DYADIC®

A TRANSFORMATIONAL PLATFORM THAT TRANSCENDS THE LIMITS OF LEGACY PROTEIN PRODUCTION TECHNOLOGIES

Dyadic Thermophilic Filamentous Fungus (C1 platform) Recombinant Production of Glycoprotein Antigen Vaccines, Antibodies & Other Therapeutic Protein Products

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BioProcess International

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Our Mission, Transforming Biomanufacturing

"To improve how we feed[1], fuel[1], and heal the world by utilizing modern biotechnology to revolutionize science, medicine, agriculture[1], and engineering. To provide a cost-effective solution to increase biomanufacturing outputs and satisfy the growing demand for protein production and unmet needs for affordable biologic drugs, vaccines and other biologic products and processes."

1 Dyadic has achieved certain ports of the above "Mission" through its twenty plus years of experience in industrial biotech. Currently, we are primarily focused on animal and human health applications while opportunistically exploring if and how we may enter/re-enter certain industrial biotechnology applications.

Company highlights

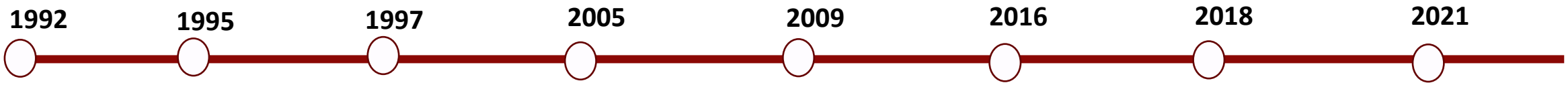
Next Generation C1 Protein Expression Biotech with Well-established Global Partners

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

History of the C1 Protein Production Platform

"C1" cells are industrially proven, hyper productive, genetically engineered thermophilic filamentous fungus – (*Thermothelomyces heterothallica* (formerly *Myceliophthora thermophila*) cells whose usages have expanded through 20 years of commercial engineering



1992

C1 wildtype strain discovered to produce commercially valuable catalytic enzymes

1995

Discovery of C1 mutation leads to commercial launch of C1 enzymes produced for industrial textile manufacturing

1997

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

2005

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

2009

Enzyme library developed

Generally Recognized as Safe (GRAS) designation by FDA

C1 launched in biofuels industry

2016

Launch of human biologics development for vaccines and biosimilars

ZAPI project initiated

2018

Proof-of-Concept collaborations with pharma industry accelerates

2021

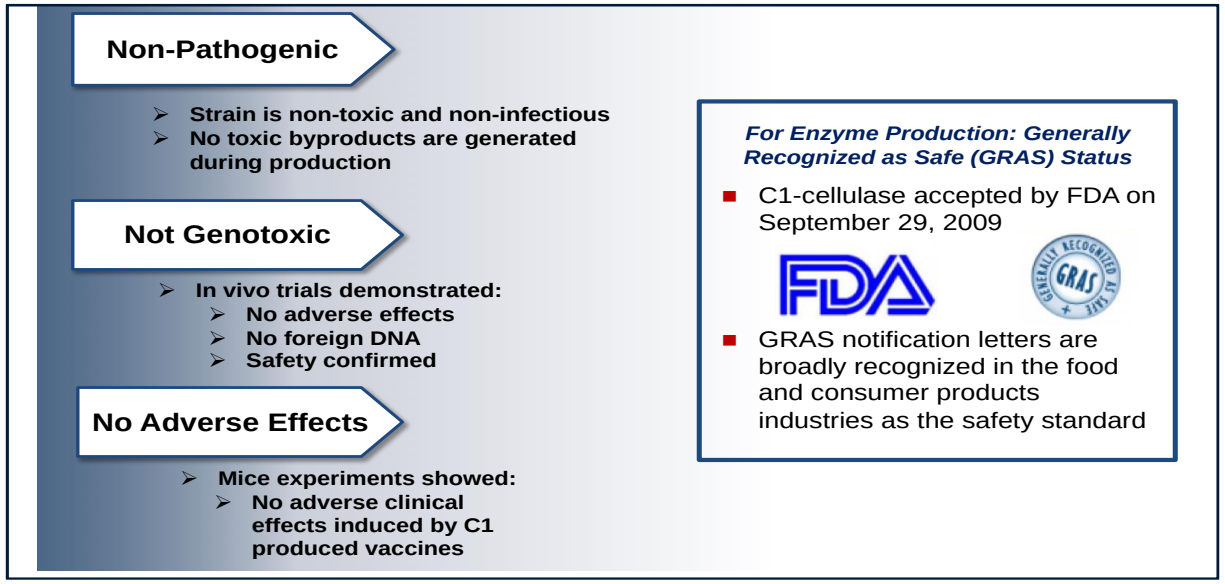
First DYAI-100 licensing agreement signed for $10M in upfront payments

Fully funded R&D trials with top pharma and leading animal health companies

First in human trials are the next step in the C1 platform's commercial evolution

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.

Advancing Towards Human Clinical Trials

SARS-CoV-2 RBD Vaccine Candidate (DYAI-100) Toxicology Study

- A repeat dose toxicology study in rabbits with the Wuhan RBD vaccine candidate is ongoing. Results suggest no adverse effects when dosed at 33 µg over a 3-week period (Days 0, 7, 14 and 21)

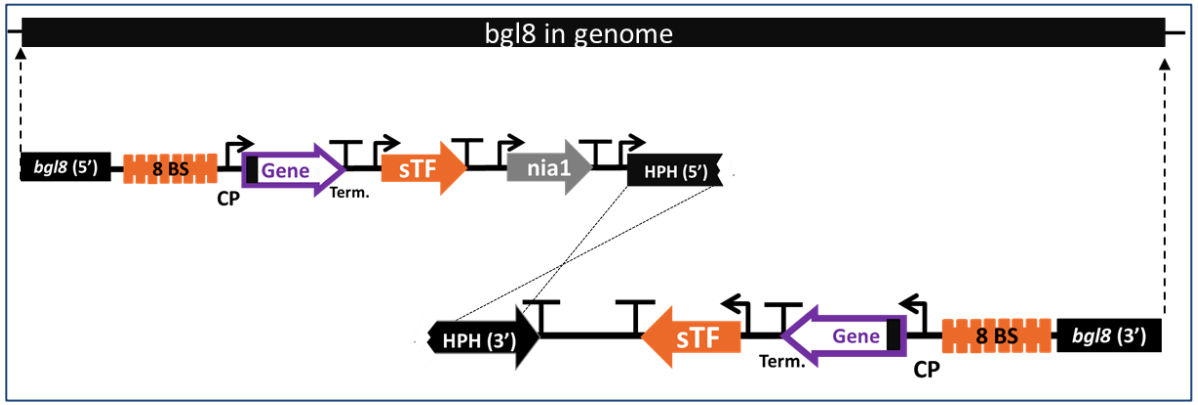
  - No mortality
  - No abnormal clinical signs
  - Normal range of food consumption
  - No local reaction in terms of edema
  - Comparable body weight gain
  - Normal appearance of blood vessels and the optic disc at the indirect ophthalmoscopy examination
  - No marked differences in urinalysis values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

  - **Final Toxicology Report Due In October 2021**

C1 Technology

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

Site specific integration for stable cell line



- **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

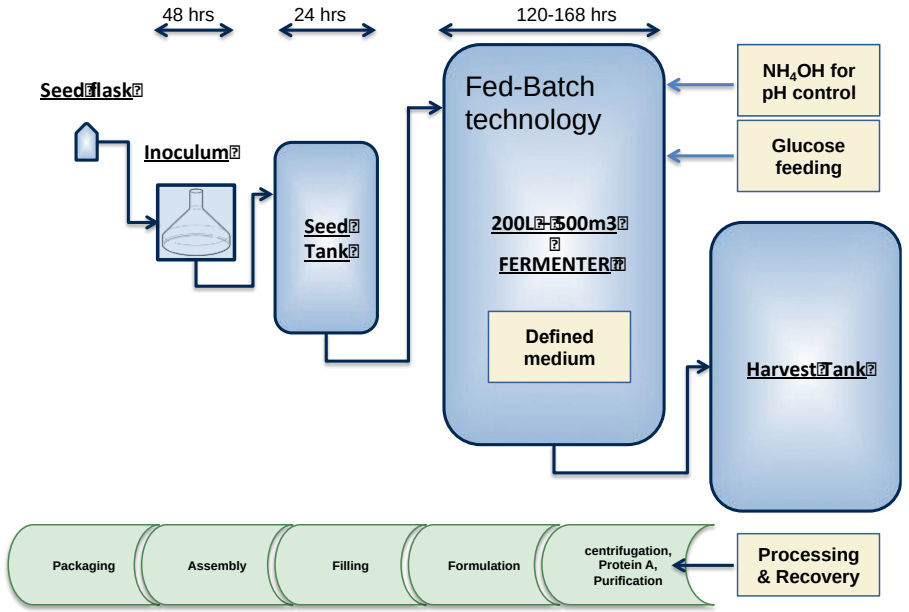
cGMP grade strain and process characterization, MCB,

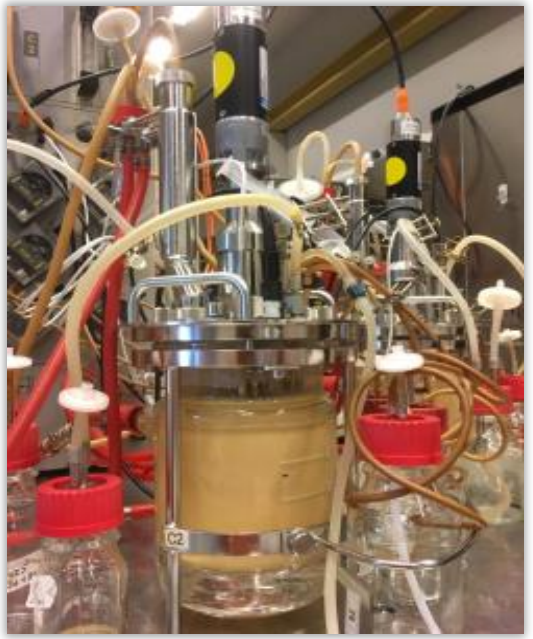
- **Rapid Development Timelines**
- **High Productivity – large quantities**
- **Purity**
- **Stability**
- **Robust Manufacturing Process**
- **Flexible Commercial Scales**
- **Low Cost**

C1 Fermentation Technology

Fed-batch Process

- fully defined cheap 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
- (typically) 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)
- protein production requires no inducer
- protein is (typically) secreted to the media
- Only 12 – 14 days between Pre-inoculum to production bioreactor



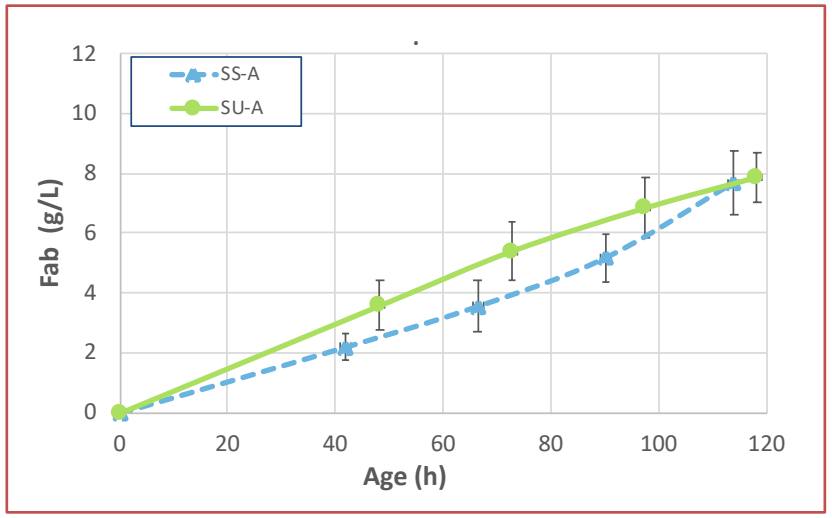


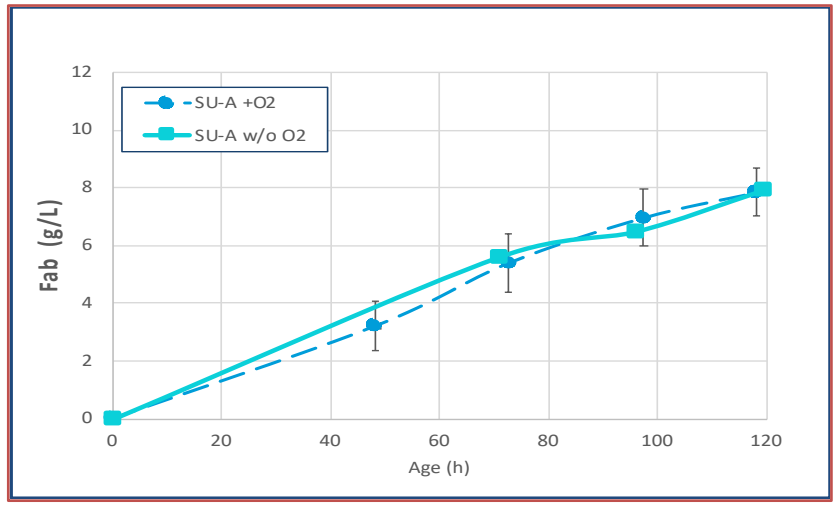
From MTP to Large scale mAbs productivity

24 wells MTP – 1mg/4ml
1L fermentor – 1.7/g/l/d
30L fermentor – 2.4 g/l/d

Certolizumab production with C1 in Single use Bioreactor (SUB)

Conditions A

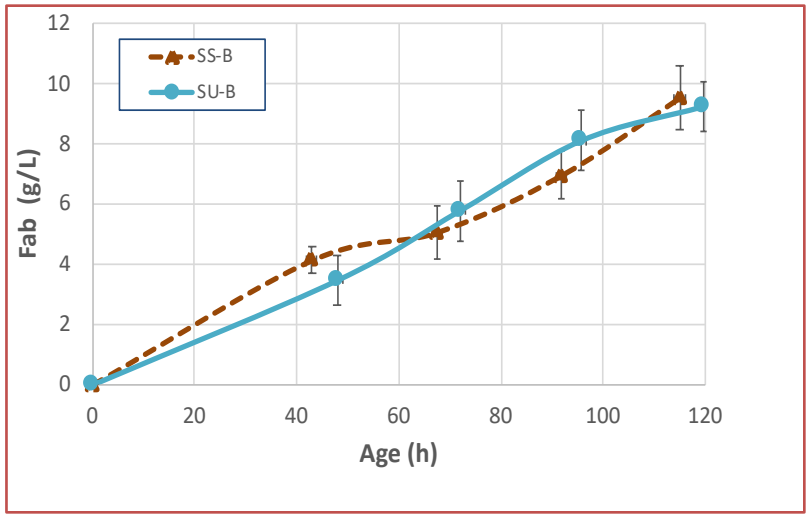


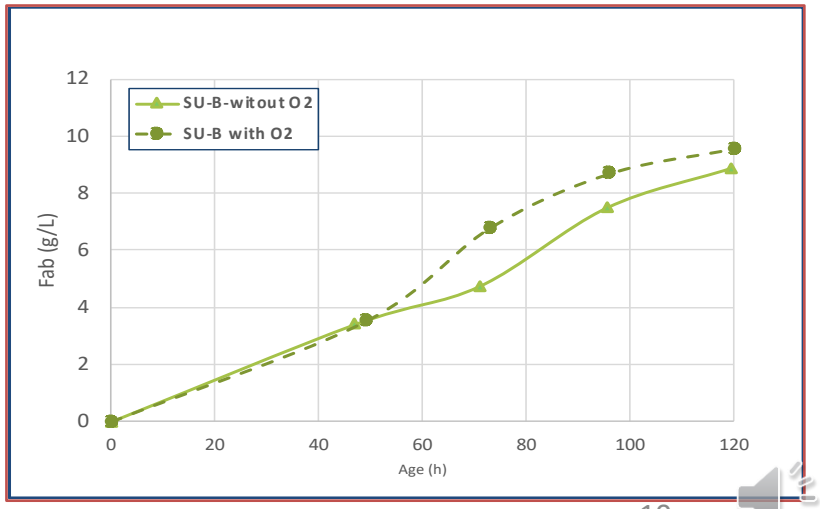


GE's Xcellerex™ XDR-50 MO

- 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

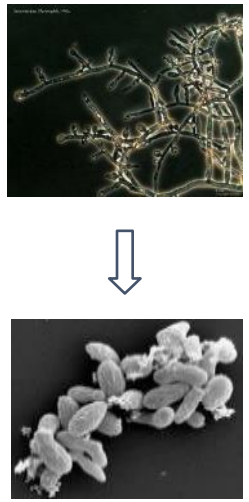
Conditions B



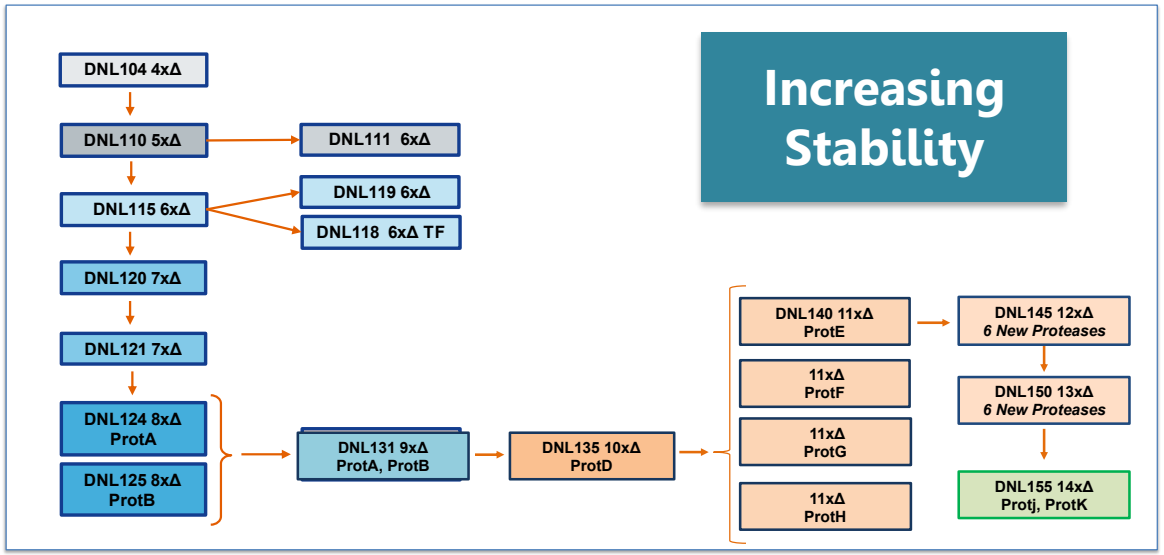


C1 Filamentous Fungus - High Level Protein Production Platform

- C1/*Thermothelomyces heterothallica* (previously known as *M. thermophila*) offers several advantages to address Therapeutic drugs and vaccine:
  - Highly productive and stable cell line that is suitable for the rapid development and production of antigens without the need for transient stage.
  - Highly efficient, robust & versatile low-cost fermentation processes
  - Rapid (fed-batch fermentation cycles with 10 – 16 days from seed flask to finished fermentation)
  - Delivering secreted proteins with high proportions of human glycoforms



C1 strain is characterized by a morphology change to a fragmented non sporulating mycelial phenotype. This morphology results in high yield/low viscosity fermentations



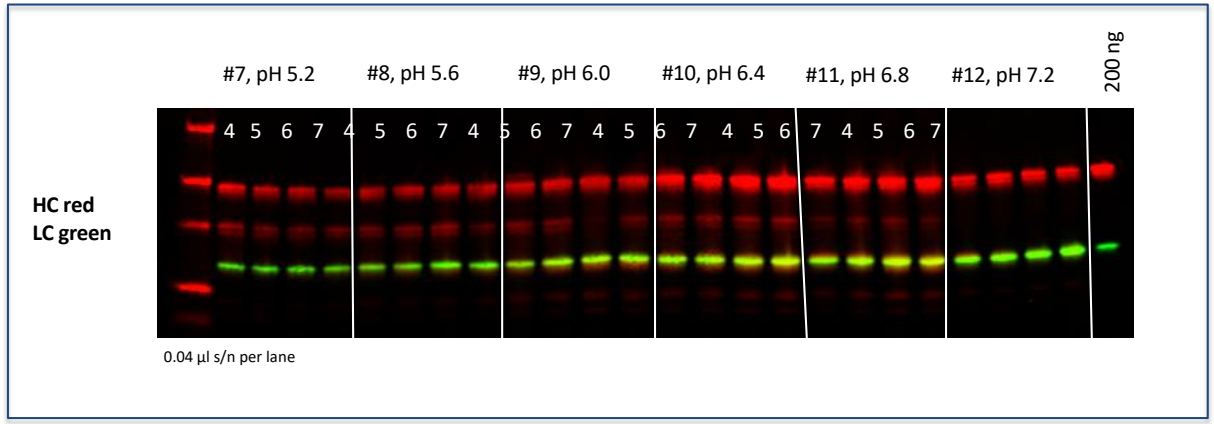
Systematic deletion of protease genes based on:

- Isolation and identification of extracellular proteases
- C1 protease library in *Pichia pastoris*
- Effect of different protease inhibitors on protease activity
- mRNA sequencing data
- Protease gene annotation

Expression of mAbX in 2 different cell lines and different pHs

Production in (Δ10)

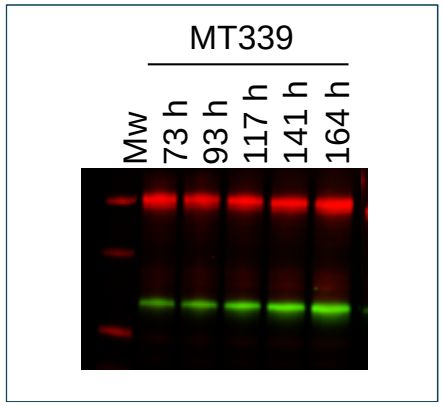
Comparison of six different pH-conditions



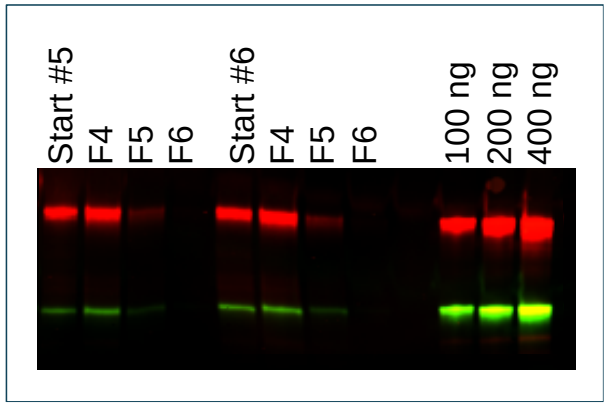
Vessel	pH	Day	Amount, g/l
ambr7	5,2	7	6,9
ambr8	5,6	7	12,5
ambr9	6,0	6	16,7
ambr9	6,0	7	14,8
ambr10	6,4	6	21,7
ambr10	6,4	7	24,5
ambr11	6,8	6	19,0
ambr11	6,8	7	18,5
ambr12	7,2	7	6,8

Production in (Δ13)

Fermentation



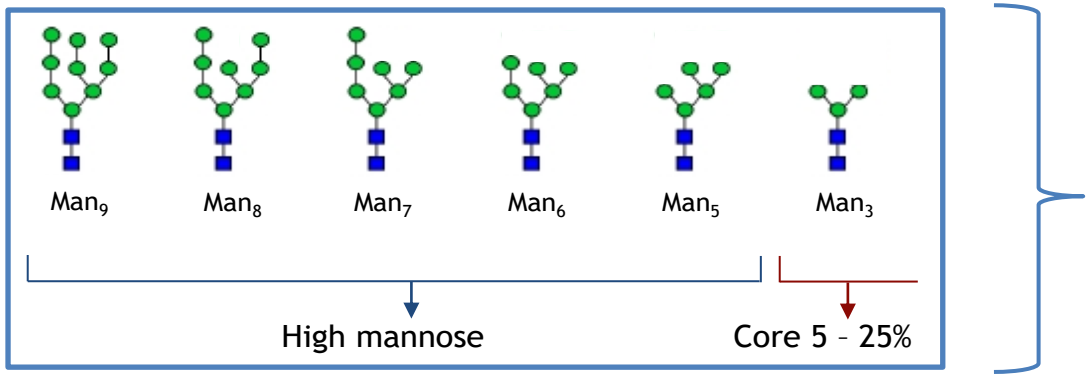
Purification



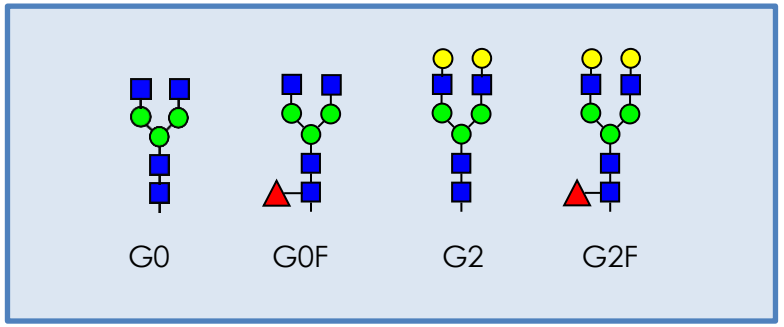
Fermentation of the mAb that was expressed in the Δ13 proteases cell line reached 16.1 g/l and 17.5 g/L in 122hrs and 145hrs respectively (2.9 g/l/day)

N-glycoengineering of C1

C1 typical Glycan structure

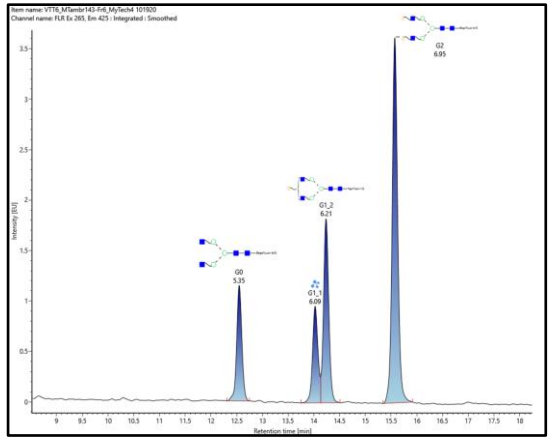


New C1 Glycan Structure



Current status

- G0 strains – best G0 levels 84% on Nivolumab in fermentation (98% in SF)
- G2/G1 strains – the best strain has 37.51% G2, 46% G1 and 16.48% G0 on Nivolumab in fermentation
- Fucosylation – the best strain has 29% G0F, 61% Man3GlcNAcFuc and 5% G0 on Nivolumab in fermentation – up to 95% Fucosylation efficiency
- G1F/G2F strains are under construction



M4092	Observed RT (min)	Glycan Units	Response	% Amount (%)
G0	15,08	5,3329	34933995	16,48
G1_1	17,6	6,0763	32315348	15,25
G1_2	17,95	6,1812	65168790	30,75
G2	20,32	6,9336	79496392	37,51
			211914525	100

C1-Cell Recombinant Protein Production: Biologics

C1-Cells Enable Commercial Manufacture Of Rapid, Cost-Effective, High Value, Safe, Effective Protein Products

High Yields and Purities Demonstrated for Therapeutic Monoclonal Antibodies (mAbs) and Vaccine Antigen GlycoProteins[1]

Fc-Fusion Products	mAb Products	Fab (Certolizumab) Product	Tri-specific Products
15.3 g/l [1]	24.5 g/l [1]	14.5 g/l [1]	6.12 g/l[1]
168 Hours	168 Hours	164 Hours	144 Hours
2.58 g/l/day	3.1 g/l/day	2.1 g/l/day	1.02 g/l/day

High Productivity for Recombinant Protein Antigen Classes Routinely Used in Vaccines

Influenza HemAgglutinin (HA) Products	Coronavirus Antigen (S-RBD) Products	Virus-Like Particle (VLP) Products
413 mg/l [1]	2,000/3,000 mg/l[1]	2,200 mg/l[1]
137 Hours	120 Hours	110 Hours
72 mg/l/day	400/600 mg/l/day	500 mg/l/day

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

Antibodies

C1 platform has potential to disrupt conventional mAb manufacturing

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

Flexible production scale; C1 media <1/20 of the cost of CHO media

No viral inactivation required

Faster Production

C1 produces product significantly faster (12-14 days) than CHO cells (41-54 days)

Higher Yields

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

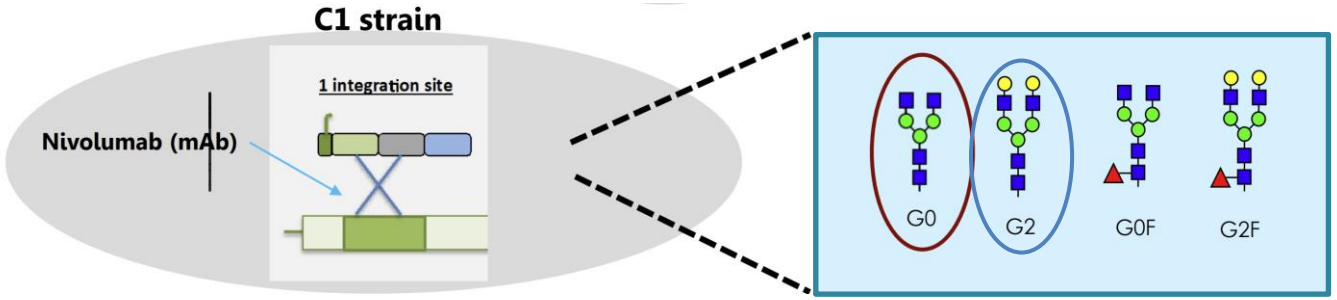
~ Potential to produce three to four batches using C1 in the same timeframe as one batch using CHO cells

Dyadic's Internal Nivolumab (Opdivo®) Biosimilar Program

Antibodies Represent a Compelling Opportunity for C1 (~ 300 Billion Market & Growing)

Nivomulab (Opdivo®), manufactured by Bristol Myers Squibb, is an immunotherapy drug indicated for metastatic cancers, including melanoma and long cancers.

- Opdivo costs about $12,500 per month or about $150,000 per year of treatment.
- Goal of program is to express nivolumab (mAb) with a glycan structure similar to nivolumab produced in CHO cells
- Dyadic has glycoengineered a C1 line with G0 levels of about 95% and G2 of about 76% as part of its glycoengineering program
- Further C1 strain and process development work is ongoing
- Important proof of concept – If we can successfully make Optivo, our C1 technology could potentially be applied to multiple monoclonal antibodies

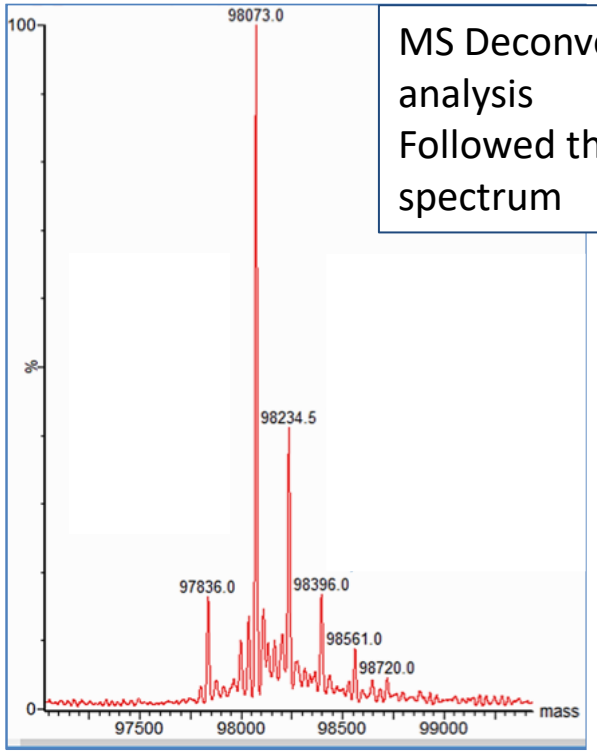


(1) Trefis team Analyst Opdivo Estimate for 2023

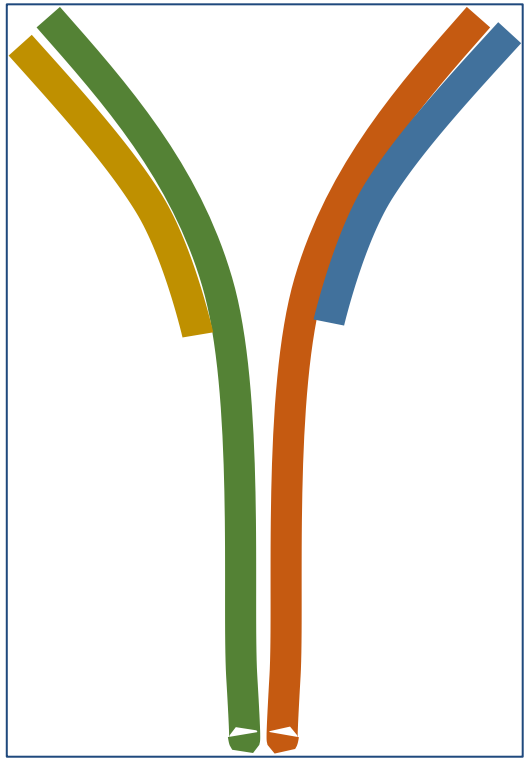
Bispecific Antibodies

- The best production level so far:
  - **Bispecific X - Day 7: 7.62 g/L**

- Bispecific X Mass spectrometry analysis indicates that vast majority of the bispecific antibody is correctly assembled.



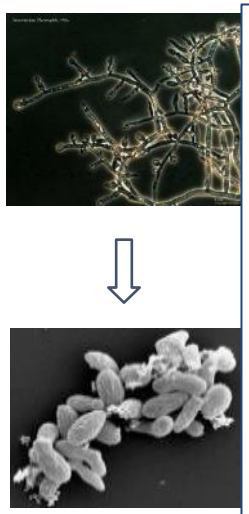
MS Deconvoluted spectrum analysis
Followed the expected spectrum



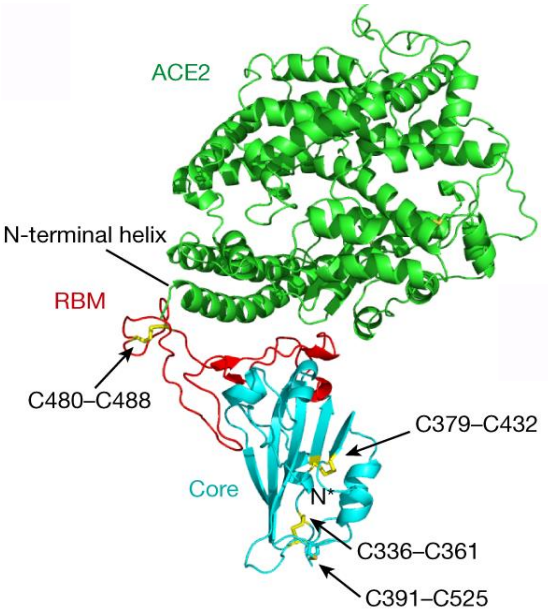
Vaccines

The C1 Expression Platform

- C1/*Thermothelomyces heterothallica* (previously known as *M. thermophila*) offers several advantages to address vaccine supply for COVID-19:
  - Highly productive and stable cell line that is suitable for antigens production without the need for transient stage.
  - Highly efficient low-cost fermentation processes.
  - Rapid (fed-batch fermentation cycles with 10 – 16 days from seed flask to fermenter).
  - Delivering proteins with high proportions of human glycoforms.
  - Development of new variants in 2 months or less without changing the genotype of the current cell line.
- Vaccine candidate: SARS-CoV-2 RBD-C-tag adjuvanted with alum alhydrogel (DYAI-100)
- Purification via glutamic acid-proline-glutamic acid-alanine (E-P-E-A) 'C-tag'
- Dyadic is a key Zoonoses Anticipation and Preparedness Initiative (ZAPI) stakeholder
- C1 offers the ability to target ANY emerging Variant of Concern:
  - Rapid production of large amounts of antigen at low cost - >1 g/L SARS-CoV-2 RBD from a single 20 L fermentation – equivalent to >600,000 human doses of 33 µg
  - 10-100 higher yield than CHO and Insect cells, rapid doubling time, etc
  - Can quickly be modified to protect against new emergent variants and future threats



C1 strain is characterised by morphology change to a fragmented mycelial phenotype. This morphology resulted in low viscosity fermentation.

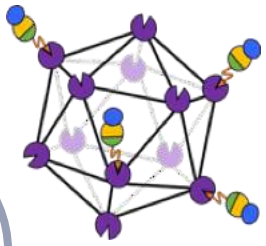


[1]Stuible M, Gervais C, Lord-Dufour S, et al. Rapid, high-yield production of full-length SARS-CoV-2 spike ectodomain by transient gene expression in CHO cells. J Biotechnol. 2021;326:21-27. doi:10.1016/j.jbiotec.2020.12.005

Success in Expressing High Level of SBV & RVFV Antigens

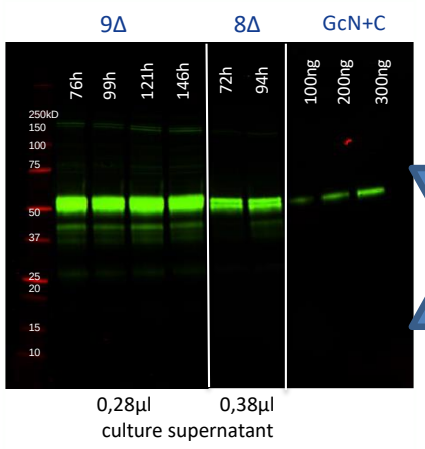
ZAPI, is a research and development program sponsored by the EU with the goal of developing a platform suitable for the rapid development and production of vaccines and protocols to fast-track registration of developed products to combat epidemic Zoonotic diseases that have the potential to effect the 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.
- **C1 was selected as the ultimate production platform for ZAPI's antigens**



SBV

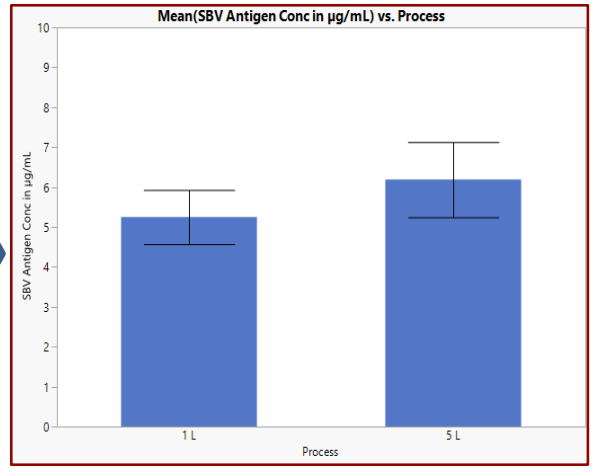
C1 Fermentation



X300

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

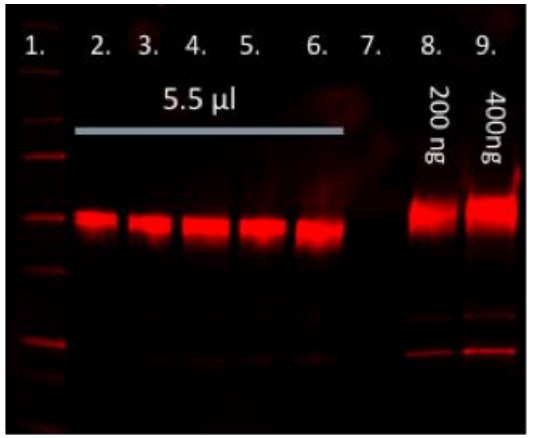
Baculovirus Fermentation



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

RVFV

RVFV Gn_DVII-SpyCatcher-C-tag

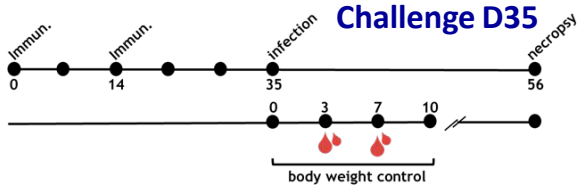


1. MW
2. Day 3
3. Day 4
4. Day 5
5. Day 6
6. Day 7
7.
8. SpyC RVFV
9. SpyC RVFV

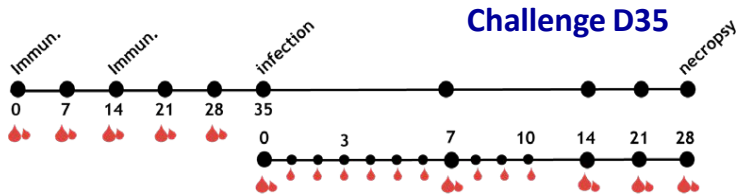
RVFV yield: **1,24 g / L**

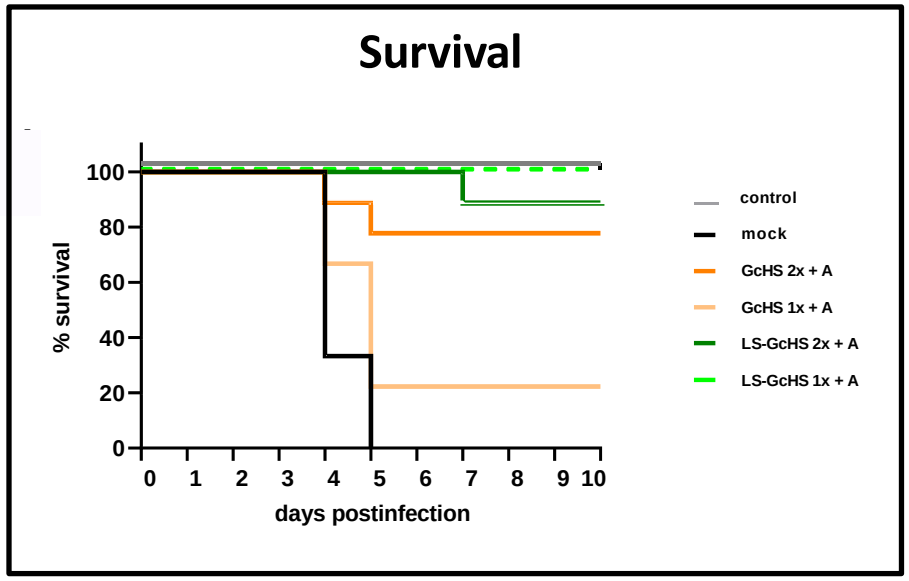
Success in Mice and Cattle Challenge Tests (SBV)

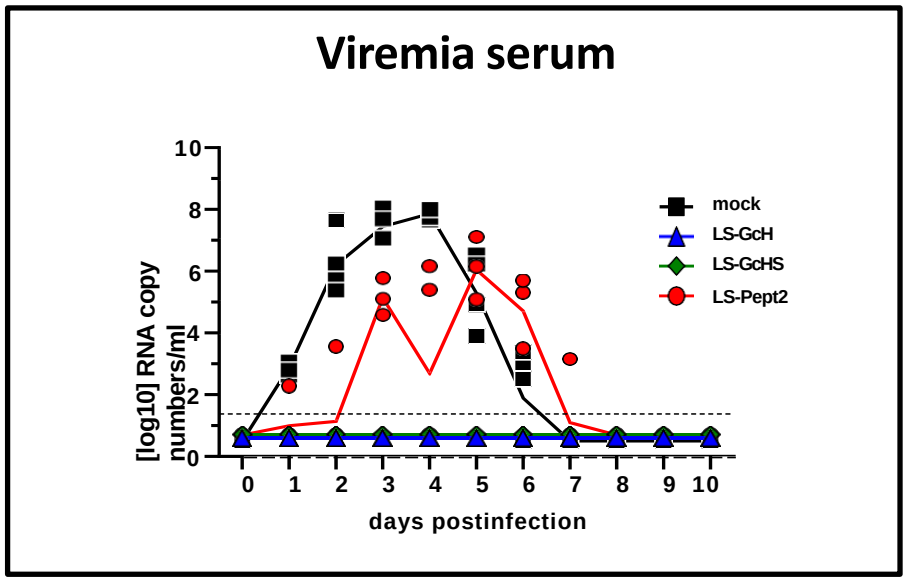
Mice trials



Cattle trials







The C1 expressed SBV antigen that was assembled to Nano-particle expression molecules was tested in animal tests:

- All immunized mice and cattle survived challenge infection without any clinical signs of disease.
- Protection was conferred even after only one immunization.

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 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:

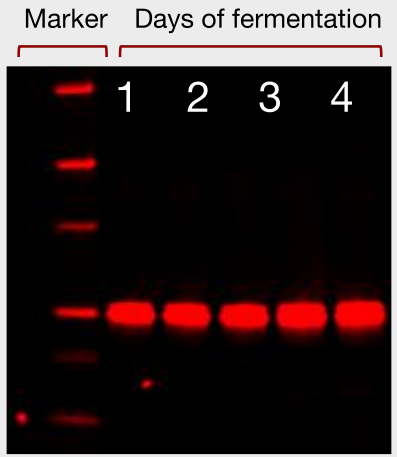
Single folded polypeptide chain

All potent neutralizing Ab target the RBD

Ag minimization -> focused immune response

Advancing Towards Phase I clinical study (estimated in 2022)

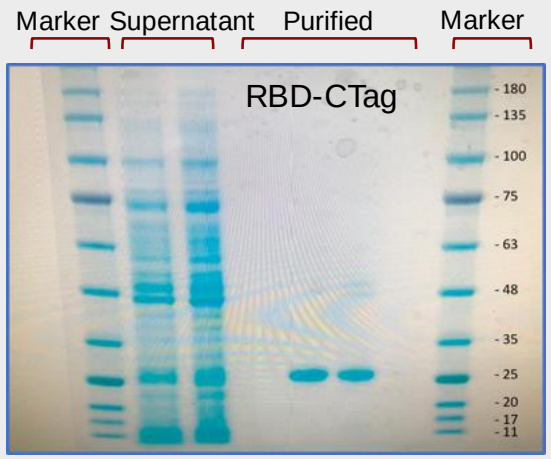
C1 cell line in Ambr250 fermenter system



C1 RBD strain was run in Ambr250 for 5 days

WB analysis off fermentation broth

C1 cell line in 1L fermenter system

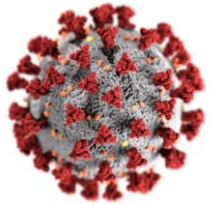


C1 RBD strain was run in 5L scale fermentation
The RBD was purified twice with CaptureSelect™ C-tag 10ml column.

98% purity
70% recovery

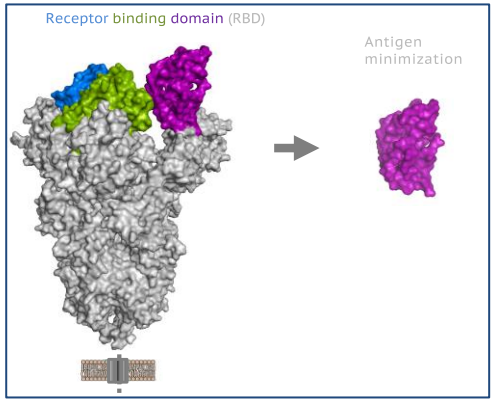
Integration of RBD Genes Into the Same Genomic Locus

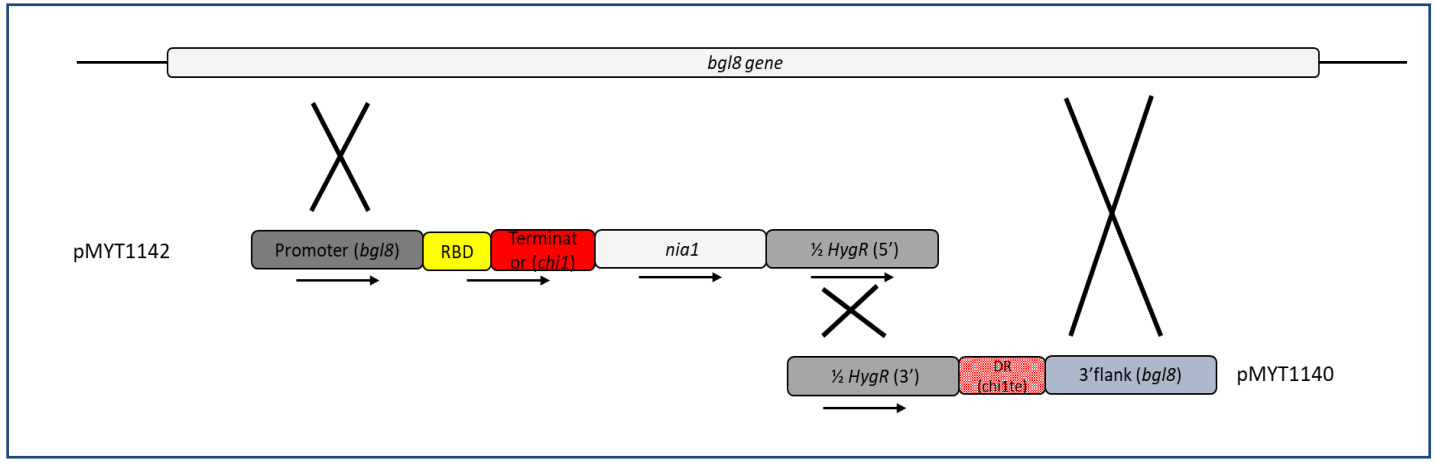
Transformation efficiency



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

- The Gene of Interest (GOI) are being integrated into the *bgl8* site.
- B*gl8* promiscuous promoter generate high expression level
- Stable single-copy or double copy integration
- No need for transient stage
- No need for induction





- Transformation procedure based on chemical (PEG) method with protoplasts or electroporation
- Frequencies for 1µg DNA: ~ 20 colonies
  - Not more than 20 transformants for site specific integration have to be screened in order to identify the right clones (usually >50% of the transformants have the expected productivity and quality

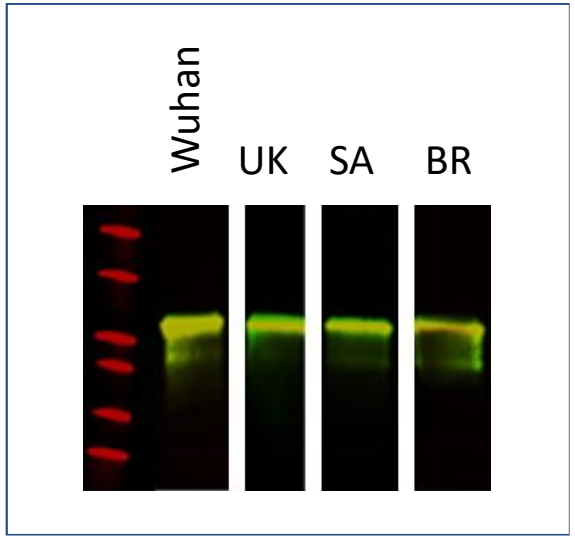
Production of SARS-CoV-2 Vaccine Protein Variants

Dyadic is expressing the following variants in C1:

- C1 strains have been engineered to express high levels of Receptor binding domains (RBDs) of the B.1.1.7 (UK or alpha), B.1.351 (South Africa or beta), P.1 (Japan/Brazil or gamma, and B.1.617.2 (Delta (Indian) variants.
- Expression constructs of the Alpha (UK), Beta (SA) and Gamma (BR) variant RBD's were individually transformed into the same C1 cell line that was used to express the Wuhan RBD to create 3 cell lines.
- C1 technology will be able to rapidly swap out genes of interest and express emerging Variants of Concern

Fermentations of variant RBD producing clones

- Initial fermentations of Alpha (UK) , Beta (SA), Gamma (BR) and Delta (IN) RBD have been successfully completed
- The expression level as it was analysed by Western Blotting revealed that the production levels of the variants are similar.

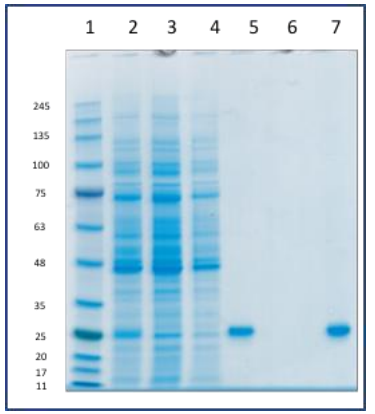


Western blotting analysis with anti-RBD antibody.
RBD from Wuhan variant produced earlier is shown as control.

Stable C1-RBD-SpyTag Production Without C-Tag

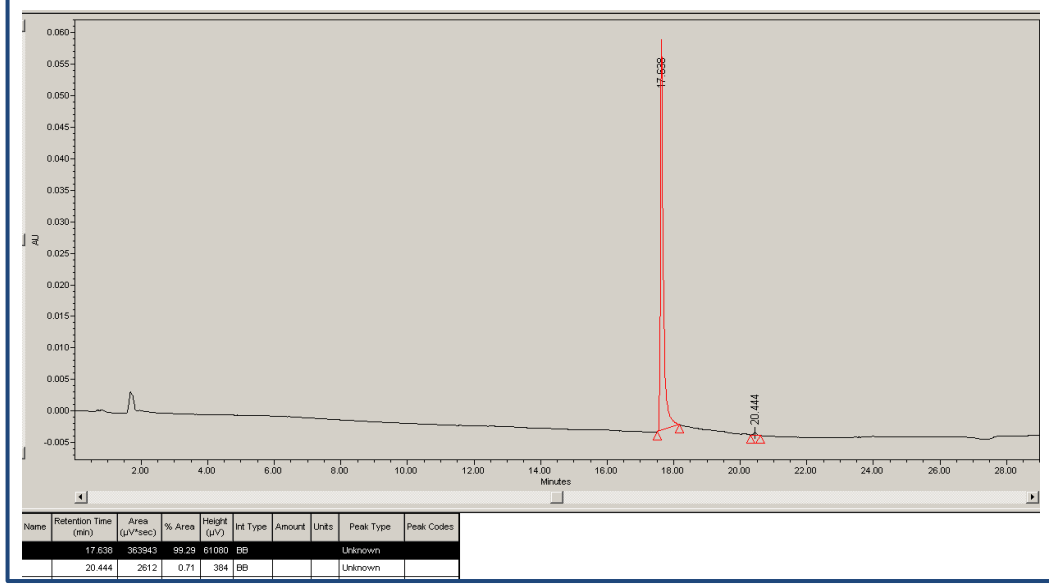
1L scale fermentation

- **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

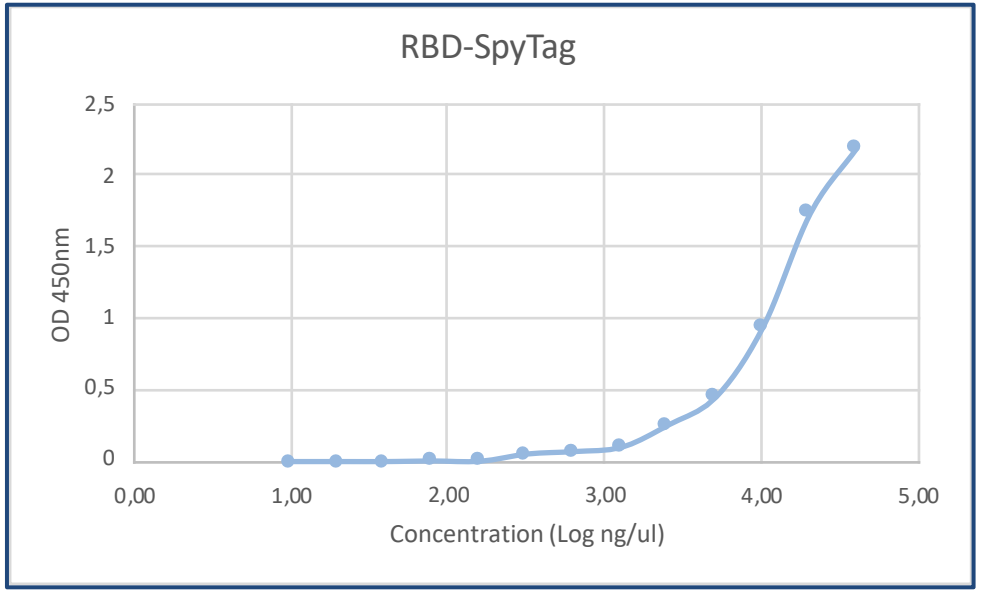


1 - Mw marker
2 – EFT 55h 8µl
3 – F.T. (15ml) 20µl
4 – Wash I 30µl
5– Elutions 1+2+3 in conc. 15µl
6 – Flow through 30µl
7 – RBD std. (2µg total) 10µl

HPLC analysis RBD SpyTag - Purity 99.29%



ELISA-ACE2 Binding Assay



C1-cells Production Platform Utilized in ZAPI Study Published in "VACCINES" a Leading Peer-Reviewed Scientific Journal

vaccines

- *Dyadic's C1-cell protein production platform selected as a fast response vaccine manufacturing model against zoonotic diseases.*
- *C1 -cell platform expressed SBV antigen exhibited efficacy, potency, and safety in veterinary target species.*
- *Demonstrated recombinant protein-based antigens can be produced from C1-cells at very high yields.*
- *C1-cell expressed antigens were successfully used to develop recombinant particle-display vaccines.*

The peer-reviewed study demonstrates the successful use of Dyadic's patented and proprietary C1-cell protein production platform to facilitate a fast, coordinated, and practical response to new infectious diseases as soon as they emerge.

Dr Jean-Christophe Audonnet, Senior Director Regional R&D Asia & EU Partnerships, IMI ZAPI Project Coordinator commented, "Dyadic and its C1-cell protein production platform far exceeded our initial expectations at the start of the program."

N-glycosylation pattern. NP-HPLC chromatography analysis

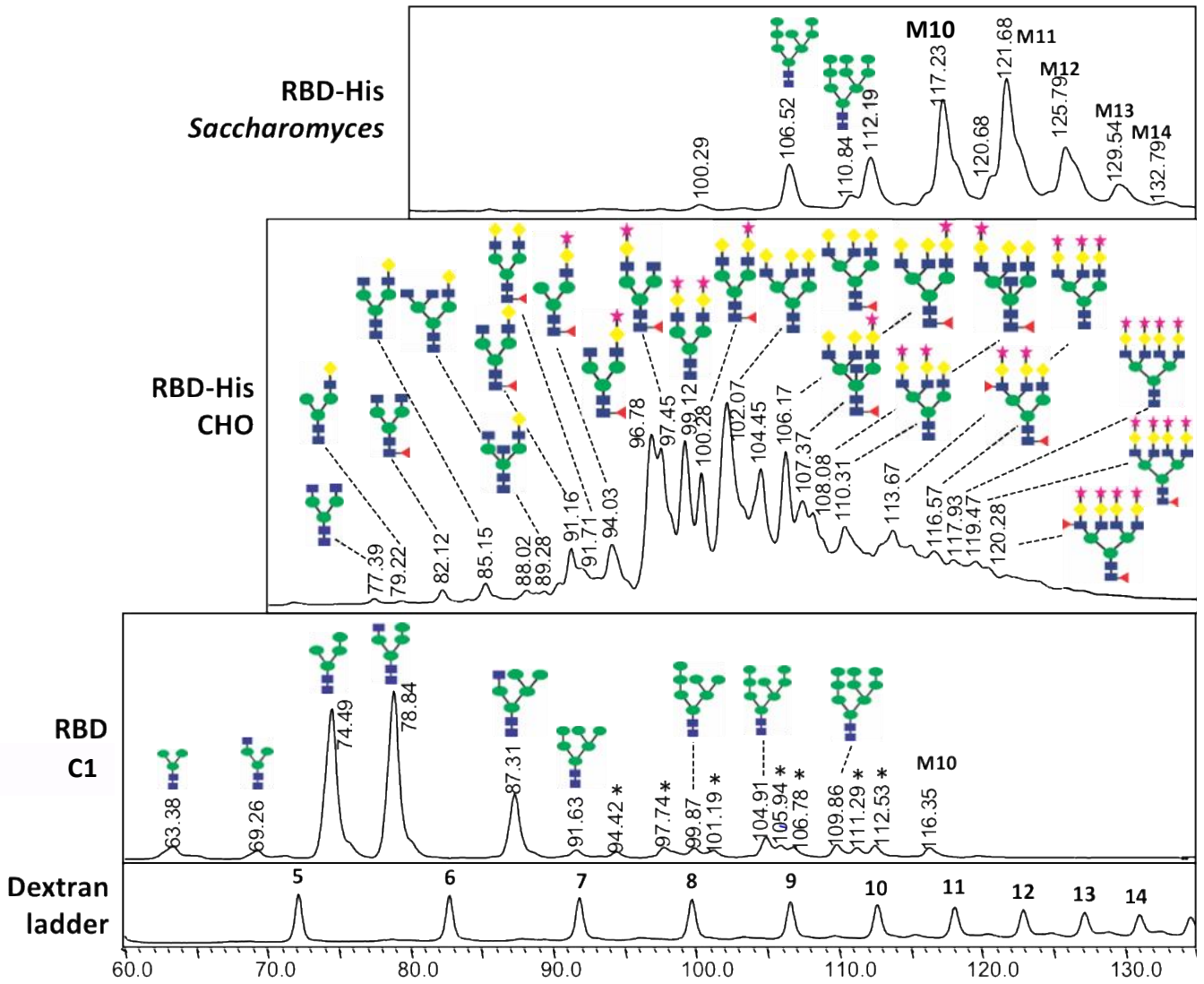
N-glycosylation pattern

Legend

- Man
- GlcNAc
- Gal
- Fuc
- NeuAc

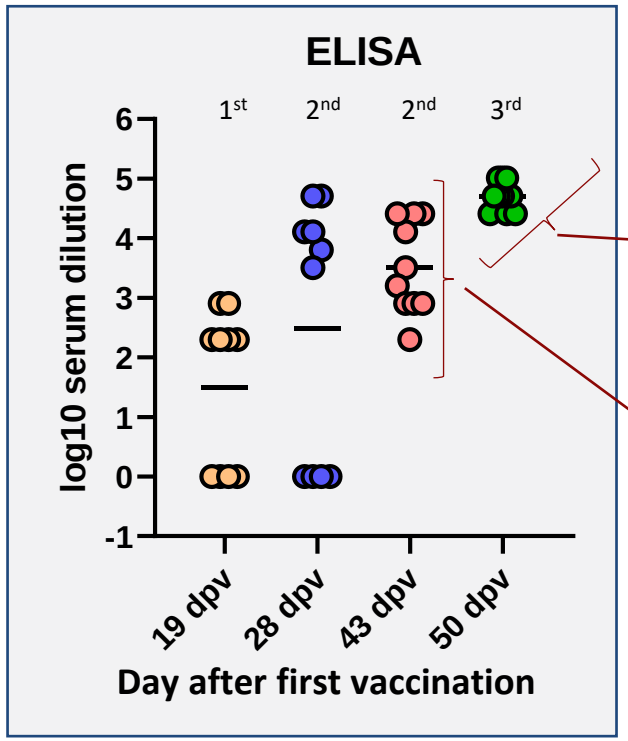
* not identified structures

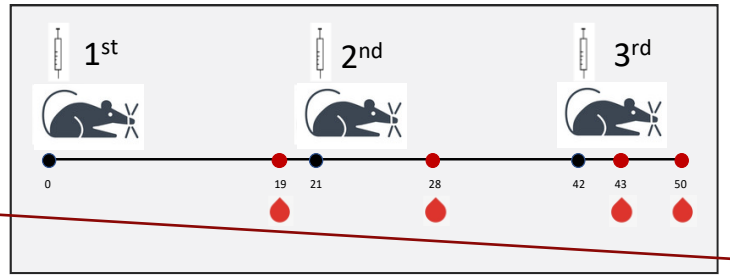
M10-M14: N-glycans bearing 10-14 mannose residues



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

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





Plaque reduction neutralization test (PRNT)

SARS-CoV-2 and Vero E6 cells

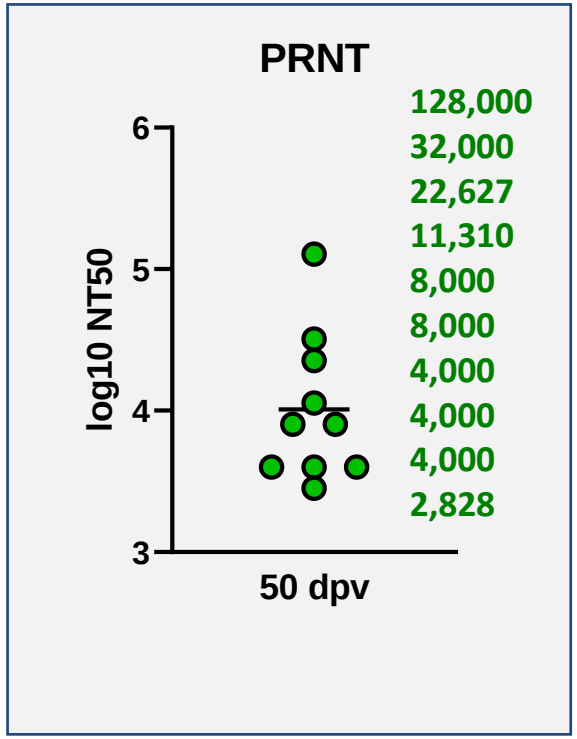
NT_{50} **Dilution** that neutralizes 50% of the virions

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

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

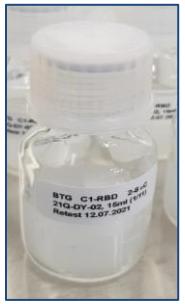
Range 2: 6,400 -12,800 (Mid) - **5,120**

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.



DYAI-100 Vaccine Candidate

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.

DYADIC

Challenge Test with ACE2 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.**

Group 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 8. - *Death prior to challenge***

Mouse	DAY 35 Anti RBD	Day 35 PRNT 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

Group 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	Day 56 PRNT Neutralizing	Challenge
1	102,400	**32,000**	**Live**
2	204,800	**64,000**	**Live**
3	204,800	**22,627**	**Live**
4	204,800	**128,000**	**Live**
5	204,800	**512,000**	ND
6	204,800	**32,000**	ND
7	25,600	**22,627**	ND
8	409,600	**512,000**	ND
Cont-1	0		Dead
Cont-2	0		Dead
Cont-3	0		ND

Commercial Scale Production of C1-RBD

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

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

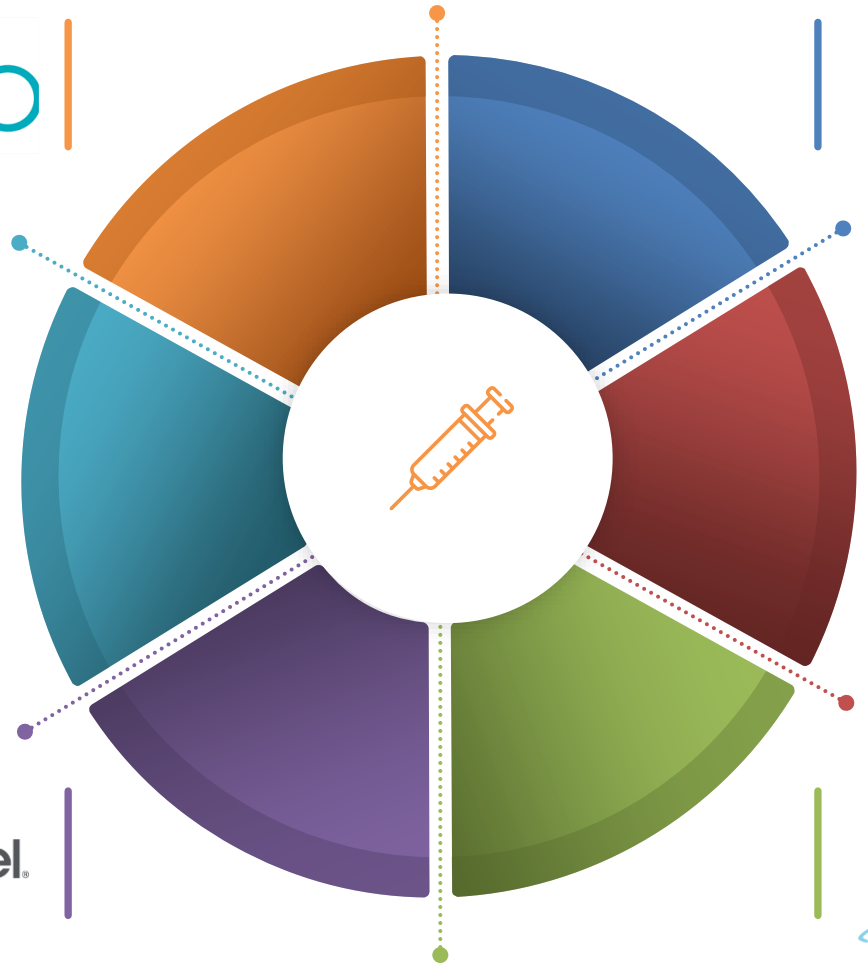
cGMP manufacturing, Fill/Finish, Toxicology, initiating and completion of an anticipated Human Phase 1 Clinical Trial Of a C1 produced SARS-CoV-2 vaccine candidate. Prove Safety & Efficacy In Humans

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

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)

sorrento THERAPEUTICS

Sorrento and Dyadic Announce Binding Term Sheet to License Dyadic's Lead COVID-19 Vaccine Candidate "DYAI-100"

Dr. Henry Ji, Sorrento Chairman and CEO, commented, "We look forward to continuing our collaboration with Dyadic, which began last year, initially with a goal of developing and commercializing a protein-based COVID-19 vaccine that can be rapidly manufactured in large quantities in our existing cGMP facilities, and stored and transported at room temperature, in order to increase access and affordability to underserved populations globally."

Syngene

A Biocon company

Dyadic announces development of COVID-19 Vaccine in India

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."

RUBIC

Dyadic Announces Technology Transfer and Licensing Agreement With South Africa's Rubic Consortium

"The need to quickly acquire and commercialize technology and manufacturing capabilities, which addresses the infrastructure necessary to deploy vaccinations for broad populations affordably and timeously has never been a more strategic imperative of African nations than today," said Shabir Madhi, professor of vaccinology, Dean Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, who is leading COVID-19 vaccine trials in South Africa.

Medytox

Dyadic and Medytox To Develop Vaccines Against COVID-19 Variants (South Korea & SE Asian Countries)

Dr. Gi-Hyeok Yang, Sr, Executive Vice President and Head of Research and Development at Medytox stated, "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."

The next potential pandemic: H5N1 and H7N9 influenza?

"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." -26 March 2021 – by KessRowe (GAVI)

- **University of Oslo re-confirms viability of C1 to express APC-targeted Influenza virus antigen (HA) protein.**
  - **A/California/4/2009 (H1N1) was successfully produced from C1-cells**

- **C1 previously successfully tested in a Sanofi Pasteur influenza vaccine trial versus baculovirus.**

  - 2015 immunogenicity study of Recombinant Hemagglutinin (HA) from the A/H1N1/New Caledonia/20/99 strain with Sanofi Pasteur demonstrated that:

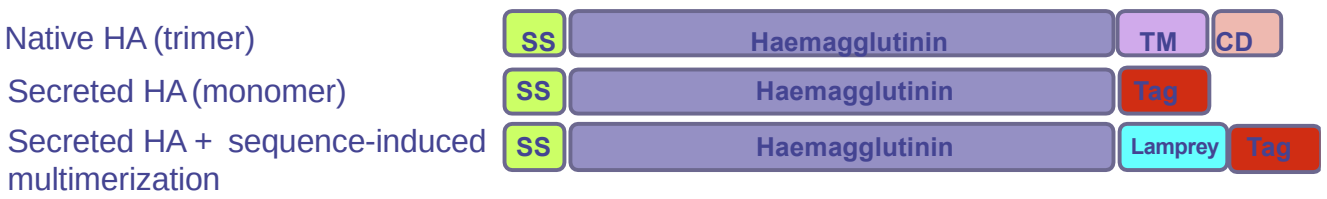
    - (1) C1 produced r-HA was safe and well-tolerated in mice; and

    - (2) the C1 produced r-HA was **at least as immunogenic in mice as the baculovirus-rHA**.

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)

Expression of Haemagglutinin (HA) in C1-cells



SS Signal Sequence (HA SS for *L talentolae* expression, C1 SS for fungi expression)
TM Transmembrane Domain
CD Cytoplasmic Domain
Tag 6X Histidine
Lamprey Sequence-induced multimerization

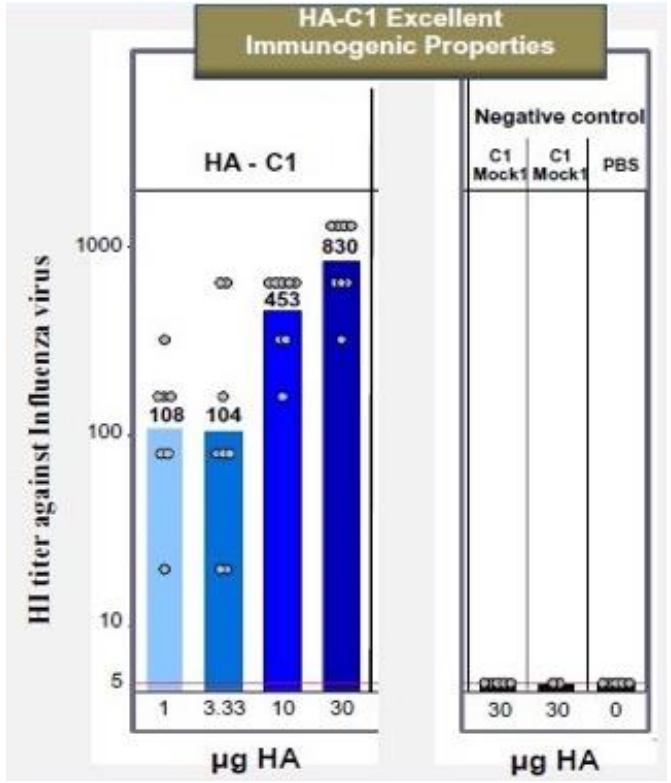
Ability to Express Biologically active HA´s

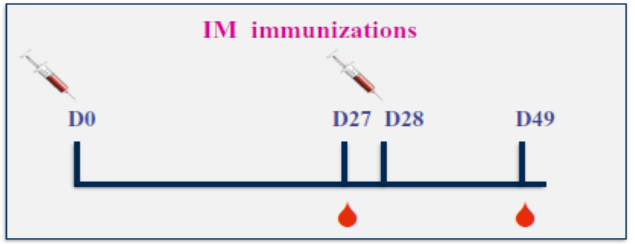
- 5 Recombinant HA´s have been expressed by C1:

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:

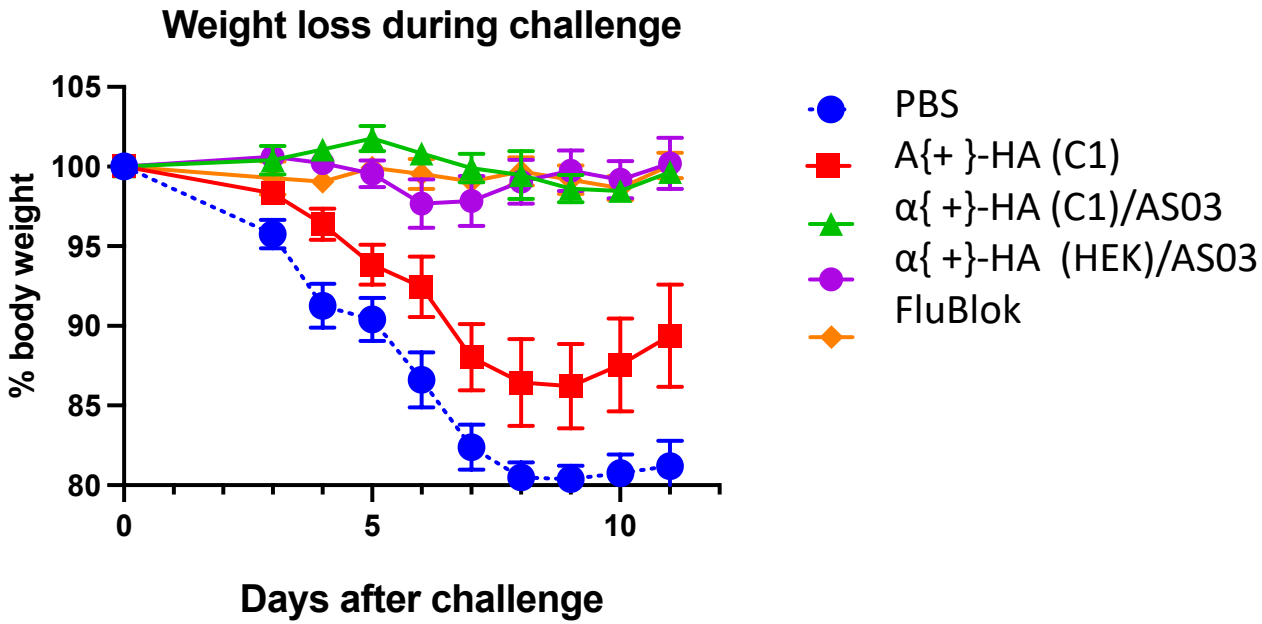
Mice Study was conducted by Sanofi-Pasteur:

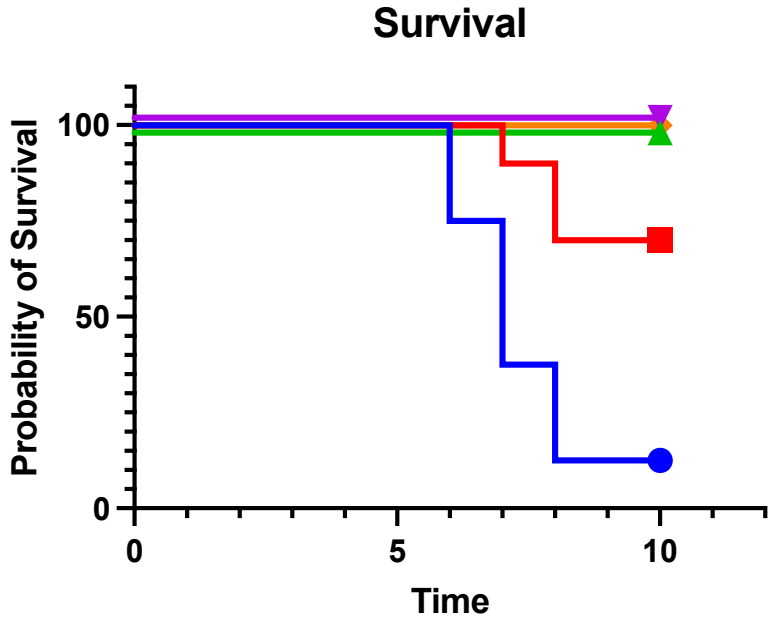




The full lengh rHA from A/New Caledonia/20/99 (H1N1) strain showed **excellent immunogenicity** properties in mice without adjuvant

Protection against influenza challenge





At week 16 post vaccination, the mice were challenged with a lethal dose of influenza A/California/4/2009 (H1N1), and monitored for weight (left). For humane reasons, mice reaching a weight loss of 20% were euthanized. As such, the survival curve (right) represent mice reaching this endoint, or not. **Interestingly, the reduced antibody responses observed following vaccination with non-adjuvanted A{ +}-HA (C1) could award some protection against the viral challenge, likely in combination with vaccine induced T cell responses.**

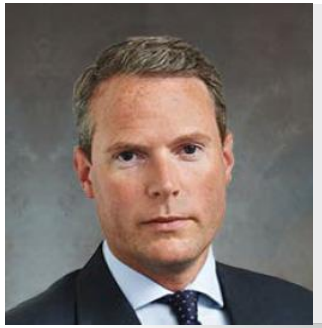
Management & Directors With Track Record Of Success/Value Creation

Highly Energized Management Team With Deep Industry Expertise & Products In Market
Active Board with Decades of Relevant Experience in Biomanufacturing

Mark Emalfarb
Founder, CEO
Serial Entrepreneur, Inventor 25+ U.S. and foreign biotechnology patents, filamentous fugal enzyme product commercialization

Ronen Tchelet
CSO
20+ years in Biopharmaceutical Industry & Recombinant Product Commercialization

Ping Rawson
CFO
20+ years of finance, accounting & international trade and business development experience



Matthew Jones
CCO
20+ years life professional management, business development and leadership of biopharma products

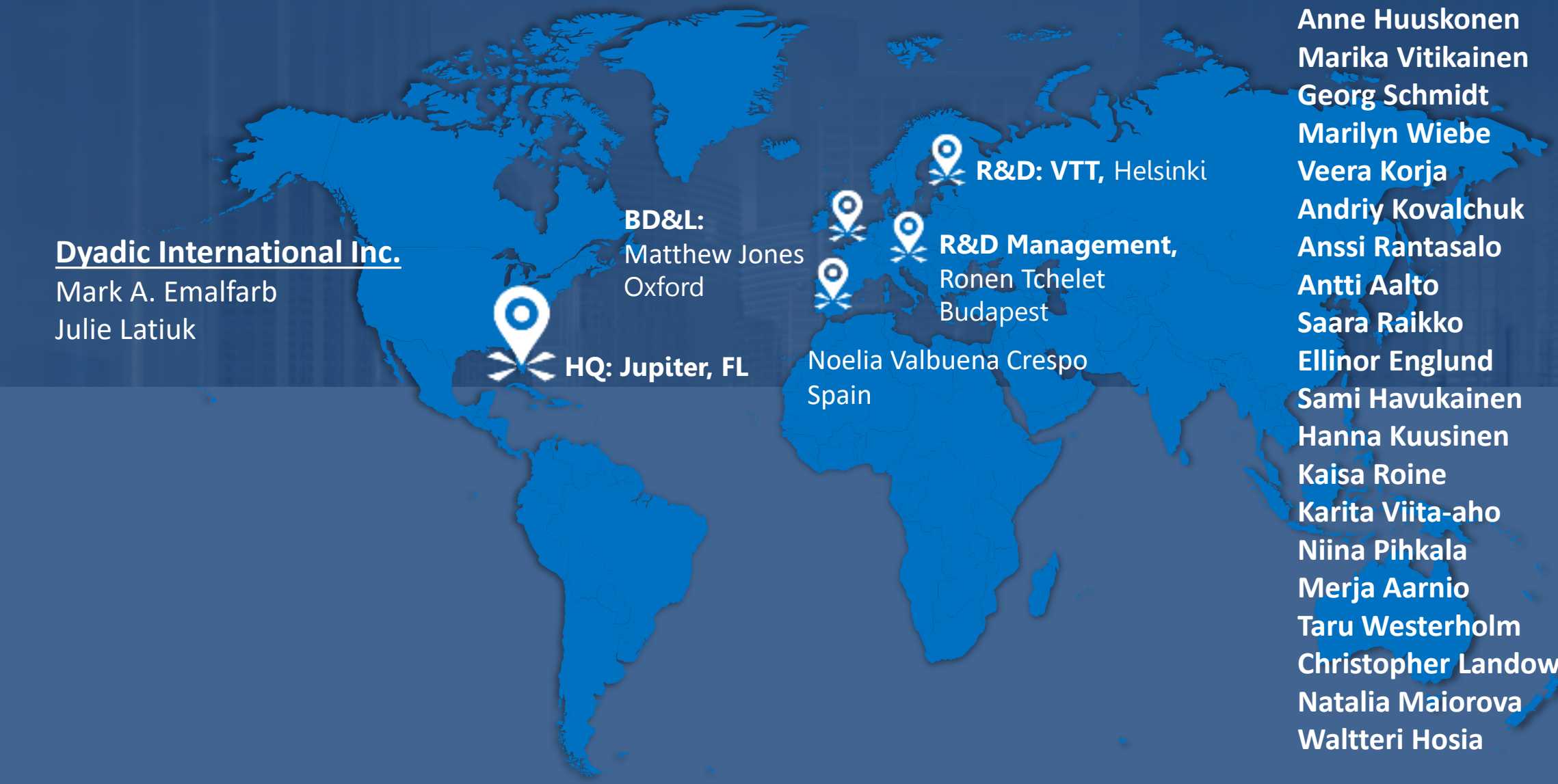
Dr. Arin Bose
Board Member
34 years bioprocess development and clinical manufacturing

Dr. Barry Buckland
Board Member
29 years R&D leadership | US National Academy of Engineering

Michael Tarnok
Board Member - Chairman
Seasoned pharma industry finance and operational executive

Patrick Lucy
Board Member
20+ years of bioprocess biotech and business development

Acknowledgements



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Anssi Rantasalo
Antti Aalto
Saara Raikko
Ellinor Englund
Sami Havukainen
Hanna Kuusinen
Kaisa Roine
Karita Viita-aho
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