SUPPLEMENTARY MATERIALS

Manuscript NO: 125111 — Jason A Brianton, Marina Yu Kochevalina, Anton P Bonartsev, Vera V Voinova — "Putative role of drug-resistant helminths in tissue engineering of gastrointestinal tract: a hypothesis" — World Journal of Stem Cells

1. SCOPE AND LITERATURE SELECTION

The principal search strings, given in PubMed syntax:

Gastrointestinal tissue engineering:

("Tissue Engineering"[MeSH] OR "Regenerative Medicine"[MeSH] OR "Organoids"[MeSH] OR scaffold*[tiab] OR decellulari*[tiab] OR "cell sheet*"[tiab] OR "organ-on-a-chip"[tiab] OR "gut-on-a-chip"[tiab] OR bioreactor*[tiab]) AND ("Gastrointestinal Tract"[MeSH] OR intestin*[tiab] OR colon*[tiab] OR esophag*[tiab] OR oesophag*[tiab] OR "Short Bowel Syndrome"[MeSH] OR "Inflammatory Bowel Diseases"[MeSH])

Microbiota and intestinal stem cell crosstalk:

("Gastrointestinal Microbiome"[MeSH] OR microbiota[tiab] OR microbiome[tiab] OR "Fatty Acids, Volatile"[MeSH] OR butyrate[tiab] OR propionate[tiab] OR lactate[tiab] OR "indole-3-propionic acid"[tiab] OR "Bile Acids and Salts"[MeSH]) AND ("Intestinal Mucosa"[MeSH] OR "intestinal stem cell*"[tiab] OR Lgr5[tiab] OR crypt*[tiab] OR "Paneth Cells"[MeSH] OR regenerat*[tiab] OR "epithelial renewal"[tiab])

Helminth immunobiology and epithelial interaction:

("Helminths"[MeSH] OR "Helminthiasis"[MeSH] OR nematod*[tiab] OR Heligmosomoides[tiab] OR Trichuris[tiab] OR Nippostrongylus[tiab] OR hookworm*[tiab] OR Ancylostoma[tiab]) AND ("Immunomodulation"[MeSH] OR "Th2 Cells"[MeSH] OR "T-Lymphocytes, Regulatory"[MeSH] OR "Interleukin-10"[MeSH] OR "excretory-secretory"[tiab] OR "Extracellular Vesicles"[MeSH] OR "Intestinal Mucosa"[MeSH] OR "Stem Cells"[MeSH] OR "Wound Healing"[MeSH])

Anthelmintic resistance:

("Anthelmintics"[MeSH] OR "anthelmintic resistan*"[tiab] OR benzimidazole*[tiab] OR "macrocyclic lactone*"[tiab] OR ivermectin[tiab] OR levamisole[tiab] OR imidazothiazole*[tiab] OR monepantel[tiab] OR emodepside[tiab]) AND ("Drug Resistance"[MeSH] OR resistan*[tiab] OR "beta-tubulin"[tiab] OR "P-Glycoprotein"[MeSH] OR "glutamate-gated chloride"[tiab] OR "faecal egg count reduction"[tiab] OR "fecal egg count reduction"[tiab]) AND (helminth*[tiab] OR nematod*[tiab])

Antimicrobial resistance and the gut resistome:

("Drug Resistance, Bacterial"[MeSH] OR "antimicrobial resistance"[tiab] OR resistome[tiab] OR "Gene Transfer, Horizontal"[MeSH] OR "antibiotic resistance gene*"[tiab] OR "Vancomycin-Resistant Enterococci"[MeSH] OR "Clostridioides difficile"[MeSH]) AND ("Gastrointestinal Microbiome"[MeSH] OR gut[tiab] OR intestin*[tiab] OR "Dysbiosis"[MeSH] OR "Fecal Microbiota Transplantation"[MeSH])

Reference lists of retrieved articles and relevant reviews were hand-searched to identify further sources (backward snowballing), and forward citation tracking was performed for the key primary studies underpinning each section. Because the review deliberately bridges literatures that are seldom indexed together, a small number of sources bridging two domains — for example, helminth–microbiota interaction and helminth immunomodulation in transplantation — were identified through this citation tracking rather than through the database searches.

Records were eligible for inclusion if they were peer-reviewed primary studies, systematic reviews, or authoritative guideline or agency documents published in English, and if they addressed at least one of: the design, fabrication or in vivo performance of GI tissue-engineered constructs; microbiota- or metabolite-mediated regulation of intestinal epithelial renewal; helminth-driven immune or epithelial modulation of the intestine; or

the epidemiology and molecular basis of anthelmintic or antimicrobial resistance relevant to the gut. Conference abstracts, preprints, non-peer-reviewed commentary, and articles without an accessible full text were excluded. Where several studies reported the same finding, the earliest definitive report and the most recent confirmatory or contradictory report were preferred.

Screening was performed by 4 authors (**Jason A Brianton, Marina Yu Kochevalina, Anton P Bonartsev, Vera V Voinova,**), who reviewed titles and abstracts independently and resolved disagreements by discussion, with **Jason A Brianton** adjudicating where consensus was not reached. Full texts of retained records were read in full before citation. Every reference cited in this review was independently verified against the published record (title, authorship, journal, year, and DOI or PMID), and each in-text citation was checked against the specific claim it supports; references that did not directly support the statement attributed to them were replaced or removed.

The different elements of this review varies greatly in directness, all substantive claims were assigned to one of five evidence tiers, defined in Table S1 and applied throughout the text.

Table S1. Evidence tiers used in this review

Tier	Definition	What this means for the present review	Example
D	Direct clinical evidence Obtained in a tissue-engineered GI construct, in vivo or in a clinical setting, with the biotic factor of interest present	No claim in this review is supported at this tier. No published study has combined helminth infection, defined microbiota manipulation and an implanted GI construct.	None available
P	Preclinical in vivo Animal models of intestinal injury, infection or regeneration, without an engineered construct	Supports most mechanistic claims about helminth-driven epithelial reprogramming and microbiota-ISC signalling. Species and model dependence limits extrapolation.	Fetal-like reversion of the ISC niche in H. polygyrus-infected mice
O	Organoid and in vitro Organoid, gut-on-chip, co-culture or cell-line systems	Supports metabolite-ISC mechanisms and helminth ESP/EV effects on epithelium. Lacks immune, vascular and luminal-flow context.	Conditioned-medium effects on organoid growth; microbiota-metabolite effects on organoid barrier function
C	Clinical, adjacent field Human studies in fields other than GI tissue engineering	Supports inferences drawn from HSCT, FMT decolonisation and helminth-therapy trials. Transferability	Microbiota diversity and mortality after allogeneic HSCT; FMT for recurrent CDI;

		to tissue engineering is assumed, not demonstrated.	Trichuris suis ova trials in IBD
V	Veterinary Livestock and companion-animal data	Supports nearly all anthelmintic-resistance claims. Parasite ecology, drug exposure and host management differ substantially from clinical regenerative medicine.	Multidrug resistance in Haemonchus contortus and Ancylostoma caninum
M	Mechanistic inference No direct test; reasoning from established biology across two or more of the above	Supports the triad framework itself and every claim linking resistance to graft outcome. These are hypotheses.	Dysbiosis plus resistant helminths compounding to impair graft integration