**SUPPLEMENTARY METHODS**

**Inclusion Criteria**

(1) Has provided written informed consent and is willing to comply with all Protocol scheduled visits, treatment plan, laboratory tests, and other trial procedures and in the opinion of the Investigator has a good understanding of the Protocol, the length of the study and the demands of the study.

(2) Aged between 18-80 (at time of consent);

(3) Have a pre-treatment histological diagnosis of Marsh grade 3 CeD;

(4) Have a pre-trial V:C >2.0;

(5) Have elevated tTG or endomysial Ab +ve pre-trial;

(6) Have been adherent to a gluten-free diet for >6 months pre-enrolment;

(7) Have a tTG <20 IU/mL (normal <15) at screening;

(8) If female, has met either of criterion “a or “b” below:

a. **If of non-childbearing potential**, has met 1 of the following – Amenorrheic for at least 2 years, or has had a hysterectomy and/or bilateral oophorectomy at least 8 weeks prior to screening, or has had a tubal ligation at least 8 weeks prior to screening.

b. **If of childbearing potential**, must be willing to use the acceptable methods of contraception.

(9) In the opinion of the Investigator is in good general health

**Exclusion criteria**

(1) Have any finding at screening that in the opinion of the Investigator or medical monitor would compromise the safety of the Participant or affect their ability to adhere to protocol scheduled visits, treatment plan, laboratory tests, and other trial procedures.

(2) Have participated in any other clinical trial and/or have received an investigational drug or device within 30 days of screening.

(3) Have history or current evidence of any of the following: compromised respiratory function (chronic obstructive pulmonary disease, respiratory depression, signs or symptoms of hypoxia at screening); thyroid pathology (unless stabilized and euthyroid for >3 months at the time of screening); hepatitis B, hepatitis C, or human immunodeficiency virus (HIV) infection; evidence of clinically significant chronic cardiac, hepatic or renal disease; psychiatric illness (poorly controlled); seizure disorder, autoimmune condition (e.g. insulin dependent diabetes mellitus or Addison’s disease) or any other chronic health issues that in the opinion of the Investigator would exclude the Participant from the trial.

(4) History of substance abuse or current substance abuse that in the opinion of the Investigator would exclude the Participant from the trial.

(5) Have a history of intolerance, allergy or hypersensitivity to the proposed placebo - Tabasco® Sauce or any of its known ingredients.

(6) Have a history of intolerance, allergy or hypersensitivity to the proposed anthelmintic – mebendazole.

(7) Have a history of intolerance, allergy or hypersensitivity to the proposed chemicals used in preparation of *N. americanus* – amphotericin B and Betadine that in the opinion of the Investigator would exclude the Participant from the trial.

(8) Current requirement for consistent use of anti-inflammatory drugs (includes prescription and OTC medication >2 doses per week, that in the opinion of the Investigator would significantly alter the Participant’s immunity), aspirin exceeding 125 mg/day or the use of immunotherapeutics;

(9) Diagnosis of cancer which has been in remission for < 5 years, excluding Participants with adequately treated or excised non-metastatic basal cell or squamous cell cancer of the skin or cervical carcinoma in situ.

(10) Poor venous access making the Participant unable to comply with the safety laboratory testing and/or endoscopy sedation requirements.

(11) Are an employee of the Sponsor, Investigator or study centre or immediate family of such employees or the Investigator.

**HLA genotyping analyses**

For HLA-DQA1 and HLA-DQB1 imputation analysis, genomic DNA was extracted from whole blood buffy coat samples using standard protocols (1). Samples were processed on the Illumina® Global Screening Array (GSA) Bead Chipsinitially to generate genotyping data for genome wide association studies. Bead chips were scanned on the Illumina® iScan System and analysed using Illumina® GenomeStudio Genotyping Module™Software v2.0. Four-digit *HLA-DQA1* and *HLA-DQB1* alleles were imputed using R package HIBAG (HLA Genotype Imputation with Attribute Bagging) (2). Imputed *HLA-DQA1* and *HLA-DQB1* alleles were combined (see **Supplementary Table 2**) to determine the HLA-DQ haplotypes and genotypes for each individual.

**Preparation of treatments**

Hookworm ova were collected from a single volunteer donor who was initially infected in 2013 with *N. americanus* L3, from a line donated by Professor David Pritchard (University of Nottingham) and maintained in-house since 2004. The appropriate number of visibly motile L3 were individually selected for inclusion in the inocula, contained with 300 µl of de-ionised water. Placebo inocula comprised of 300 µl of de-ionised water containing approximately 5 µl of Tabasco® sauce. Inocula tubes were maintained at ambient temperature (15-30°C) under temperature logged conditions from the time of production until time of inoculation (typically 2-14 days). The inoculation procedure involved dispensing the solution to a non-absorbent dressing pad that was placed onto the participant’s forearm.

**Blood, histopathology and symptom analyses**

Complete blood counts were measured in Australian and New Zealand accredited clinical pathology laboratories. Serum IgA-tTG titres in samples from all 4 sites were measured using a multiplex flow immunoassay (BioPlex® 2200 Celiac IgA and IgG) using a single pathology provider. Endoscopies were performed under sedation by experienced gastroenterologists (co-authors T.R., J.C., J.M., R.B.G., C.W. and M.N.). Four biopsies were collected from the mid-duodenum at each endoscopy and were processed for analysis of intraepithelial CD3+ T cells (IET) per 100 enterocytes after staining for CD3, Vh:Cd score and Marsh score as previously described (3, 4), using a single histopathology facility and histopathologist (G.M.). CSI and QoL analyses were performed as previously described (5, 6). Adverse events were documented throughout the study and suspected causality was recorded.

**SUPPLEMENTARY TABLES**

**Supplementary Table 1: Participant Demographics**

|  |  |  |
| --- | --- | --- |
|  | **Allocation** |  |
|  | **Placebo (n= 7)** | **L3-20 (n= 38)** | **L3-40 (n= 9)** | **Total (n=54)** |
| **Mean age (SD), years** | 43 (10) | 46 (13) | 45 (10) | 45 (12) |
| **Female, n (%)** | 7 (100%) | 27 (71%) | 7 (78%) | 41 (76%) |
| **Weight, mean (SD), kg** | 81 (23) | 78 (16) | 76 (13) | 78 (16) |
| **Height, mean (SD), cm** | 168 (5) | 173 (10) | 172 (5) | 172 (9) |
| **BMI, mean (SD), kg/m2** | 28.7 (8.7) | 25.9 (4.8) | 25.7 (4.1) | 26.2 (5.3) |

**Supplementary Table 2: HLA DQ haplotype determination**

|  |  |  |
| --- | --- | --- |
| ***HLA-DQB1*** | ***HLA-DQA1*** | **DQ haplotype** |
| 02:02 | 02:01 | DQ2.2 |
| 02:01 | 05:01 | DQ2.5 |
| 02.02 | 03:03 | DQ2.3 |
| 03:01 | 05:05 | DQ7.5 |
| 03:01/03 | 03:02 | DQ8 |

**Supplementary Table 3: HLA DQ haplotype determination**

|  |  |
| --- | --- |
| **HLA-DQ genotype** | **Study samples (*n*=50)** |
| DQ2.2/DQ2.2 | 1 |
| DQ2.2/DQ7.5 | 1 |
| DQ2.2/DQ8 | 2 |
| DQ2.5/DQ2.2 | 15 |
| DQ2.5/DQ2.5 | 2 |
| DQ2.5/DQ7.5 | 4 |
| DQ2.5/DQ8 | 2 |
| DQ2.5/DQX | 14 |
| DQ8/DQ7.5 | 3 |
| DQ8/DQ8 | 2 |
| DQ8/DQX | 4 |
| DQ2.3/DQ7.5 | 0 |
| **Total** | **50** |

DQX = Not DQ2.2, DQ2.5, DQ2.3, DQ7.5 or DQ8

**Supplementary Table 4: Relative changes in secondary outcome parameters in each study group between baseline and week 42**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Placebo** | **L3-20** | **L3-40** |
| **Vh:Cd ratio, mean (95% CI)** | -0.6 (-1.3–-0.2) | -0.5 (-0.8–-0.2) | -1.1 (-1.8–-0.4) |
| **IET%, median (IQR)** | 8 (6–14) | 21 (10–36) | 36 (21–48) |
| **tTG (U/ml), median (IQR)** | 2 (0-3) | 0 (0-5) | 2 (0-4) |
| **CSI units, mean (95% CI)** | -4.0 (-11.4 - 3.4) | -0.2 (-1.8 – 1.4) | -1.7 (-3.3– -0.1) |
| **QoL units, mean (95% CI)** | -16.2 (-32.7 – 0.3) | -5.2 (-9.8 – -0.5) | -7.3 (-14.9– -0.3) |

**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure 1. Hookworm infection was not associated with anaemia in trial participants.** Serum (**A**) hemoglobin and (**B**) iron levels in the 3 clinical cohorts, determined at each clinic visit. Greyed area indicates the normal ranges for each parameter. Each individual data point is shown along with mean ± 95% CI.

**SUPPLEMENTARY REFERENCES**

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