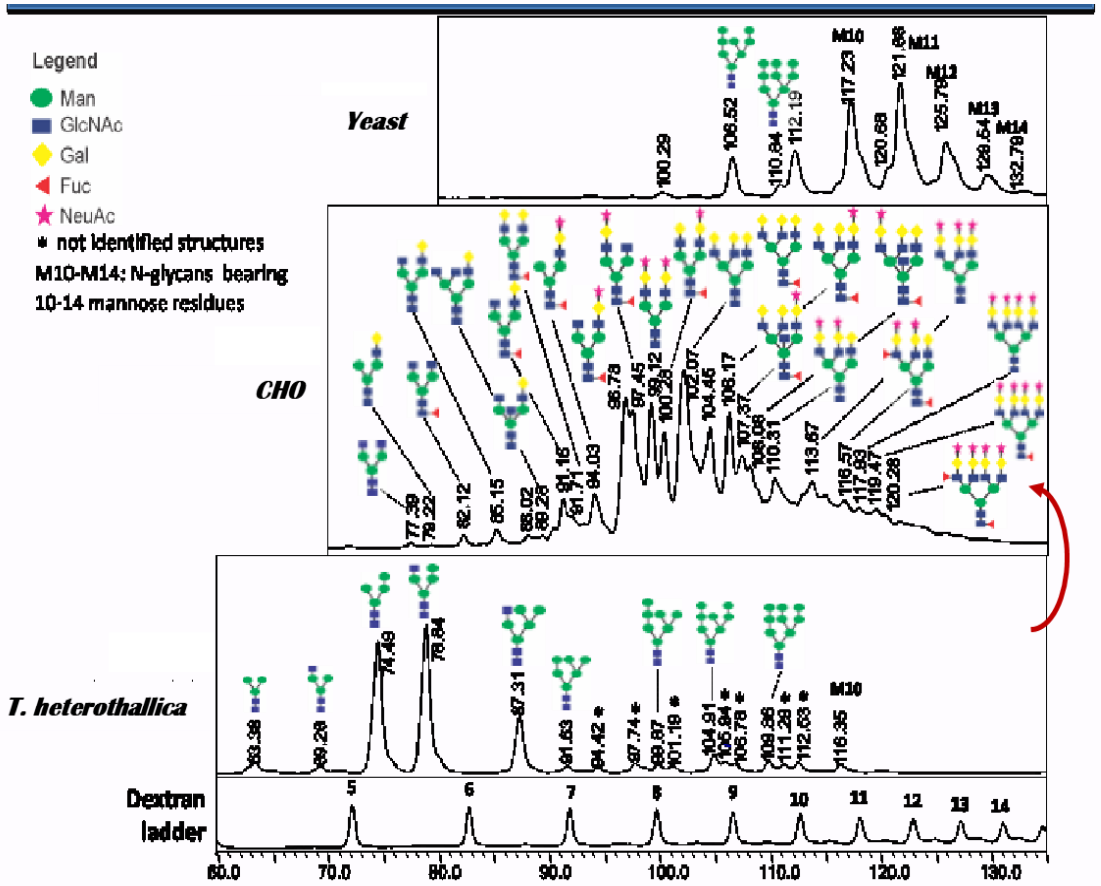


*Direct fluoresence-based assay with casein substrate

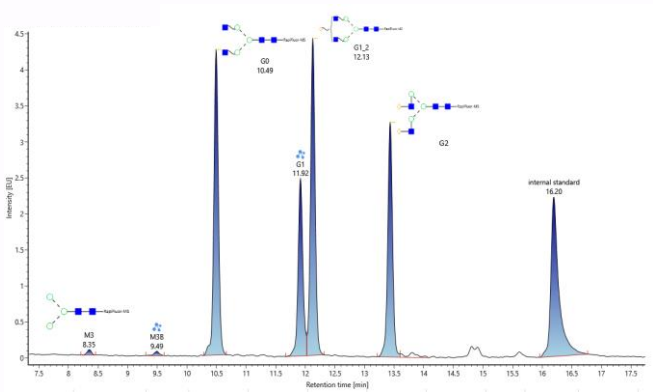
mAbs stability is increased with proteases deletion



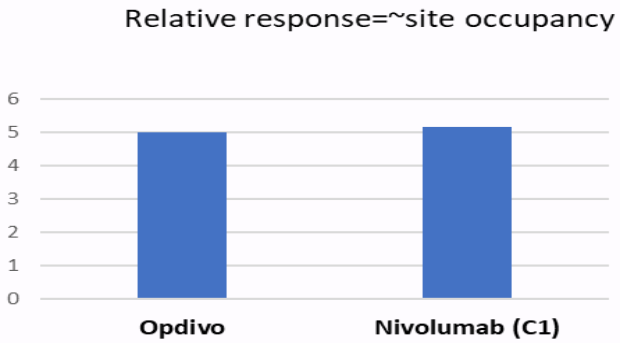
C1-cell glycosylation pattern(s) modified to improve vaccine efficacy



C1 Humanized Proteins for Monoclonal Antibodies



Glycan	Observed RT (min)	Amount (%)
M3	8,35	0,35
M3B	9,49	0,45
G0	10,49	32,54
G1	11,92	17,21
G1_2	12,13	29,51
G2	13,43	19,94
	Sum w/o IS	100
	Response w/o IS	101843184
	All human-like	99,2

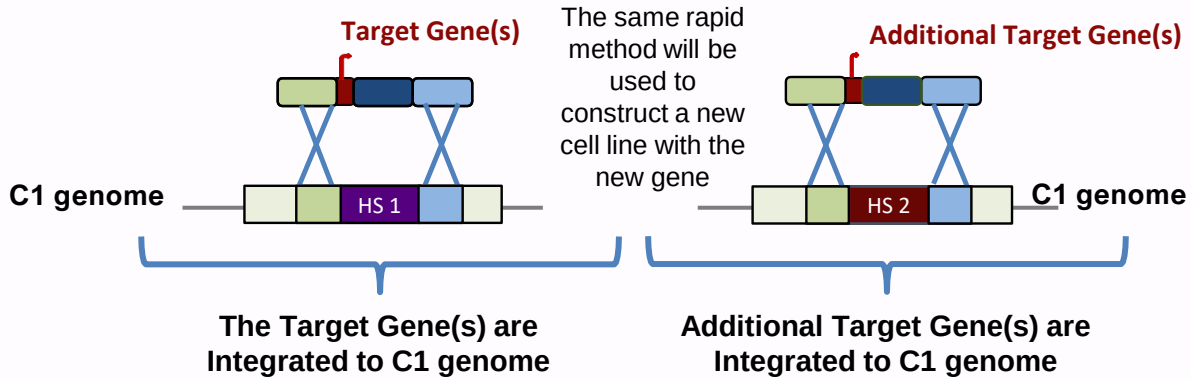


- Native glycosylation pattern from C1 has been glycoengineered to produce glycan structures that resemble human structure corresponding to CHO
- Several rounds of C1 glycoengineering steps:
  - Removal of detrimental activities
  - Over-expression of different activities:

- Nearly all human-like N-glycans; over 99 %, the remaining percentage is Man3-species
- Site-occupancy: relative response close to the values of reference Opdivo

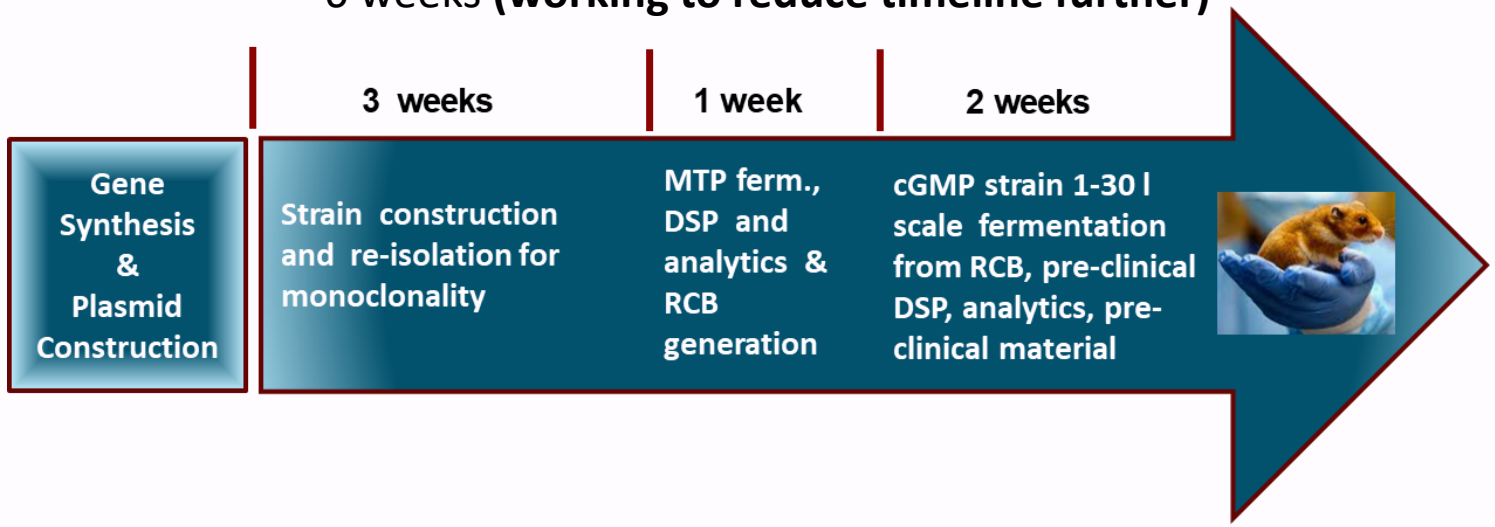
Rapid generation of purified antigens expressed from highly productive stable C1 cell lines

Site Directed Integration:



- The Genes are located in 1 Hot Spot gene locus (HS 1)
- Additional Genes are inserted at another HS locus (HS 2).
- The C1 Site Directed Transformation method leads to quick generation of stable C1 cell lines and purified protein (~6 weeks)

~6 weeks **(working to reduce timeline further)**



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

Rapidly develop vaccine, antibody, other therapeutic protein candidates which can be manufactured in microbial single use or stainless-steel bioreactors at flexible commercial scales in larger quantities more affordably.

DYADIC® INSIDE™

C1 Protein Production System

Ideal choice for recombinant protein vaccines

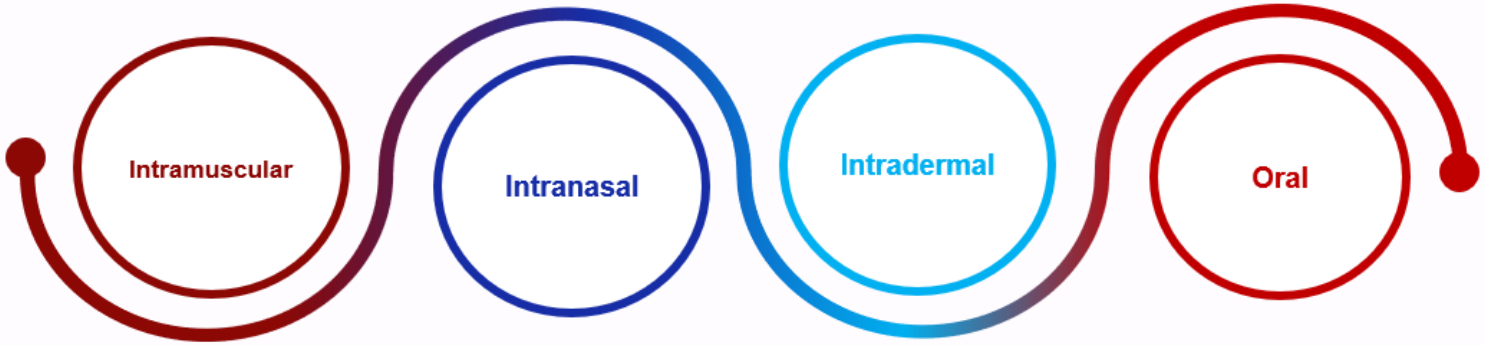
- C1 Platform meets the needs for global demand and response
- Rapid production of RBD, Full Spike, Nanoparticle and other complex molecules
- Currently in Phase 1 with RBD COVID vaccine, no SAE's reported

Characteristic	Pichia	Baculovirus	CHO	C1
Complex proteins	Difficult	Yes	Yes	Yes
Secreted	Yes	Yes	Yes	Yes
Endotoxins	No	No	Yes	No
Viral Clearing	No	Yes	Yes	No
High Yield (g/L)	Low/Med	Very Low	Low/Med	High
Production Speed	12-14d	17-20d	50-54d	12-14d
Multiple Cell Types	No	No	No	Yes
Human Trials	Yes	Yes	Yes	Yes

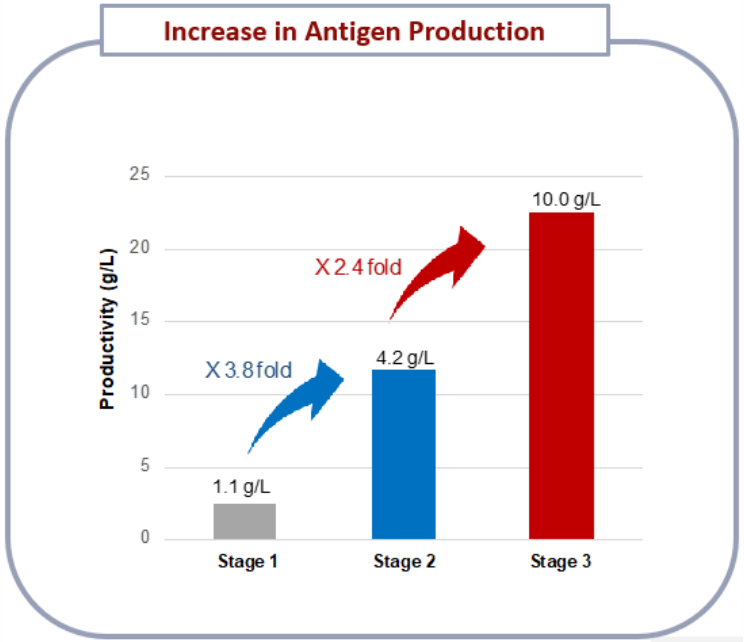
C1 Protein Production System

True global solution for pandemic and infectious diseases

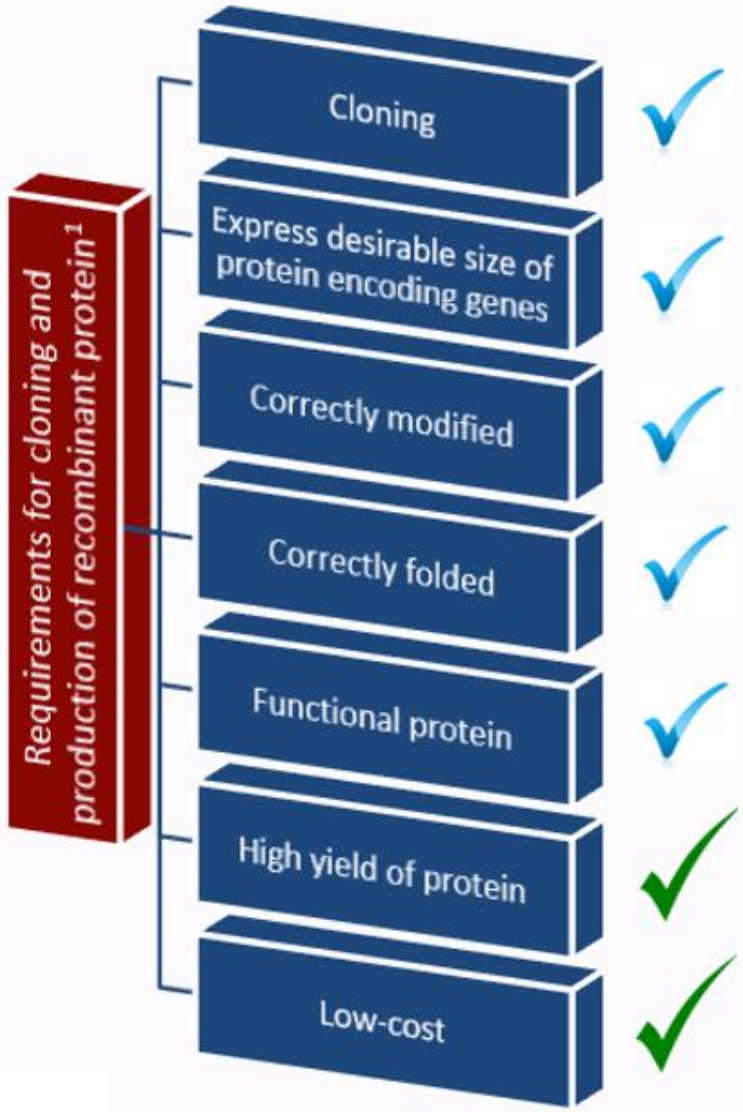
- Route of Administration Agnostic



- Unparalleled Productivity



- **Global Solution Requirements:**
  - Ability to produce standard or complex proteins
  - Sufficient quantity and quality
  - Affordable and Adaptable to ALL REGIONS!



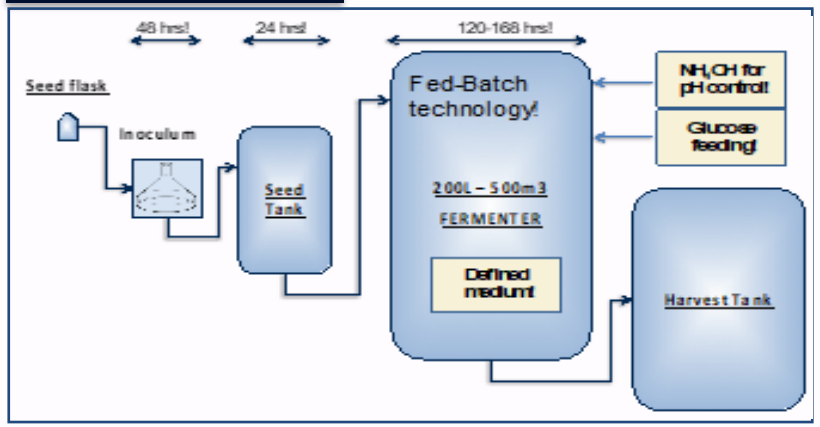
[1]Basic and Applied Aspects of Biotechnology. 2016 Oct 23 : 77–101

C1 Up/Downstream processes: straightforward, adaptable and economical

Fed-batch Process

- Fully defined low-cost 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-to-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)
- Protein production requires no inducer
- Protein is (typically) secreted to the media

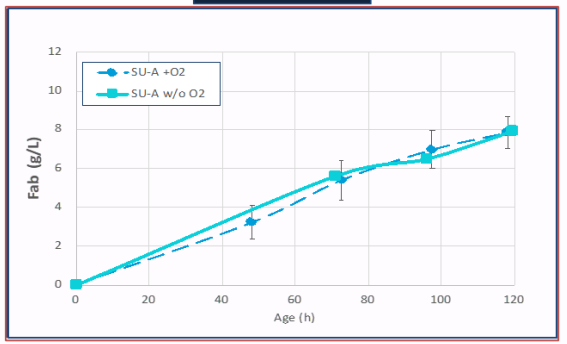
Fermentation



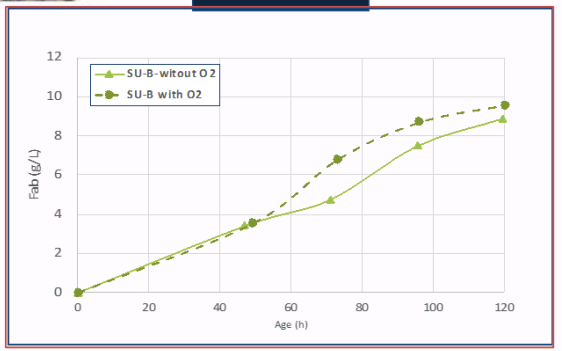
GE's Xcellerex™ XDR-50 MO



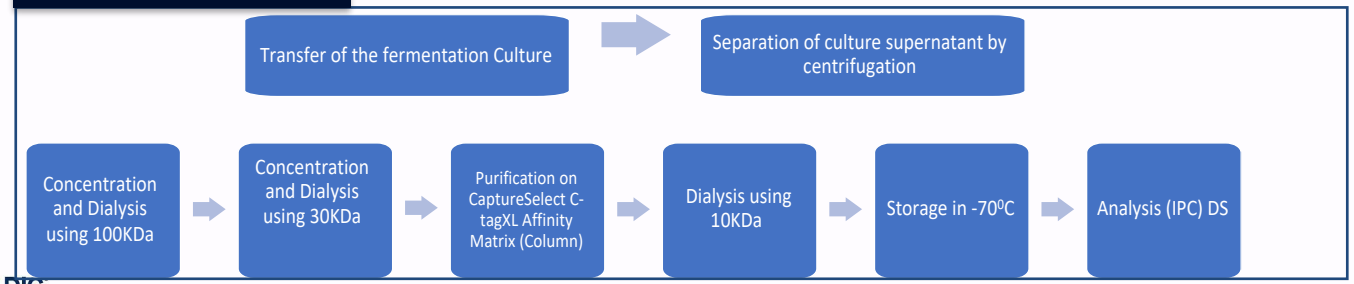
Conditions A



Conditions B



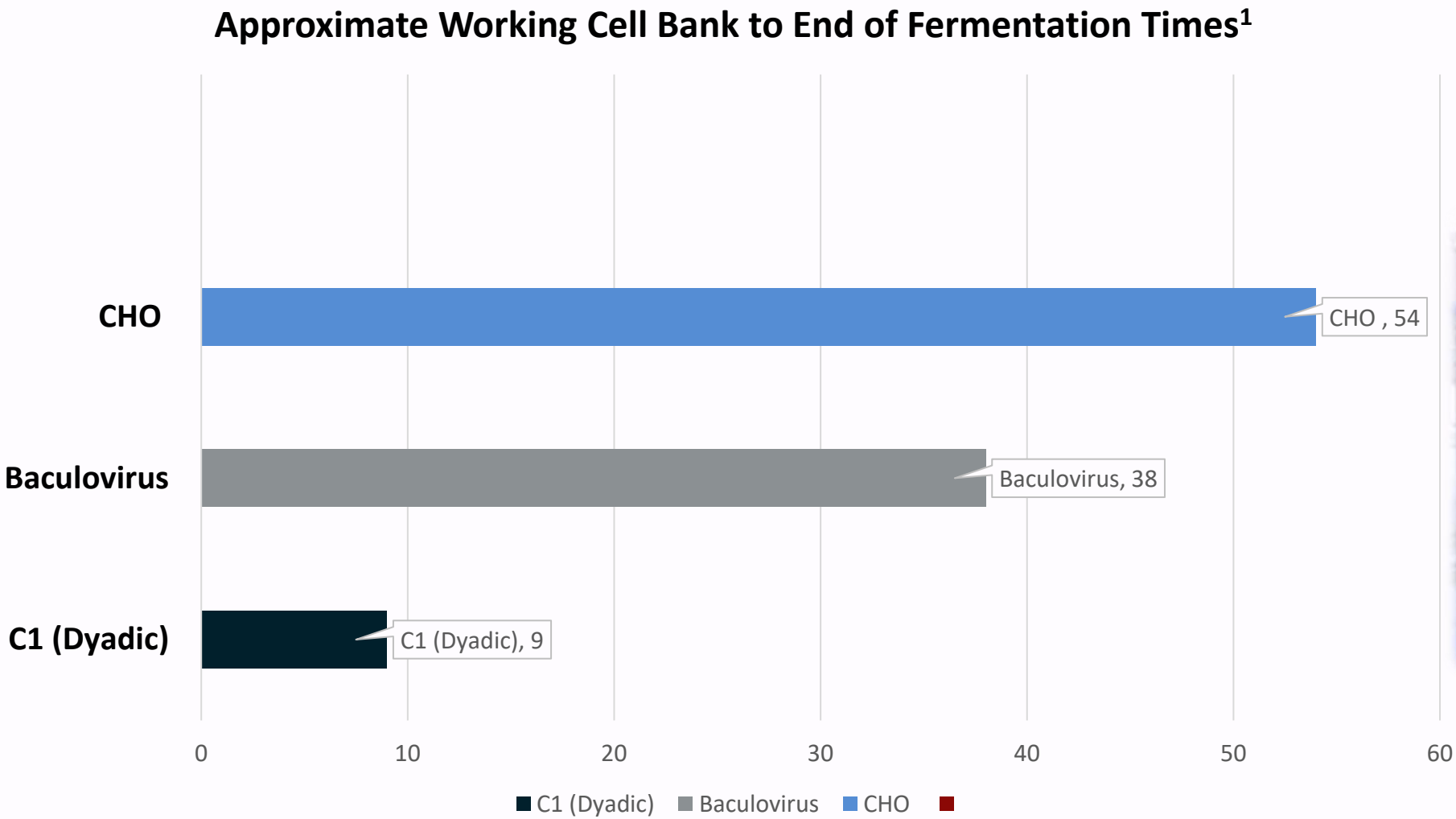
Purification



fAb production kinetics in SSB or SUB under 2 different operating conditions

- 6 batches w-w/o O_2 supplementation.
- Conditions B more productive in SSB & SUB.
- Supplementation of O_2 slightly improves productivity

Working cell bank to end of fermentation times significantly favor C1



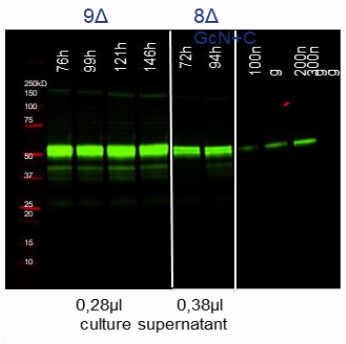


[1] Estimated Ranges: C1 6-9 days; Baculovirus 28-38 days; CHO 41-54 days

C1-cells ~ 300 times higher productivity than Baculovirus

In ~3+ Weeks Less Time, No Viral Clearing Needed

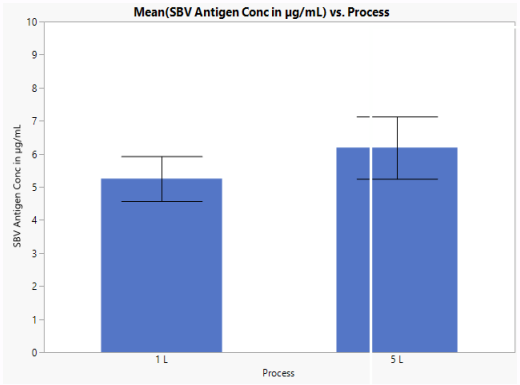
C1 Fermentation



X300

SBV yields: 1800 mg/L (time point 121h)

Baculovirus Fermentation

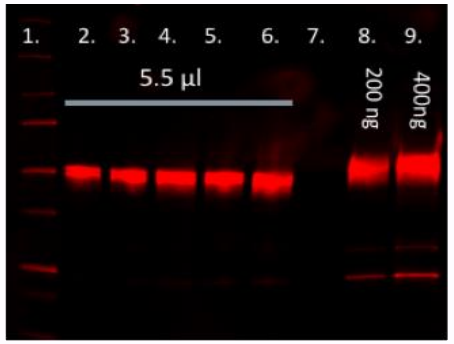


SBV yields: 6 mg/L (time point 192h)

- An antigen against SBV was developed by ZAPI group and was expressed by C1.
- Production level reached **1.8 g/L in 7 days fermentation – 300-fold higher than in Baculovirus.**

C1 Expressed High Level of RVFV (Rift Valley fever virus) Antigen

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

1L fermentation culture and downstream purification

Purification yield: 1,240 mg / L

- An antigen against RVFV was developed by ZAPI group and was expressed by C1.
- Production level reached **1.2 g/L in 7 days fermentation – 300-fold higher than in Baculovirus.**

ZAPI: EU Sponsored R&D program with 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.

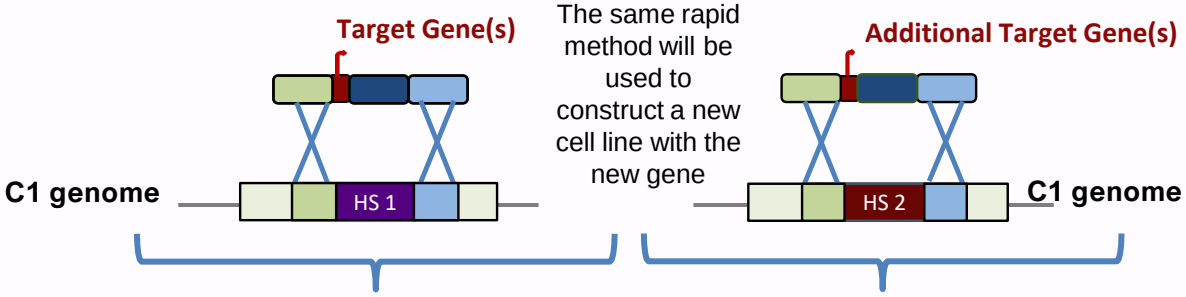
DEVELOPMENT OF PHASE 1 STUDY TO DEMONSTRATE SAFETY & EFFICACY OF ANTIGENS EXPRESSED FROM C1-CELLS

PHASE 1 Trial / DYAI-100 COVID-19 BOOSTER VACCINE

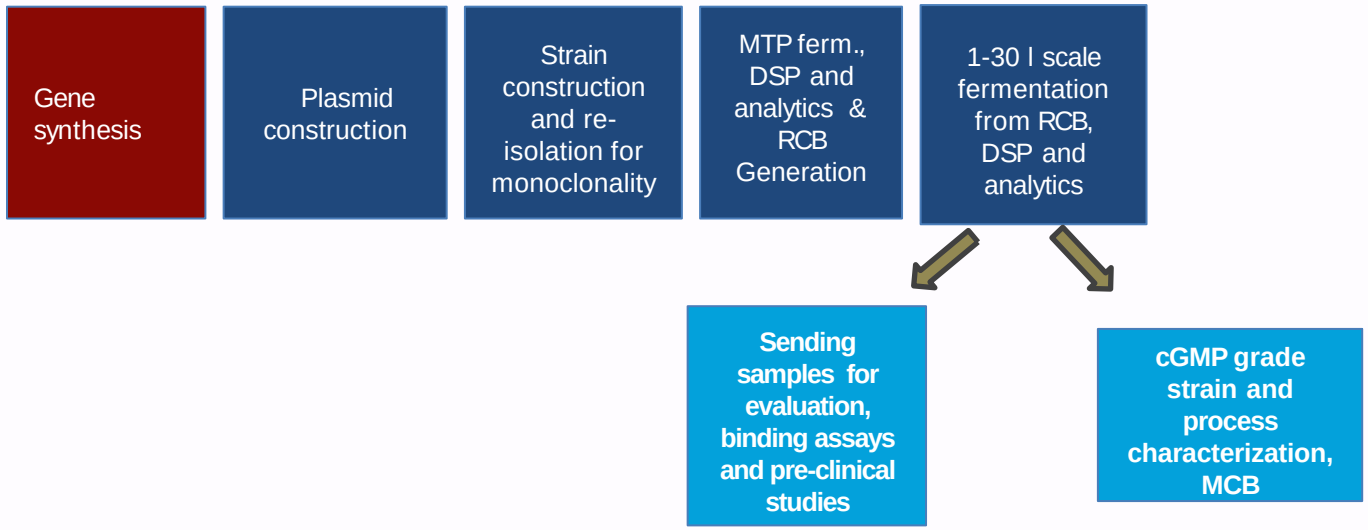
- Rapid High Yield Stable Strain construction ✓
- Mice Studies ✓
- Challenge Study ✓
- Toxicology Study ✓
- GMP production 99% purity ✓
- Ethic committee approved protocol, IMPD submitted ✓
- Completed Dosing in Clinical Phase I trial in South Africa Q1 2023 ✓

Rapid generation of highly productive stable C1 cell lines

Site Directed Integration:



1 week **3 weeks** **1 week** **2 weeks**

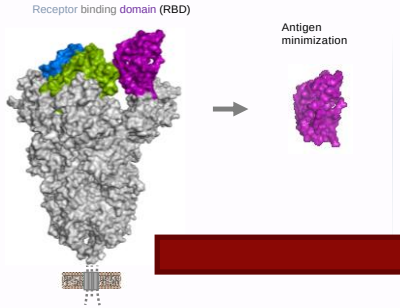


- The Genes are located in 1 Hot Spot gene locus (HS 1)
  - **DYAI-100 one copy of Wuhan gene**
- Additional Genes are inserted at another HS locus (HS 2).
- The C1 Site Directed Transformation method leads to quick generation of stable C1 cell lines and purified protein (~ 7 weeks)

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

Rapidly develop vaccine, antibody, other therapeutic protein candidates which can be manufactured in microbial single use or stainless-steel bioreactors in larger quantities more affordably.

Development of DYAI-100 Covid-19 vaccine candidate with SARS-CoV2 spike RBD:
A key target for induction of potent neutralizing mAbs

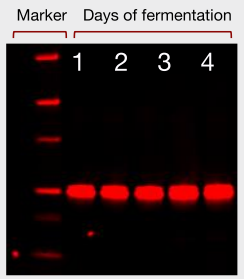


C1 produced vaccine antigens tested for safety in animal trials with cattle, lambs, chicken, rabbits, hamsters and mice in a variety of infectious diseases

Spike Protein Antigen Minimization Advantages

- Single folded polypeptide chain
- All potent neutralizing Ab target the RBD
- Ag minimization -> focused immune response
- Efficient induction of neutralizing antibodies
- RBD is much easier to produce 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)

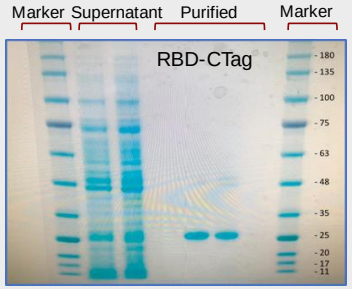
C1 cell line in Ambr250 fermenter system



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

Advancing Towards Phase 1 Clinical Study

- Developed C1 cell line expressing the RBD (23kDa) of SARS-CoV-2 spike protein in ~2 mos.
- Originally expressed at a level of ~ 1 g/L– no need for transient stage
  - Fermentation optimization +/- 2 g/l in 5 days in 22L fermenter
- 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
- Rabbit Tox study demonstrated no adverse events

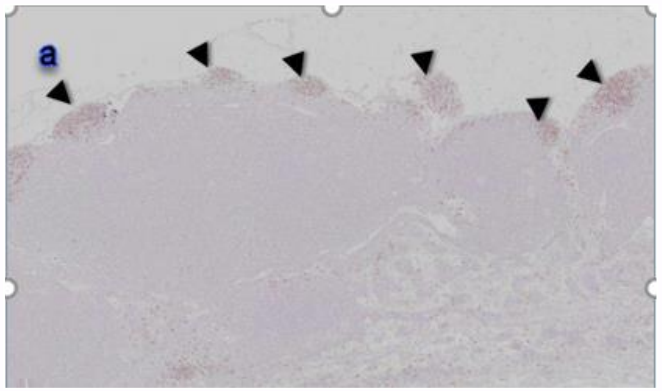
No adverse events observed & antigenic stimulation continued in rabbit toxicology study

Protocol: 2 groups of 20 rabbits each (10 males and 10 females per Test Group) and were subjected to ×4 injections

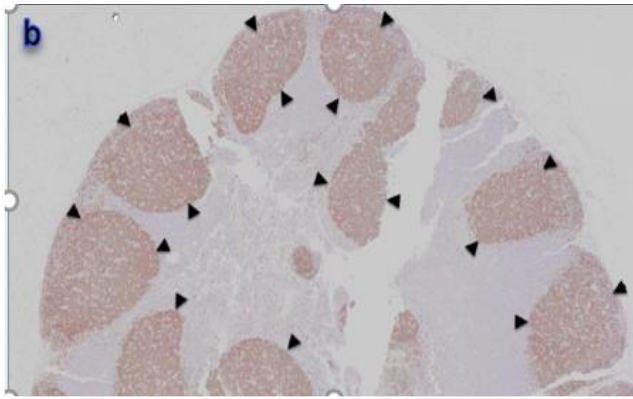
- No mortality
- No abnormal clinical signs
- No local reaction in terms of edema
- Comparable body weight gain
- Normal range of food consumption
- Normal appearance of blood vessels and the optic disc at the indirect ophthalmoscopy examination
- No marked differences in urinalysis values
- No gross pathological abnormal findings were noted

Histopathology Analysis:

a. The iliac lymph node from an animal injected with the Alhydrogel®'85', Placebo (Group 1, control item), sacrificed 42 days post first dosing (Recovery phase). Note, arrowheads - no evidence of germinal centers



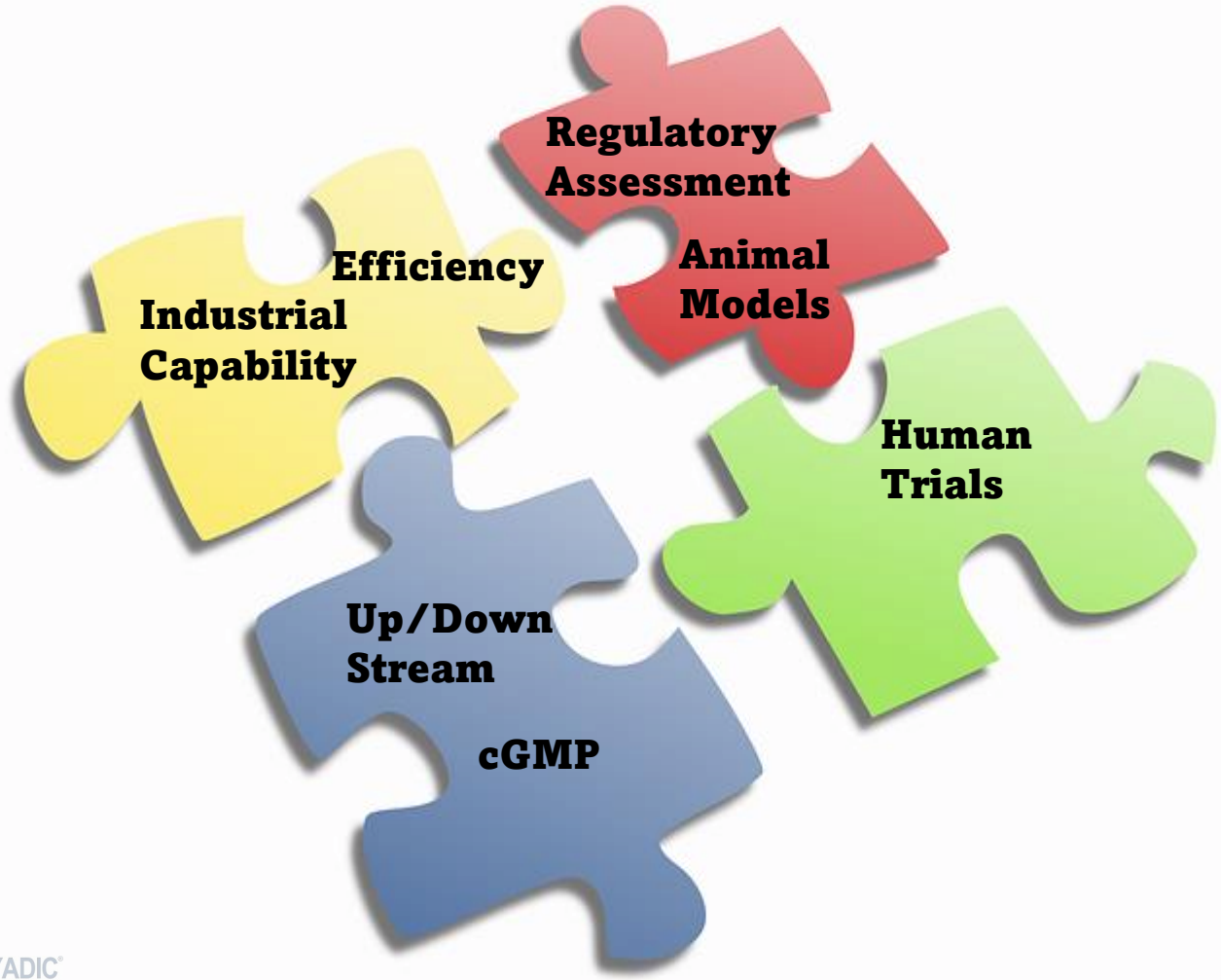
b. The iliac lymph node from an animal injected with C1-RBD Vaccine (Group 2, test item), sacrificed 42 days post first dosing (Recovery phase). The lesions (arrowhead) consist of mild germinal centers increased lymphocytic cellularity (i.e., follicular hyperplasia).



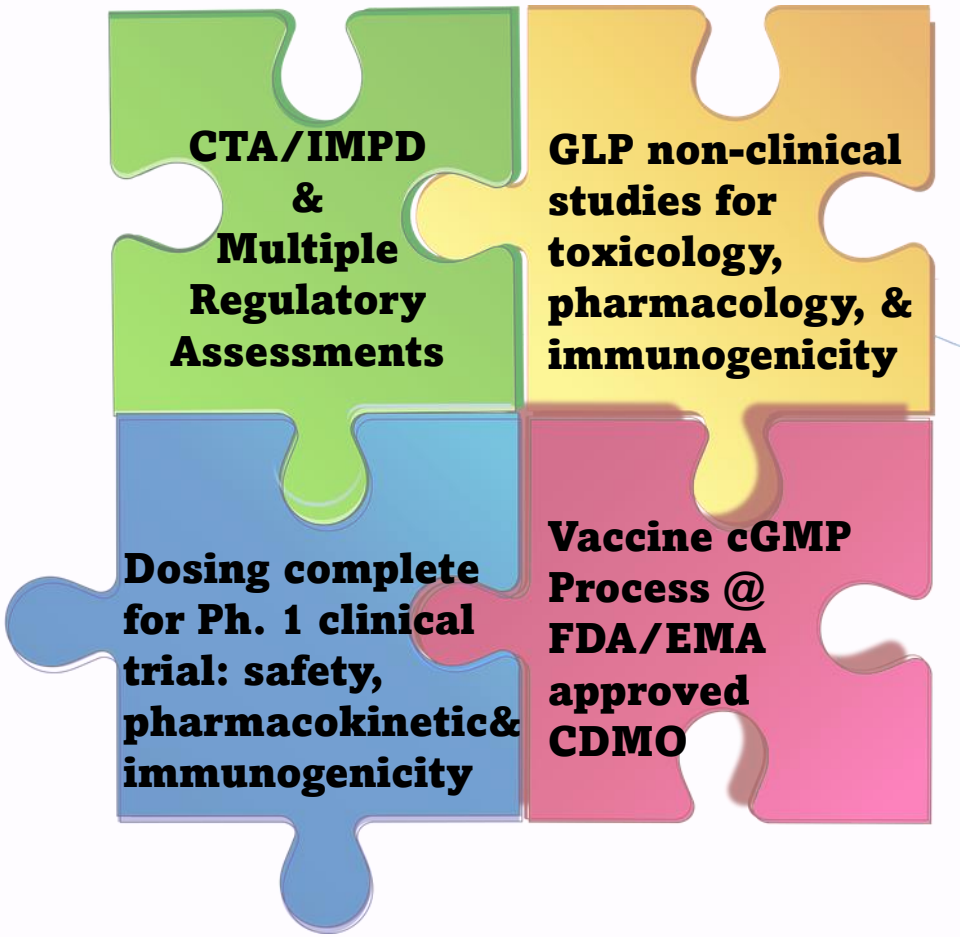
The fact that the changes persisted following 42 days post-1st-dosing session is suggested to reflect a major beneficial effect of the vaccination, i.e., continued, and not declining antigenic stimulation, for relatively long duration, without any local or systemic adverse effect.

The pieces fit / C1 recombinant protein platform - global vaccine readiness

Pre-Phase 1 Clinical Trial



TLR 6 Readiness



Next Generation Recombinant Protein-Based COVID-19 Vaccines

Preparing annual cocktail of multi-valent antigen COVID-19 vaccines

Wuhan/Omicron Vaccine Under Development

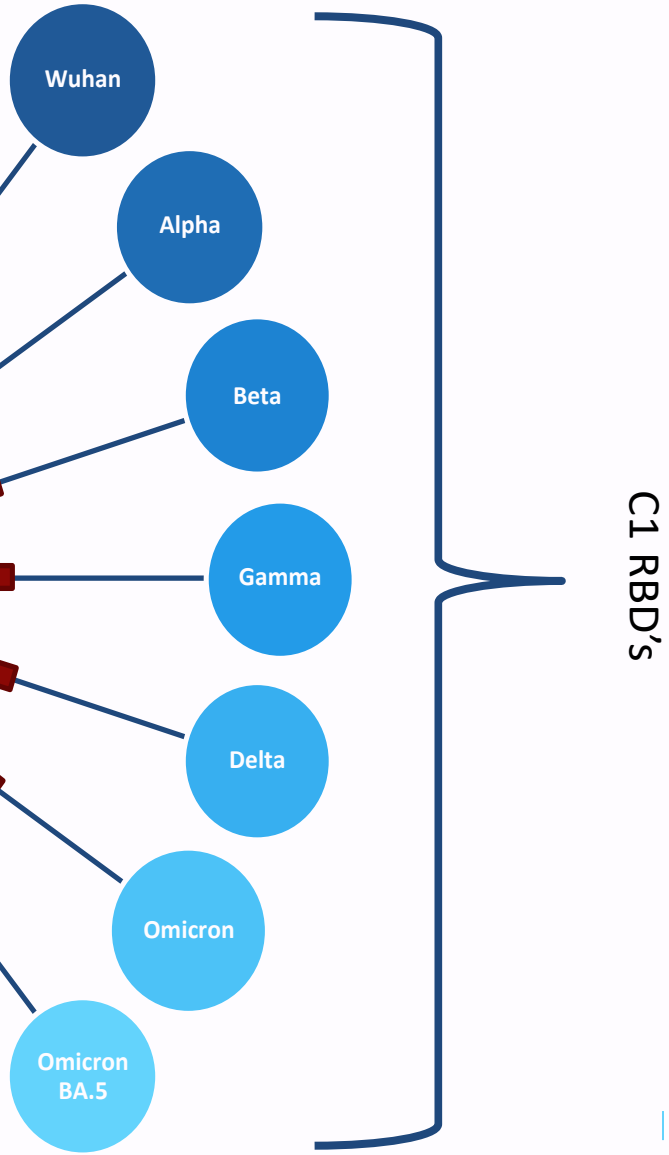
Responses to:

- ***Global &Regional Annual vaccine demands***

C1 cell lines that produce:

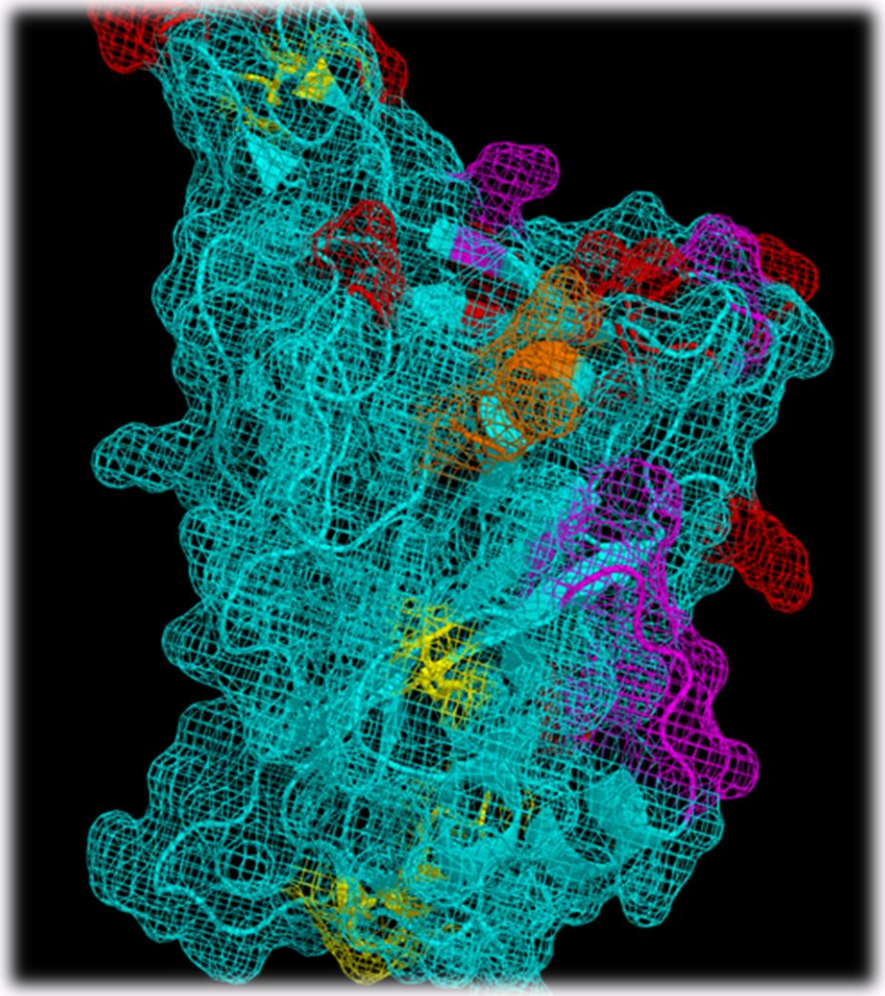
- ***Complex Antigens***
- ***Neutralizing mAbs***





C1 can prepare vaccine cocktail with selected variants to address specific needs

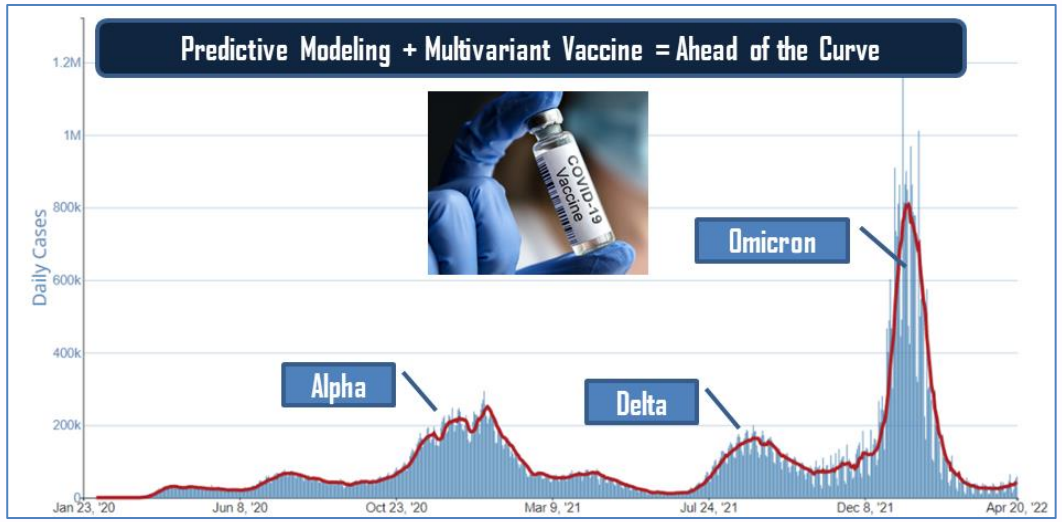
Next Generation COVID Vaccines: “Universal” SARS-CoV-2 RBD



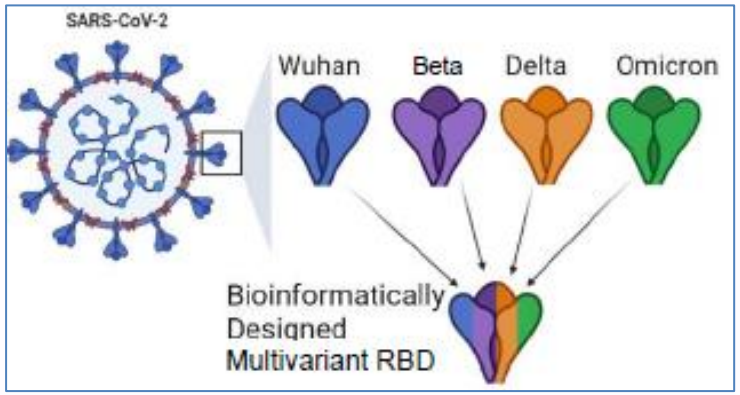
Model of Universal SARS-CoV-2 RBD

- Designing next generation SARS-CoV-2 RBD antigen to induce broad and long-lasting neutralizing antibodies against future COVID-19 variants.
  - Successfully expressed from stable C1-cells as a RBD ferritin nanoparticle antigen.
  - Animal trials ongoing, initial readout expected May 2023.

Next Generation COVID Vaccines: CHIMERIC SARS-CoV-2 RBD MULTIVARIANT



Predictive Chimeric Multivariant RBD



- Combining predictive modeling and the ability to design multivariant vs. multivalent vaccines to respond to new emerging variants with rapid and efficient production may enable a wide-range of broadly protective and fast-response vaccines[1].

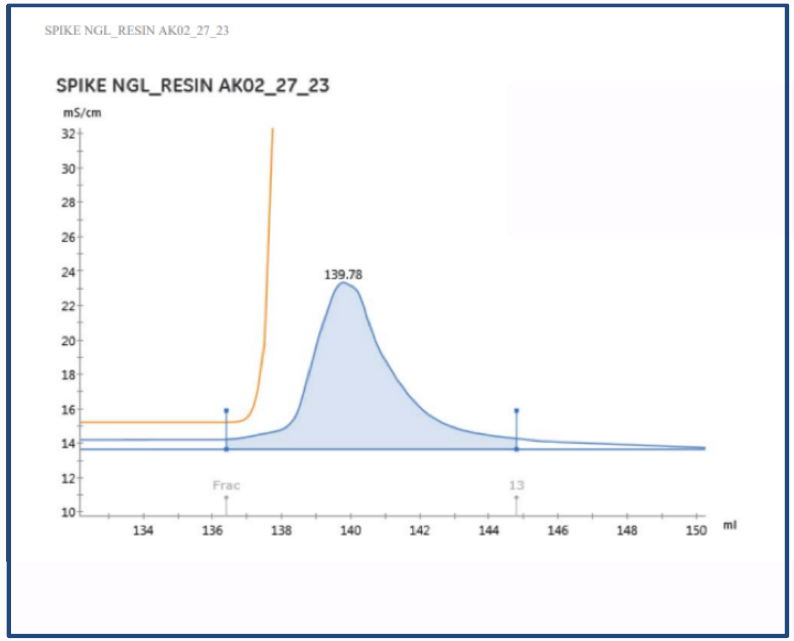
  - Results demonstrate that the variant design approach used yielded a potential multivariant antigen.
  - RBD sera significantly inhibit binding of each of the six tested RBDs to ACE-2 Receptor at least as effectively as homologous antibodies
  - Results demonstrate that the variant design approach used yielded a potential multivariant antigen.
  - Potential utility for other viruses

1. In collaboration with independent third party

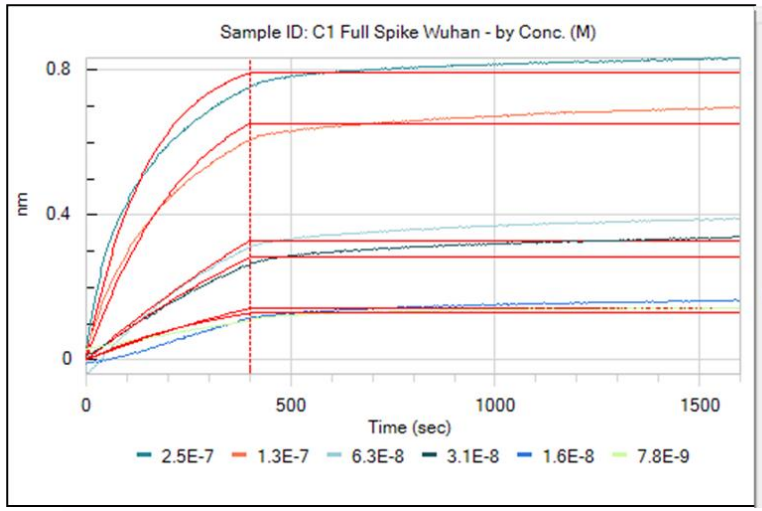
SARS-CoV-2 spike protein expressed by C1

The full spike protein was produced and secreted at a level of 1 g/L in 5 days (before any optimization)

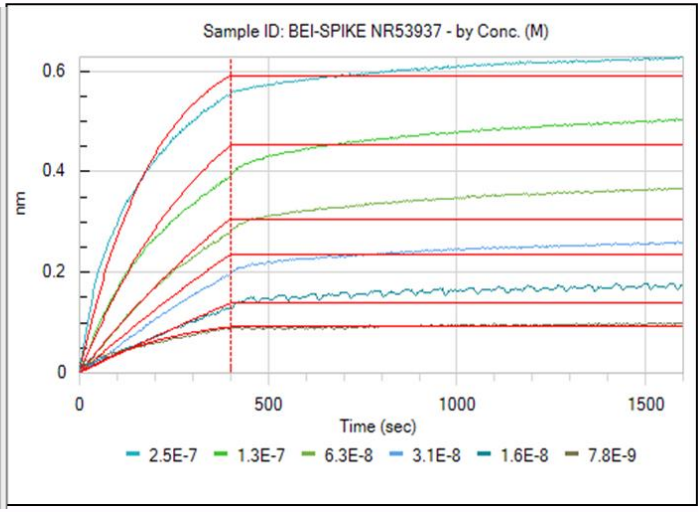
Purification - intact protein analysis



Octet Titer & Binding Results



Fitting Results
Model Equation:
Response = Rmax*Conc. /(KD + Conc.)
Y Weighting: None
Results:
Chi^2/DoF = 0.0055
R^2 = 0.5283
RMax = 0.8495 ± 0.038
KD = 1.2E-09 ± 5.4E-10M

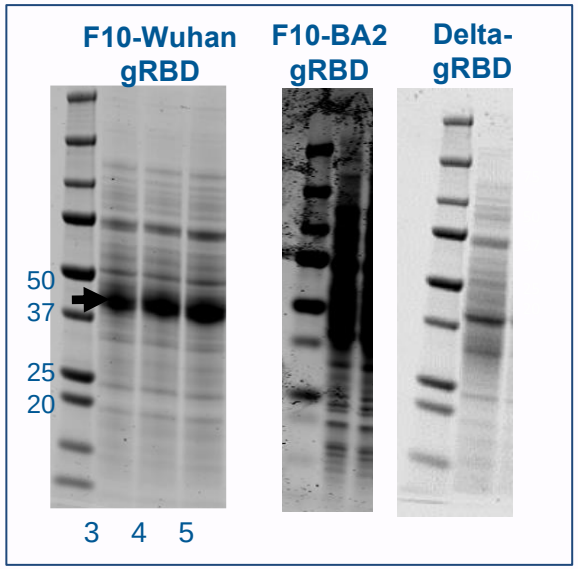


- ***Binding behavior of spike protein and receptor binding domain of the SARS-CoV-2 virus analysis using Bio-layer interferometry (BLI)***

- **The binding curves of C1 Spike and BEI Reference CHO spike share similar features with KD of 1.2 μM**

C1 expressed ferritin nanoparticles, keeping pace with advancing science

Coomassie staining gel from culture supernatants:



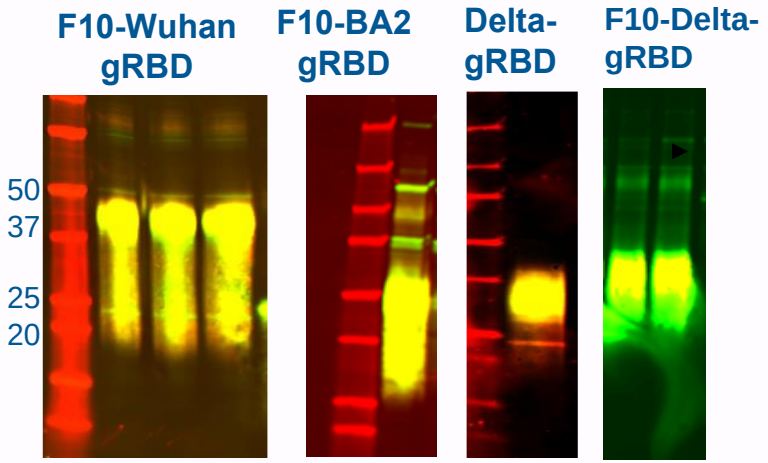
Fermentation at higher scale shows clearly bands of the target proteins in Coomassie staining gels.

Titers after 5 days of production:

Target	Titer (5 days)*
F10-Wuhan-gRBD	3.48 g/L
F10-Omicron BA2-gRBD	0.7 g/L
Delta-gRBD	2.47 g/L

*Bioreactor cultivations (prior to optimization)

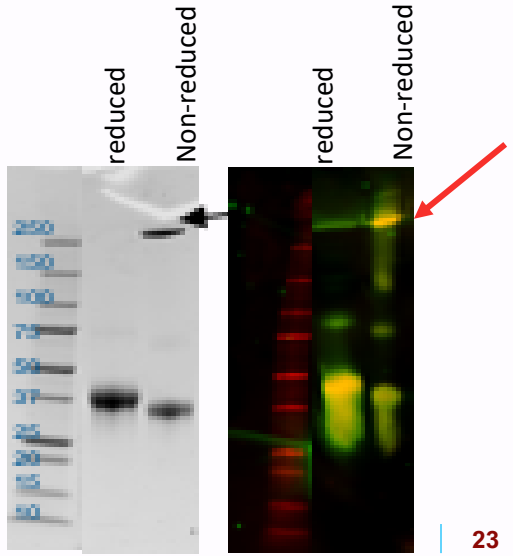
Western blot from culture supernatants:



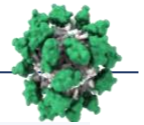
Dual detection with polyclonal anti-RBD and anti Ctag antibodies

Purified F10-Wuhan-gRBD:

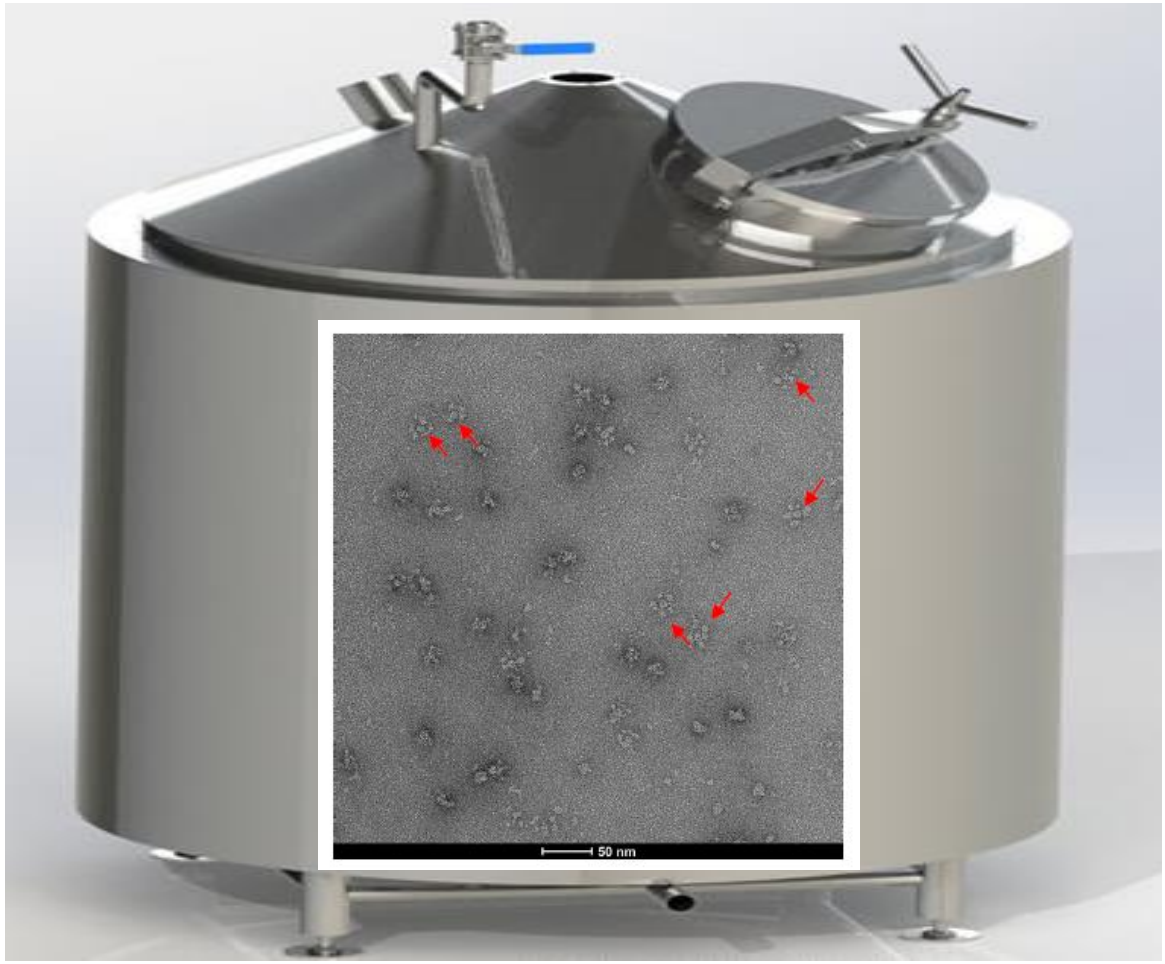
All F10-RBD variants shows high molecular weight bands under non-reduced conditions indicating putative oligomerization forms



C1 expressed ferritin nanoparticles in a single fermentation



C1 F10-Wuhan-gRBD



- **Past approach was less efficient and more costly and time intensive to create the antigen and nanoparticle separately then combine**

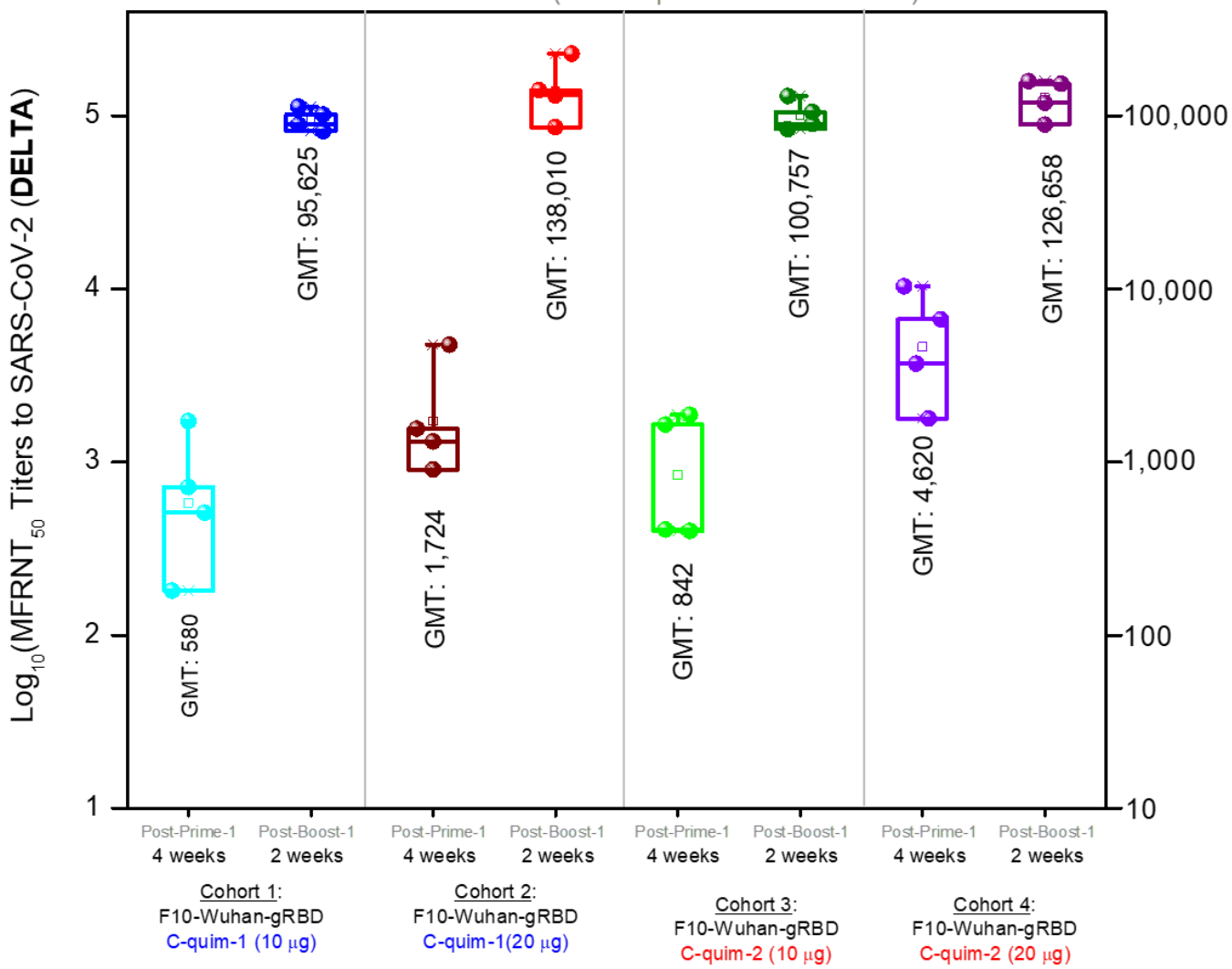
Antigen



Nanoparticle

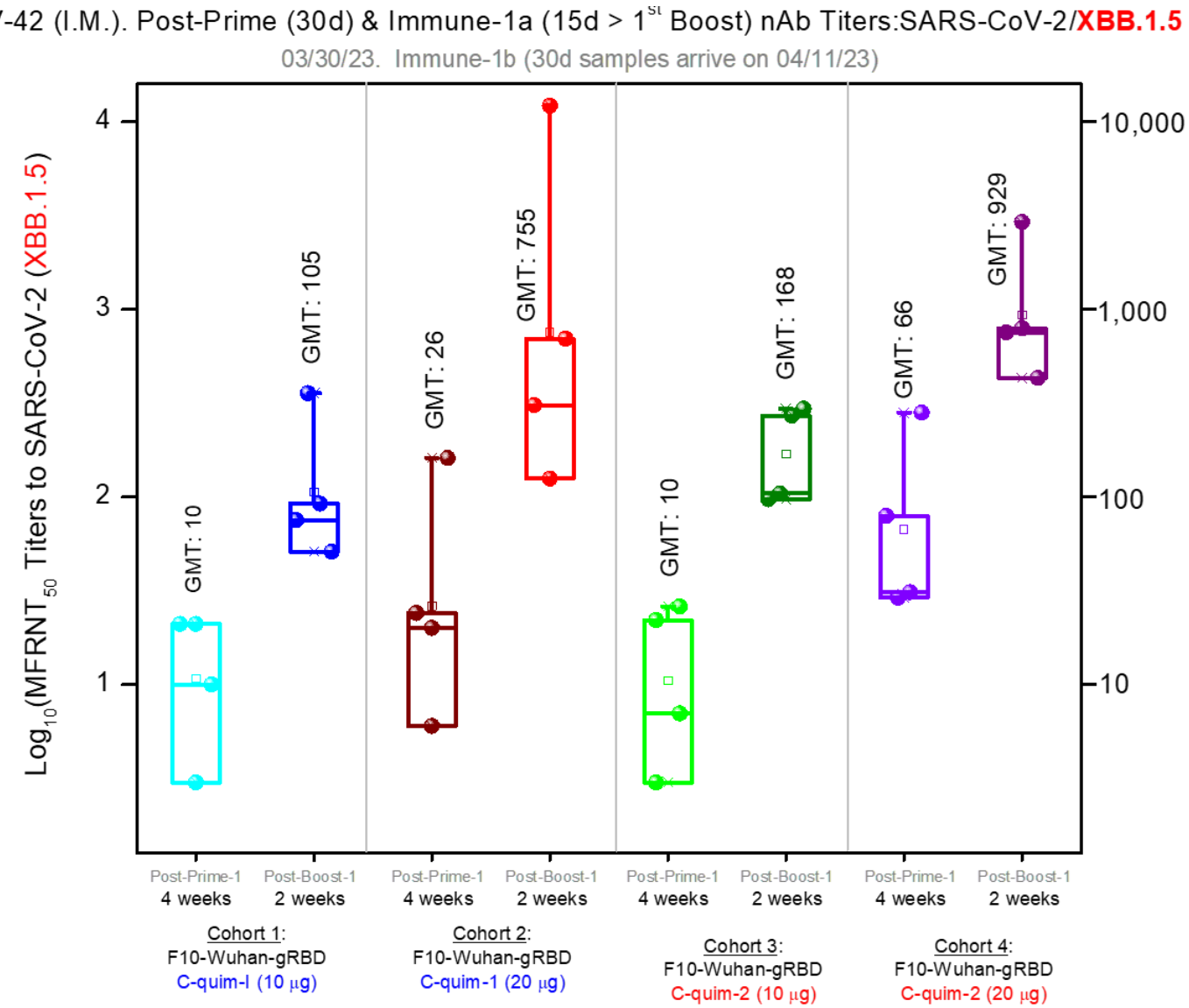


2. Rabbit Intramuscular Subunit Vaccine – DELTA



Delta. NR-55672. SARS-CoV-2 Isolate hCoV-19/USA/MD-HP05647/2021 (Lineage B.1.617.2; Delta variant)

2. Rabbit Intramuscular Subunit Vaccine – XBB.1.5.

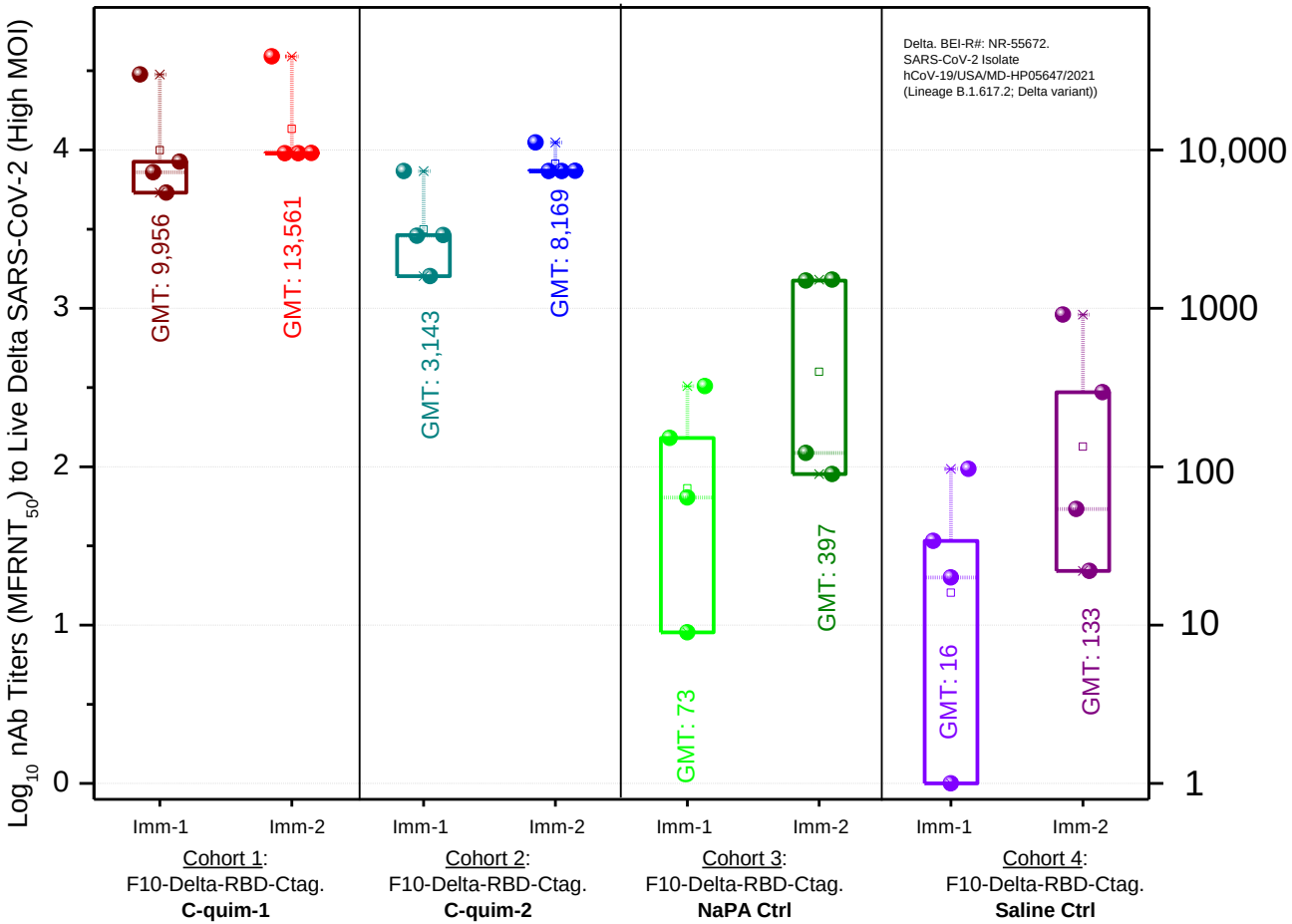


Omicron XBB.1.5. NR-59104. SARS-CoV-2 Isolate hCoV-19/USA/MD-HP40900/2022 (Lineage XBB.1.5; Omicron Variant).

Rabbit Intranasal Subunit Vaccine – DELTA

01/26/23. Summary. VV-39 (Rabbit, Intranasal): F10-Delta-RBD-Ctag // Cquims-1, -2

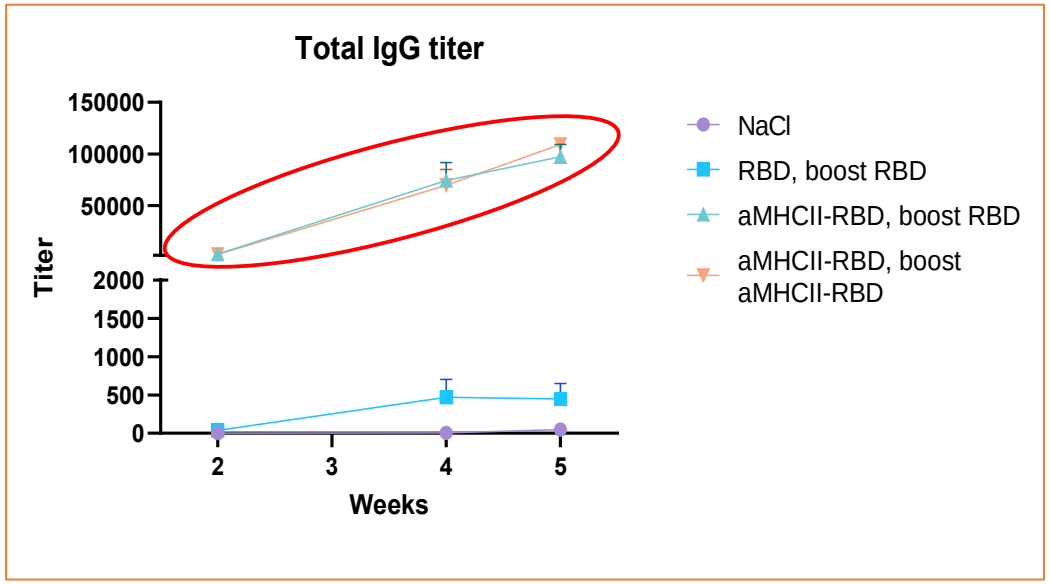
Systemic Immune-1 nAb Titers Against Delta Live Virus after Intranasal Prime + One (Immune-1) or Two (Immune-2) Boosts

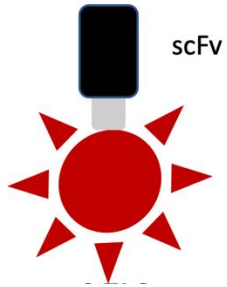


FDA/EMA Guidance for Mucosal Vaccines: Examine Systemic nAb Titers.

C1 aMHCII-RBD COVID-19 prime elicits very high IgG antibody titers

RBD fusion to scFv direct the vaccine protein specifically towards MHCII receptors expressed on antigen presenting cells (APC).





Experiment 1 (32 mice)(ongoing, 11 weeks):

Groups:

A: NaCl

B: Prime with RBD, boost with RBD

C: Prime with aMHCII-RBD, boost with RBD

D: Prime with aMHCII-RBD, boost with aMHCII-RBD

Aim is to examine the impact of MHCII-targeting for boosting.

Readout: antibodies in sera for 6-8 weeks by ELISA. EliSpot of cellular responses at termination (in vitro stimulation with peptide pool/Spike).

Mice were vaccinated once intradermally with 1µg of the indicated protein vaccines, mixed 1:1 with AS03 adjuvant and boosted with 6 weeks after the first vaccination. Following vaccination and boost, sera were evaluated in ELISA for IgG antibodies against Delta Spike (homologous to RBD in protein vaccines).

April 13, 2023, USPTO Issues Notice of Allowance - Production of Flu Vaccine Produced From C1-cells

"Type A influenza virus not only poses one of the largest threats to the modern world, but the risk of spill-over of avian influenza from poultry to humans is growing." – Kess Rowe (GAVI) 03/2021

Influenza progress:

- C1 expressed HA successfully tested by **Sanofi Pasteur** versus HA expressed from Baculovirus
- Immunogenicity study of Recombinant Hemagglutinin (HA) from A/H1N1/New Caledonia/20/99 strain:
  a) C1 produced r-HA was **safe and well-tolerated in mice**; and
  b) the C1 produced r-HA was **at least as immunogenic in mice as the baculovirus-rHA**

EXPRESSION SYSTEM	DOSE OF RHA 1 U/G	DOSE OF RHA 3.3 U/G	DOSE OF RHA 10 U/G	DOSE OF RHA 30 U/G
C1	50% (4/8)	57% (4/7)	100% (8/8)	100% (8/8)
Baculovirus	62% (5/8)	12% (1/8)	50% (4/8)	75% (6/8)

- University of Oslo re-confirms viability of C1 to express APC-targeted Influenza virus antigens
  a) H1N1, H5N1, H7N9 & Neuraminidase - ALL successfully produced from C1-cells
  b) Plan to partner and advance development or seek government funding

Demonstrated consistent successful expression of broad array of potent antigens & mAbs from C1-Cells

❑ SARS-CoV-2 S-RBD

- Alpha (B.1.1.7)
- Beta (B.1.351)
- Gamma (P.1)
- Delta B.1.617.2
- Omicron B.1.1.529
- Omicron BA.5

❑ SARS-CoV-2 gRBD Ferritin Nanoparticles

- Wuhan gRBD Ferritin nanoparticle
- Omicron gRBD Ferritin nanoparticle

❑ Infectious Disease Monoclonal Antibodies

- COVID-19
  - Neutralizes All SARS-CoV-2 Variants of Concern, Including Omicron BA.1/2.
  - Hamster & Non-Human Primate Studies
- ZIKA
- RVFV
- ANDV

❑ Other Antigens

- RABIES
- INFLUENZA HA (H1N1, H5, H7)
- INFLUENZA NA
- WN (West Nile)
- POWASSAN
- SBV (Schmallenberg)
- RVFV (Rift Valley Fever)
- MERS (Middle East Respiratory Syndrome)
- IBVD-VLPs (Infectious Bursal Virus Disease)
- Others under development

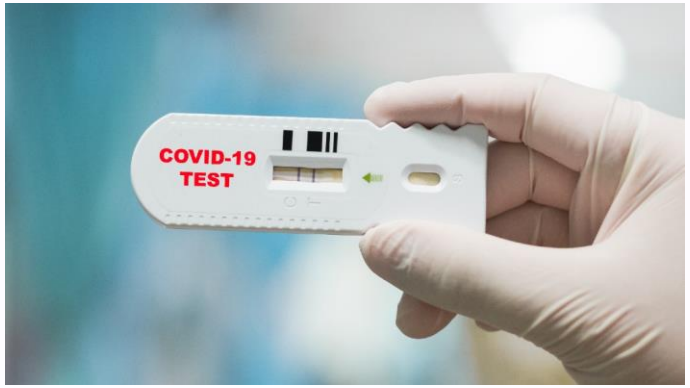
❑ Of Note:

- S-RBD / Spy Tag (For Nanoparticles)
- Full Spike / Spy Tag (For Nanoparticles)
- Full Spike
- aMHCII Delta RBD
- Delta-gRBD
- Fc-RBD
- Others in Development

Supply Chain Solution: Enzymes & Proteins for Biopharmaceutical Use

DIAGNOSTICS

- Diagnostic Testing
  - Antigens
  - Antibodies



DEVELOPMENT/PRODUCTION

- Recombinant Albumin
  - Protect challenging drug, cell and vaccine products from:
  - Aggregation
  - Non-specific adsorption
  - Oxidation
  - Improves solubility, batch-to-batch consistency



ENZYME CATALYSTS

- Endonucleases
  - DNASE-1 (2g/L)
  - DNA template removal during development manufacturing of mRNA therapeutics and vaccines



DYADIC®

A VACCINE OR ANTIBODY THAT IS UNAVAILABLE OR UNAFFORDABLE IS 100% INEFFECTIVE

THANK YOU

APPENDIX I: MONOCLONAL ANTIBODIES

Human and Animal Therapeutic Proteins (mAbs)

Proof of Concept, Nivolumab

High improvements in yield production of mAbs (Nivolumab) have been obtained using novel genetic elements, transformation technology and approaches:

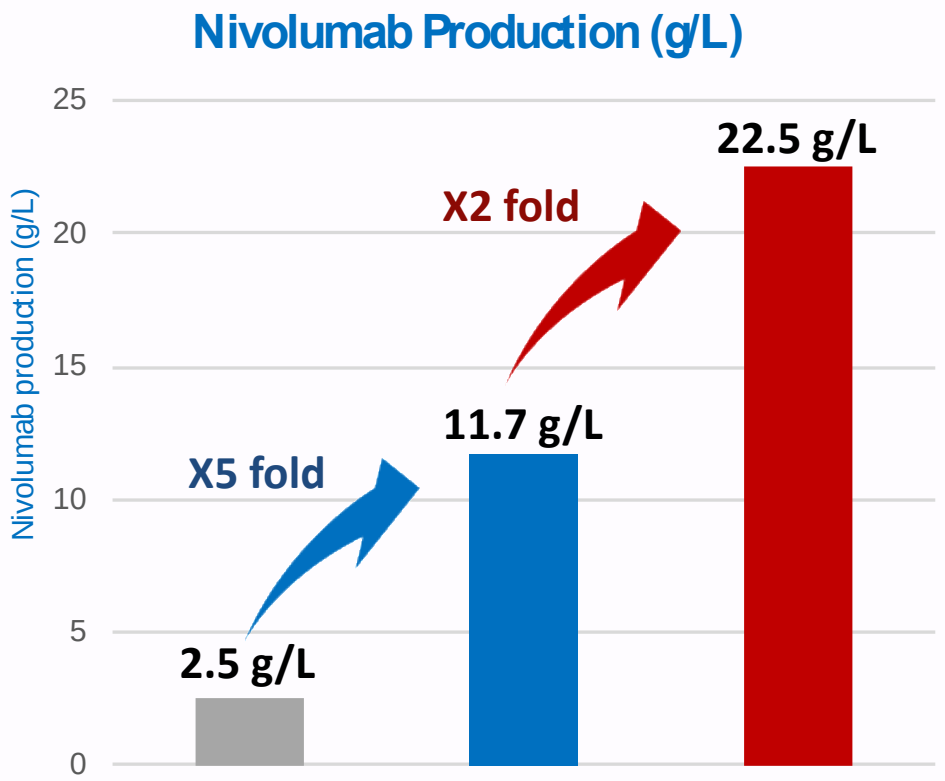
Standard transformation in Dyadic microbial platforms:

- Site directed integration into specific hot spots in the genome
- One/two copies integration per transformation round
- One hot spot per transformation round

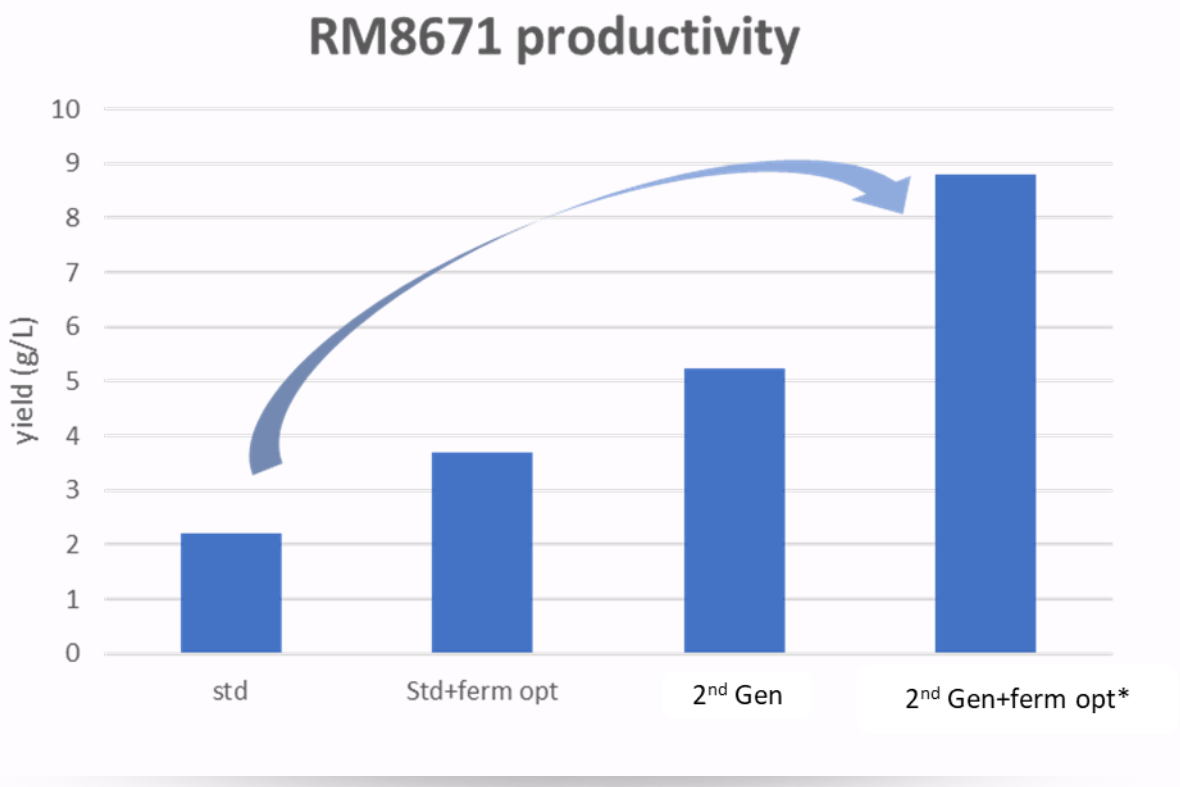
Random transformation in Dyadic microbial platforms:

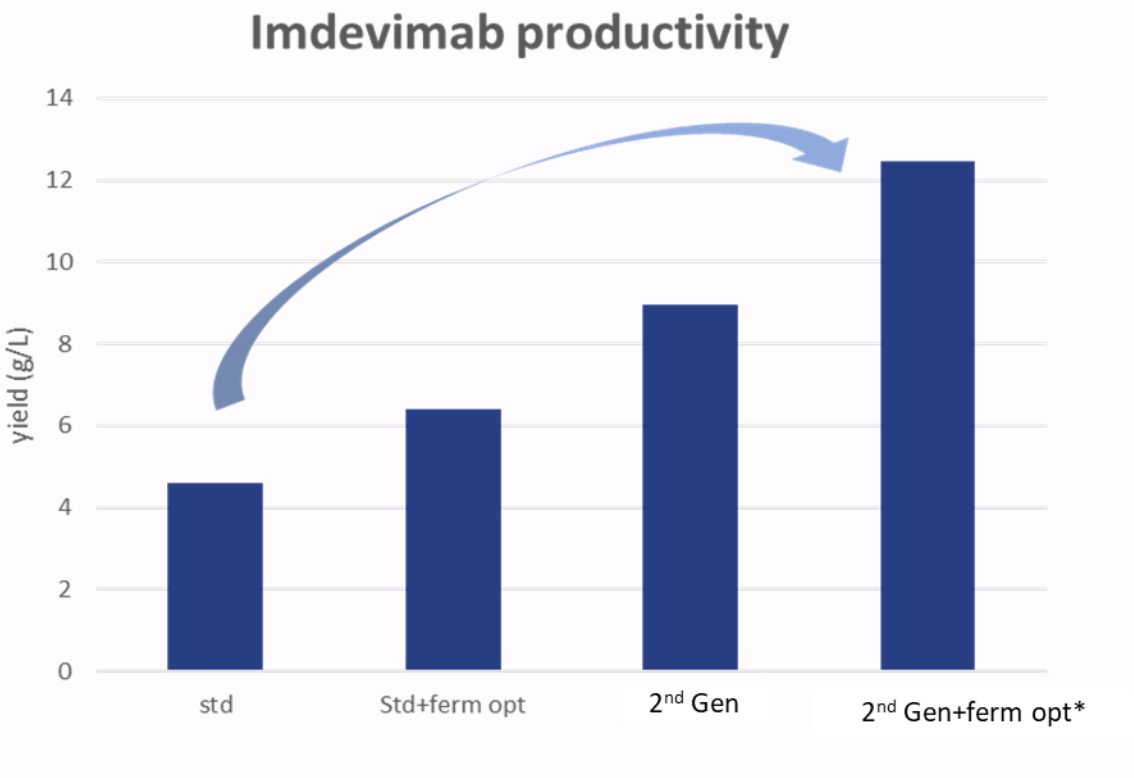
- Expression cassettes are not site directed integration
- Random integration along the Host cell genome
- Several copies can be integrated

Novel genetic elements, transformation technology and approaches in Dyadic microbial platforms



Expression & fermentation optimization of RM8671 and Imdevimab in C1

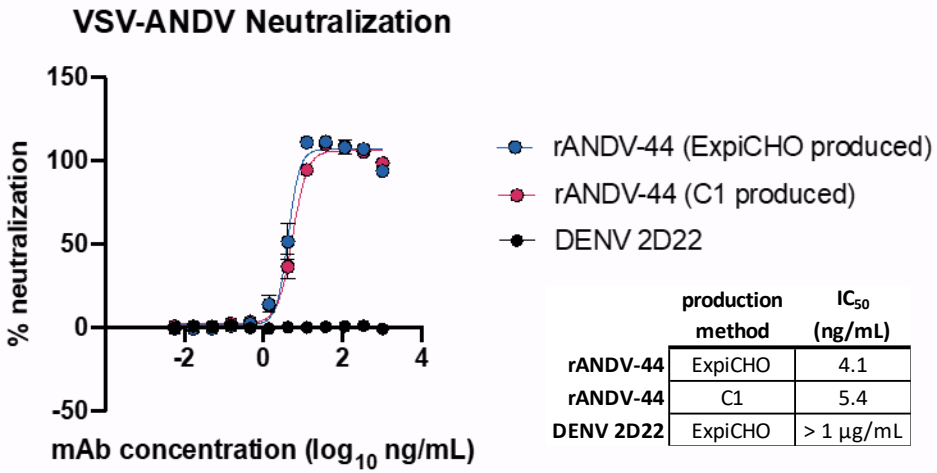




*Expected improvement considering previous fermentation optimization

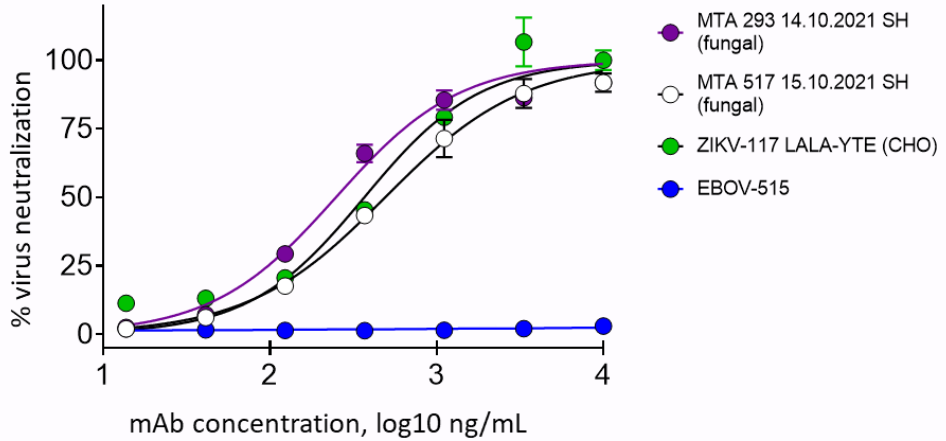
CHO-like neutralization and binding, but faster, larger quantities more affordably

ANDES VIRUS

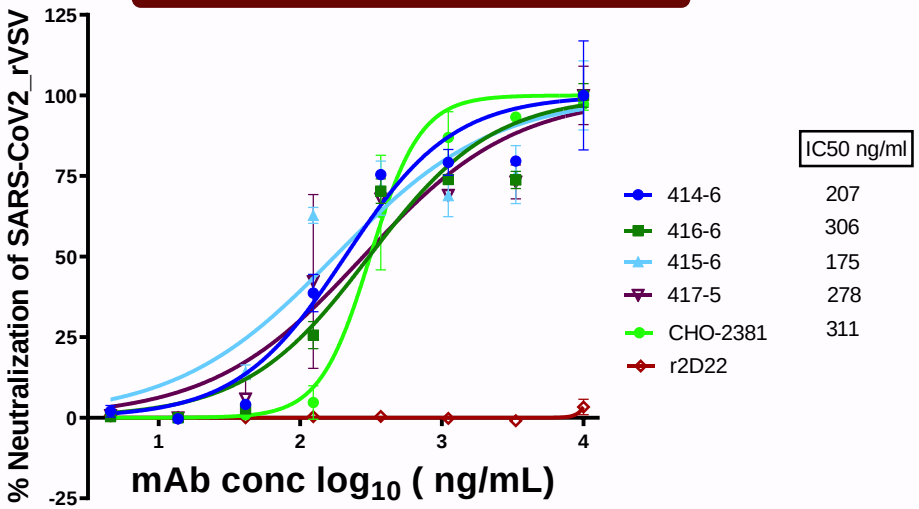


	production method	IC_{50} (ng/mL)
rANDV-44	ExpiCHO	4.1
rANDV-44	C1	5.4
DENV 2D22	ExpiCHO	> 1 µg/mL

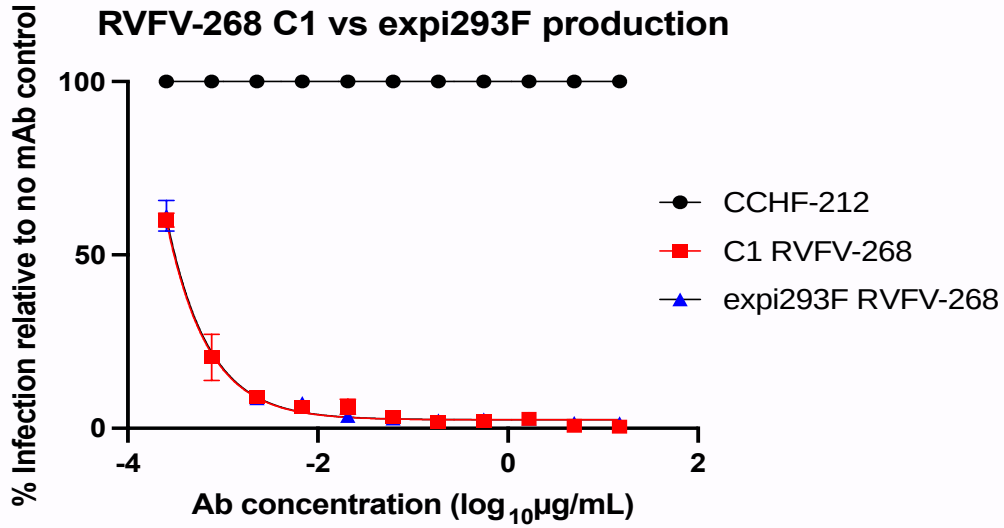
ZIKA



SARS-COV2



RIFT VALLEY



C1 87G7 mAb - Same safety & efficacy profiles as CHO mAb in animal studies
87G7 mAb was produced from native (non glycoengineered) C1-cells

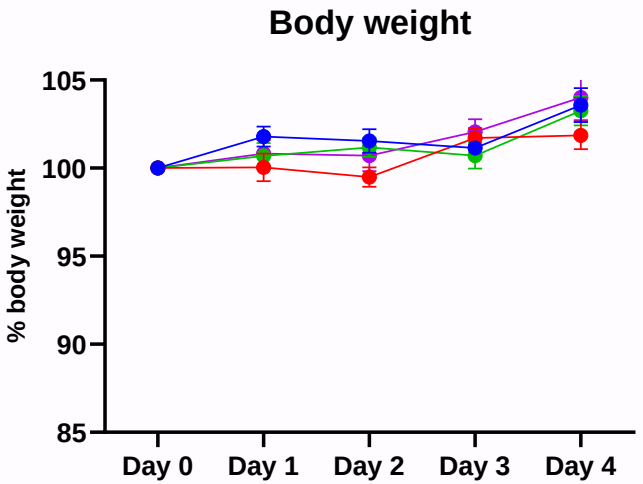
87G7 mAb NHP Study



- Challenge with 10^4 TCID50 SARS-CoV-2 Delta VOC
- RT-qPCR carried out on throat and nasal swab samples.
  - *No SARS-CoV-2 RNA detected in animals pre-treated with 87g7.*
  - *Animals treated with the control antibody (Palivizumab) had RNA in the swab samples.*
- VI from lung, nasal tubinates and other tissues confirmed RT-qPCR data for each sample.
- Histopathological changes corresponded with RT-qPCR and VI data
- **NB: No signs of antibody mediated enhancement**

87G7mAb Hamster Study





C1 platform: Increase mAb access faster more affordably globally

C1 platform produces comparable therapeutic proteins as CHO while overcoming key production limitations

C1 produces stable and correctly folded mAbs that have binding and neutralizing properties similar to those produced from CHO cells

Lower Cost, faster release

Flexible production scale, single use and/or stainless-steel bioreactors

C1 media <1/20 of the cost of CHO media

No viral inactivation required; limited infrastructure investments

Faster Development, Production and Release Time

Stable cell lines in 5-7 weeks enabling rapid development and response

C1 produces product significantly faster (12-14 days) than CHO cells (41-54 days); ~ Potential to produce three to four batches using C1 in the same timeframe as one batch using CHO cells, and release treatment sooner

Higher Yields

C1 has the potential to produce more product per batch and larger overall quantities

~ Potential to produce three to four batches using C1 in the same timeframe as one batch using CHO cells