Dyadic International (NASDAQ: DYAI)

Next Generation Proteins for World Health

C1 Vaccine Technology Presentation

Mark Emalfarb, Founder & CEO

Vaccine Technology Summit
September 15-16, 2022
London UK

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

Dyadic's Mission: Greater Quantities of Lower Cost Proteins for World Health

Meeting the growing demand for proteins worldwide for human and animal health with highly productive scalable microbial biomanufacturing platforms.

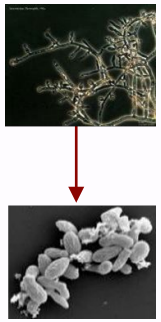
To improve how we feed, fuel, and heal the world by utilizing modern biotechnology to revolutionize science, medicine, agriculture, nutrition and food.

Mark Emalfarb

Founder, CEO

Proven entrepreneur, inventor 25+ U.S. and foreign biotechnology patents, filamentous fungal enzyme product commercialization

Over $100MM and 30 Years of Commercial Engineering Invested in C1



"C1" (*Thermothelomyces heterothallica*) is an exceptional genetically engineered fungal strain
Broad application of C1 has expanded through decades of commercial engineering

1992

C1 strain discovered to produce commercially valuable enzymes

Commercial launch of C1 enzymes produced for industrial textile manufacturing

C1 molecular toolkit optimized for broad development across other industries including human therapeutic applications

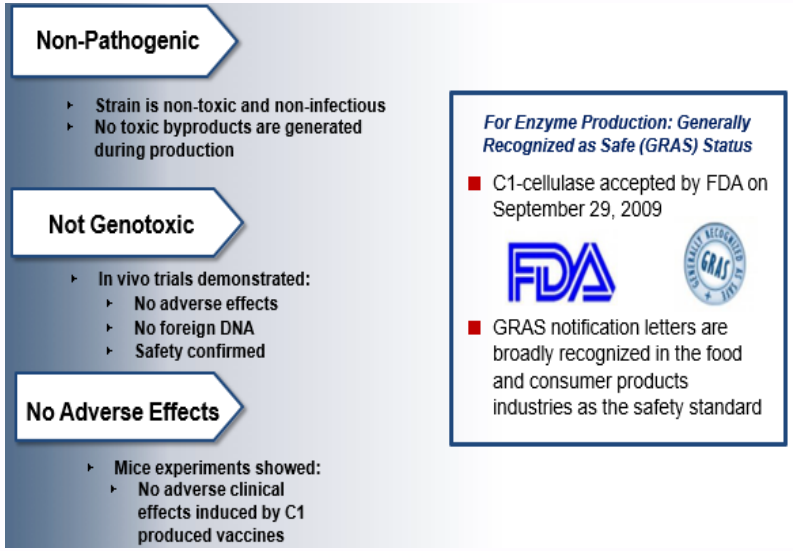
Genome sequenced and cell line refined for high purity in industrial manufacturing

2005

Enzyme library developed

C1 launched in biofuels industry

Generally Recognized as Safe (GRAS) designation by FDA



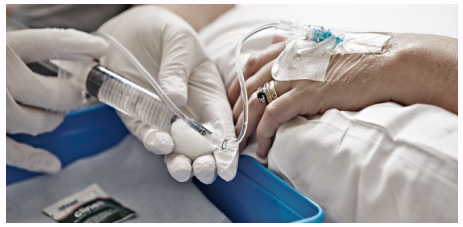
2016

Launch of human biologics development for vaccines and biosimilars

Proof-of-Concept collaborations with pharma industry accelerate

Global partnerships and R&D projects with top pharma and leading animal health companies

Today



Preparations for 1st in human trials: DYAI-100 COVID Vaccine moving into in Phase 1 Clinical Trial in South Africa & 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
- Phibro

C1 Protein Production Capability has Key Advantages vs. Other Platforms

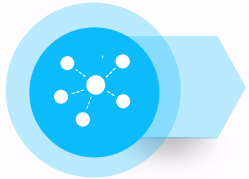
Potential to disrupt conventional manufacturing platforms by overcoming key production limitations

Purity

- High retention of target secreted protein through downstream processing
- No requirement for viral (i.e., CHO) or endotoxin (i.e., E.coli) inactivation

Productivity

- Robust & versatile growth conditions
- High yields of secreted protein
- Low viscosity due to C1's unique morphology



Robustness

- Broad commercial scale size, ranging from laboratory microtiter plates, shake flasks, single use and/or stainless-steel microbial bioreactors.
- Stable and correctly folded mAbs; Binding and neutralizing properties similar to CHO cells

Speed

- Develop stable C1 cell lines in ~ 7 weeks
- Production time savings of ~30 days over CHO-cell production (C1: 12-14 days vs CHO: 41-54 days)
- Potential to make ~ 3-4 batches of mAbs in the same time it takes to make 1 batch using CHO cells

Cost

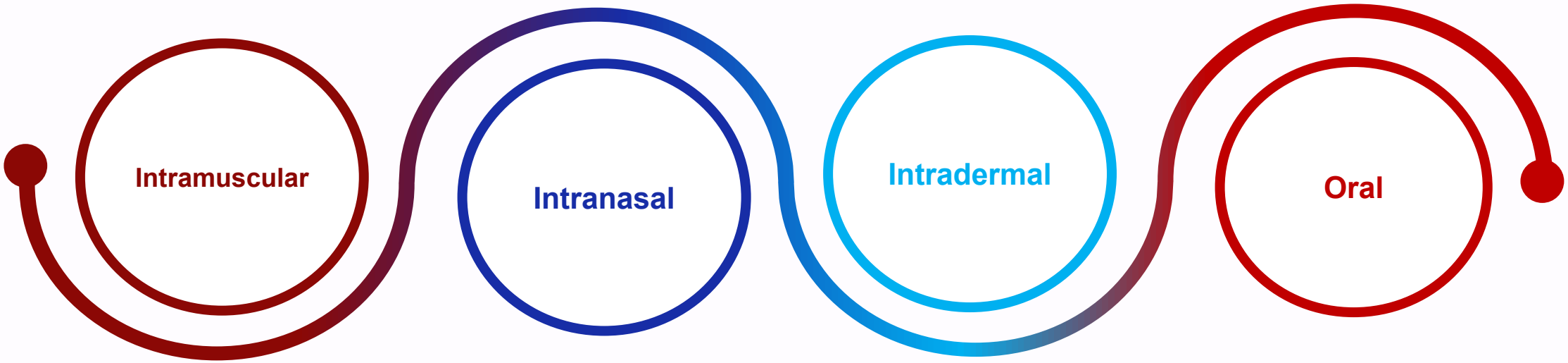
- High yields and rapid production cycle times reduce cost and shrink manufacturing footprint
- Requires only low-cost cGMP synthetic media; C1 media <1/20 of the cost of CHO media
- No requirement for viral or endotoxin inactivation, simplifies processing compared to CHO & E.coli saving time, money

C1 Uniquely Positioned to Address Vaccine and Biologic Drug Challenges

Manufacturing Challenges		C1 Manufacturing Advantages
Material and Equipment	Shortened timeframes and shortage of raw materials, equipment, consumables, lipid nanoparticles due to high demand Examples: Cell culture media, Bioreactor bags, vials, tangential flow filters	20+ years of proven industrial large-scale manufacturing Conventional stainless-steel fermenters or bioreactor bags Low cost, widely available media
Process Complexity	Inherent challenges include time to produce new cell lines, drug substance, process optimization, aseptic techniques, cold manufacturing	Stable cell lines in ~ 60 days: From gene synthesis to stable strain for pre-clinical testing Broad conditions for growth & High Yield
Capacity Constraints	Infrastructure and production requirements, cold storage and distribution challenges, geographic challenges	No unique cold storage requirements for manufacture or distribution -Unparalleled scalable capacity from 5L to 500K L

C1 produced antigens are agnostic to the route of vaccine administration

Dyadic has Results and/or Collaborations Across Different Routes Of Administration



Intramuscular

An intramuscular injection is a technique used to deliver a medication deep into the muscles. This allows the medication to be absorbed quickly.

Intranasal

Intranasal is administered into each nostril using a manufacturer-filled nasal sprayer.

Intradermal

Intradermal injections (ID) are injections administered into the dermis, just below the epidermis.

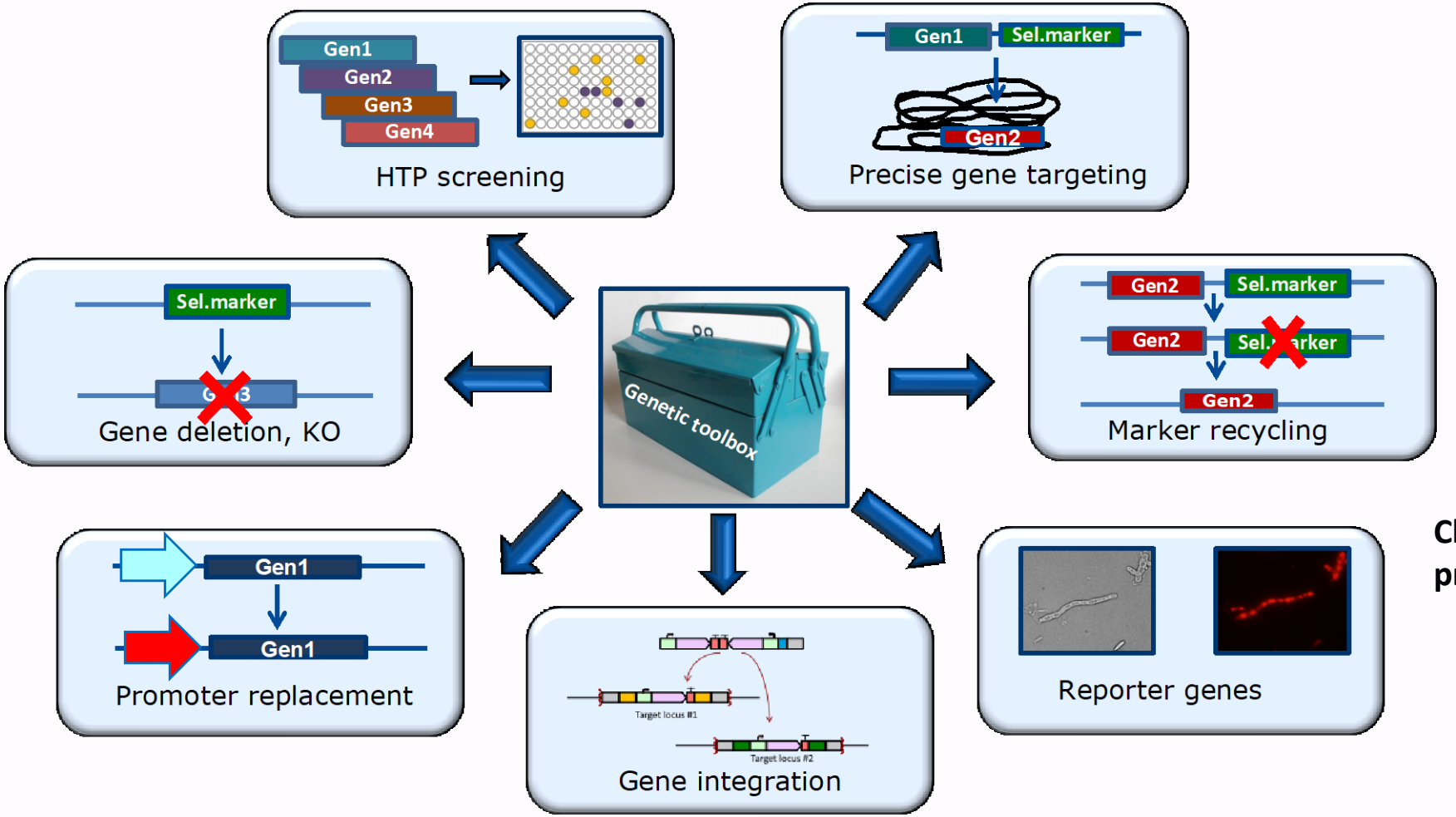
Oral

The oral route of administration provides a more efficient and convenient method of administration relative to injectable vaccines.

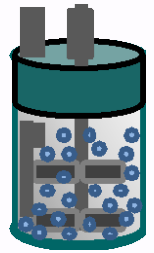
RAPID, STABLE, & HIGH YIELD C1 STRAIN DEVELOPMENT

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

The advanced genetic toolkit for synthetic biology

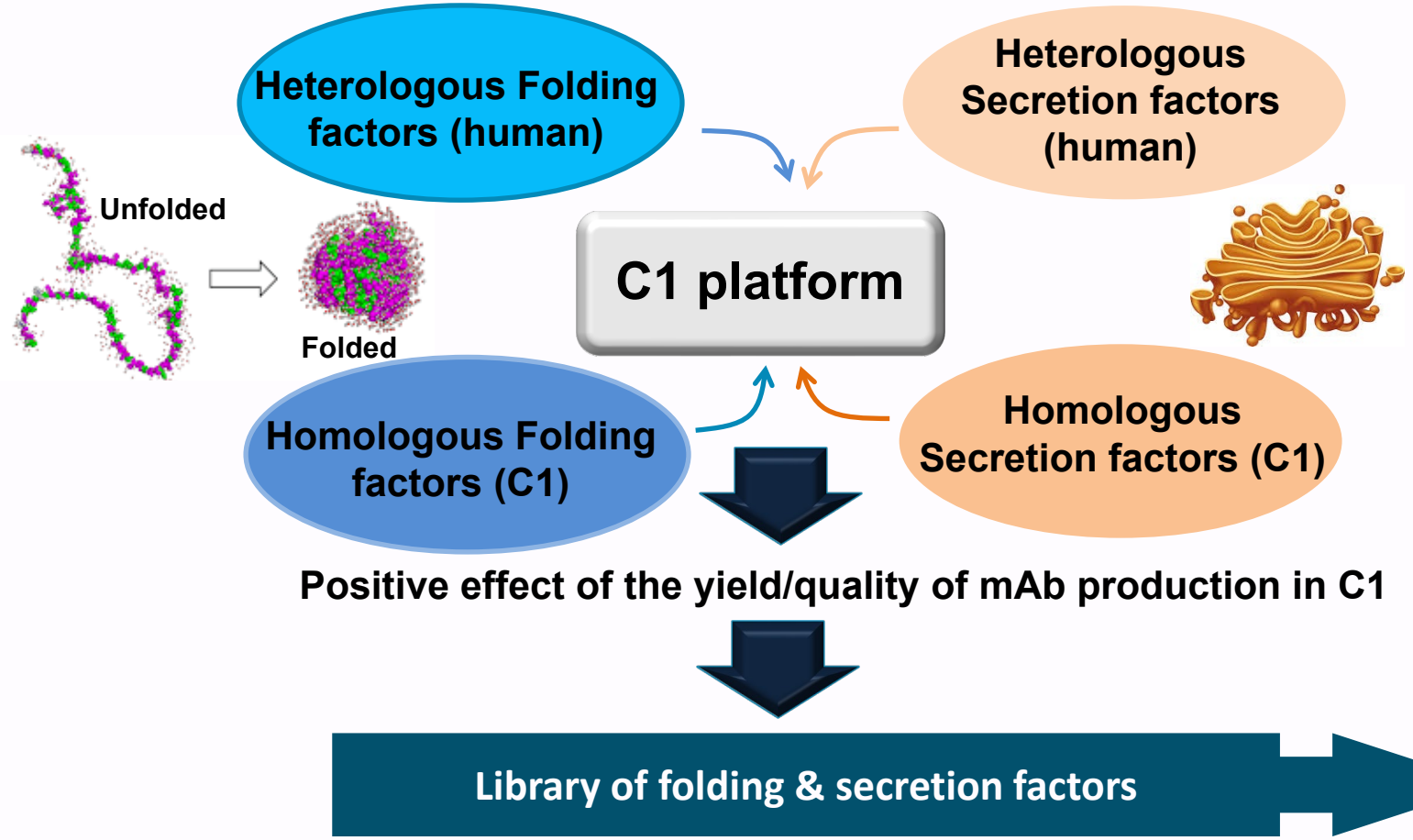


Change in morphology led to hyper-productivity and low viscosity.



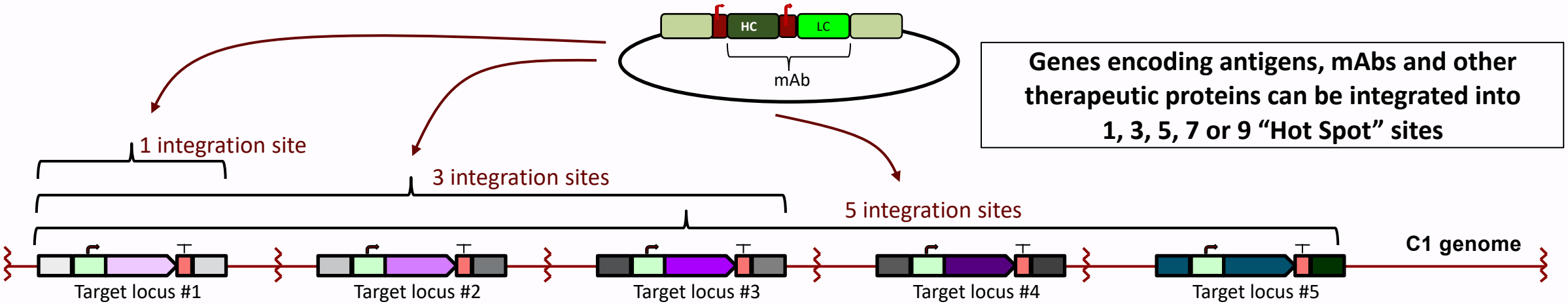
Library of secretion and folding factors genes

Selection of secretion and folding factors genes improves the productivity and stability



- Potentially enhance secretion and/or folding in C1 expression platform
- The factor genes of the library were selected based on
  - Bibliography
  - RNA-seq data
  - Proteomic analysis

Rapid generation of highly productive and stable C1-cell lines



Genes encoding antigens, mAbs and other therapeutic proteins can be integrated into 1, 3, 5, 7 or 9 "Hot Spot" sites

Gene synthesis → Plasmid construction and Transform-ation → Strain construction and re-isolation for monoclonality → DSP and analytics & RCB generation → 1-30 l scale fermentation from RCB, DSP and analytics

- **Sending samples for evaluation, binding assays and pre-clinical studies**
- **cGMP grade strain and process characterization, MCB,**

C1 Transformation leads to rapid generation of stable C1 cell lines and purified protein in as short as 7 weeks [1]

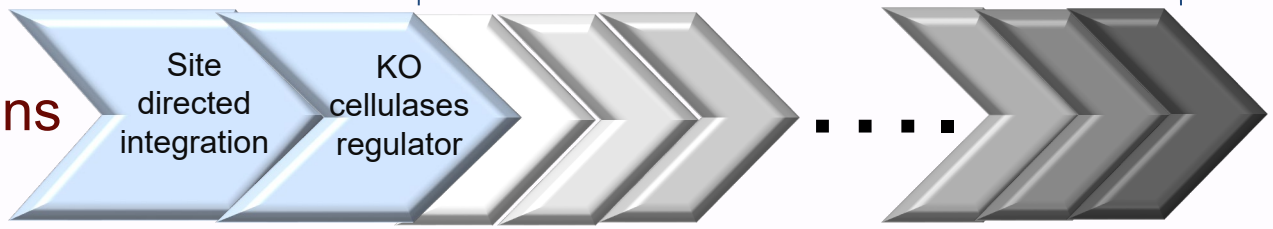
1 Depending on site specific or random integration approach

Rapid Development of Highly Productive Stable C1 Cell Lines

Systematic deletion of active proteases in C1 host to improve stability

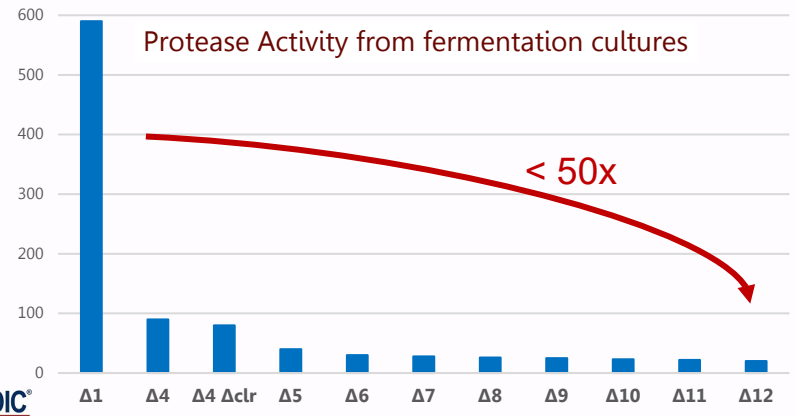
KO proteases and main secreted proteins (15 genes knocked out of C1 cell)

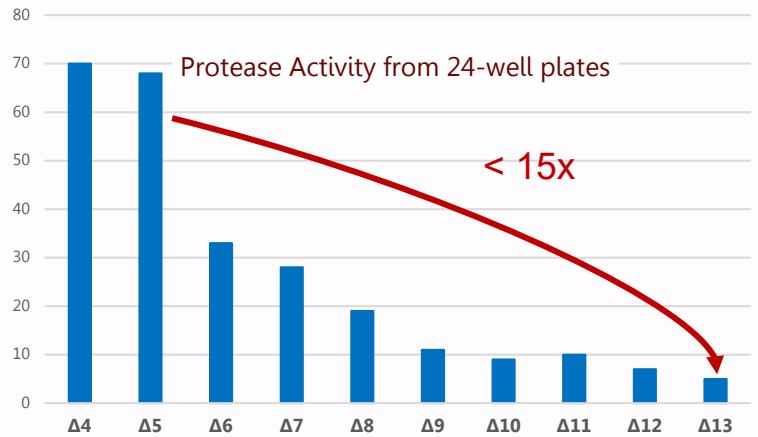
Pharma C1 strains



Systematic deletion of proteases genes based on:

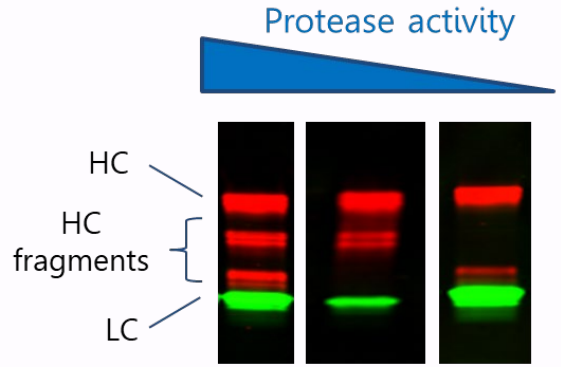
- Isolation and identification of extracellular proteases
- C1 protease library in Pichia pastoris that allows to test more than 50 proteases
- Effect of different protease inhibitors on protease activity
- mRNA sequencing data
- Protease gene annotation



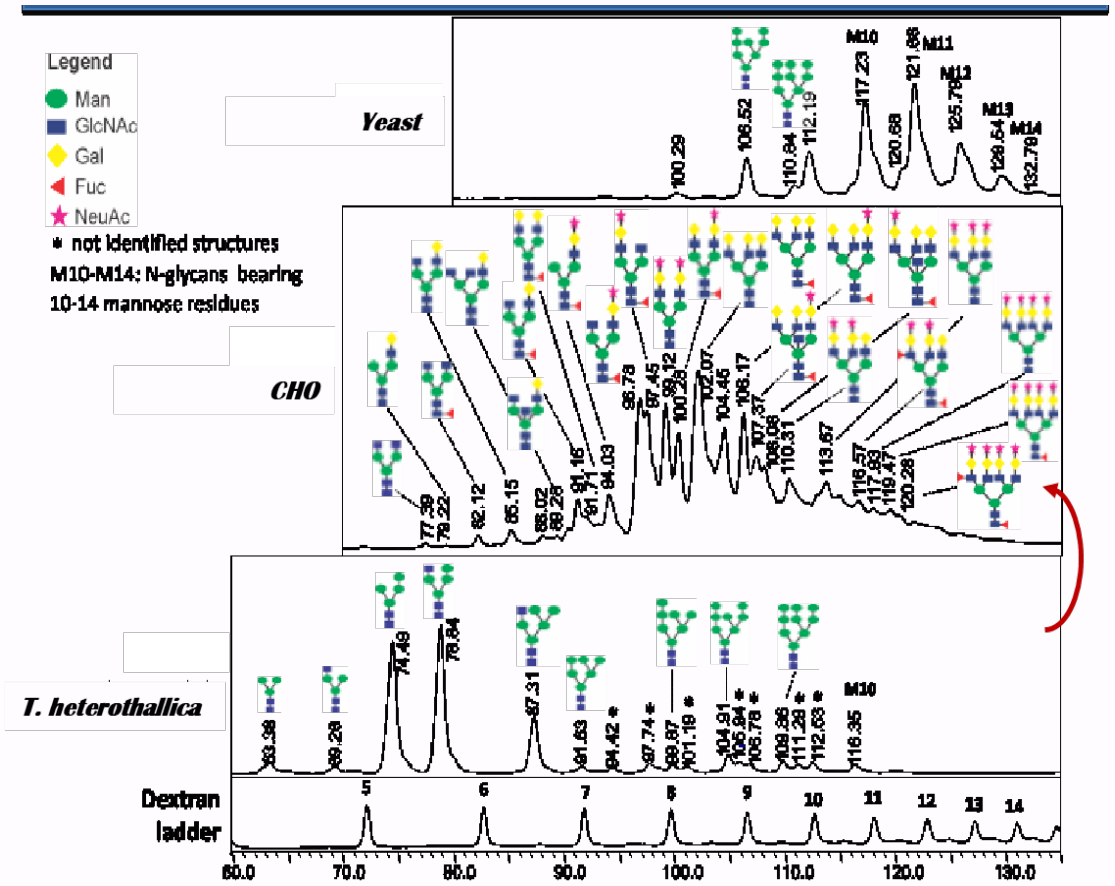


*Direct fluoresence-based assay with casein substrate

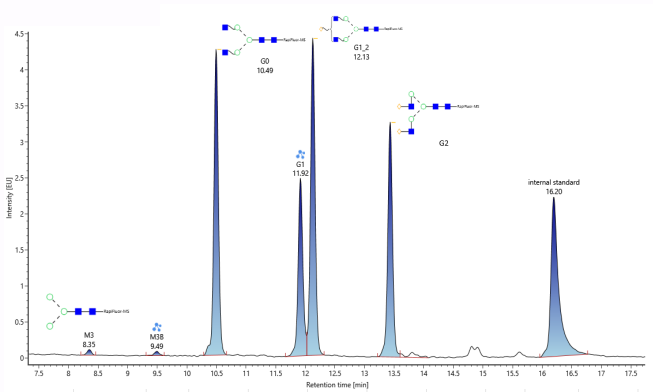
mAbs stability is increased with proteases deletion



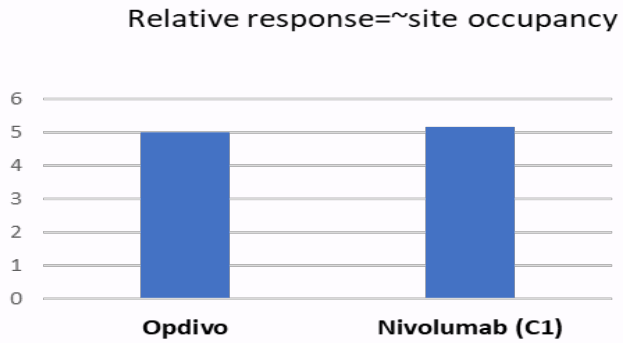
Native C1 glycosylation pattern is more similar to human glycan structures than yeast



C1 Humanized Proteins



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

DYADIC®

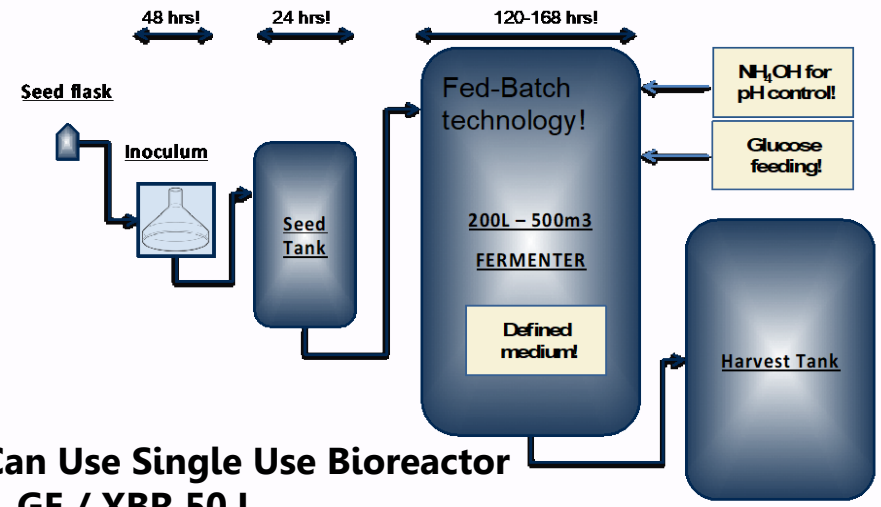
HIGH YIELD & ROBUST VACCINE MANUFACTURING

C1 Fermentation – Up and Downstream Processes

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

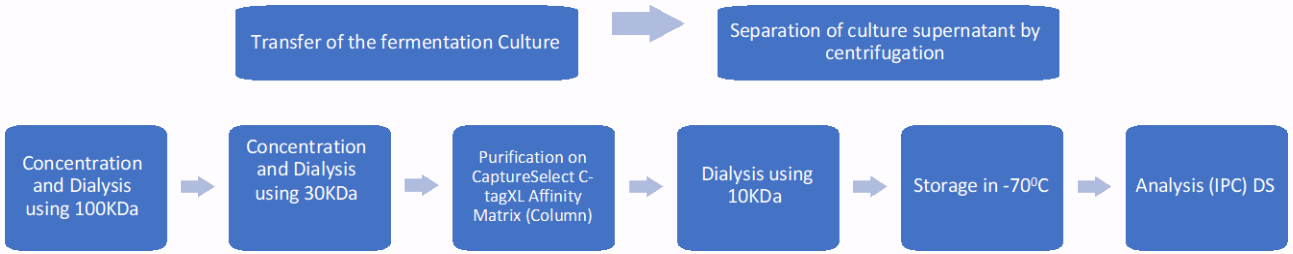
Fermentation



Can Use Single Use Bioreactor

- **GE / XBR 50 L**

Purification



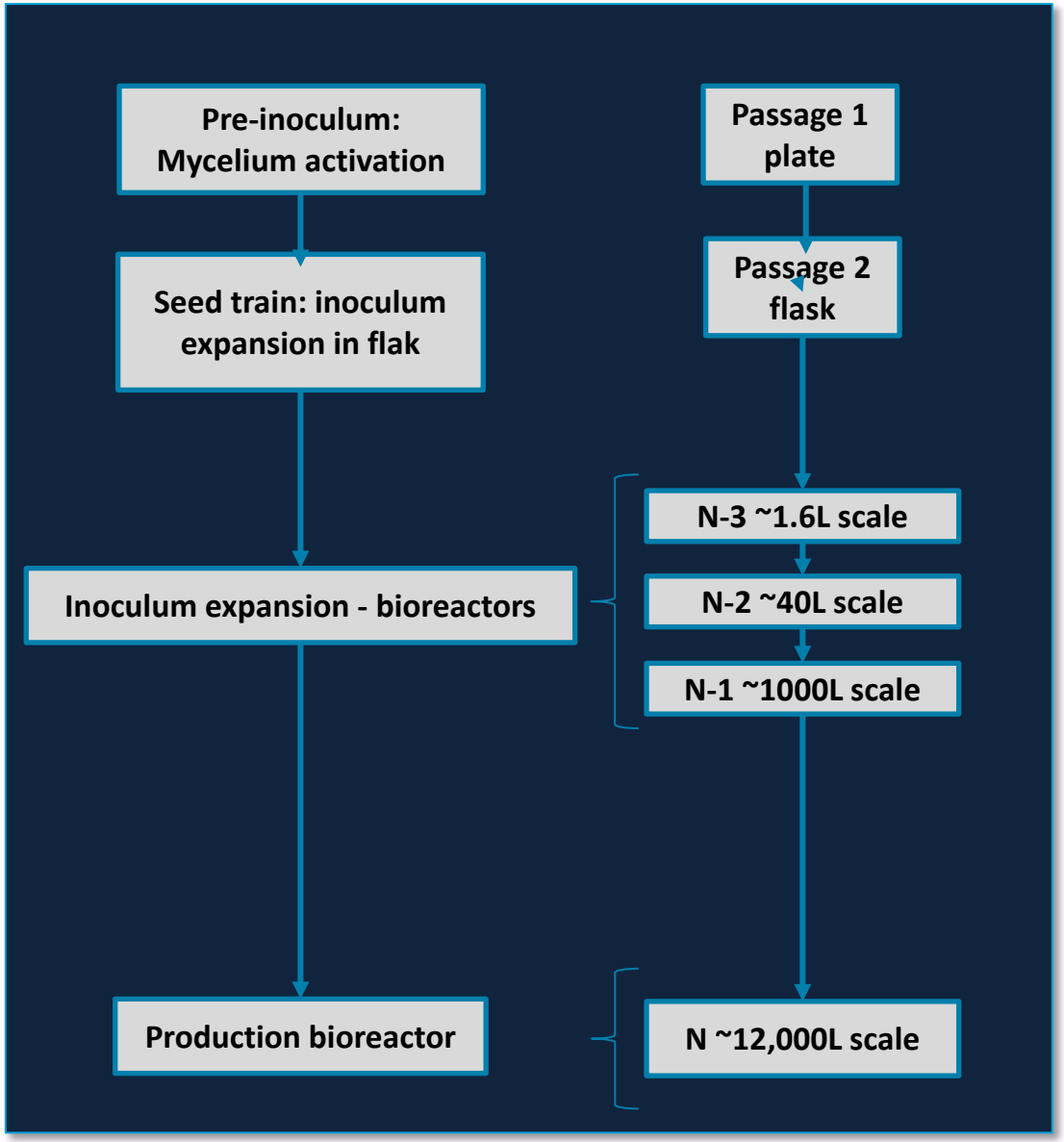
Generic Process Flow Chart for C1 of 12-14 days vs. CHO at 41-54 Days

Generic Process Flow Chart for CHO is 41 – 54 days

1 – 1,5 days

0,75 -2,5 days

5 days



Rapid commercial manufacture of affordable, high value, safe and effective protein products

HUMAN HEALTH

High Yields and Purities for Therapeutic Proteins[1]

Vaccine Antigen Glycoproteins & mAbs	Total Yield	Yield per Day
Fc-Fusion Products	15.3 g/L	2.58 g/L
mAb Products	24.5 g/L	3.1 g/L
fAb Product	14.5 g/L	2.1 g/L
Tri-Specific	6.12 g/L	1.02 g/L

High Productivity for Classes Routinely Used in Vaccines[1]

Recombinant Protein Antigens	Total Yield	Yield per Day
Influenza HemAgglutinin (HA)	413 mg/L	72 mg/L
Influenza A California (H1N1)	490 mg/L	160 mg/L
Neuraminidase (NA) Products	800 mg/L	114.2 mg/L
Coronavirus Antigen (S-RBD)	2000-3000 mg/L	400-600 mg/L
Coronavirus Antigen (Ferritin-S gRBD)	3000 mg/L	600 mg/L
Virus-Like Particle (VLP) Products	2,200 mg/L	500 mg/L

ANIMAL HEALTH

High Productivity for Animal Vaccines

Recombinant Protein Antigens	Total Yield	Yield per Day
Avian Livestock	10.0 g/L	1.43 g/L
Rift Valley Fever Virus (RVFV)	1.6 g/L	.32 g/L
Schmallenberg Virus (SBV)	1.8 g/L	.36g/L

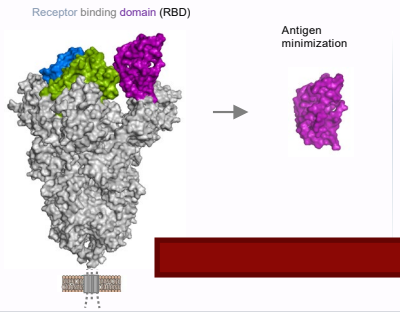
1. Data from non-glycoengineered C1-cells using different protease deficient C1-cells

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

CASE STUDY / 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 ✓
- Expect to initiate Clinical Phase I trial in South Africa 2022

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

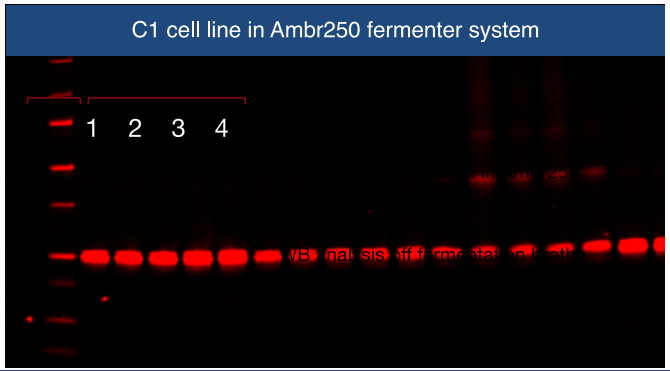


C1 produced vaccine antigens tested for safety in animal trials with cattle, lambs, chicken, rabbits, hamsters and mice in a variety of infectious diseases

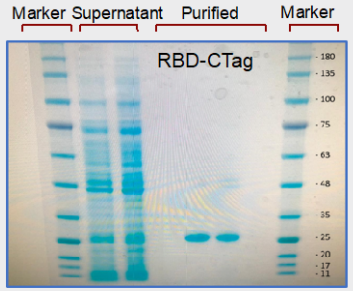
Spike Protein Antigen Minimization Advantages

- 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 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

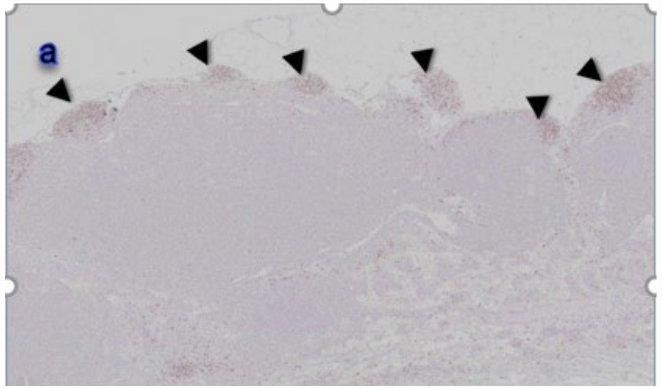
No adverse events observed & antigenic stimulation continued in rabbit toxicology study

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

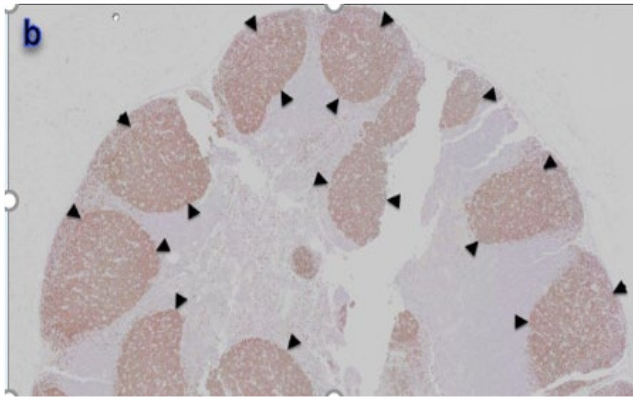
- No mortality
- No abnormal clinical signs
- No local reaction in terms of edema
- Comparable body weight gain
- Normal range of food consumption
- Normal appearance of blood vessels and the optic disc at the indirect ophthalmoscopy examination
- No marked differences in urinalysis values
- No gross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

Dyadic investing in validation of C1 Platform for human clinical trials

Platform Evaluations

Regulatory

- -DYAI-100 regulatory assessment performed by Parexel with **no significant deficiencies identified**
- -DYAI-100 CMC technical package, pre-clinical animal and toxicology studies evaluated by Paul-Ehrlich-Institut (PEI)
  - **-PEI conclusion data package and studies supported submission of Clinical Trial Application (CTA)**
- -DYAI-100 IMPD and IB completed, CTA submitted with CMC requirements to South African Health Products Regulatory (SAHPRA)
  - **-Human Ethics Committee approved protocol, SAHPRA comments received, awaiting final approval for Phase 1 study**

CMC

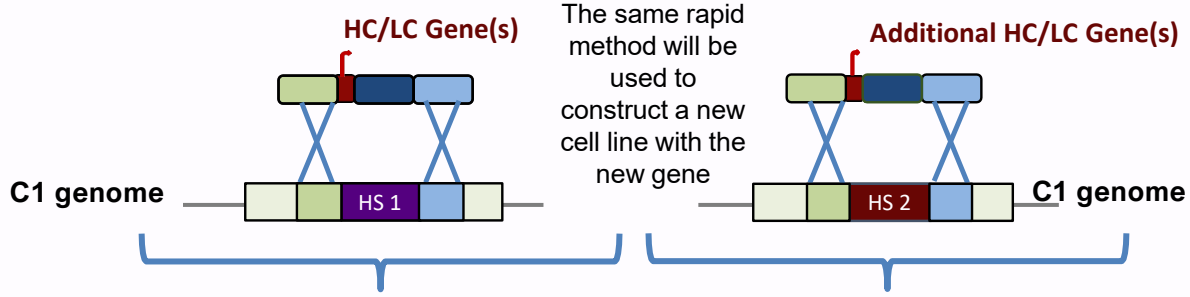
- -Completed cGMP manufacturing of toxicology and clinical trial material
- -Developed full regulatory CMC technical package for Drug Product & Drug Substance, Submitted CTA
- -DYAI-100 fill/finish completed, product on long term stability
  - **-CMC technical package completed under review with SAPHRA, no issues noted to date**

Toxicity/Safety

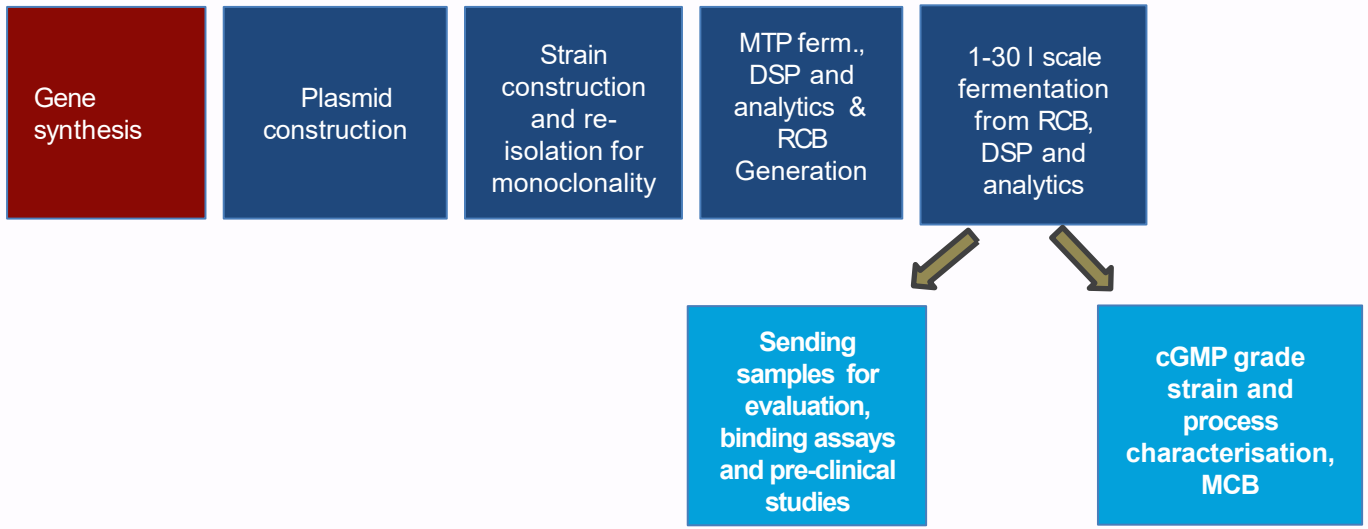
- -Vaccines produced from C1 proteins tested for safety in animal trials with cattle, lambs, chicken, rabbits, hamsters and mice
  - **-Demonstrated safety and efficacy in broad range of animal models for infectious diseases (influenza, COVID, RVFV)**
- -DYAI-100 safety studies in mice, hamster studies and rabbit toxicology study
  - **-DYAI-100 Rabbit Tox Study observed no adverse events**

Rapid generation of highly productive and stable C1-Cell lines

Site Directed Integration:



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



- The HC/LC Genes are located in 1 Hot Spot gene locus (HS 1)
- Additional HC/LC 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.

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

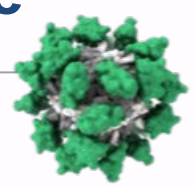
- **SARS-CoV-2 S-RBD**
  - Wuhan
  - Alpha (B.1.1.7)
  - Beta (B.1.351)
  - Gamma (P.1)
  - Delta B.1.617.2
  - Omicron B.1.1.529
  - Omicron BA.5
- **SARS-CoV-2 gRBD Ferritin Nanoparticles**
  - * Wuhan gRBD Ferritin nanoparticle
  - * Delta gRBD Ferritin nanoparticle
  - * Omicron gRBD Ferritin nanoparticle
- **Other Antigens**
  - INFLUENZA HA (H1N1, H5, H7)
  - INFLUENZA NA
  - WEST NILE
  - RABIES
  - SBV (Schmallenberg) ; RVFV (Rift Valley Fever) - ZAPI
  - MERS
  - IBVD-VLPs
- **INFECTIOUS DISEASE MONOCLONAL ANTIBODIES**
  - COVID-19
    - Neutralizes All SARS-CoV-2 Variants of Concern, Including Omicron *
      - Hamster & Non-Human Primate Studies
  - ZIKA
  - RVFV
  - ANDV

Of Note:

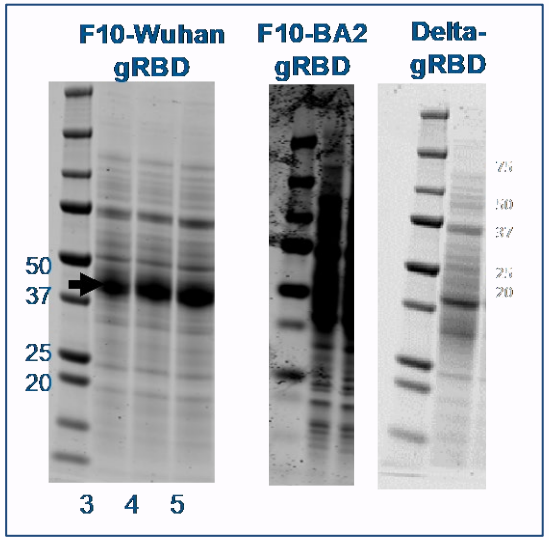
- S-RBD / Spy Tag (For Nanoparticles)
- Full Spike / Spy Tag (For Nanoparticles)
- Full Spike
- Delta aMHCII RBD
- Delta gRBD
- FC RBD
- Others in Development

* Tested Against Omicron 1 & 2

C1 expressed ferritin nanoparticles[1], keeping pace with advancing science

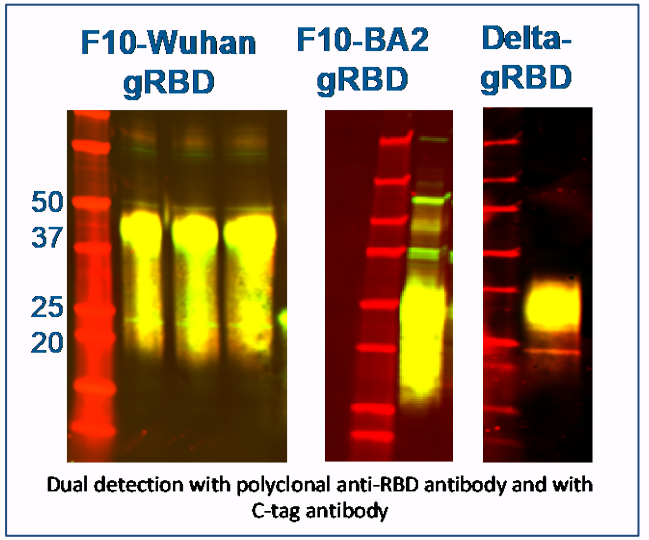


Coomassie staining gel from culture supernatants:



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

Western blot from culture supernatants:



Titers after 5 days of production:

Target	Titer (5 days)
F10-Wuhan-gRBD	3.48 g/L
F10-Omicron BA2-gRBD	0.6-1.1 g/L
Delta-gRBD	2.47 g/L

* Production of F10-Delta-gRBD is ongoing. Titer not available

[1]Bioreactor cultivations (prior to optimization)

Manufacturing larger quantities of affordable cocktails of mAbs & antigen treatments / vaccines

C1 Neutralizing mAbs

87G7 mAb neutralize **ALL** CoV-2 variants through Omicron BA.2

87g7

New Variant

Variant CoV-2 mAb can be added to 87G7 to develop mAb cocktail to neutralize BA.4/5 …..

Wuhan

Alfa

Beta

Gamma

Delta

Omicron

Omicron BA.5

C1 RBDs

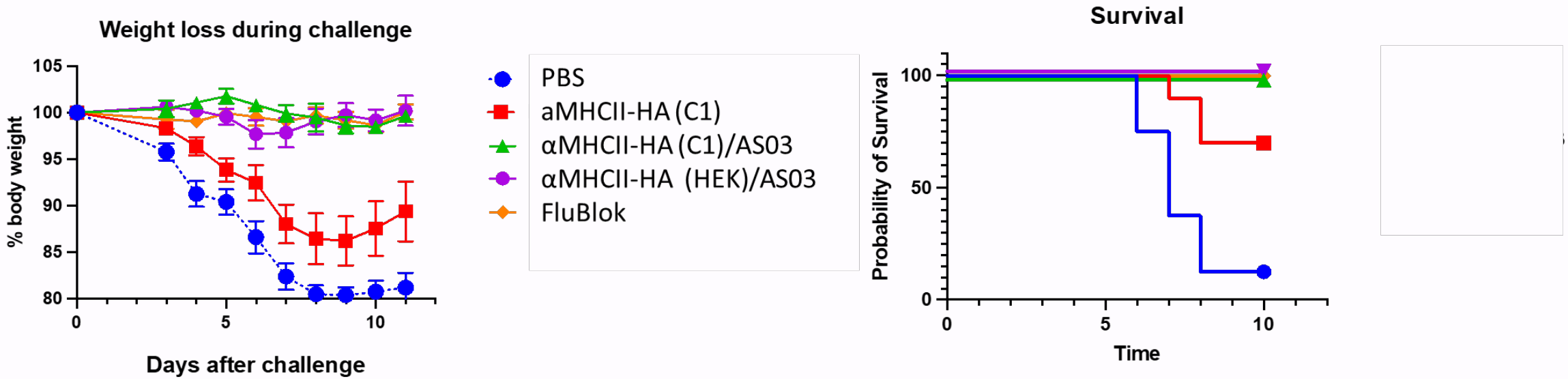
- **The C1 recombinant protein production platform can be used to rapidly respond to regional and global antibody & vaccine demand.**
- **C1 cell lines produce neutralizing mAbs or antigen cocktails faster, in larger quantities, and more affordably using low-cost media in standard microbial bioreactors**

INFLUENZA: C1 EXPRESSED NEURAMINIDASE (NA) / HEMAGGLUTININ (HA

Influenza Challenge: C1 produced HA protects against lethal dose

Influenza A/California Study (Oslo University)

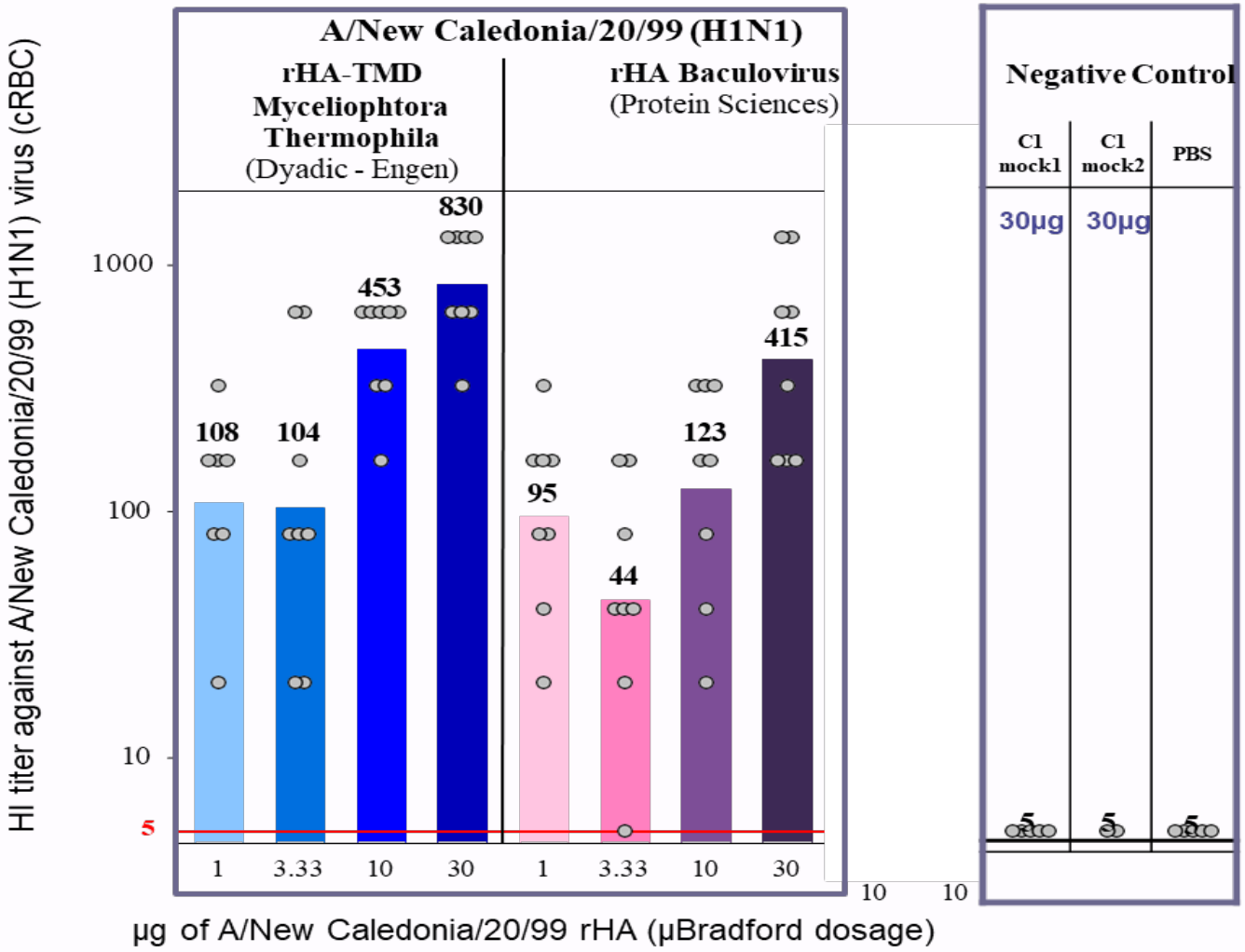
- **Mice were challenged with lethal dose of influenza A/California/4/2009 (H1N1) at wk 16 post vaccination**
  - Monitored for weight
  - Mice reaching a weight loss of 20% were euthanized
  - Survival curve represents mice reaching,or not reaching, this endoint

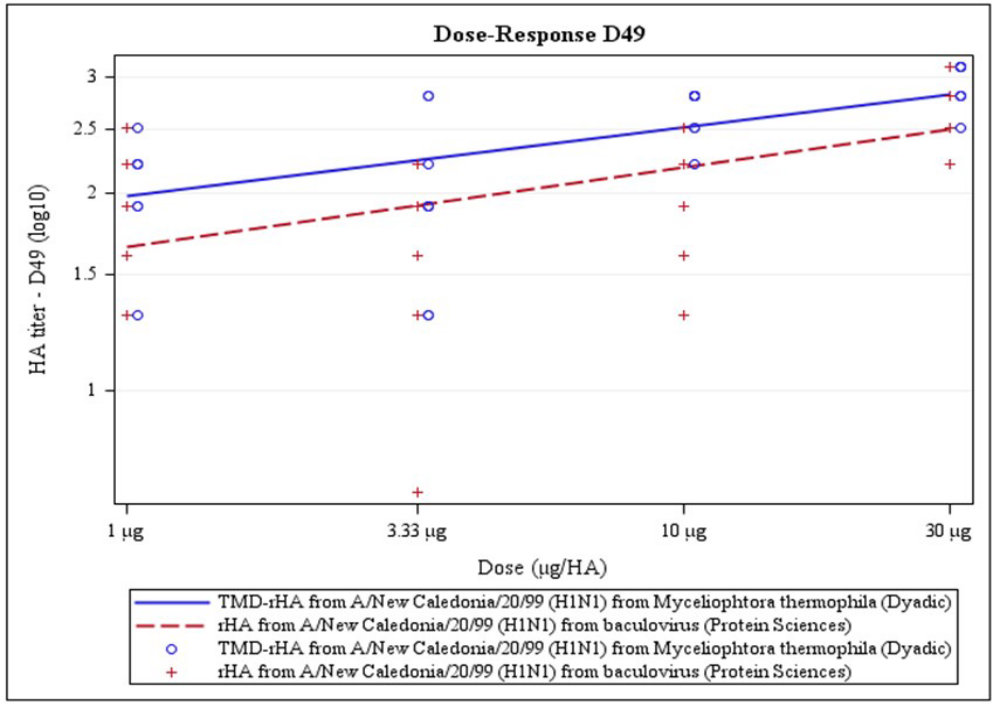


Reduced antibody responses following vaccination with non-adjuvanted aMHCII-HA (C1) could award some protection against viral challenge, likely in combination with vaccine induced T cell responses

Influenza Advangtage: C1-rHA displayed better response than Baculo-rHA

Significant difference : C1-rHA >baculo-rHA





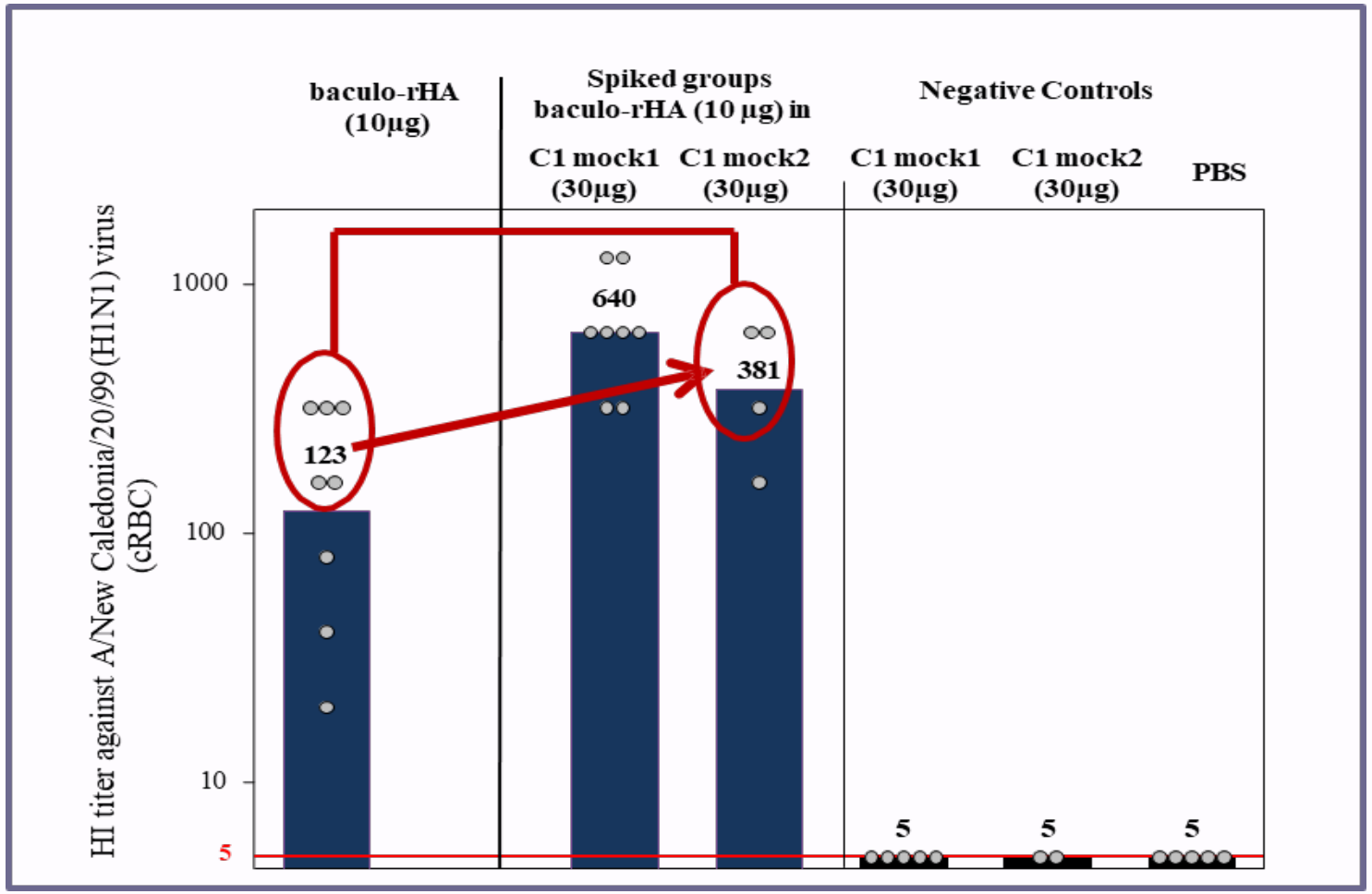
HI response after **2 IM** immunizations in mice:

- **Significant 2-fold difference in favor of C1-rHA TMD (p-value: 0.006)**

Sanofi Pasteur C1 Project Data

Influenza Advantage: C1-rHA Displayed Adjuvant-Effect over Baculo-rHA

Significant difference: Adjuvant-effect with both C1-mock preparations



Mock2 most representative C1 mock

HI response after **2 IM** immunizations in mice:

- **Significant adjuvant-effect with both C1-mock preparations (30µg) on the baculo rHA (10µg)**
  - **with C1-mock 1 (p-value: 0.0005)**
  - **with C1 mock-2 (p-value: 0.0439)**

Sanofi Pasteur C1 Project Data

Dyadic influenza progress: Pandemic Preparedness…H5N1 and H7N9 influenza, the next pandemic?

"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:

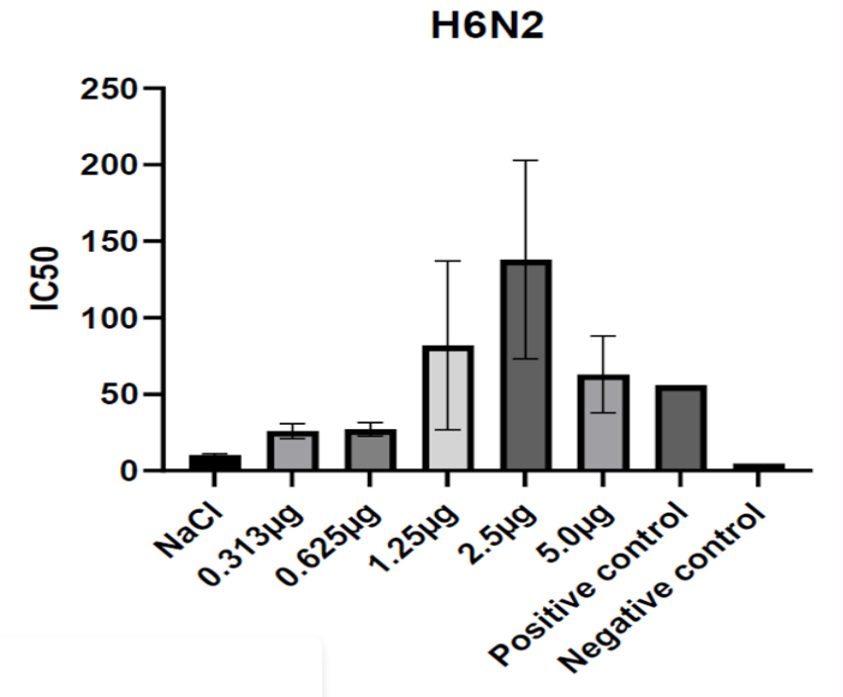
- C1 expressed HA successfully tested by Sanofi Pasteur versus HA expressed from baculovirus
- Immunogenicity study of Recombinant Hemagglutinin (HA) from A/H1N1/New Caledonia/20/99 strain:
  a) C1 produced r-HA was **safe and well-tolerated in mice**; and
  b) the C1 produced r-HA was **at least as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

- University of Oslo re-confirms viability of C1 to express APC-targeted Influenza virus antigen (HA) protein
  a) A/California/4/2009 (H1N1) was successfully produced from C1-cells
  b) Plan to partner and advance development or seek government funding

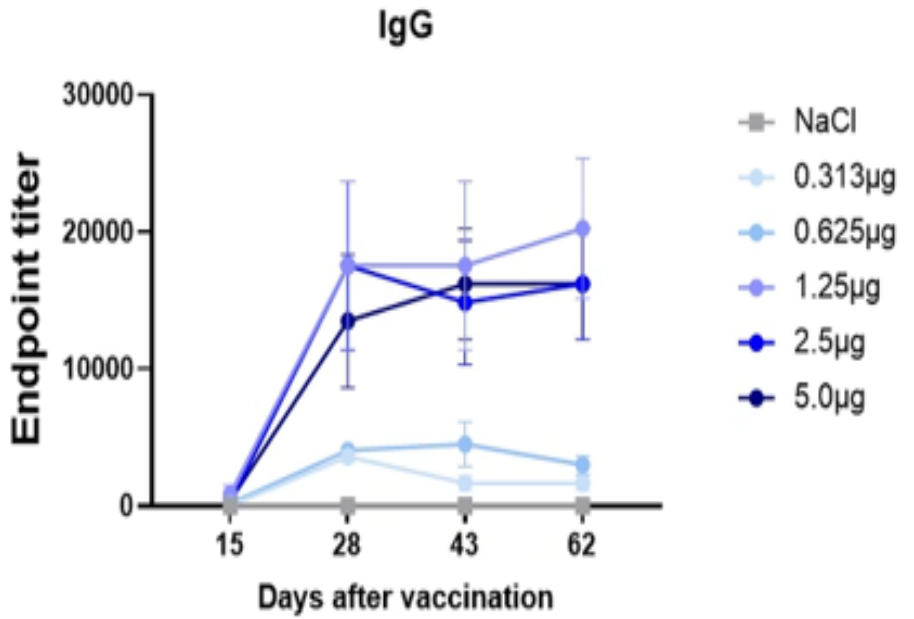
EXPRESSION SYSTEM	DOSE OF RHA 1 U/G	DOSE OF RHA 3.3 U/G	DOSE OF RHA 10 U/G	DOSE OF RHA 30 U/G
C1	50% (4/8)	57% (4/7)	100% (8/8)	100% (8/8)
Baculovirus	62% (5/8)	12% (1/8)	50% (4/8)	75% (6/8)

C1 Neuraminidase: High neutralizing response and high yield enabling more potent influenza vaccines

ELLA Assay



IgG Titer through day 62



- Difference seen at day 15 difference not visible in chart due to formatting.
- Suggested starting dose of 1µg for testing based on these results

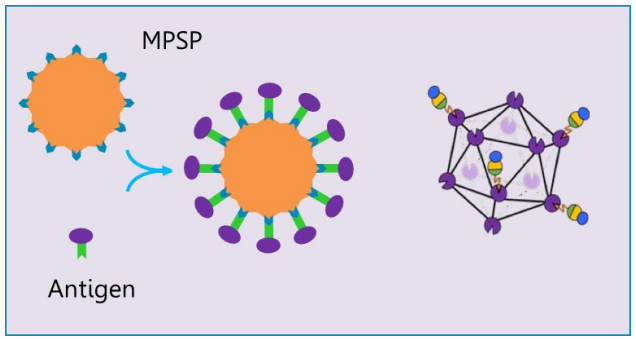
Recombinant Protein Antigens	Total Yield	Yield per Day
Influenza HemAgglutinin (HA)	413 mg/L	72 mg/L
Influenza A California (H1N1)	490 mg/L	160 mg/L
Neuraminidase (NA) Products	800 mg/L	114.2 mg/L

C1 VACCINE DEVELOPMENT: Virus Like Particles (VLP)

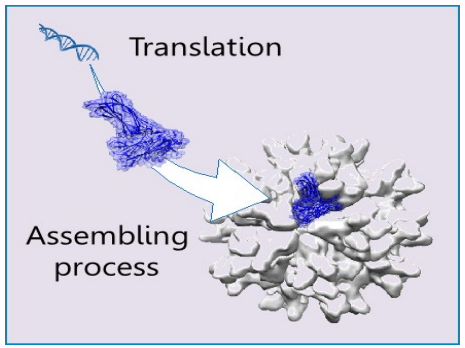
Growing number of vaccines in development based on nanoparticles, which include VLPs

- **Advantages nanoparticle-based**
  - High specificity and efficiency, good pharmacokinetic characteristics
- **2 primary methods for efficiency and potency:**
  - Genetically fused a peptide epitope in the self-assembling VLP/MPSP
  - Plug-and-display strategy and conjugated heterogeneously produced antigens to the pre-assembled scaffold

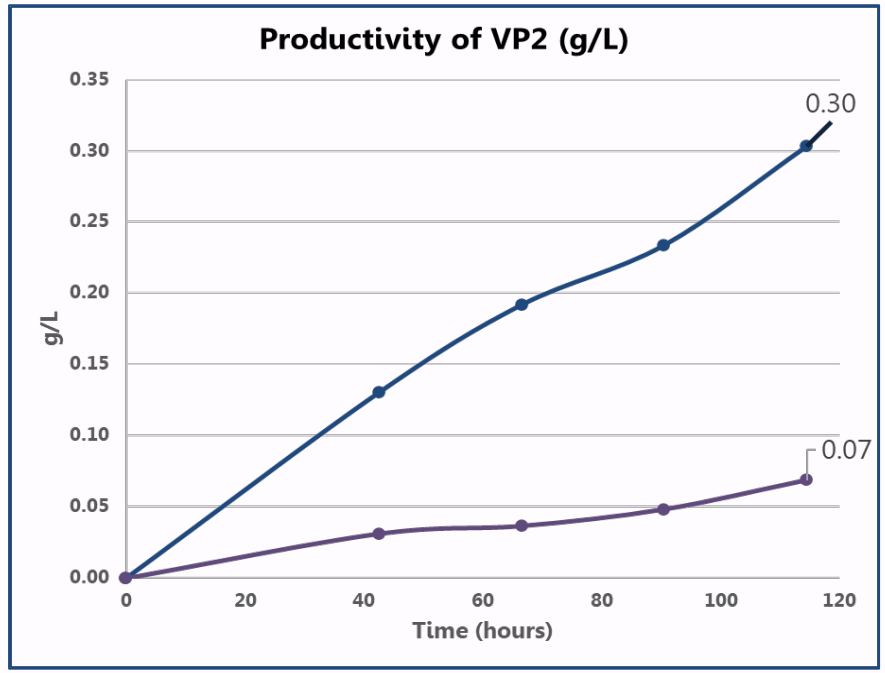
In Vitro Antigen Assembly



Genetically Fused Antigen



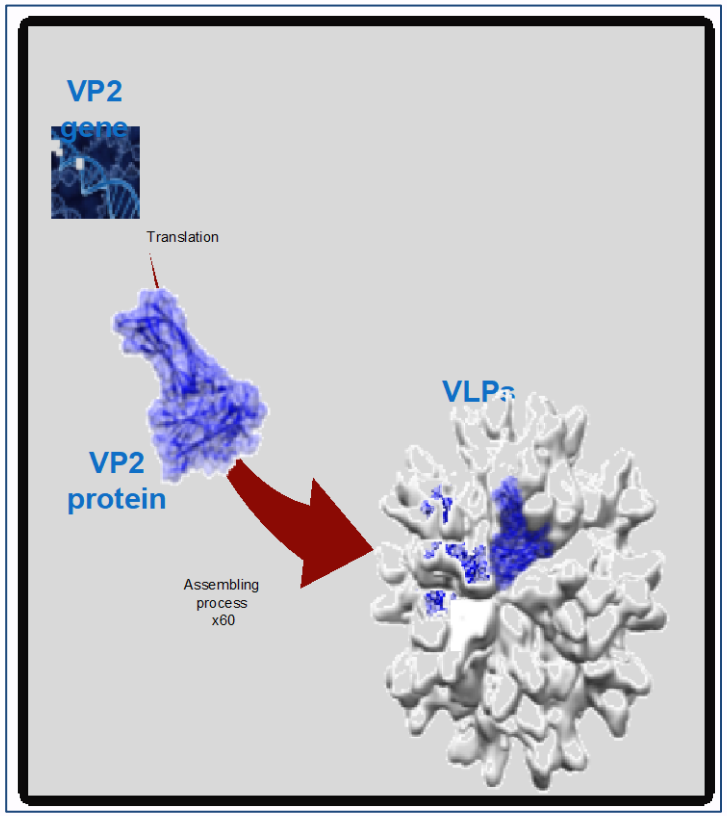
Both types were expressed by C1



- **In C1 The production level of the extracellular VLP produced was 300 mg/L, while intracellular VLP expression was 70 mg/L**
- **In S. cerevisiae, the VLP could not be secreted, intracellular titers were comparable to C1**
- **There are more C1 technology options to increase the productivity to a higher level because this titer was obtained without any optimization**

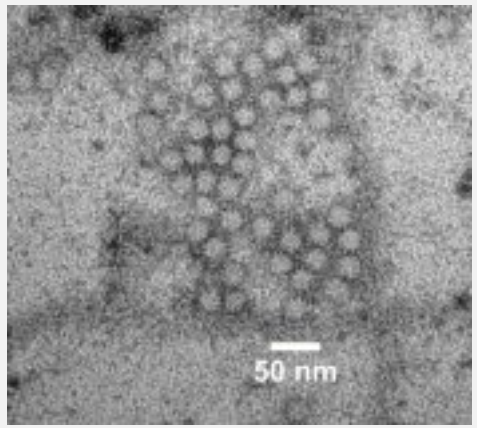
C1 Production of Genetically Fused VLP

- VP2 protein is a 60-mer structural protein of the Infectious Bursitis virus (IBDV; Gumboro)
- VLP size: 3000 kDa / 25 nm diameter



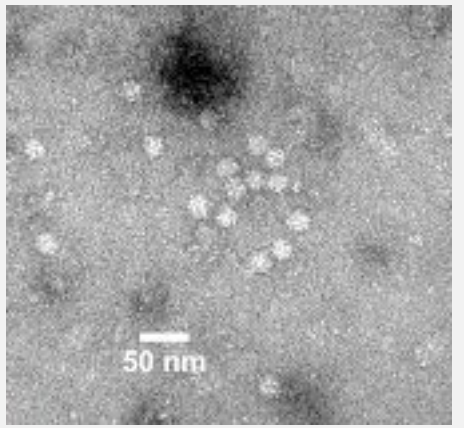
VLP produced by C1

Extracellular fraction



300mg/L (112,5H)

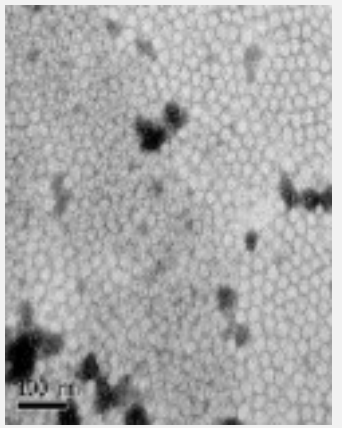
Intracellular fraction



70mg/L (112,5H)

Control

***S. Cerevisiae* Intracellular**



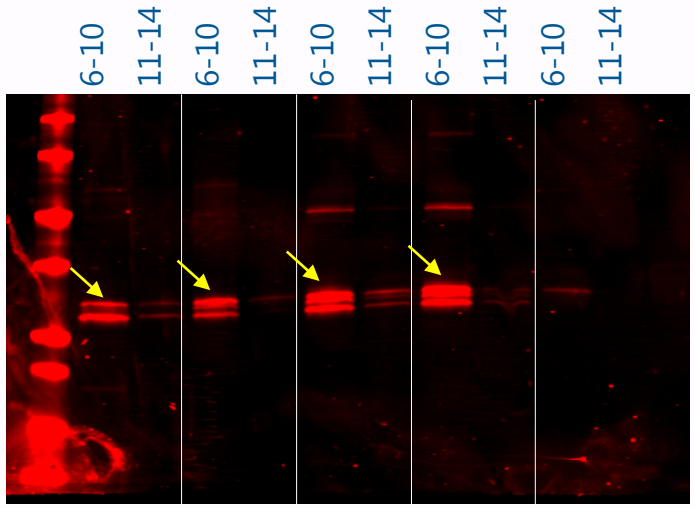
70mg/L

Observation of VLPs by transmission electro microscopy

Foot-and-mouth disease virus VLPs are successfully produced and secreted by C1 platform

Western Blot

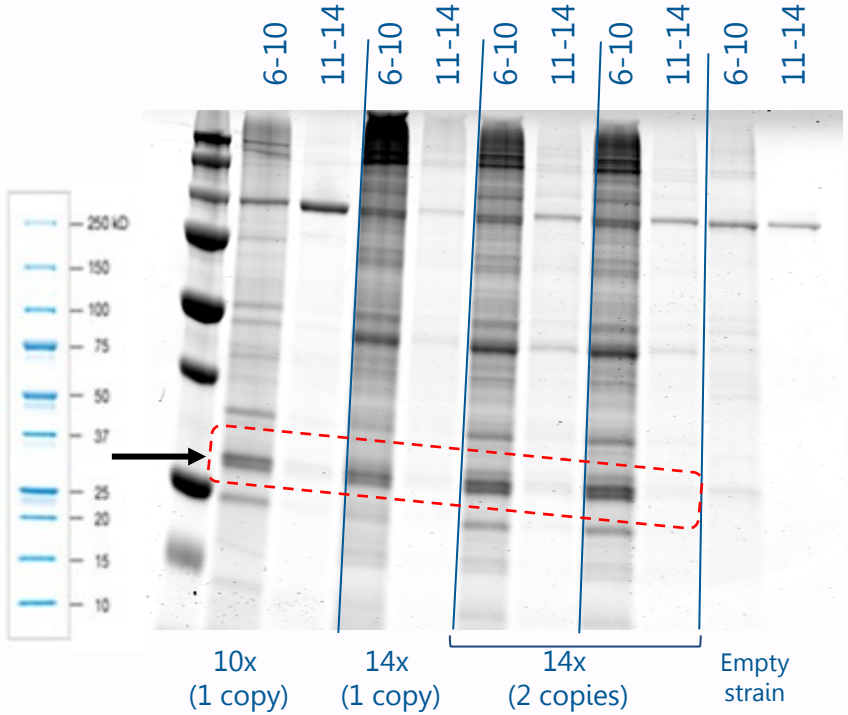
Pools after sucrose gradient:



Detection:
Anti-VP1 rabbit antibody (orb499995, raised against type O1)
+ goat anti-rabbit IRDye680 (LiCor)

Coomasie staining

Pools after sucrose gradient:



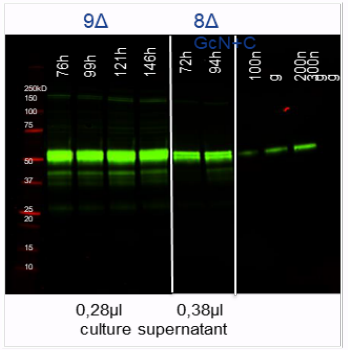
Strain	Productivity in the fermentation broth
10x 1 copy	336 mg/L
14x 1 copy	25 mg/L
14x 2 copies	195 mg/L

- Titer 200-300mg/L exceeds levels obtained in other systems
  - (i.e. insect cells)

- **FMDV VLPs are produced by C1 and secreted to the extracellular broth (O1 Manisa and Asia/Shamir)**
- VLPs from culture supernatant were separated by sucrose gradient. Fractions were pooled 6-10 and 11-14
- Fractions were analyzing by Western blotting
- VLPs were present in pool 6-10

Successful Plug and Display MPSP for Expressing High Level of SBV & RVFV Antigens

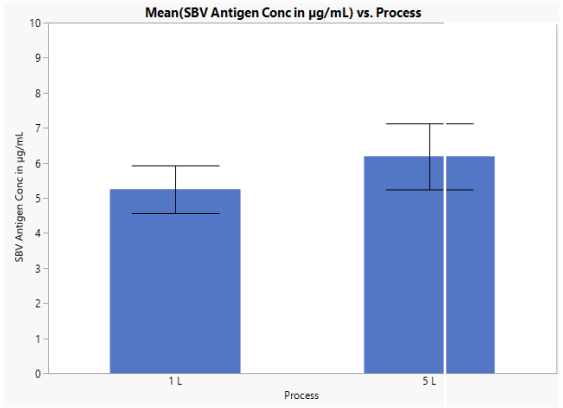
C1 Fermentation



X300

SBV yields: 1.8 g/L (time point 121h)

Baculovirus Fermentation

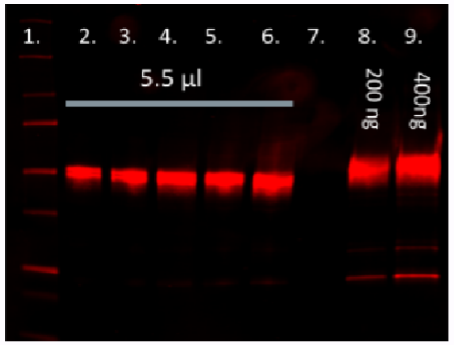


SBV yields: 6 mg/L (time point 192h)

- SBV (Schmallenberg Virus) causes congenital malformations and stillbirths in cattle, sheep, goats, and alpaca.
- 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: 1.24 g / L

- Rift Valley fever (RVF): Viral disease of humans and livestock that can cause mild to severe symptoms
  - Mild symptoms may include fever, muscle pains or headaches
  - Severe symptoms may include loss of sight, severe headaches or liver problems
  - Animals such as cows, sheep, goats, and camels may be affected
- An antigen against Rift Valley Fever Virus (RVFV) was expressed by C1.

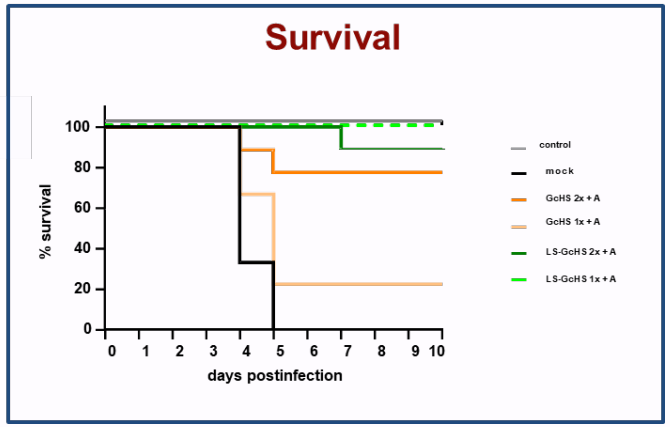
DYADIC INSIDE

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.

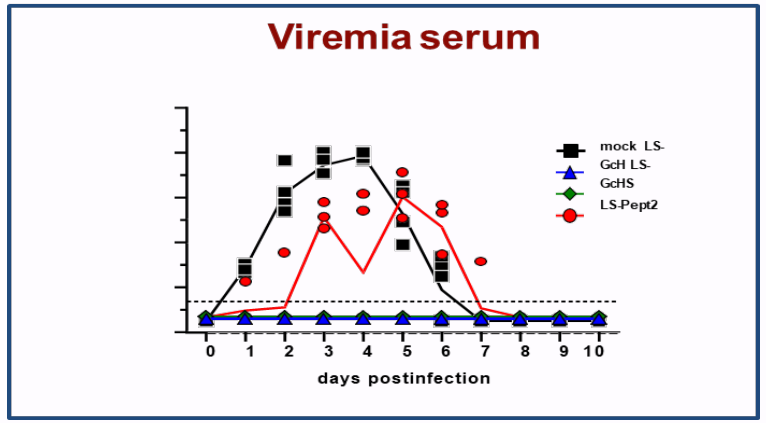
Success in Mice and Cattle Challenge Tests (SBV and RVFV)

C1 expressed SBV and RVFV antigen assembled to Nano-particle expression molecules tested in animal tests:

Mice trials

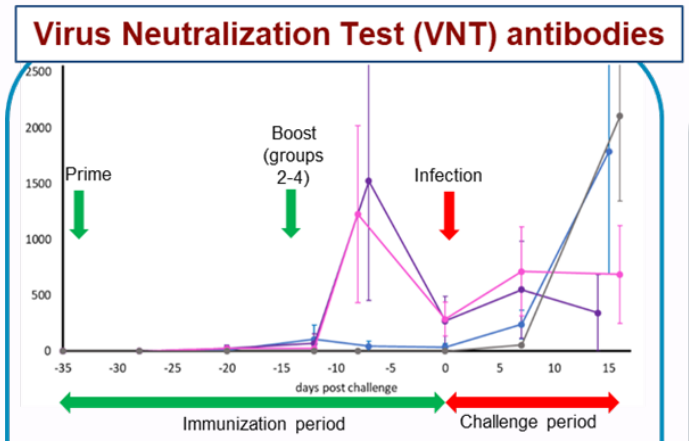


Cattle trials

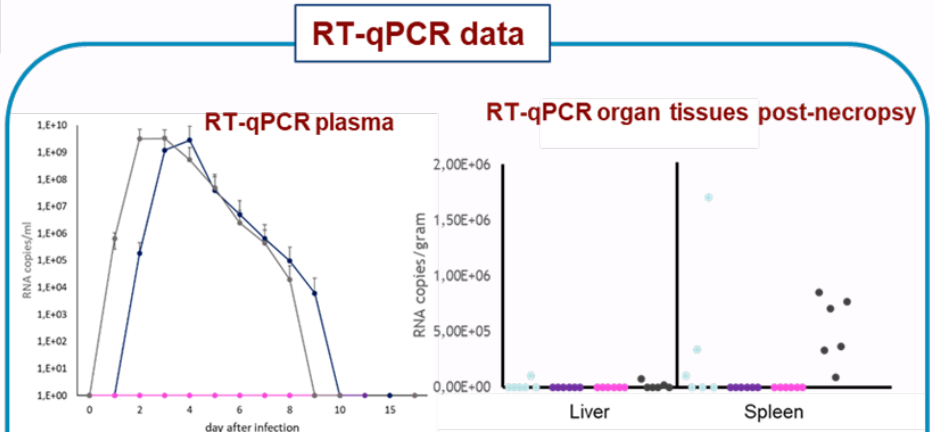


➢ **All immunized mice, lambs and cattle survived challenge infection without any clinical signs of disease**

Lamb trials



Good response in VNT antibodies with 2-shots strategy



No viral RNA is detectable in plasma of animal immunized twice

C1 is the Right Platform for Rapid Development of Vaccines at High Yields

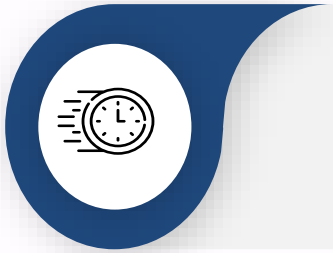
RIGHT TIME, RIGHT PLATFORM

C1 Platform

Antigens in higher yields and less time for more efficiency

Simple development process with well known and available media and infrastructure

Lower COGS provide enhanced pricing flexibility

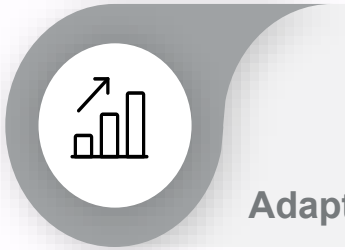


Regulatory

Rapidly generate stable cell lines to respond to emerging strains

Ability to quickly develop mono or multivalent vaccines

C1 faster production and higher yields can speed development



Adaptable and Scalable

Demonstrated ability to express wide variety of antigens

Testing in multiple ROA's with experience in HA,NA, RBD, and VLP

"Traditional" vaccines to target vaccine hesitant and special populations

Industrial background enables rapid scale up experience

Dyadic International (NASDAQ: DYAI)

Next Generation Proteins for World Health

APPENDIX 1: MONOCLONAL ANTIBODIES

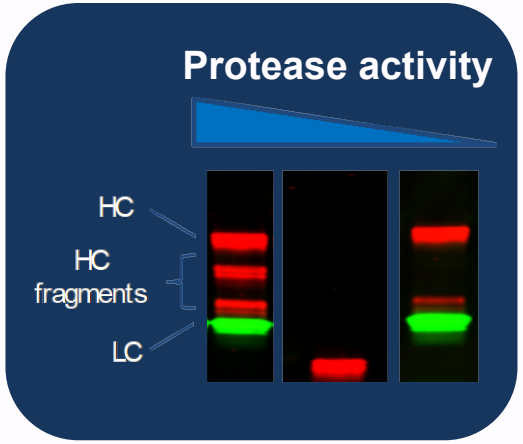
C1 mAb Proof of Concept: Nivolumab

➢ ***Nivolumab strain development for high production level, high stability and Human glycan structure***

Productivity

Best productivity 22.3 g/L in 7 days fermentation[1]

Stability



N-Glycan

Glycan	Amount (%)
M3	0,35
M3B	0,45
G0	32,54
G1	17,21
G1_2	29,51
G2	19,94
Sum w/o IS	100
Response w/o IS	101843184
All human-like	99,2

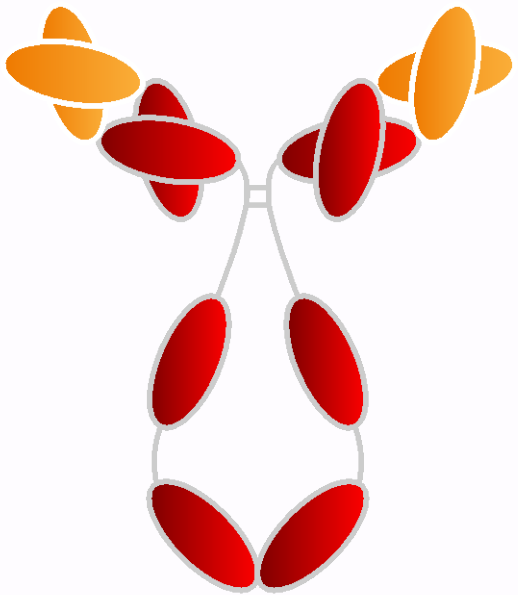
Constructing Commercial Strains:

Developing highly productive (>20g/L), stable with human type of N-Glycan structure

1. Data from non-glycoengineered C1-cells using different protease deficient C1-cells

C1 produced mAbs have virtually identical structure, properties and activities to CHO produced mAbs

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



C1 produced mAbs have virtually identical properties and activities to CHO produced mAbs

Neutralization

C1 mAbs neutralize viruses virtually identical to CHO produced mAbs

Binding

C1 mAbs are correctly folded, bind virtually identical to CHO produced mAbs

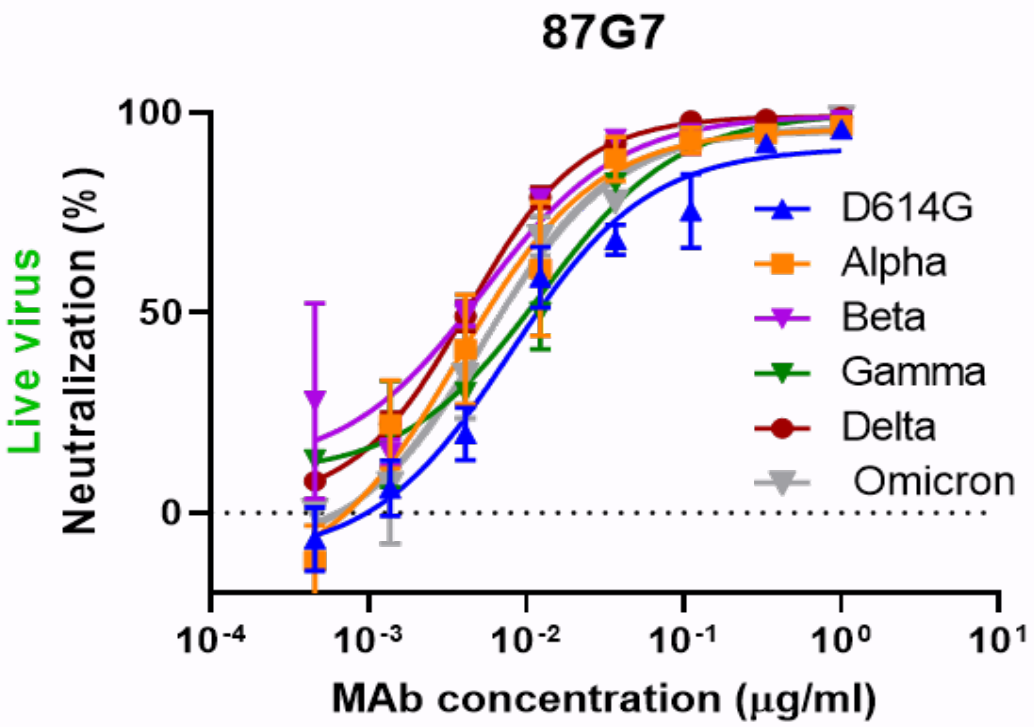
Glycan Structure

C1 native glycans are more human than yeast

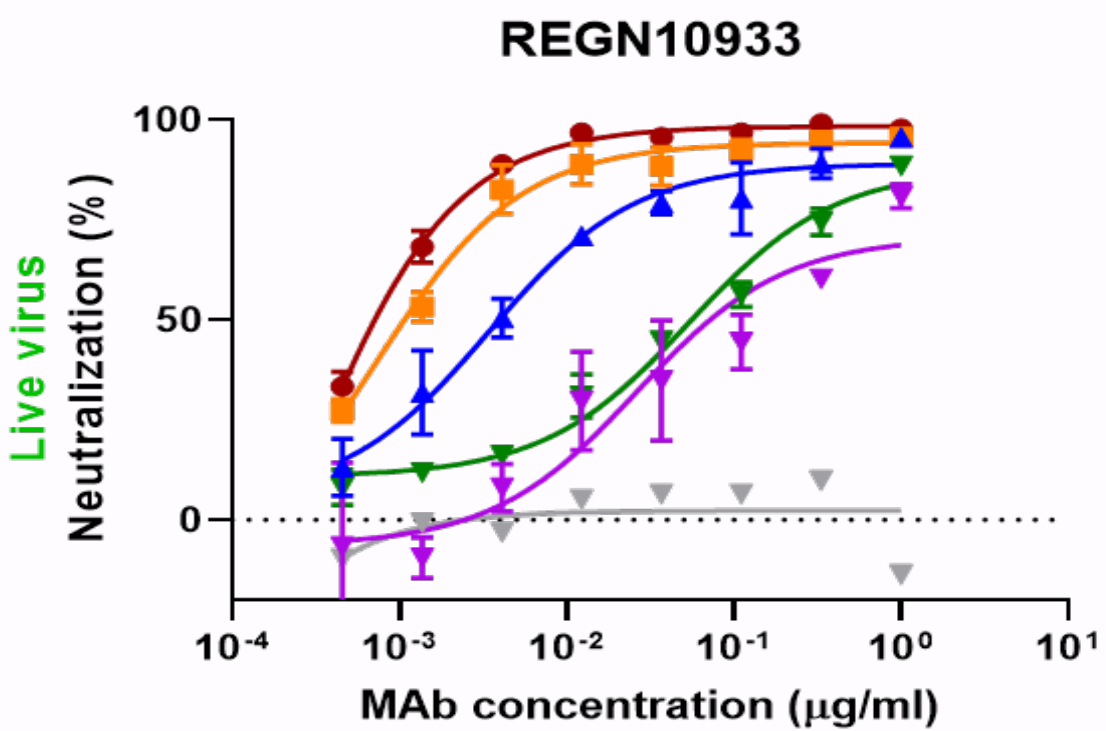
C1 cells have been glycoengineered to express mAbs with human like glycan structures (~99%)

Broad Neutralization of SARS-CoV-2 Variants of Concern mAb

C1 Expressed SARS-COV2 mAb



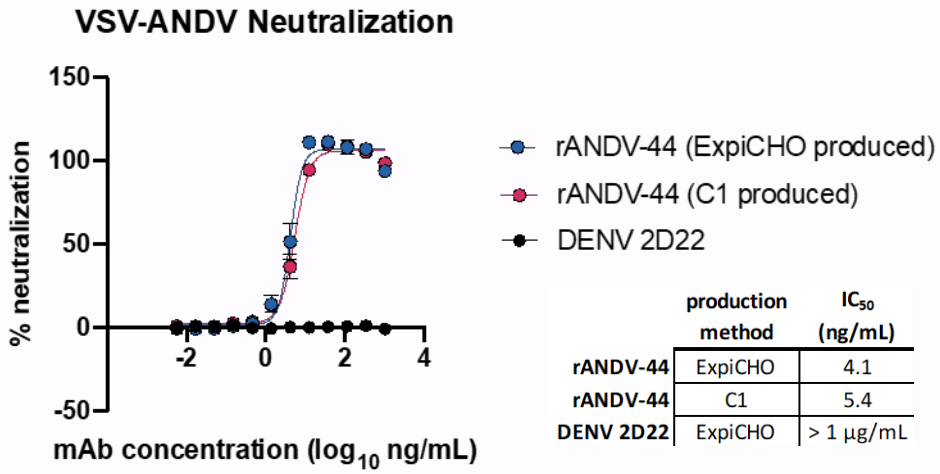
Regeneron SARS-COV2 mAb



- C1 SARS-CoV-2 mAb neutralizes all variants up to and including Omicron 1 & 2
- Expected 3Q readout for ongoing non-human primates

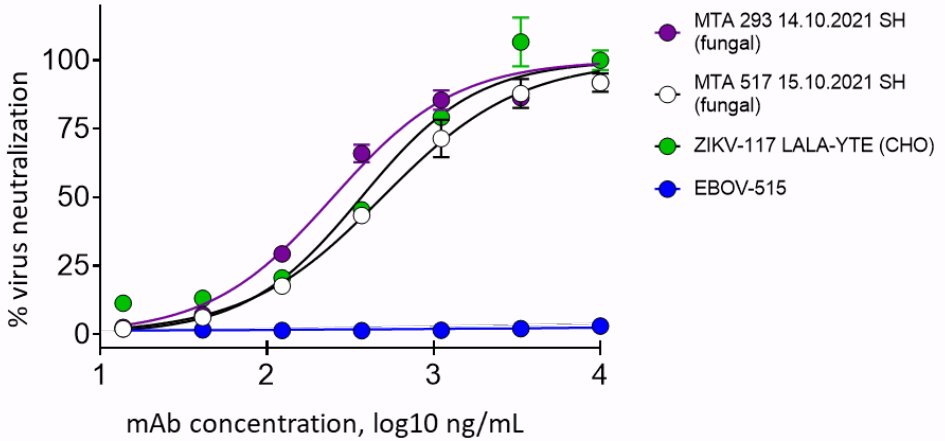
CHO Like Neutralization and Binding, Faster and More Affordable

ANDES VIRUS

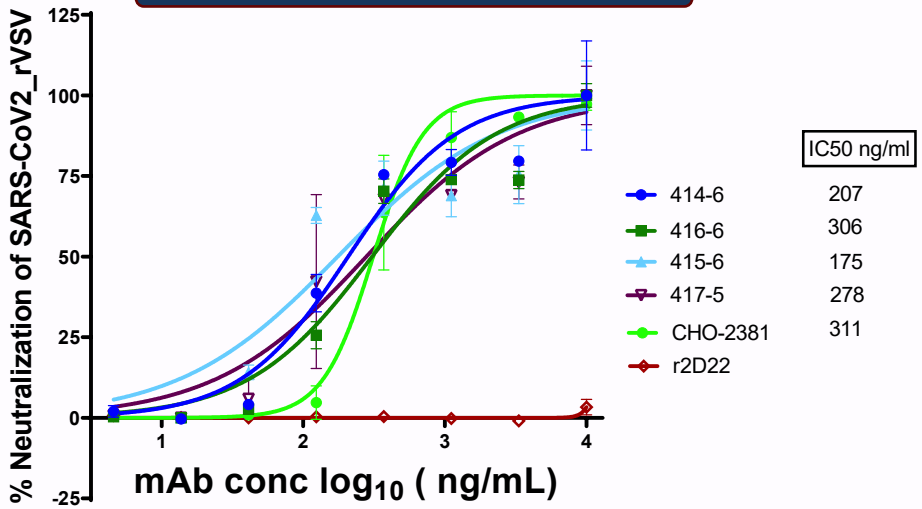


	production method	IC_{50} (ng/mL)
rANDV-44	ExpiCHO	4.1
rANDV-44	C1	5.4
DENV 2D22	ExpiCHO	> 1 µg/mL

ZIKA

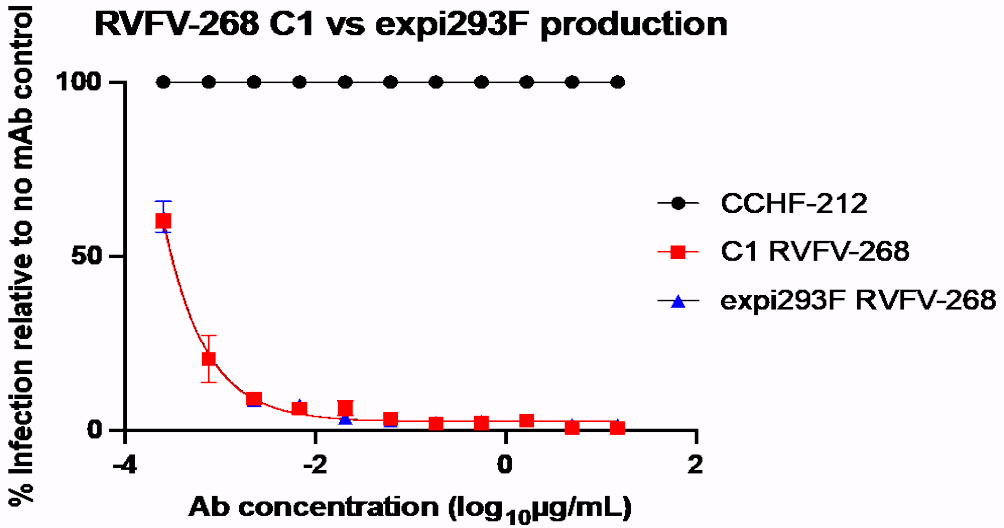


SARS-COV2



	IC50 ng/ml
414-6	207
416-6	306
415-6	175
417-5	278
CHO-2381	311

RIFT VALLEY



DYADIC®

DELETED SLIDES

Dyadic Investments for Validation of the C1 Platform

Platform Evaluations

Regulatory

-Parexel presented proposed CMC technical package pre-clinical animal and toxicology studies on DYAI-100 to PEI for evaluation and support of Clinical Trial Application (CTA).
-DYAI-100 IMPD and IB completed, CTA submitted with CMC requirements to South African Health Products Regulatory (SAHPRA)

CMC

-Completed cGMP manufacturing of toxicology and clinical trial material
-Developed full regulatory CMC technical package for DP/DS and incorporation into CTA
-DYAI-100 fill/finish completed and product on stability

Toxicity/Safety

-Infectious diseases vaccines produced from C1 proteins tested for safety in a range of animal trials with cattle, lambs, chicken, rabbits, hamsters and mice
-DYAI-100 safety studies in mice studies and rabbit toxicology study

C1 Platform Validation

-PEI agreed CMC package, clinical plan and supporting studies supported CTA

-PEI supported ability to switch antigens w/o additional tox studies if all other excipients remained the same

-Received protocol Human Ethics approval

-Comments received, awaiting final SAPHRA approval

-CMC technical package completed under review with SAPHRA, no issues noted to date

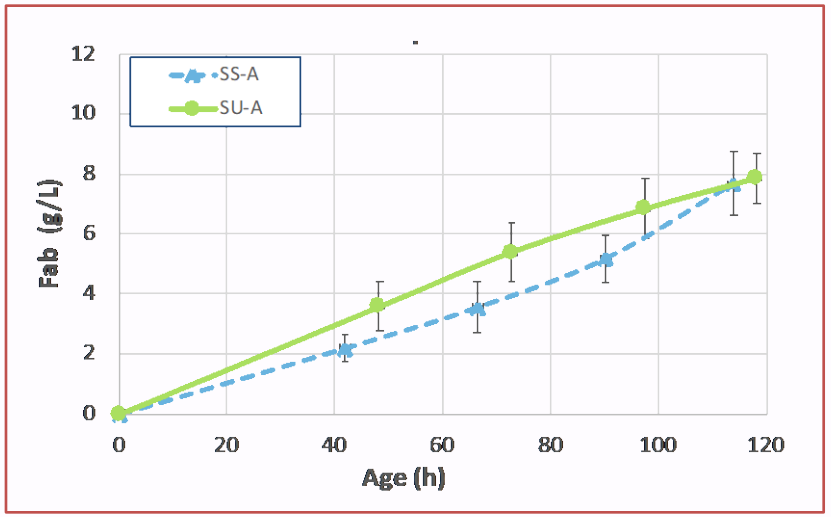
-Demonstrated capability of C1 to run under cGMP specs

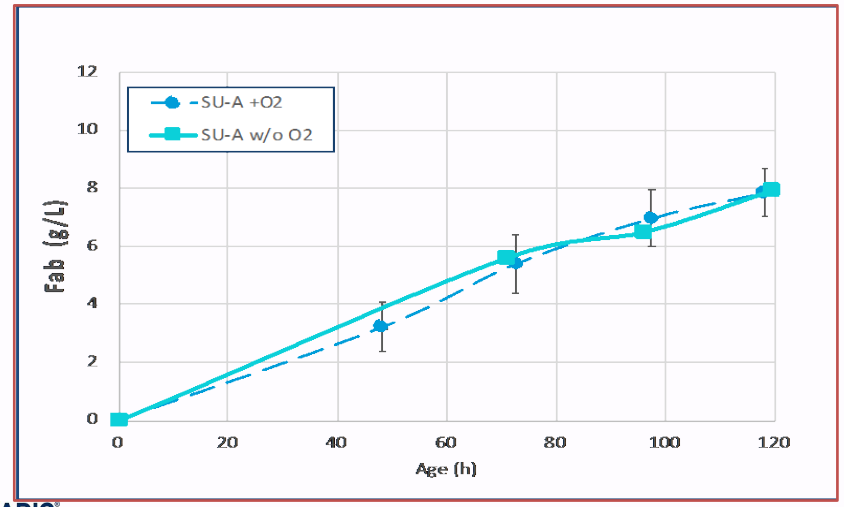
-C1 produced vaccines demonstrated safety and efficacy in broad range of animal models for infectious diseases (influenza, COVID, RVFV)

-DYAI-100 Rabbit Tox Study observed no AE's

Comparison of fAb (certolizumab) Production in Single use Bioreactor (SUB)under different conditions

Conditions A



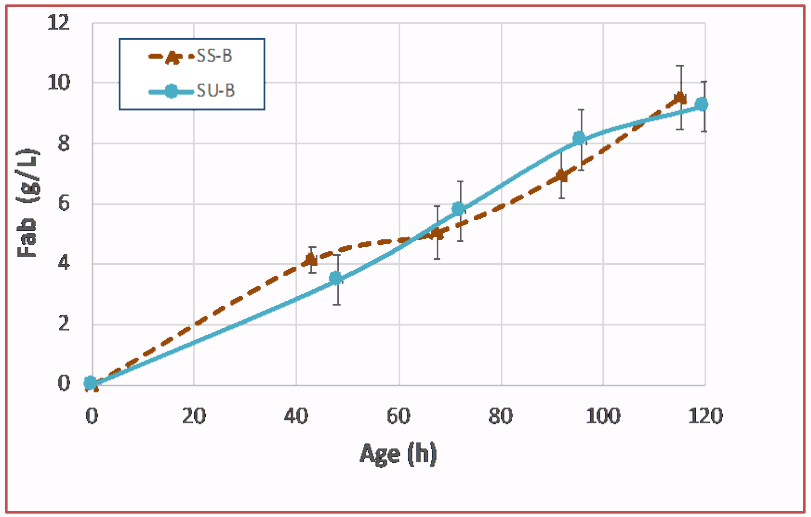


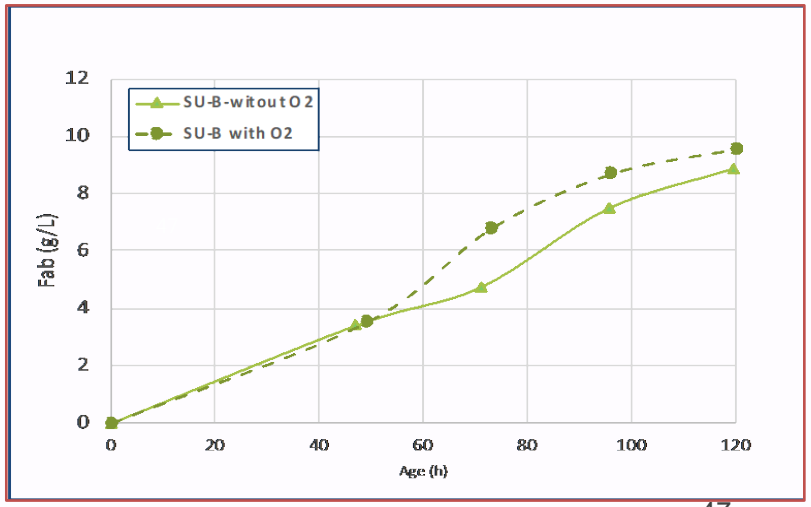
GE's Xcellerex™ XDR-50 MO

fAb production kinetics in either SSB or SUB under two different operating conditions

- 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





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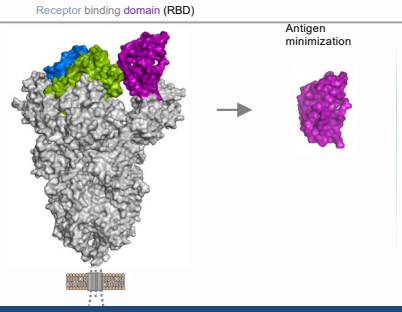
SARS-CoV2 Spike RBD: A Key Target For Induction Of Potent Neutralizing mAbs

Covid-19 Vaccine Candidate DYAI-100 Phase 1 Clinical Trial Expected 2022

Receptor Binding Domain

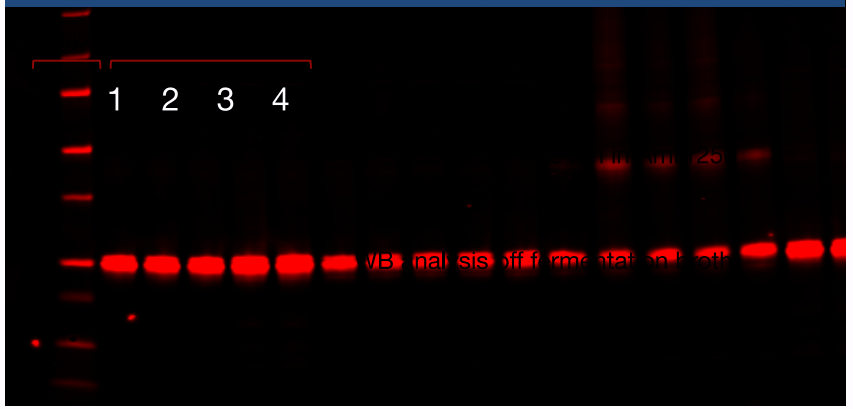
- Single folded polypeptide chain
- All potent neutralizing Ab target the RBD
- Ag minimization -> focused immune response



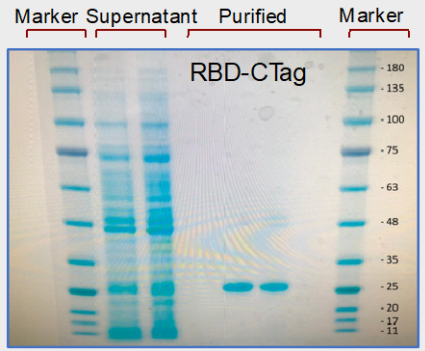
Spike Protein Antigen Minimization Advantages

- 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)

C1 cell line in Ambr250 fermenter system



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

- In ~2 months, 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 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

Rapid Development & Production of SARS-CoV-2 Vaccine Protein Variants

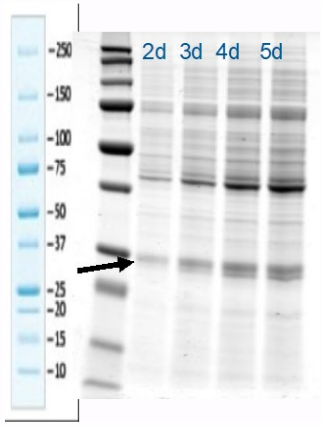
Dyadic is expressing the following variants in C1:

- C1 strains expresses high levels of RBDs of the SARS-CoV-2: (Wuhan, Alpha, Beta, Gamma, Delta, Omicron) variants
- Variants individually transformed into same C1 cell line used to express Wuhan RBD creating 6 producing cell lines
- C1 technology will be able to rapidly swap out genes of interest and express emerging Variants of Concern

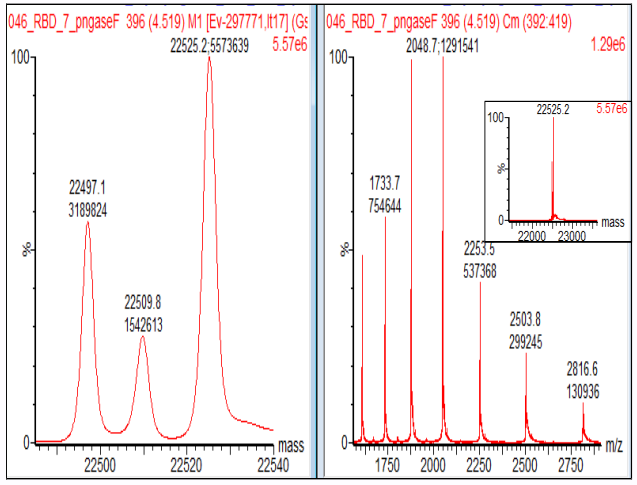
Expression of Three RBD variants in A Single Strain (potential for 4[1]):

- Strain simultaneously expressing Alpha, Beta and Gamma RBD variants has been successfully constructed
- Expression constructs were integrated into two defined genomic loci
- Expression ratio of different variants showed good reproducibility in fermentation runs

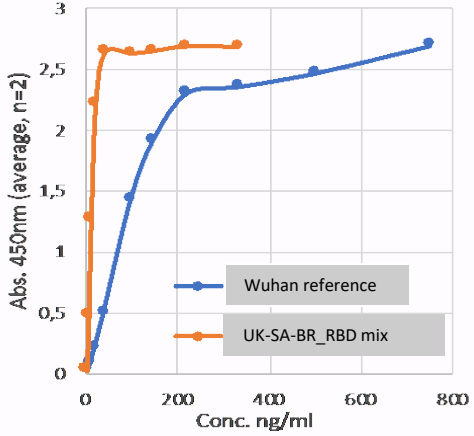
Coomassie staining gel



Intact LC-MS



Ace2 ELISA Potency Assay



RBD-Ctag mix has nearly 9x activity compared to Wuhan

1. Potential for six or more genes can be expressed simultaneously in a single C1 cell

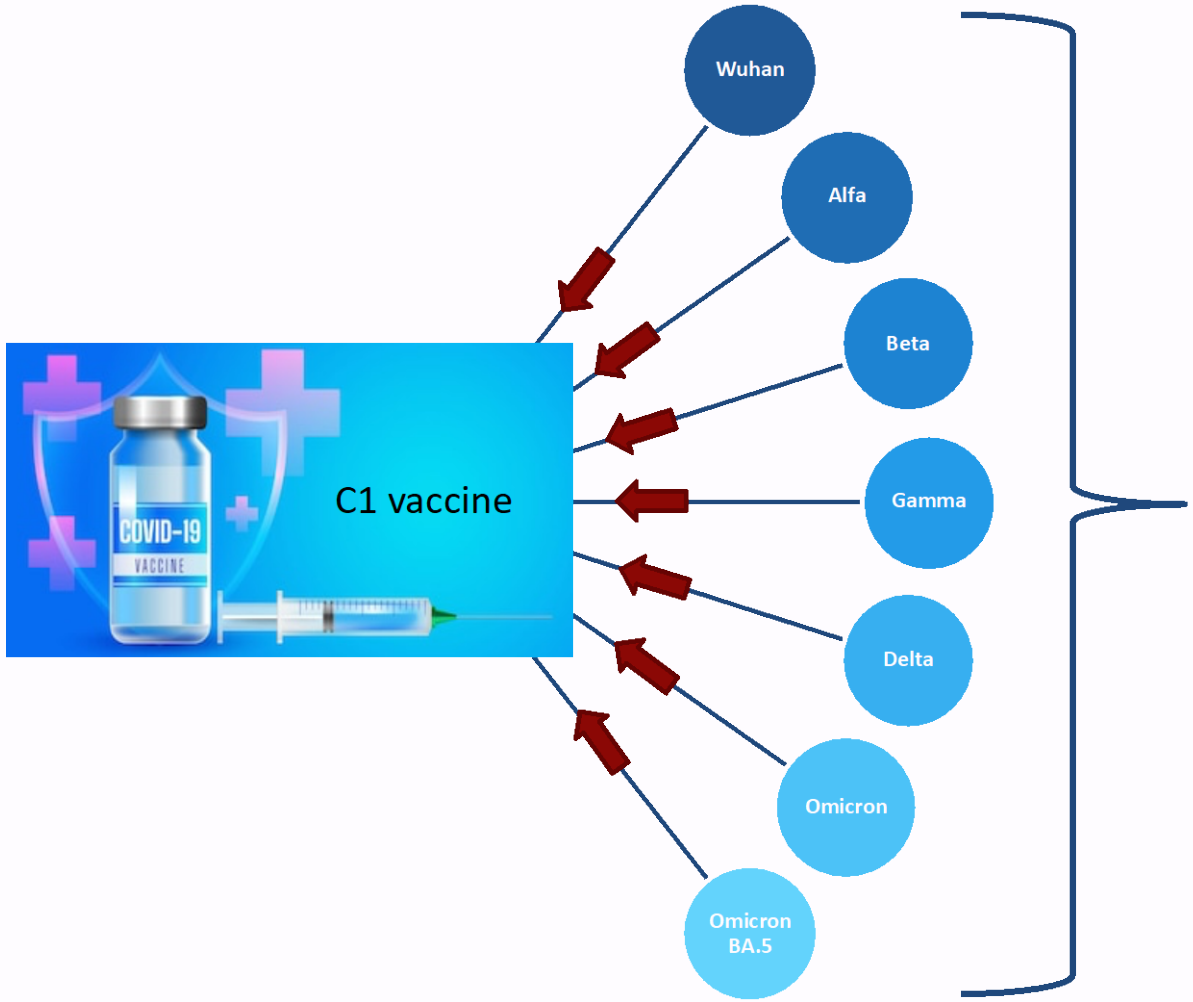
Manufacturing larger quantities of affordable cocktails of mAbs & antigen treatments / vaccines

- *Dyadic's C1 recombinant protein production platform can be used to rapidly respond to regional and global antibody & vaccine demand.*
- *C1 cell lines produce neutralizing mAbs or antigen cocktails faster, in larger quantities more affordably using low-cost media in standard microbial bioreactors*

C1 Neutralizing mAbs

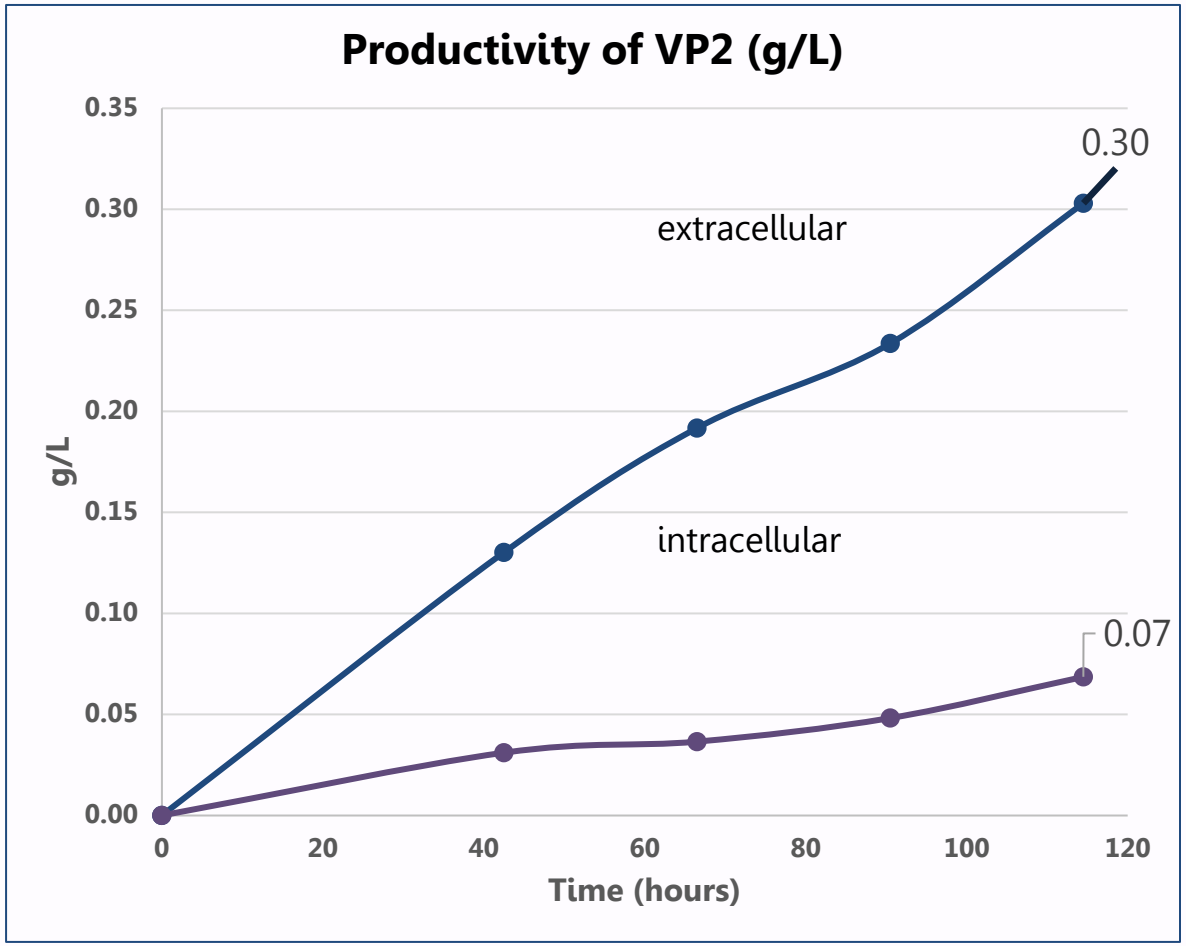
87G7 mAb neutralize **ALL** CoV-2 variants through Omicron BA.2

Variant CoV-2 mAb can be added to 87G7 to develop mAb cocktail to neutralize BA.4/5 …..



C1 RBDs

In S. cerevisiae, the VLP could not be secreted, intracellular titers were comparable to C1



- In C1 The production level of the extracellular VLP produced was 300 mg/L, while intracellular VLP expression was 70 mg/L
- In S. cerevisiae, the VLP could not be secreted, intracellular titers were comparable to C1
- There are more C1 technology options to increase the productivity to a higher level because this titer was obtained without any optimization