Parasitic Helminths: New Perspectives in Biology and Infection

Hotel Bratsera, Hydra, Greece

Abstracts for Oral Presentations

4 September 2023

Monday 4 September

09:00-10:40 Session 1. Host-Parasite Interactions. Chair Niki Gounaris

09:00	**Maria Duque-Correa**	**Cambridge, UK**	**Unravelling the whipworm niche at the host intestinal epithelia**
09:40	Omer Bay	Manchester, UK	The first genome-scale metabolic model of parasitic whipworm: gateway to the rapid discovery of novel host pathogen interactions and therapeutic targets
10:00	Simone Haeberlein	Giessen, DE	Combined single-cell and spatial transcriptomics provide unprecedented molecular insights valuable for basic and applied research on liver flukes
10:20	Kerstin Fischer	Washington, USA	Deep visual proteomics of the protein inventory of *Onchocerca volvulus* neoplasms

10:40 – 11:10 Coffee Break

11:10 – 12:50 Session 2. Immune Effector Mechanisms. Chair Rick Maizels

11:10	**Lida Derevnina**	**Cambridge, UK**	**Cyst nematodes counteract immunity by inhibiting activation of central nodes of a Solanaceae immune receptor network**
11:50	Unnati Sonawala	Cambridge, UK	Understanding the HYPervariability of HYP effectors in potato cyst nematodes
12:10	Alexandra Ehrens	Bonn, DE	Microfilariae-induced eosinophil ETosis is NOX- and inflammasome-dependent
12:30	Nicolas Pionnier	Manchester, UK	Natural Killer cell activation and memory-like phenotype development following helminth infection

16:00 – 18:00 Session 3. Drug Development. Chair Thomas Spangenberg

16:00	Andrew Fraser	Toronto, CA	Closing in on new anthelmintics that target rhodoquinone-dependent metabolism
16:20	Daniel Sprague	Wisconsin, USA	Developing novel flatworm ion channel ligands to treat various neglected tropical diseases
16:40	Hala Fahs	New York, USA	Multi-species nematode screening uncovers new classes of broad-spectrum anthelmintic compounds.
17:00	Wannaporn Ittiprasert	Washington, USA	All-in-one lipid nanoparticle delivery of programmed gene knock-in in *Schistosoma mansoni*
17:20	**Poster Pitches 1-17 (2-minutes each)**		

1) The first genome-scale metabolic model of parasitic whipworm: gateway to the rapid discovery of novel host pathogen interactions and therapeutic targets

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Trichuris trichiura, human whipworm, is one of the most common soil-transmitted helminths of man and often modelled in laboratories using the closely related mouse whipworm, *Trichuris muris*. The immune response to mouse whipworm has been extensively studied but relatively little is known about the detailed biology and biochemistry of the parasite. Genome-scale metabolic models are widely used to enhance our understanding of metabolic features of organisms, host-pathogen interactions and to identify novel therapeutics for diseases. In our study, we developed the first genome-scale metabolic model of the mouse whipworm *Trichuris muris*, iTMU798. The model demonstrates the metabolic features of *T. muris* and allowed the prediction of metabolic steps essential for its survival. Specifically, that the enzyme Thioredoxin Reductase (TrxR) was essential for worm survival, a prediction we validated *in vitro* with the drug auranofin. Furthermore, our observation that the *T. muris* genome lacks *gsr-1* encoding Glutathione Reductase (GR) but has GR activity that could be inhibited by auranofin indicates a novel mechanism for the reduction of glutathione by the TrxR enzyme in *T. muris.* Overall, iTMU798, as the first whipworm genome-scale metabolic model, is as a powerful tool to study not only the *T. muris* metabolism but also other *Trichuris spp.* in understanding host parasite interactions and the rationale design of new intervention strategies.

2) Combined single-cell and spatial transcriptomics provide unprecedented molecular insights valuable for basic and applied research on liver flukes

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Fasciolosis is a food-borne trematode infection caused by the liver fluke *Fasciola hepatica* and related species. Comprehensive knowledge on the parasite's cell types and cell-specific gene expression repertoire is missing to date, but would boost research e.g. on drug target genes or developmentally important genes. We applied single-cell (sc) transcriptomics and spatial transcriptomics (ST) technologies by 10x Genomics on *F. hepatica* to establish a cell and tissue atlas of gene expression in this parasite. scRNA-seq was performed on single-cell preparations and ST on cryosections from adult worms. The ST method involves capturing and barcoding of transcripts *in situ* using oligonucleotide-coated glass slides. The positional information from barcodes allows to visualize gene expression spatially resolved and within the original morphological context. By performing differential gene expression analysis, we successfully identified 15 cell clusters by scRNA-seq that represent distinct cell types, including gastrodermal cells expressing cathepsins, neoblasts expressing nanos2, and neuronal cells. With ST we could discriminate eight different tissues, such as intestine, tegument and reproductive organs. Marker genes for each sc and ST cluster were identified and their expression validated by *in situ* hybridization. Single-cell RNA velocity analyses revealed marker genes defining a germline lineage. Furthermore, our new expression atlas enabled us to reveal several drug target genes (such as ß-tubulins, protein kinases and calcium channels), drug resistance genes, and transcription factors with cell type- or tissue type-specific expression. Functional characterization using RNA interference and small-molecule inhibitors identified a tegumental calcium channel and a tegumental protein kinase with importance for worm survival. Taken together, this work provides the first transcriptomes for the liver fluke *F. hepatica* on a single-cell and spatial resolution. Our expression atlas serves as playground for the discovery of tissue-type and cell-type specific genes in this parasite.

3) Deep visual proteomics of the protein inventory of *Onchocerca volvulus* neoplasms

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The nematode *Onchocerca volvulus* is targeted by WHO for elimination. Main strategy for elimination is mass treatment with ivermectin. The drug is microfilaricidal, but weak macrofilaricidal effects have been reported. A fraction of adult *O. volvulus* contain pleomorphic neoplasms, and their development is more common after ivermectin. We analyzed 428 females by histologicaly of paraffin embedded nodule from a trial of ivermectin combination treatments. Neoplasms were present in 5.6% of these worms. The purpose of this study was to compile protein inventories of adult worm tissues to identify protein profiles associated with neoplasms. We used digital image analysis of tissue sections, laser capture microscopy and highly sensitive mass spectrometry (ThermoScientific, Eclipse). Neoplasm tissue from three females was analyzed, and compared with normal tissues from the body wall, uteri and intestine of these worms and also tissues from a healthy female without neoplasm. Unlike healthy females, females with neoplasms did not show any signs of embryogenesis. Protein were called present, if supported by 2 peptides and found in at least 2 of 3 replicates. In worms with neoplasms, we detected 151 proteins in the body wall, 215 proteins in the intestine, 47 proteins in the uterus and 1577 proteins in the neoplasms. The uterus of the healthy female with embryogenesis had a high number (1710) of proteins detected that was similar to that of neoplasms. A majority of the 20 most abundant proteins detected in neoplasms was conserved, and only two proteins were nematode specific. Proteins that were found in neoplasms but not in the other analyzed tissues included peroxiredoxins, proteases, proteins related to signal transduction, and ribosomal or proteasome activity. In conclusion, we have successfully used deep visual proteomics to analyze the proteome of individual *O. volvulus* tissues in nodule sections and identified proteins that are overexpressed in neoplasms.

4) Understanding the HYPervariability of HYP effectors in potato cyst nematodes

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Plant-parasitic nematodes have evolved a large repertoire of effectors. Like many effectors, HYPs are secreted into the host and are necessary for infection. HYP genes consist of a 'hyper-variable' domain characterized by variable number, organization, and subfamily-specific repeats. The hyper-variable domain is flanked by 410 and 94 nucleotides that have remained 95% identical for ~30 million years of evolution. The objective of this research is to understand how it is possible for the genome of an animal to permit such variability in a single domain of a gene family, while maintaining the stability of the genome in general, and HYPs in particular. In order to capture the entire HYP gene in a single read we sequenced the genomes of *Globodera pallida* and *Globodera rostochiensis* using long-read sequencing technologies. Strikingly, we found that the dominant majority of HYP variation is allelic. Furthermore, through direct mRNA sequencing we discovered that this genomic diversity is also expressed at the transcriptional level. To unravel the extent of such unprecedented diversity in an allelic series of a single gene, we have performed Cas9-based targeted Nanopore sequencing to enrich for HYP gene containing locus. Using statistical analysis, we estimate this to be close to a thousand alleles. Additionally, we have performed amplicon sequencing of multiple individuals across the lifecycle using Pacbio HiFi sequencing to understand when and how HYP variation is introduced. Latest results from these efforts at understanding the extent and nature of HYP diversity, and potentially yet unknown biology underlying HYP variation will be presented.

5) Microfilariae-induced eosinophil ETosis is NOX- and inflammasome-dependent

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Eosinophils are important for protective immunity against parasitic filarial nematodes. Granulocytes possess different effector mechanisms such as the extracellular DNA trap cell death (ETosis). Thereby, intracellular DNA is explosively released, entrapping and killing pathogens. However, its contribution to protective immunity against filariae remains unclear. We demonstrated that microfilariae (MF) of the rodent filaria *Litomosoides sigmodontis* induce eosinophil ETosis (EETosis) *in vitro*, which is mediated by the dectin-1 receptor. *In vivo*, the *L. sigmodontis* infection increases the local and peripheral DNA concentration in dependence of eosinophils. Moreover, DNA traps facilitate the clearance of MF from the peripheral blood, indicating that EETosis is an essential protective mechanism by eosinophils. However, the role of ETosis in pathology development is less clear. Immune responses by granulocytes towards dead MF have been shown to contribute to dermatitis and vision impairment in onchocerciasis patients, while viable MF rarely elicit inflammation. Interestingly, we observed that viable and dead MF induce different EETosis signaling cascades. While viable MF induce EETosis in a NADPH oxidase (NOX)-dependent manner, dead MF trigger a Ca^{2+}-dependent EETosis. Therefore, we determined calcium influx, ERK, p38 and Pyk2 phosphorylation, inhibited calcium, determined histone citrullination and trap formation using wild-type and NOX knockout mice during MF-induced EETosis. Moreover, the canonical inflammasome pathway is involved during MF-induced EETosis. The canonical inflammasome assembles after activation of a sensor molecule, which recruits pro-caspase-1 and the adaptor molecule ASC. As an executing caspase, caspase-1 is able to cleave GSDMD, which forms pores in the cellular membrane. During MF-induced EETosis, active caspase-1 was detected in DNA traps. Inhibition of caspase-1 as well as eosinophils generated from caspase-1, ASC and GSDMD knockout mice failed to release DNA in response to MF. Using eosinophils from AIM2 knockout mice, we identified AIM2 as the responsible inflammasome sensor. Moreover, we visualized GSDMD pores during EETosis.

6) Natural Killer cells activation and memory-like phenotype development following helminth infection

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Helminths are the most common infectious agents worldwide, with more than 400 million people suffering from lymphatic filariasis or schistosomiasis. Type 2 inflammation is a hallmark of nematode tissue infection and is implicated both in immunopathology and eosinophil-dependent immunity. Type-2 innate lymphoid cells (ILC2) are usually associated with the initiation of type 2 inflammation in helminth infection. However, we noticed that they surprisingly failed to expand following *Brugia malayi* experimental peritoneal filarial infections. Conversely natural killer (NK) cells, usually associated with a Type 1 immune response, rapidly expanded, and represented over 90% of the ILC population in the first week of infection. Interestingly, specific ablation or depletion of the NK cell compartment in RAG2 or RAG2gc immunodeficient mice using anti-NKp46 or asialo GM1 antibody injections led to increased susceptibility to chronic adult *B. malayi* infection and impaired granulocyte recruitment to the site of infection. We have also demonstrated that in RAG2 deficient mice, drug clearance of a primary *B. malayi* infection followed by challenge infection led to resistance against early larval *B. malayi* establishment. This innate resistance was associated with bolstered NK and eosinophils whereby NK cells expressed markers of memory-like/enhanced activation (increased expression of IFNg and Ly6C). Our data thus promotes a novel functional role for NK cells in immunoprotection against experimental primary and secondary filarial infection which can proceed in the absence of adaptive immune regulation. Interestingly, we also observed similar NK cell expansions following murine *Schistosoma mansoni* helminth infections over a 15 weeks' time-course longitudinal experiment. NK cell activation data is currently being analysed and will inform on potential wider scope of NK cell functional fates than initially thought and on their role to play in innate-memory protection from *S. mansoni* infection.

7) Closing in on new anthelmintics that target rhodoquinone-dependent metabolism

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Soil-transmitted helminths (STHs) are major human pathogens, infecting over a billion people worldwide. STHs can survive for months in the highly anaerobic conditions of the human gut and do this by using an unusual form of anaerobic respiration. This requires rhodoquinone (RQ), an electron carrier that is only made and used by the parasite and not by the animal host. RQ is thus an ideal drug target: blocking RQ synthesis or use should kill the parasite while leaving the host unharmed.

We established a simple assay for RQ-dependent metabolism in the nematode *C.elegans* and used this to dissect the pathway for RQ synthesis. We showed that RQ synthesis requires 3-hydroxyanthranilate (3HA) as a precursor. This is generated by metabolism of tryptophan via by the kynurenine pathway and we also showed that mutations or drugs that block the kynurenine pathway inhibit RQ synthesis establishing the kynurenine pathway as a key anthelmintic target. We have now screened >50k compounds and natural products to identify drugs that block RQ-dependent metabolism. We have found a new class of benzimidazoles that are extremely potent species-selective Complex I inhibitors — excitingly some of these Complex I inhibitors are potent anthelmintics in *H.bakeri*. We will present the data from our drug screens including specific screens for kynurenine pathway inhibitors that have yielded multiple classes of potent compounds that also affect RQ levels. We will also present new data showing that the microbiome can have strong effects on RQ-dependent metabolism suggesting that altering the microbiome could affect STHs in vivo. We believe that we are making concrete progress towards new classes of anthelmintics that specifically target RQ-dependent metabolism and RQ synthesis and are excited to present the recent advances.

8) Developing novel flatworm ion channel ligands to treat various neglected tropical diseases

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Praziquantel (PZQ) is the mainstay for treatment of parasitic flatworm infections. After ~40 years of clinical usage, a putative target for PZQ was identified: a transient receptor potential ion channel in the melastatin subfamily ($TRPM_{PZQ}$). This channel is conserved across all profiled parasitic flatworm species, and *in vitro* potency at each ortholog mirrors the known clinical sensitivity of each parasite. Notably, *Fasciola* spp. are insensitive to PZQ mirroring the lack of activation of *Fasciola* spp. $TRPM_{PZQ}$ *in vitro*. It was discovered that there are residues in the binding pocket of $TRPM_{PZQ}$ responsible for the observed differential sensitivity, and this knowledge prompted the development of molecules that could tolerate this variation. Given the increasing resistance of *Fasciola* spp. to triclabendazole, the discovery of new fasciolicidal targets is timely. Therefore, for proof-of-principle the decision was made to interrogate the druggability of *Fasciola hepatica* $TRPM_{PZQ}$ (*Fh*.$TRPM_{PZQ}$). Employing a target-based screen of >600,000 small molecules against both *Sm*.$TRPM_{PZQ}$ and then *Fh*.$TRPM_{PZQ}$, a series of chemotypes that activate both channels were identified, and the pharmacophore shown to be most potent at *Fh*.$TRPM_{PZQ}$ was selected for further development. A library of molecules was synthesized to interrogate structure-activity relationships around the core of this molecule resulting in **S55**, a new molecule with submicromolar potency at both channels. When applied *ex vivo* to *Schistosoma mansoni*, **S55** produced rapid contraction of the flatworm with concomitant tegument damage, phenocopying PZQ. Likewise, **S55** produced an identical phenotype on freshly excised triclabendazole-sensitive and triclabendazole-resistant *Fasciola hepatica*. **S55** was non-toxic in HepG2 assays and is active and well-tolerated *in vivo*. Given these results, the data show that there is differential druggability among flatworm $TRPM_{PZQ}$ orthologs and demonstrate that *Fh*.$TRPM_{PZQ}$ is a druggable target that warrants further exploration.

Multi-species nematode screening uncovers new classes of broad-spectrum anthelmintic compounds

9) Hala Fahs[1], Fathima S. Refai[2], Suma Gopinadhan[2], Patricia G. Cipriani[1,2], Robert White[2], Yasmine Moussa[2], Stephan Kremb[2], Xin Xie[2], Yanthe Pearson[2], Glenn L. Butterfoss[2], Gennaro Esposito[2], Rick Maizels[3], Antony Page[3], Fabio Piano[1,2], Kristin C. Gunsalus[1,2]

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Parasitic helminths are a major global health threat, infecting nearly one-fifth of the human population and causing significant losses in livestock and crops. New anthelmintic drugs are urgently needed, as resistance to existing drugs is emerging. Using the NYUAD HTS platform, we screened ~50,000 compounds for broad anthelmintic properties while being non-toxic to human cells. The screen identified most known anthelmintics and additionally ~150 compounds with no previously reported anthelmintic activity. Among these, four related natural compounds caused dose-dependent lethality in *Caenorhabditis elegans* and *Pristionchus pacificus* across all developmental stages, including dauer and embryos, while being relatively well tolerated in mammalian cells. These molecules also caused mortality in direct testing on three veterinary parasitic nematode species: the multidrug resistant *Haemonchus contortus* UGA strain (ruminants), *Teladorsagia circumcincta* (sheep and goat) and *Heligmosomoides polygyrus* (rodents). *In vivo* preclinical trials in mice infected by *Heligmosomoides polygyrus* helminths further showed that these compounds cause a consistent and significant reduction in *H. polygyrus* egg laying and adult worm load. In-depth phenotypic characterization in *C. elegans* revealed abnormal lipid accumulation, mitochondrial defects, reduced oxygen consumption, diminished ATP levels, and increased reactive oxygen species. Together with genetic and pharmacological perturbations, these results point to lipid metabolism as the key target pathway of this novel cluster of anthelmintic compounds.

10) All-in-one lipid nanoparticle delivery of programmed gene knock-in in *Schistosoma mansoni*

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CRISPR/Cas performs site-specific gene disruption, repair, and modification *via* DNA repair pathways. The omega-1 ribonuclease secreted by the schistosome eggs plays a central role in pathogenesis and disease transmission. Previously, we reported that CRISPR is active in schistosomes and, specifically, catalyzed non-homologous end joining (NHEJ) and homology directed repair (HDR) at the targeted *omega-1* (*ω1*) locus in *Schistosoma mansoni*, when CRISPR was delivered by lentiviral or by ribonucleoprotein (RNP) systems. Although lentiviral delivery is a convenient, hands-off approach, random chromosomal integration of the proviral transgene and its maintenance and potentially continuous expression of Cas9 and guide RNA (gRNA) are drawbacks. Delivery by RNPs along with the donor DNA template requires that all CRISPR components reach the same target cell, the success of which is stochastic and unpredictable. By contrast, CRISPR delivery using lipid nanoparticles (LNPs) offers advantages over both these approaches because all these CRISPR elements, i.e., Cas nuclease, gRNA and donor template are complexed within a single LNP. Here, we investigated delivery of CRISPR by LNPs. All-in-one delivery to the schistosome egg was undertaken for Cas9 mRNA, gRNA and donor DNA [termed LNP-mRNA] and for RNP and donor DNA [termed LNP-RNP]. In terms of outcomes, successful programmed editing was achieved as indicated by the absence of *ω1* transcripts at day five post LNP delivery while programmed KO of *ω1* was confirmed by analysis of NGS reads, which revealed indel levels of 23.4% and 22.8% at *ω1* with LNP-mRNA and LNP-RNP, respectively. Similar performance was seen with LNP-mRNA and LNP-RNP using either electroporation or soaking of the eggs with the LNPs. Details of HDR and protein expression of omega-1 will also be presented. The findings contribute to improving the efficacy in CRISPR/Cas KO and gain-of-function knock-in and represent continued progress towards heritable transgenesis in schistosomes.