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DEVELOPMENT OF THE FILAMENTOUS FUNGUS *Thermothelomyces heterothallica* C1 INTO A NEXT-GENERATION PRODUCTION PLATFORM FOR HUMAN AND ANIMAL VACCINES

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C1 protein production platform has been developed through more than 20 years of commercial genetically engineering. The thermophilic fungus *Thermothelomyces heterothallica* is a robust and versatile fungal expression system for the rapid production of proteins at very high levels. In the last 6 years, the C1 protein production platform has been further improved to become a safe and efficient expression system with the prime objective of speeding up the development and production of commercial scale human and animal vaccines, monoclonal antibodies, biosimilars, as well as other therapeutic proteins at larger quantities and lower cost.

C1 is a very efficient platform to produce antigens, even to generate multicomponent vaccines. The production levels of engineered C1 strains are similar in terms of yield and purity, reaching in some cases more than 2.5 g/L (in 4-5 days). In contrast to other vaccine platforms, C1 has a higher safety profile, and production can be scaled up in a more cost-effective manner using standard microbial *E. coli* fermenters. Stable cell lines have been developed to produce different antigens as influenza, neuraminidase, west Nile, rabies, rift valley fever..etc.
Here we present the production of Dyadic´s DYAI-100 vaccine candidate against the Covid-19 pandemic, based on the ~25 kilodalton-large Receptor Binding Domain (RBD) within the S1 subunit that was identified as the most effective SARS-CoV-2 neutralizing antibodies described to date. The use of C1 as vaccine production platform against the SARS-CoV-2 offers several advantages as follows:
(i) The C1 platform can support the global immunization strategies that are needed against emerging new SARS-Covid-2 variants. In 7 weeks, new RBD variant's genes can be inserted into the same C1 cell line (same genotype) to develop stable cell lines which can be used to produce antigens at the same production levels (1-2 g/L) in 4-5 days. So far, the following variants were successfully produced; Alpha (UK), Beta (SA) Gamma (BR) Delta (Ind) and Omicron (B.1.1.529) at levels and qualities that are similar to the Wuhan RBD – DYAI-100.
(ii) Safety and Persistence - the results show that DYAI-100 elicits safe, effective, and protective immune responses in several successful mice studies including challenge study with hACE2-transgenic C57BL/6J mice that demonstrated full protection without adverse events. Importantly, a rabbit toxicology study demonstrated that the C1-SARS-CoV-2 RBD vaccine candidate is safe and has the potential to be an effective vaccine with a safety and tolerability profile suitable for evaluation in humans. In addition, the study suggested a major beneficial effect of the vaccination demonstrating continued, and not declining antigenic stimulation, for relatively long duration, without any local or systemic adverse effect.

This DYAI-100 vaccine candidate has been produced under GMP standards (99% purity) and proposed for further testing in safety and efficacy clinical trials.

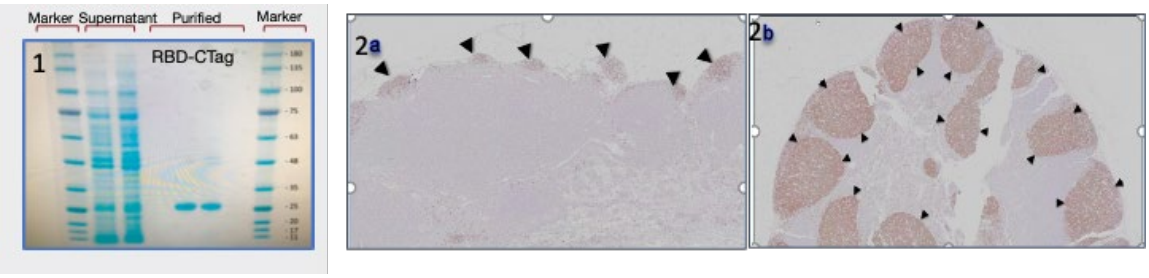

Figure 1: C1 RBD strain was run in 5L scale fermentation. The RBD was purified through CaptureSelectTM Ctag column rendering 98% purity, 70% recovery. Figure 2: (2a) The iliac lymph nodes from an animal injected with the Alhydrogel®'85' (Placebo) and (2a) and with C1-RBD Vaccine (2b) after sacrificed 42 days post first dosing (Recovery phase). Note, arrowheads in 2a- no evidence of germinal centers. The lesions (arrowhead in 2b) consist of mild germinal centers increased lymphocytic cellularity (i.e., follicular hyperplasia).