Absynth Biologics: A Novel Prophylactic Vaccine to Prevent *Clostridium difficile* Infections

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***C.difficile* infection (CDI)** causes severe diarrhoea in hospital patients when normal gut flora is disrupted by broad-spectrum antibiotics and despite antibiotic treatment, recurrence rates are 10–30% with mortality at ~20%. Vaccination could be effective but none is currently available and although three toxin-based products are in clinical development a significant proportion of patients do not response still highlighting a need for alternatives

Absynth Biologics is focused on the discovery and development of vaccines and antibodies to combat bacterial pathogens based on an innovative approach. Here Absynth presents evidence in support of their antigens providing a novel platform for a vaccine formulation for *C.difficile*:

INTRODUCTION: UNIQUE & ESSENTIAL ANTIGENS

- Absynth's platform of vaccine targets are based on essential, highly conserved antigens and have demonstrated vaccine mediated efficacy in Absynth's *S. aureus* programme
- Following the identification of *S.aureus* Ant2 and Ant3 orthologous sequences in *C.difficile (*C.diff-Ant2 and C.Diff-Ant3) , Absynth has successfully cloned and expressed these genes in *E.coli* – generating protein of acceptable purity and endotoxin levels for use in vaccine studies; potentially providing a non-toxin based alternative vaccine
- Initial proof of concept studies have been conducted to demonstrate efficacy in a mouse model of CDI

Murine *C.difficile* challenge: Survival endpoint - day 6

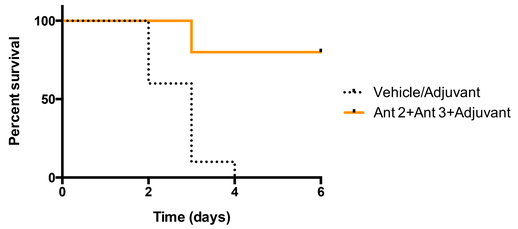

Fig. 1
Mice vaccinated with Absynth's Cdiff-Ant2+Cdiff-Ant3+Adjuvant are protected against *C.difficile* infection as illustrated in these experiments by an absence of weight loss in the treated animals (orange symbols) compared to the control treated with the adjuvant alone (black symbols). [Infective dose 6.33 x 10^7 CFU/mL] Log rank wilcoxon test p=0.0001 for hour or day survival data for high dose challenge

MATERIALS AND METHODS

- *In vivo* vaccination and challenge studies were performed by our outsource provider Evotec
- Briefly, vaccine formulated with both antigens and mixed with adjuvant was administered subcutaneously to C57/BL6 mice (n=10) (approx. 18-20g), including a control group with adjuvant alone. Schedule of dosing: prime (day 0), 1st Boost (day 14) and 2nd Boost (day 21)
- Following vaccination, mice were pre-treated for 10 days (cefoperazone) in drinking water with an additional treatment with clindamycin IP 24hrs prior to infection, before being inoculated with vegetative bacterial (dose ranges from 5x10^6 to 6 x 10^7 CFU/ml)
- Disease progression was monitored for up to 6 days: weight loss, temperature, diarrhoea, lethargy, mild dehydration. Mice were humanely euthanized at reaching the limits of disease progression. At necropsy CFUs were measured in the gut and faecal pellets included macro observation of gross gut pathology

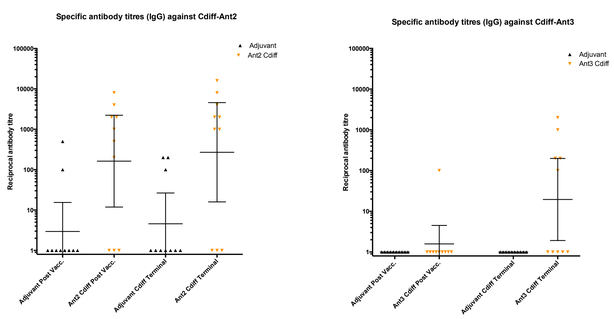

Fig. 2 Mice vaccinated with Absynth's Cdiff-Ant2+Cdiff-Ant3+Adjuvant showed specific antibody response as measured by ELISA. Data are individual animal endpoint titres with the geometric mean and 95%CL highlighted as the black bars. Endpoint titres were defined by the lowest dilution that resulted in three times greater than the background value for wells containing only conjugate

RESULTS

- Vaccination using C.diff-Ant2 and C.diff-Ant3 protects mice against *C.difficile* infection in a murine survival model as assessed by statistically significant survival (**Fig. 1**)
- Antibody responses assessed using a specific ELISA assay for Cdiff-Ant2 and Cdiff-Ant3 showed specific responses in serum for both in vaccinated animals (**Fig. 2**)
- *C.difficile* spore counts were isolated from the gut tissue (at necropsy) and faeces collected at time points post-infection, and expressed as mean CFU/ml or mean CFU/g (**Fig. 3, 4**). These data showed a difference between loads in vaccinated and control groups in the caecum and in faeces post-infection
- Post mortem analysis of macroscopic pathology using blinded visual scoring of the gut revealed that vaccinated animals appeared to show less observable pathology (**Fig. 5ab**).

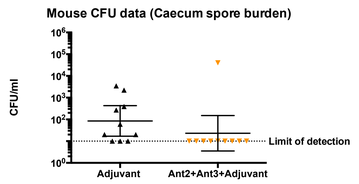

Fig. 3 Following *C.difficile* infection mice vaccinated with Absynth's Cdiff-Ant2+Cdiff-Ant3+Adjuvant had a lower spore burden in most organs (orange symbols) compared to the control treated with the adjuvant alone (black symbols). Statistical significance in caecum spore burden between groups measured by Mann-Whitney test (p=0.0198)

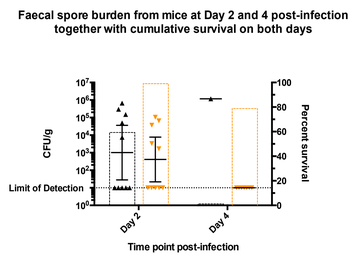

Fig. 4 Spore burden in the faeces: mice vaccinated with Absynth's Cdiff-Ant2+Cdiff-Ant3+Adjuvant had a lower spore burden post infection (orange symbols) compared to the control treated with the adjuvant alone (black symbols)

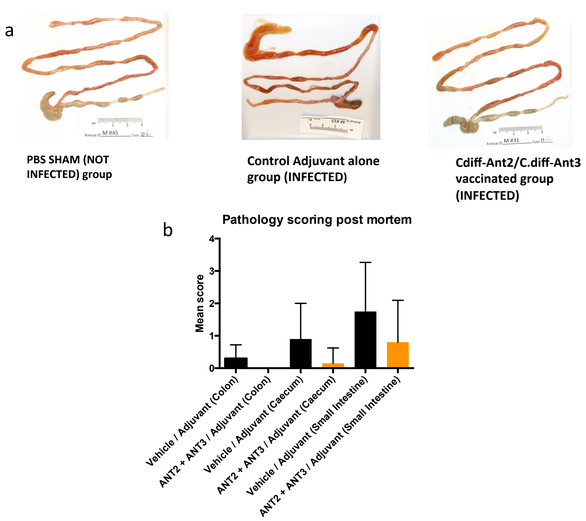

Fig. 5 (a) Images taken of the whole gut isolated from mice at necropsy. **(b)** Pathology scoring from analysis of whole gut: animals analysed blinded to treatment group and scored by an arbitrary pathology score index. Mice vaccinated with Absynth's Cdiff-Ant2+Cdiff-Ant3+Adjuvant appear to have less inflammation and pathology than control groups by macroscopic analysis.

CONCLUSIONS:

- Absynth has demonstrated vaccine-mediated protection using their proprietary antigens in mice challenged by *C.difficile*
- Current ongoing work is understanding the immunological and physiological responses to infection and vaccination-mediated protection, and enhancement of the efficacy and robustness of protection (for example optimization of adjuvant/antigen formulation)
- Absynth is currently advancing it's pipeline programmes towards partnering and clinical development