Questionnaire - Background Study Information for Histopathology Requests

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Basic concepts:
- What was the study's hypothesis?
  - Mice colonized with fecal microbiota derived from human patients with autoimmune thyroiditis have a more severe thyroiditis compared to mice colonized with fecal microbiota from human healthy donors.
- What specific scientific questions would you like to answer with the histological staining?
  - Severity of spontaneous autoimmune thyroiditis (SAT) scored of FFPE sections of the thyroid gland.
- What disease is your animal model intended to recapitulate?
  - Autoimmune thyroiditis
- What are the model's strengths? Weaknesses?
  - This is currently the only well-characterized murine model which spontaneously develops autoimmune thyroiditis.
  - Weaknessess: 60% of female NOD.H-2h4 mice, 20–24 weeks of age, develop salivary gland infiltration (Sjogren's syndrome, SS). However, in human, Hashimoto’s autoimmune thyroiditis can be present of 30% of SS symptoms.
- Any other important details?

Animal model:
- What is the model? NOD-H.2h4
- What species? Mice
- Age? 13 to 19 weeks old
- Gender? Female
- Genotype?
- If a unique genotype(s), what are knock out / knock in traits?

Treatments: (if any)
- What it the primary treatment?
  - Group A) no antibiotic pretreatment, no fecal microbiota transplantations (FMTs)
  - Group B) no antibiotic pretreatment, sham FMTs (PBS only)
  - Group C) antibiotic pretreatment, autologous FMTs (e.g. murine feces)
  - Group D) antibiotic pretreatment, FMTs from healthy human donors
  - Group E) antibiotic pretreatment, FMTs from human patients with autoimmune thyroiditis
What is the treatment regimen (route of administration, vehicle concentration & volume, number of treatments, treatment intervals, time interval between last dose and death)?

- Antibiotic pretreatment consists of 5 consecutive days of 10 mg/ml metronidazole, ampicillin, and neomycin, and 5 mg/ml vancomycin given via oral gavage (200 microL per gavage per mouse)
- FMTs treatment consists of once weekly gavage of 200 microL per mouse

What is primary treatment mechanism of action? If unknown, what about MOA of another compound in the same drug family?

- A perturbed microbiome composition may promote the development of an autoimmune disease via a reduced integrity of intercellular junctions (leaky gut), via the generation of self-antigens by posttranslational modification of proteins, via lipopolysaccharide (LPS)-induced Toll-like receptor 4 activation, and/or induction of a type1 (Th1) to type 2(Th2) T helper cell shift. (ref: Fröhlich, E. & Wahl, R. Microbiota and Thyroid Interaction in Health and Disease. Trends Endocrinol. Metab. 30, 479–490 (2019)).

What are the target organs of the drug?
- Thyroid
- Gut microbiome composition

Are there known off target effects?

What are the other study variables (i.e. time points, genotypes, diets)?

- At day 4 – all mice received 0.05% NaI (sodium iodine) in their drinking water for the rest of the study
- Normal chow diet

Study design:

What were the in-life biomarkers?
- (Fresh) fecal samples are collected once weekly (on the day on the FMT treatment)

Were there any clinical signs of toxicity/reaction to materials? Any clinical differences between the genotypes?

What other endpoints were assessed? If so, what are your findings to date?

Where there matched controls? (ie, normal diet, untreated and/or normal diet-sham injected, etc?)

- Matched controls are group A and group B (see above)

How many tissue samples total? 96 mice

How many of each tissue type? 6 tissues each: thyroid, salivary gland, thymus, spleen, liver, ileum, colon