parasitology IN PORTUGAL

FMUL 5th March 2026

ABSTRACT BOOK

Organization

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Welcome to the first Parasitology in Portugal Symposium

Foreword

Welcome to the Symposium “Parasitology in Portugal” 2026.

Parasitology in Portugal has grown markedly over the last few years – through increased critical mass, thematic diversity, and scientific and translational impact. We believe the time is right to come together and make the most of our proximity: to share knowledge, connect perspectives, and strengthen our community. This will be the first meeting of the Portuguese Parasitology community since 2014, and we have been delighted by the strong interest from researchers, clinicians, and students across institutions and disciplines.

This symposium is endorsed by the Portuguese Society of Microbiology, with the special participation of its President, Professor Jorge Pedrosa. This reinforces the meeting’s role as a bridge between parasitology, microbiology, public health, and tropical medicine. Portugal has a long and distinguished tradition in this field – built on both historical contributions and outstanding contemporary research – and this event also aims to recognize and amplify that legacy by highlighting the excellent work currently being conducted nationwide on human and veterinary parasites; their vectors and evolution; their role in ecology and immunology; and the development and implementation of diagnostics, therapeutics and control strategies.

The meeting is co-organized by colleagues from the Católica Biomedical Research Centre, the Gulbenkian Institute for Molecular Medicine, the Faculty of Veterinary Medicine of the University of Lisbon, and the Institute of Hygiene and Tropical Health. It is designed as a space for genuine exchange: a stimulating scientific programme, ample time for discussion, opportunities for networking, and dedicated moments to foster collaborations – between laboratories, institutions, and across generations. A central priority is also to showcase early-career researchers, ensuring that emerging ideas and new networks can take root and grow.

We hope this symposium will be as inspiring as it is timely, and that it will mark the beginning of a renewed tradition of regular national meetings, reflecting the energy, ambition, and excellence of parasitology in Portugal.

The Organising Committee

(Andreia Wendt, Henrique Silveira,
Luísa Figueiredo, Luís Madeira de Carvalho,
Miguel Prudêncio, Sara Silva Pereira, Silvana Belo)

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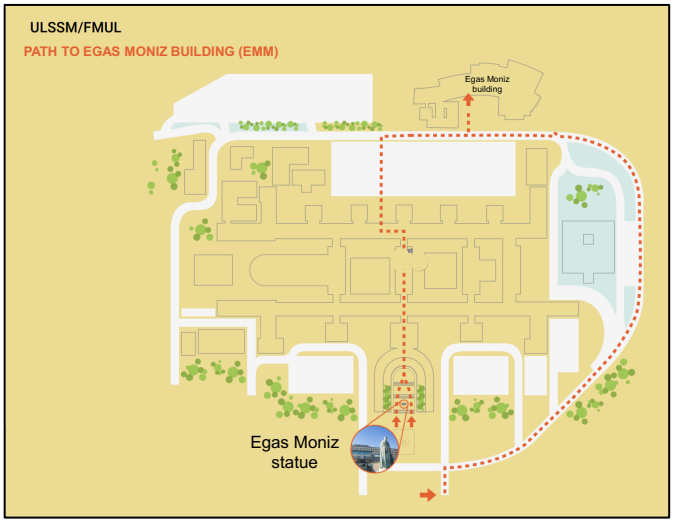
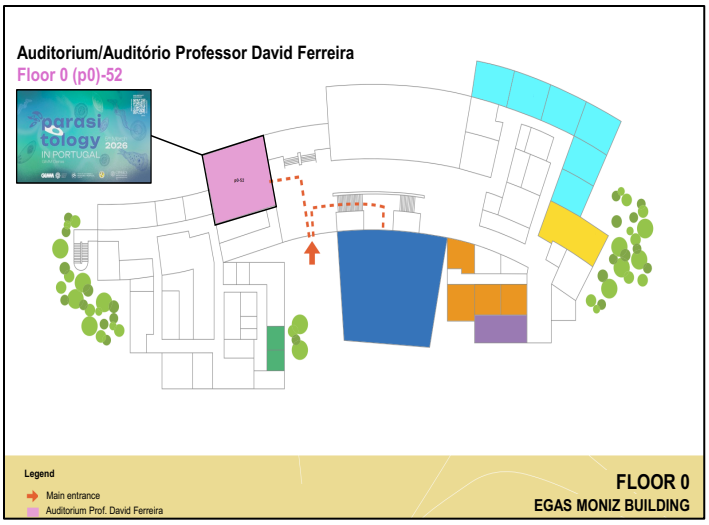
Venue

Address: Edifício Egas Moniz, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal
Coordinates: 38.74864512475682, -9.160971770558406

The oral sessions will take place in Auditorium “Professor David Ferreira” (room P0-52).
The poster sessions and coffee breaks will be in the Foyer.





Parking: Parking within the hospital premises is possible, but extremely limited. There is paid street parking in Av. Prof. Egas Moniz (green zone) and a guarded car park (SABA Estádio Universitário).

How to get there by public transport:

- **Metro Linha Amarela (Metro de Lisboa)** — get off at **Cidade Universitária** station. From there it’s a short walk to the the main entrance of Hospital Santa Maria.
- Several Carris bus lines stop very close to Hospital Sta Maria. Examples:

Autocarro 701 (Carreira dos Hospitais):
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Autocarro 55:
Av. Paulo VI > Sete Rios

Autocarro 63:
Cidade Universitária > Alto da Damaia

Autocarro 68:
Quinta Alcoutins > Cidade Universitária

Our sponsors

This meeting has been generously supported by several institutions that recognize both the significance of our work and the timeliness of this initiative. We sincerely thank all those who contributed to making this event possible and to ensuring it remains inclusive and free of charge for all participants.

Program

08h30-09h00 Registration

09h00-09h10 Welcome Session

09h10-09h20 Presentation of the Portuguese Society for Microbiology Prof. Jorge Pedrosa, President of SPM

09h20-10h35 Session I: ***My Lab in a Snapshot – Research Labs in Portugal***

1. Ana Domingos, IHMT
2. Bishara Marzook, GIMM
3. Isabel Pereira da Fonseca, FMV-UL
4. Joana Marques, IHMT
5. José Lourenço, CBR
6. Joana Tavares, i3S/ICBAS
7. Luísa Figueiredo, GIMM
8. Mª Isabel Veiga, ICVS
9. Miguel Prudêncio, GIMM
10. Nuno Santos, GIMM/FMUL
11. Ana Tomás, i3S/ICBAS
12. Sara Silva Pereira, CBR
13. Sofia Cortes, IHMT
14. Ana Paula Arez, IHMT
15. Márcia Medeiros, IHMT
16. Manuela Calado, IHMT
17. Isabel Maurício, IHMT
18. Maria Rebelo, FMUL
19. Ricardo Silvestre, ICVS
20. Henrique Silveira, IHMT
21. Moritz Treeck, GIMM
22. Pedro Ferreira, ICVS
23. Fátima Nogueira, IHMT

10h35-11h35 Coffee Break and Poster Session A

11h35-13h15 Session II: ***Host-Parasite Interactions and Mechanisms of Pathogenicity***

II.1 Juliana Inês Weber, IHMT: Understanding canine Chagas disease through immune profiling
II.2 Carolina Vieira, CBR: Glycosomal phosphoenolpyruvate carboxykinase disruption affects metacyclogenesis and infectivity of *Trypanosoma cruzi*
II.3 Nuno Santarém, i3S: Cross-species analysis of plasma extracellular vesicles in *Leishmania* infection models
II.4 Miguel Patricio, Champalimaud Foundation: Immunity-Matched cDCpoiesis: The balance of cDC subsets in type 1 vs type 2 immunity
II.5 Jane Munday, University of Glasgow: Challenging paradigms of trypanosome antigenic variation in chronic cattle infections
II.6 Leonor Loira, CBR: The role of a *T. congolense* divergent cathepsin B in the interaction with the endothelium
II.7 Jaiganesh Jagadeesh, GIMM: Resolving the transcriptional landscape of *Trypanosoma brucei* adipose tissue forms at the single-cell level
II.8 Ângelo Ferreira Chora, GIMM: Role of reticulocytes in protection from severe malaria
II.9 Kyle Cunningham, Champalimaud Foundation: Helminth-induced noradrenergic regulation of cDC2 output in the bone marrow
II.10 Sandra Trindade, GIMM: Direct suppression of host gluconeogenesis by *Trypanosoma brucei*

13h15-14h15 Lunch

14h15-15h00 Keynote Talk by Prof. Richard McCulloch, University of Glasgow

"Variant Surface Glycoprotein expression for immune evasion in *Trypanosoma congolense* occurs in the absence of monoallelic transcription"

15h00-16h45 Session III: *Diagnostics, Epidemiology and New Therapeutic Strategies in Parasitology*

III.1 Ricardo Agrícola, FMV-UL: Endoscopic diagnosis of duodenal myiasis caused by *Gasterophilus pecorum* in a horse from Portugal: parasitological and epidemiological relevance
III.2 Eyob Addise Workneh, GIMM: Assessing transmission-blocking immunity elicited by whole-sporozoite vaccines for *Plasmodium vivax*
III.3 Bruno Freitas, ICVS: African field mutation I416V in K13's BTB/POZ domain confers artemisinin tolerance in *Plasmodium falciparum*
III.4 Margarida Duarte, i3S: Metabolic rewiring in *Leishmania* Lacking PRX1/2 reveals hidden vulnerabilities for drug targeting
III.5 Maria Teresa Bispo, IHMT: Zoonotic snail-borne parasites in freshwater ecosystems: the case of lake Alqueva
III.6 Raquel Soares, IHMT: From malaria to babesiosis: repurposing antimalarial drugs to tackle an emergent disease
III.7 Adriana Gonçalves, ICVS: *Plasmodium falciparum* ubiquitin-proteasome system (UPS) modulating antimalarial resistance
III.8 Fernando Cardoso, IHMT: Recombinant antigens for rapid *Pneumocystis jirovecii* detection
III.9 Catarina de Almeida Marques, University of Glasgow: Single-cell transcriptomics analysis of cell cycle and life cycle gene expression dynamics during in vitro growth of two *Leishmania* species
III.10 Diana Moita, GIMM: Unveiling the impact of gut microbiota on vaccination against malaria

16h45-17h45 Coffee Break and Poster Session B

17h45-18h45 Session IV: *Parasites in the Global World: Ecology, Evolution and One Health*

IV.1 Gonçalo Seixas, GIMM: Strengthening national capacity in medical entomology: establishment of an *Aedes mosquito* insectary at the Gulbenkian Institute for Molecular Medicine (GIMM)
IV.2 Filipa Neves, i3S: Understanding the role of secreted protein with an altered thrombospondin repeat (SPATR) in malaria sporozoite infectivity
IV.3 Rebecca Pabst, IHMT: Trait-based assessment of the invasion potential of disease vector mosquitoes
IV.4 Vasco Nandubé, IHMT: Prevalence of triple, quadruple, quintuple, and sextuple mutant *Plasmodium falciparum* parasites resistant to sulfadoxine-pyrimethamine in Guinea-Bissau
IV.5 Maria Carvalho, IHMT: Bacterial modulation of malaria transmission: insights from *Pseudomonas*–mosquito interactions
IV.6 Sandra Antunes, IHMT: Toward genetic manipulation of *Babesia ovis*: genome sequencing and promoter evaluation

18h45-19h00 Closing Session and Award Ceremony

Poster session A

Session A: Please put your poster up by 9am and remove it before the lunch break.

SESSION	#	NAME	SURNAME	AFFILIATION	TITLE
A	1	Ana	Balau	IHMT	Selective uptake of external 2,3-diphosphoglycerate and metabolic secretion profile in *Plasmodium falciparum in vitro* cultures
	2	Virlânio	Oliveira-Filho	IHMT/UNICAMP	Expanding beauvericin's antileishmanial activity against Old World *Leishmania* spp.
	3	Jane	Dias	GIMM	Decoding trypanosome interactions with the host vasculature
	4	Vitória	Baptista	Univ. of Glasgow	Deciphering Mitochondrial Complex III in *Plasmodium falciparum*
	5	David	Ferreira	GIMM	Understanding how *T. brucei* induces lipolysis in adipocytes
	6	Luis	Constantino	Inst. Superior de Lisboa	Dynamic forces shaping the *Anopheles* mosquito microbial community across its lifespan
	7	Gwendolin	Fuchs	GIMM	Navigating the Surface: Deciphering Divergent Trafficking Pathways in *P. falciparum*
	8	Manuela	Calado	IHMT	Assessment of Soil Contamination by *Toxocara* spp. in the City of Praia, Santiago Island – Cape Verde: A Pilot Study
	9	Christian	Gnann	GIMM	To be or not to be (cleared) - How single cell heterogeneity shapes *Toxoplasma* infection outcome
	10	Madalena	Rodolfo	CBR	Exploring the kinetics of *T. congolense* sequestration
	11	Ereso	João	IHMT	Evaluation of *Strongyloides venezuelensis* antigens in immunodiagnosis of strongiloidosis
	12	João	Lucas	IHMT	Beyond the Usual Suspects: Evidence of *Colpodella* in an Avifauna-Diverse Site and Taxonomic Ambiguity in *Rhipicephalus* Ticks
	13	Daniela	Matias	IHMT	Resistance profiling of *Plasmodium falciparum* infections in Mozambique using custom dual indexing and Illumina next-generation amplicon sequencing
	14	Maria Teresa	Novo	IHMT	mosquitoWEB: Citizens detection of *Aedes allbopictus* presence.
	15	Teresa	Leão	i3S	Zinc-based regulation of the *Trypanosoma brucei* ZIP3 transporter.
	16	Ana	Dias	ULSLO	Copy number variation of plasmepsin2 and multidrug resistance-1 genes in *Plasmodium falciparum* in Luanda before and after ACTs implementation
	17	Michal	Malecki	GIMM	Nanopore profiling of polyadenylation in *T. brucei*
	18	Eva	Dias	GIMM	Design of a Broad-Spectrum Antiviral Strategy Against Flaviviruses
	19	Ana	Matias	GIMM	It takes two to tango: novel insights into phospho-STAT6 signaling in *T. gondi*i infected cells
	20	Ruth	Esho	ICVS	Lipid-dependent heme detoxification in *Plasmodium falciparum*: Localization and functional Studies
	21	Ricardo	Monteiro	i3S	Pulmonary infection by *Leishmania infantum*: implications for treatment efficacy
	22	Beatriz	Fonseca	ICVS	Multistage Steroid Derivatives as Next-Generation Antimalarials
	23	Inês	Morais	IHMT	Nature's Toxins as Tools: Antimalarial Activity of Snake Venom Fractions and Venom-Derived Peptides
	24	Eunice	Kaizeler	IHMT	Galleria mellonella: an invertebrate model with potential for *Leishmania* research

Poster session B

Session B: Please put your poster up at lunch break and remove it at the end of your session.

SESSION	#	NAME	SURNAME	AFFILIATION	TITLE
B	1	Andreia	Mósca	GIMM	MHV68 reshapes the hepatic microenvironment and reduces *Plasmodium* liver infection
	2	Stephanie	Nofal	GIMM	Unravelling the role of exported effectors on host cell remodelling during *Plasmodium falciparum* gametocyte infection
	3	Maria	Mascarenhas	Inst. René Rachou/UM	Disentangling the Roles of pfmdr1 Copy Number Variations and SNPs in *Plasmodium falciparum* Antimalarial Resistance
	4	Margarida	Leonardo	IHMT	Quinazoline Derivatives as Potential Anti-malarial Drugs: a Drug Design Approach
	5	Isabel	Maurício	IHMT	Evaluation of environmental DNA passive samplers for detection of trematodes and snail intermediate hosts
	6	Emmanuela	Rodrigues	IHMT	The impact of *Plasmodium falciparum* infection on host glycolysis: a focus on Rapoport-Luebering shunt
	7	Karla	Menezes	Univ. Federal da Paraíba	Preliminary antiplasmodial activity of new quinoline derivatives
	8	Armanda	Rodrigues	IHMT	Decoding mammal blood-parasite interaction in *Trypanosoma cruzi* infection
	9	Eduarda	Machado	UFRRJ	Repositioning of antimalarial drugs in ovine babesiosis: in vitro evaluation
	10	Marta	Tiago	CBR	N-glycosylation is required for *T. congolense* cytoadhesion
	11	Paula	Moreira	IHMT	In vitro antileishmanial activity of snake venom fractions
	12	Christoph	Wenzl	IHMT	Poly(A) polymerases and the dynamic regulation of VSG mRNA in *Trypanosoma brucei*
	13	Mariana	Pinto	IHMT	Isothermal Nucleic Acid Amplification with Recombinase Polymerase for Rapid Detection of Drug Resistance in *Plasmodium* Parasites: A Feasible Point-of-Care Technology
	14	Maria	Zorrinho-Almeida	CBR	Characterization of host-parasite interactions in the bovine blood-brain barrier in the context of *Trypanosoma congolense* infection.
	15	Jaime	Nina	UEI Clínica - IHMT	Parasitic Infections in transplanted and other severely immunocompromised patients
	16	Franziska	Hildebrandt	GIMM	From monkeys to men - *Toxoplasma gondii* as a model to study the evolution of host-pathogen interactions
	17	Mª dos Anjos	Valente	FFUL	Assessment of the biological activities and phytochemical profile of the root of *Vernonia britteniana*
	18	Sophie	Marcus-Wade	GIMM	Exploring the role of Major Surface Protease A in *T. brucei* adipose tissue colonization
	19	Maria	Silva	IHMT	Identification of novel dual-stage antiplasmodial hits using high-throughput phenotypic screening
	20	Leonardo	Moerbeck	IHMT	Tick-borne pathogens harbored by hard ticks and their microbiota in mainland Portugal
	21	Margarida	Marques	IHMT	Genomic and phenotypic characterization of bacteria isolated from *Anopheles* mosquitoes midgut microbiota
	22	Milena	Souza Silva	IHMT	Venoms against malaria: in search of the next generation of drugs.
	23	Manuel	Vueba	ISPP-Kilamba	Development of a computer-assisted QSAR model for predicting antiparasitic activity

Keynote Lecture

Prof. Richard McCulloch
Centre for Parasitology School of Infection and Immunity, University of Glasgow

Prof. Richard McCulloch completed his PhD in Genetics in 1993 from the University of Glasgow. From 1994 to 1997, he was a Wellcome Trust International fellow at the Nederlands Kanker Instituut, in Amsterdam, and then a Royal Society University Research Fellow at the Wellcome Trust Centre for Molecular Parasitology (University of Glasgow) until 2006. He was then appointed Senior Lecturer and Reader until 2019, after which he became Professor of Molecular and Cellular Parasitology at the Wellcome Centre for Integrative Parasitology. His laboratory focuses on DNA recombination, replication, repair and antigenic variation in trypanosomatid parasites.

Variant Surface Glycoprotein expression for immune evasion in *Trypanosoma congolense* occurs in the absence of monoallelic transcription

Antigenic variation is a ubiquitous process for pathogen evasion of mammalian adaptive immunity, involving the continuous change of exposed antigens. In African trypanosomes, antigenic variation relies on expression of Variant Surface Glycoprotein (VSG), and considerable work in *Trypanosoma brucei* has revealed the machinery that means only one of ~15 VSG expression sites is actively transcribed at a time. In the closely related African trypanosome, *T. congolense*, we have no such understanding of VSG expression control or dynamics during antigenic variation. Here, we have examined the patterns of VSG expression at the transcript and protein level in populations of *T. congolense*, revealing much greater diversity of VSG expression than seen in *T. brucei*. Such diversity is unaltered in mutants that impair homologous recombination. Using single cell transcriptomics, we find no evidence for monoallelic transcription of VSGs in *T. congolense* but show instead that the parasite dynamically encodes up to 30 different VSG transcripts in a single cell. Co-transcription of a range of VSGs reflects the absence of dedicated VSG expression sites, with VSG transcription occurring across the genome. Thus, comparing two parasites that rely on the same surface antigen for immune evasion has revealed highly distinct mechanisms for generating antigen diversity.

Best Talk and Best Poster Awards

We are pleased to announce that **Chaperone** has generously sponsored one *Best Talk Award* and one *Best Poster Award* for Early Career Researchers.

Chaperone is an online career development platform specifically designed to support scientists and scientific institutions. Its mission is to democratize access to high-quality career support in science, helping researchers navigate complex career decisions, advance professionally, and connect with experienced career consultants.

Each awardee will receive **two complimentary one-to-one career consultation vouchers** on the Chaperone platform, with no consultant price limit.

We sincerely thank Chaperone for supporting early career researchers and contributing to their professional development.

Abstracts

Session II: Host-Parasite Interactions and Mechanisms of Pathogenicity

Mechanistic insights into how parasites interact with, manipulate, and evade their hosts at molecular, cellular, and immunological levels.

II.1 Understanding canine Chagas disease through immune profiling

Juliana Inês Weber[1], Cláudia Moreno[1] , Micheli Ferla[1] , Marta Monteiro[2,4], Vânia Vieira[1], Joana Palma-Marques[1], Telmo Nunes[5], Wilson Antunes[6], Graça Alexandre-Pires[2.4], Rui Ferreira[3], Inês Cardoso[3], Isabel Pereira da Fonseca[2.4], Armanda Rodrigues[1], Gabriela Santos Gomes[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon
3 Banco de Sangue Animal, Porto, Portugal
4 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)
5 Microscopy Center, Faculty of Sciences, Campo Grande, 1749-016 Lisboa, Portugal
6 Instituto Universitário Militar (IUM), Centro de Investigação, Desenvolvimento e Inovação da Academia Militar (CINAMIL), Unidade Militar Laboratorial de Defesa Biológica e Química (UMLDBQ), Lisboa

Chagas disease, caused by *Trypanosoma cruzi*, is a vector-borne parasitic infection with dogs serving as important reservoirs. Without effective vaccines or treatments for animals, understanding parasite-driven modulation of the canine immune response is essential. This study aimed to investigate the immunomodulatory effects of *T. cruzi* conditions on canine immune cells. Two in vitro cell models derived from peripheral blood of healthy dogs were used: Whole Blood Model and a Monocyte-derived Macrophage and Lymphocyte co-culture. Cells were stimulated with epimastigotes, antigen (Ag), or extracellular vesicles (EVs) of *T. cruzi*. Flow cytometry showed that Ag and EVs induced significant MHCI upregulation and $CD8^+$ T-cell modulation at 24 h in both models, indicating cytotoxic activation. EVs and Ag also significantly increasedTLR2, NOD1, IL-12, and TNF-α gene expression analyzed by qPCR. Images obtained by Scanning Electron Microscopy showed macrophages exposed to parasites and EVs displayed marked morphological changes. These findings highlight early immune activation mechanisms in canine Chagas disease and provide a basis for further investigation of host–parasite interactions.

II.2 Glycosomal phosphoenolpyruvate carboxykinase disruption affects metacyclogenesis and infectivity of *Trypanosoma cruzi*

Carolina Silva Dias Vieira[1,2], Wei Wang[2], Fernando Sanchez-Valdez[2], Jihyun Lim[2], Brooke White[2], Camilla Garcia da Silva Souza[1], Rick L Tarleton[2], Marcia Cristina Paes[1,3], Natália Pereira de Almeida Nogueira[1,2,3]

1 Laboratório de Interação de Tripanossomatídeos e Vetores, IBRAG, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brasil;
2 Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, USA;
3 Instituto Nacional de Ciência e Tecnologia, Entomologia Molecular (INCT-EM), Brasil.

Trypanosoma cruzi, the causative agent of Chagas disease, possesses glycosomes, unique organelles that house key metabolic enzymes and contain several promising therapeutic targets. Among them, phosphoenolpyruvate carboxykinase (PEPCK) plays a central role in succinic fermentation, the main glycosomal route for NAD+ regeneration. Using CRISPR/Cas9 editing, PEPCK gene was disrupted in *T. cruzi*, producing only single-allele knockout epimastigotes (TcPEPCK-sKO) with reduced gene expression and enzyme activity. This impairment led to a decrease in glucose consumption and mitochondrial respiration, especially related to oxidative phosphorylation, reducing the parasite dependence on mitochondrial ATP production in glucose-rich conditions. Although TcPEPCK-sKO epimastigotes exhibited a slight reduction in proliferation, their differentiation to the infective stage (metacyclogenesis) and invasion capacity were severely compromised. However, once inside the

host cell, TcPEPCK-sKO amastigotes increased their replication, leading to enhanced trypomastigote production. The same phenotype was observed in in vivo infection, where TcPEPCK-sKO infection in IFNγ-deficient mice caused uncontrolled parasitemia and severe pathology, initially indicating a more virulent phenotype of mutant parasite. However, an intact immune system effectively contained TcPEPCK-sKO infection. Altogether, PEPCK showed to be important for *T. cruzi* energy metabolism, enabling the parasite differentiation within the insect vector and controlling the infection of mammalian host cells.

II.3 Cross-species analysis of plasma extracellular vesicles in *Leishmania* infection models

Nuno Santarém[1,2], Inês Costa[1,2], Sofia Esteves[1,2], Clara Lima[1,2] , Ana Isabel Pinto[1,2], Cátia Caldas[3], Hugo Osório[1,4,5], Carmen Fernandez-Becerra[6,7,8] and Anabela Cordeiro-da-Silva[1,2]

1 Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal.
2 Serviço de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia da Universidade do Porto, Portugal.
3 Departamento de doenças infeciosas, Centro Hospitalar e Universitário de São João, Faculdade de Medicina da Universidade do Porto, Porto, Portugal.
4 Ipatimup - Institute of Molecular Pathology and Immunology of the University of Porto, University of Porto, 4200-135 Porto, Portugal.
5 Department of Pathology, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
6 ISGlobal, Barcelona Institute for Global Health, Hospital Clínic-Universitat de Barcelona, Carrer Rosselló 149-153, CEK Building. E-08036 Barcelona, Spain.
7 IGTP Institut d'Investigació Germans Trias i Pujol, Badalona, Spain, Ctra. de Can Ruti. Camí de les Escoles, S/n, 08916 Badalona (Barcelona), Spain.
8 CIBERINFEC, ISCIII-CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, Spain.

Extracellular vesicles (EVs) are emerging as key mediators of host–pathogen interactions and a promising source of biomarkers. To investigate their systemic relevance in infectious diseases, we used Leishmania infantum, a well-characterized protozoan capable of infecting mice, dogs, and humans, as a multispecies model. EVs were isolated from infected THP-1 macrophages and plasma samples from all three hosts using Size Exclusion Chromatography, characterized by Dynamic Light Scattering and Electron Microscopy, and subjected to proteomic profiling. Host proteins with significantly altered abundance compared to non-infected controls were analyzed for functional enrichment using STRING. In THP-1 cells, infection reduced proteins linked to antigen presentation and oxidative phosphorylation, consistent with a metabolic shift toward glycolysis. Despite plasma's complexity, networks associated with Leishmania infection were detectable, including impaired haematopoiesis (all models), reduced ferroptosis and antigen presentation (dogs), and additional pathways warranting investigation. Comparative analysis revealed marked divergence in EV protein profiles between mice and the other hosts. These findings demonstrate that plasma EV proteomics provides dynamic insights into host–pathogen interactions and may distinguish host-specific from pathogen-driven responses. Extending this approach to other pathogens could enable personalized strategies for disease monitoring and management.

II.4 Immunity-matched cDCpoiesis: The balance of cDC subsets in type 1 vs type 2 immunity

Miguel Â. Magalhães-Patrício[1,*]; Sahar S.H. Tehrani[1,*]; Robert W. Baber[1]; Hannah S. Blumtritt[1]; Mariana Henriques[1]; Vasco Correia[1]; Maria V. Pires[1]; Kyle T. Cunningham[1]; Neuza Sousa[2]; Frank Brombacher[3]; João C. Guimarães[4]; Joana Neves[2]; Pedro Sousa-Victor[2]; Andreas Wack[5]; Bart N. Lambrecht[6]; Judith E. Allen[7]; Carlos Minutti[1]
* Co-first authorships
1 Champalimaud Foundation, Champalimaud Centre for the Unknown, Immunoregulation Lab, Lisbon, Portugal
2 Instituto Gulbenkian de Medicina Molecular, The Aging & Tissue Repair lab, Lisbon, Portugal
3 Division of Immunology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa.
4 Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
5 The Francis Crick Institute, Immunoregulation Lab, London, UK

6 Laboratory of Mucosal Immunology, VIB-UGent Center for Inflammation Research, Ghent University, Ghent, Belgium.
7 Faculty of Life Sciences, University of Manchester, UK

Conventional dendritic cells (cDCs) are immune sentinels, bridging innate and adaptive immunity. Among them, cDC2s comprise two functionally distinct lineages, cDC2A and cDC2B, which instruct divergent T helper cell responses. While this division of labour is well described in peripheral tissues, it remains unclear whether such functional specialization is pre-determined earlier during haematopoiesis. Here, we hypothesize that the bone marrow functions as a central immune-sensory organ that dynamically tailors cDC2 output in an immunity-matched manner, selectively expanding specific cDC2 lineages at precursor stage in response to distinct infectious cues. By using viral and parasitic infection models coupled with lineage tracing, and single-cell transcriptomic profiling, we show that hematopoietic output toward cDC2 lineages is infection-specific. Specifically, Influenza A virus infection preferentially expands $LysM^+$ pre-cDC2Bs in an interferon-mediated manner. In contrast, *Nippostrongylus brasiliensis* challange promotes expansion of $T\text{-}bet^+$ pre-cDC2As in an IL-4–dependent fashion.Together, our findings demonstrate that cDC2 functional regulation is not merely a peripheral adaptation but is pre-configured during hematopoiesis through cytokine-driven precursor selection. This immunity-matched cDCpoiesis provides a framework for rapid and tailored immune responses before antigen encounter, uncovering an unappreciated layer of immune regulation.

II.5 Challenging paradigms of trypanosome antigenic variation in chronic cattle infections

Stephen Larcombe[1*], **Jane C Munday[2*]**, Guy Oldrieve[1], Marija Krasilnikova[2], Craig Lapsley[2], Craig Duffy[3], Christina Vrettou[4], Edith Paxton[4], Monica Mugnier[5], Andrew Jackson[3], Liam J Morrison[4], Richard McCulloch[2] and Keith R Matthews[1]

1. Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, UK
2. The University of Glasgow Centre for Parasitology, School of Infection and Immunity, University of Glasgow, Glasgow, UK.
3. Institute of Infection, Veterinary & Ecological Sciences, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, UK
4. Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK
5. Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St, Baltimore, MD 21205, USA

Trypanosoma brucei exploits an extreme form of antigenic variation to escape the mammalian immune response. This involves the progressive expression of antigenically distinct variant surface glycoproteins (VSGs) on the surface of individual parasites in the population, generating waves of parasitaemia, each successively resolved by host antibodies. In vitro studies and high parasitaemia acute infections in rodents have established the current paradigms for antigenic variation, but disease-relevant natural infections in bovines are characterised by their sustained low parasitaemias and chronicity. Here, we compared the infection dynamics of isogenic parasites in mice and cattle in blood during early and chronic infections, quantitating their VSG expression diversity within and between hosts, their persistence in vivo and their timing of appearance. This revealed enhanced antigenic diversity in cattle but with a surprisingly reproducible temporal hierarchy for the expression of related VSGs between independent chronic infections. The VSG genomic archive also exhibited exceptional diversification within infections invoking high levels of microhomology-based recombination to evolve the antigen repertoire. This diversity was restricted but not eliminated in RAD51 and BRCA2 recombination mutants, which could nonetheless sustain chronic infections. These data challenge existing paradigms for trypanosome antigenic variation.

II.6 The role of a *T. congolense* divergent cathepsin B in the interaction with the endothelium

Leonor Loira[1], Meriem Ben Cheikh[1], Henrique Carmona[1], Carolina Vieira[1], Bill Wickstead[2], Catarina Gadelha[2], Luísa Figueiredo[3], Sara Silva Pereira[1]

1 Católica Biomedical Research Centre, Católica Medical School, Universidade Católica Portuguesa

2 School of Life Sciences, University of Nottingham
3 Gulbenkian Institute for Molecular Medicine

Trypanosoma congolense is a unicellular, intravascular parasite that causes animal African trypanosomiasis. This is a wasting disease that impacts animal welfare and the socioeconomic development of African economies. The most common clinical signs are fever, anemia and weight loss. Inside the host, *T. congolense* parasites cytoadhere to the endothelial cells in a process named sequestration. Previously, the lab showed that sequestration is a virulence factor that determines disease severity, but the mechanisms of sequestration remain unknown. We performed an in vivo RNAi-based genetic screen to identify genes important for sequestration. We identified a divergent cysteine peptidase of the cathepsin-B family (TcCbc6) that reduced sequestration in vivo when silenced. Using static and in-flow cell binding assays, mRNA sequencing and enzymatic inhibition assays, we show that TcCbc6 modulates endothelial cells to make them more prone to cytoadhesion by *T. congolense* parasites. Given that sequestration is important for parasite division, we propose TcCbc6 as a promising drug target for animal African trypanosomiasis.

II.7 Resolving the transcriptional landscape of *Trypanosoma brucei* adipose tissue forms at the single-cell level

Jaiganesh Jagadeesh[1], Lara Lopez-Escobar[2], Abdulbasit Amin[1], Vincent Gardeux[3], Michal Malecki[1] and Luisa Figueiredo[1]

1 Gulbenkian Institute for Molecular Medicine, Lisboa, Portugal
2 Institute of Tropical Medicine, Antwerp, Belgium
3 École Polytechnique Fédérale de Lausanne, Switzerland

Trypanosoma brucei exhibits remarkable developmental plasticity to adapt to distinct host environments. Upon colonizing adipose tissue, the parasite adopts a specialized form — termed the Adipose Tissue Form (ATF) — that differs markedly from the canonical Bloodstream Form (BSF). Previous studies have shown that ATFs grow more slowly, activate β-oxidation pathways, and display substantial population heterogeneity. To investigate the molecular basis of these adaptations, single-cell RNA sequencing (scRNA-seq) was used to compare the transcriptomic landscapes of ATFs and BSFs. Our analyses reveal that BSFs display a relatively homogeneous transcriptome, largely comprising replicative slender forms and parasites differentiating into stumpy forms. In contrast, ATFs exhibit extensive transcriptional heterogeneity, particularly among parasites in the G_0 state. Notably, by day 7 post-infection, a distinct transcriptional trajectory emerges from these G_0 parasites, separate from the canonical stumpy differentiation pathway. This branch may represent a quiescent or “persister-like” population. To better characterize this decision point in which G0 cells become either stumpy forms or persister-like, we are currently analysing scRNA-seq data from a *T. brucei* ZC3H20 knockout line. ZC3H20 is an RNA-binding protein required for the slender-to-stumpy transition in bloodstream parasites. We postulate this protein will also prevent the formation of stumpy forms in the adipose tissue, providing no choice to parasites and they all become persister-like forms. By integrating computational analyses with functional validation, this work aims to uncover the regulatory networks that control key decision points in the life cycle of *T. brucei*. These findings underscore the power of scRNA-seq in revealing parasite heterogeneity and provide insights into tissue-specific adaptations that could inform new therapeutic strategies for African trypanosomiasis.

II.8 Role of reticulocytes in protection from severe malaria

Mariana Sousa[1], Maria M. Mota[1], **Ângelo Ferreira Chora[1]**

1 Gulbenkian Institute for Molecular Medicine

In endemic settings, naturally acquired immunity to Plasmodium, the causative agent of malaria, supports host survival while enabling parasite transmission [1]. However, the mechanisms governing the balance between host immunity and parasite virulence remain poorly understood. In mice, splenic production of reticulocytes during the blood stage of Plasmodium berghei infection protects against severe outcomes [2], suggesting a critical role for

this erythroid subset in modulating parasite virulence. Using a biotin-based labeling strategy to track both endogenous and adoptively transferred erythroid cells, we demonstrate that Plasmodium exhibits a strong tropism for reticulocytes. During infection, parasites exclusively invaded reticulocytes while these cells were available. Once reticulocytes are depleted through successive rounds of invasion and egress, parasites switched to invading normocytes, a transition associated with the onset of experimental cerebral malaria. Transcriptional profiling revealed an accumulation of sequestration-prone schizonts in normocytes, which associated with an extended asexual developmental cycle.Our findings provide mechanistic insights into how reticulocytes promote host survival by modulating parasite development and virulence. These results underscore the intricate interplay between Plasmodium and its host, offering new perspectives on the pathogenesis of malaria.

II.9 Helminth-induced noradrenergic regulation of cDC2 output in the bone marrow

Kyle T. Cunningham[1], Simão A. Nogueira[1], Vasco Correia[1], Maria V. Pires, Miguel Â. Magalhães-Patrício[1], Sahar S.H. Tehrani[1], Robert W. Baber[1], María Martínez-López[2], Judith E. Allen[3], Henrique Veiga-Fernandes[2], Carlos M. Minutti[1]

1 Immunoregulation Lab, Champalimaud Foundation, Champalimaud Centre for the Unknown, Immunoregulation Group, Lisbon, Portugal
2 Immunophysiology Lab, Champalimaud Foundation, Champalimaud Centre for the Unknown, Immunoregulation Group, Lisbon, Portugal
3 Lydia Becker Institute of Immunology and Inflammation, School of Biological Sciences, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Manchester UK

Conventional dendritic cells (cDCs) are essential for the robust induction of innate and adaptive inflammatory responses, and for the successful induction of sustained T and B cell memory. The rapid differentiation, activation, and mobilization of precise cDC subsets are highly dependent on this success. This differentiation process, known as cDCpoiesis, is driven by various mediators, including host factors and parasite-derived molecules during parasitic worm infection. In addition to these influences, the bone marrow is highly innervated by the sympathetic nervous system, which has been demonstrated to aid in progenitor cell mobilization. Preliminary research from our lab has identified through scRNA-Seq that direct cDC precursor cells (pre-cDCs) differentially express the β2 adrenoreceptor (ADRB2), a key receptor for the sympathetic neurotransmitter noradrenaline (NA). Therefore, we investigated ADRB2 signaling specifically within the cDC2 lineage during helminth infection. Through the integration of in vitro assays, confocal imaging, spectral cytometry, and in vivo pharmacological and genetic manipulation of NA signaling, we have found a distinct expansion of the pre-cDC2A during infection with *Nippostrongylus brasiliensis*. This expansion coincides with increased NA in the bone marrow and is replicated ex vivo. These findings illustrate a novel mechanism for the induction of immunity to helminths and beyond.

II.10 Direct suppression of host gluconeogenesis by Trypanosoma brucei

Abdulbasit Amin[1], João Colaço[1], Henrique Machado[2], Helena Nunes-Cabaço[1], **Sandra Trindade[1]**, Philipp Scherer[3], Sara Silva Pereira[4], Luísa Figueiredo[1]

1 Gulbenkian Institute for Molecular Medicine
2 ICVS-Life and Health Sciences Research Institute, School of Medicine, University of Minho
3 The University of Texas Southwestern Medical Center
4 Católica Biomedical Research Centre, Católica Medical School, Universidade Católica Portuguesa

The mechanisms underlying hypoglycaemia during African trypanosomiasis are poorly defined. Using a mouse model, we show that *Trypanosoma brucei* infection does not impair intestinal carbohydrate processing, glucose absorption, or peripheral glucose utilisation, but instead markedly limits endogenous glucose generation. Transcriptomic profiling of infected livers revealed broad suppression of metabolic programmes, including gluconeogenesis, alongside strong induction of inflammatory pathways. Experiments in Rag2-deficient mice indicated that adaptive immune responses play only a minor role in this metabolic disruption. In contrast,

elimination of circulating parasites fully restored blood glucose levels and gluconeogenic capacity despite ongoing immune activation, implicating parasite presence as the dominant driver. Consistently, direct exposure of primary hepatocytes to *T. brucei* in vitro was sufficient to repress gluconeogenic gene expression. Importantly, supplementation with glycerol, a gluconeogenic substrate, enhanced glucose production, improved glycaemia, and prolonged survival without altering parasite burden. Together, these findings identify direct inhibition of host gluconeogenesis as a previously unappreciated strategy by an extracellular eukaryotic parasite and highlight metabolic support as a potential avenue to improve disease tolerance.

Session III: Diagnostics, Epidemiology and New Therapeutic Strategies in Parasitology

Advances in disease detection, resistance surveillance, epidemiological monitoring, and the development of innovative intervention strategies.

III.1 Endoscopic diagnosis of duodenal myiasis caused by *Gasterophilus pecorum* in a horse from Portugal: parasitological and epidemiological relevance

R. Agrícola [1,2,3,4], J. Prazeres[1,4], G. Ribeiro [1,4,5], A. Resendes[4,5], J. Lozano[2,3], L. Carvalho[1,4], J. Borges[1,4,6], J. Correia[2,3], M. Oliveira[2,3,7], L. Madeira de Carvalho[2,3]

1 HVE - Equine Veterinary Hospital, Santo Estêvão, Portugal.
2 CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.
3 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal.
4 I-MVET Research in Veterinary Medicine, Lusófona University, Lisbon Portugal.
5 CECAV - Animal and Veterinary Science Center, Lusófona University, Lisbon, Portugal.
6 MED - Mediterranean Institute for Agriculture, Ambiente e Desenvolvimento, Évora, Portugal.
7 Centre for Ecology, Evolution and Environmental Changes (cE3c), Global Change and Sustainability Institute (CHANGE), Faculty of Sciences, University of Lisbon

Equine gastrointestinal (GI) myiasis caused by *Gasterophilus spp.* is an underdiagnosed parasitic condition with important implications for health, welfare and athletic performance. Larval attachment to the GI mucosa induces inflammatory and erosive lesions that can mimic other causes of poor performance and abdominal discomfort. *Gasterophilus pecorum* is considered one of the most pathogenic species, although it is regarded as rare in Portugal.
A 6-year-old Lusitano mare used for sport was presented for gastroduodenoscopy because of poor performance and girth-related pain. Endoscopic examination revealed mild equine squamous gastric disease (grade 1/4), pyloric erosions, and moderate to severe, diffuse inflammatory and diphtheric lesions of the duodenal mucosa, with multiple larvae firmly attached and directly visualised. Larval morphological characteristics were consistent with the species *G. pecorum*, and histopathology confirmed lymphoplasmacytic and eosinophilic enteritis, supporting a parasitic aetiology. Moreover, faecal analysis using Mini-FLOTAC showed a moderate nematode egg count (400 EPG). Endoscopic larval removal followed by treatment with ivermectin (200 µg/kg b.w.) resulted in marked clinical improvement.
This case underlines the diagnostic value of endoscopy for direct detection of Gasterophilus spp. and supports its inclusion in the work-up of horses with unexplained GI signs.

III.2 Assessing Transmission-Blocking Immunity Elicited by Whole-Sporozoite Vaccines for *Plasmodium vivax*

Eyob A. Workneh (1), Diana Fontinha (1), Wouter Graumans (2), Kjerstin Lanke (2), Geert-Jan van Gemert (2), Blandine Franke-Fayard (3), Teun Bousema (2), Benjamin Mordmuller (2), Miguel Prudêncio (1,4)

1 Gulbenkian Institute for Molecular Medicine
2 Department of Medical Microbiology, Radboud University Nijmegen Medical Centre
3 Leiden University Medical Center, Leiden
4 Faculdade de Medicina da Universidade de Lisboa

Malaria, caused by *Plasmodium* parasites, remains a major global health threat, with *P. falciparum* (Pf) and *P. vivax* (Pv) causing most cases. Despite progress, subunit vaccines like RTS,S/AS01 and R21/Matrix-M target only Pf and offer limited protection, while Pv—the most widespread malaria parasite—remains neglected. Pv forms dormant liver-stage hypnozoites that cause relapses, hindering elimination and highlighting the need for effective Pv vaccines. Whole-sporozoite (WSp) vaccines offer a promising approach by providing broad antigenic exposure and inducing robust immune responses. However, the inability to continuously culture Pv in the

laboratory limits WSp vaccine development. To overcome this, a rodent *P. berghei* (Pb) platform engineered to express human-infective antigens has been developed as a surrogate. This approach was clinically validated with PbVac, a Pb parasite expressing PfCSP, which showed safety and strong biological effects. Here, we used PbViVac, a transgenic Pb line co-expressing the pre-erythrocytic PvCSP and the transmission-blocking (TB) antigen Pvs25. PbViVac showed normal sporogonic development in *Anopheles stephensi* and retains full infectivity both in-vitro and in-vivo. Antigen expression was confirmed, and immunized mice generated antibodies against PvCSP and Pvs25. Importantly, sera reduced mosquito infectivity in DMFA, demonstrating TB activity. These results support PbViVac-Pvs25 as a promising model for testing Pv TB candidates and developing multistage vaccines

III.3 African field mutation I416V in K13's BTB/POZ domain confers artemisinin tolerance in *Plasmodium falciparum*

Bruno Freitas[1,2], Vitória Baptista[1,2], Maria Eduarda Pereira Mascarenhas[1,2,3], Nuno S. Osório[1,2], Romain Couppée[4], Maria Isabel Veiga[1,2]

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho
2 ICVS/3B's - PT Government Associate Laboratory
3 Instituto René Rachou/Fiocruz Minas, Belo Horizonte, MG, Brasil
4 Université de Rouen Normandie

Artemisinin tolerance in Plasmodium falciparum is clinically defined by delayed parasite clearance following artemisinin-based combination therapy and is primarily associated with non- synonymous mutations in the parasite's pfk13 gene, encoding the Kelch13 (K13) protein. To date, all World Health Organization–validated tolerance-associated mutations map to the Kelch domain of the protein, however, emerging evidence suggests that variants outside this region may also affect artemisinin susceptibility.In a case of severe malaria imported from Mozambique, we identified a single-nucleotide polymorphism (I416V) within the BTB/POZ domain of pfk13. Using CRISPR–Cas9 genome editing, we generated an isogenic 416V mutant line which displayed a delayed early-stage parasite development and increased survival following exposure to the clinical dose of dihydroartemisinin, the hallmarks of an artemisinin-tolerant phenotype. Furthermore, in silico structural modeling suggested that this aminoacid change alters the tertiary structure of the protein, likely leading to the observed phenotype.Together, these findings are the first to validate this mutation as a molecular marker of artemisinin tolerance and identify one of the first native African K13 variants conferring reduced susceptibility to artemisinin derivatives. They further support a broader structural basis for arteminisin tolerance beyond the Kelch domain and highlight the clinical relevance of BTB/POZ domain variants.

III.4 Metabolic rewiring in *Leishmania* lacking the cytosolic peroxiredoxins PRX1/2 reveals hidden vulnerabilities for drug targeting

M Duarte[1], JA Rodrigues[2], C Lubbers[1], V De Boeck[1], DSC Bispo[2], AM Gil[2] and AM Tomás AM[1,2]

1 i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto
2 CICECO-Aveiro Institute of Materials, Department of Chemistry, University of Aveiro
3 ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Leishmaniasis remains a neglected tropical disease with limited treatment options and increasing rates of drug resistance. Therapeutic failure is often linked to the parasite's remarkable ability to adapt and survive under oxidative and pharmacological stress. While redox metabolism has emerged as a promising target for intervention, the complexity of the *Leishmania* antioxidant network and its integration with core metabolic pathways remain incompletely understood. Elucidating how *Leishmania* adapts to redox imbalance could reveal novel mechanisms that sustain parasite fitness and offer alternative targets for therapy.

Peroxiredoxins (PRXs) are central players in redox homeostasis, with *Leishmania* uniquely expressing two cytosolic isoforms, PRX1 and PRX2. These enzymes are capable of sensing hydrogen peroxide and oxidizing protein targets, suggesting roles in both detoxification and redox signaling. Surprisingly, a double knockout mutant lacking both PRX1 and PRX2 remains viable and shows no overt fitness defect in vitro, despite increased sensitivity to hydrogen peroxide [1]. Using this genetically engineered mutant as a tool and a combination of redox-sensitive probes, phenotypic assays, and metabolic profiling techniques—including proteomics and metabolomics—we aim to uncover the compensatory mechanisms underlying this unexpected resilience. Our findings suggest that *Leishmania* can activate alternative pathways to maintain redox balance and drug tolerance, in the absence of canonical antioxidant enzymes PRX1 and PRX2. Indeed, ablation of PRXs does not affect parasite survival or infectivity. Aside from ascorbate/cytochrome c peroxidase, a mitochondrial located enzyme, other peroxidases do not appear to change expression in the mutant. PRX-KO parasites rewire metabolism, relying more on the respiratory chain than glycolysis, yet maintain similar ROS levels to wild type, even upon short exposure to a lethal dose of miltefosine. We propose that these adaptations represent novel metabolic vulnerabilities that can be exploited for therapeutic intervention, either alone or in combination with existing anti-leishmanial drugs.

III.5 Zoonotic snail-borne parasites in freshwater ecosystems: the case of lake Alqueva

MT Bispo[1], IL Maurício[1], PM Ferreira[1]; S Belo[1]; M Calado[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Environmental changes associated with climate change are expanding the distribution of snail-borne parasites across Europe, many of which have zoonotic potential. In Portugal, however, the epidemiology of these trematodes affecting wildlife, domestic animals, and humans remains poorly characterized, despite suitable ecological conditions. This study investigated the occurrence of zoonotic trematodes in Lake Alqueva, a major freshwater ecosystem in southern Portugal, through malacological surveys and molecular analyses. Between May 2023 and October 2025, a total of 7,125 freshwater snails were collected from 25 sampling sites, mainly Physella acuta and Radix auricularia. Molecular screening using PCR and DNA sequencing of COI and ITS regions revealed the presence of three zoonotic trematode genera. *Trichobilharzia franki*, the causative agent of cercarial dermatitis in humans, was detected in *R. auricularia*. *Posthodiplostomum spp*. were identified in *P. acuta*, while *Echinostoma spp.*, with the ability to infect birds, mammals, and humans, were found in both snail species. Phylogenetic analyses suggested similarities with lineages reported in central and northern Europe, indicating a potential role of migratory birds in parasite dispersal. These findings underscore the need for continuous monitoring of freshwater ecosystems under environmental change.

III.6 From Malaria to Babesiosis: repurposing antimalarial drugs to tackle an emergent disease

Raquel Soares[1], Sandra Antunes[2,3], Ana Domingos[2,3], Gustavo Cassiano[3]

1 NOVA Medical School – Universidade NOVA de Lisboa
2 Instituto de Higiene e Medicina Tropical – Universidade NOVA de Lisboa
3 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Babesiosis is an emerging tick-borne disease caused by protozoan parasites of the genus Babesia, with significant economic, veterinary and public health impacts worldwide. Among the more than 100 described species, *Babesia ovis* is the most pathogenic in small ruminants, causing high mortality rates and substantial economic losses. Despite the existence of treatment, particularly diminazene aceturate and imidocarb dipropionate, the limitations and challenges associated with these therapies underline the urgent need for new, safer and more effective therapeutics options. Given the evolutionary proximity between *Babesia* and

Plasmodium species, several antimalarial compounds have been explored as potential alternatives for babesiosis treatment. In this study, fifteen antimalarial compounds, including quinoline derivatives, antifolates, artemisinin-based compounds, and novel compounds, were evaluated for their in vitro activity against *Babesia ovis* using an optimized SYBR Green I fluorescence assay. Compounds showing the highest inhibitory effects were selected for further IC_{50} determination and viability assessment. Of all tested compounds, four demonstrated notable inhibitory activity, with cipargamin emerging as the most effective. These findings suggest that repurposing antimalarials may be a promising strategy for the treatment of babesiosis and underline the value of fluorescence-based screening as a powerful tool for identifying candidate compounds in antiparasitic drug discovery.

III.7 *Plasmodium falciparum* Ubiquitin-proteasome system (UPS) modulating antimalarial resistance

Adriana F. Gonçalves[1,2], Ana Lima-Pinheiro[1,2], Gustavo Capatti Cassiano[3], Pedro Cravo[3] and Pedro E. Ferreira[1,2]

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho
2 Life and Health Sciences Research Institute (ICVS)/ Biomaterials, Biodegradables and Biomimetics Research Group (3B's)-PT Government Associate Laboratory
3 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Drug resistance in malaria remains a major challenge. Although artemisinin resistance is mainly associated with K13 mutations, other components of the ubiquitin–proteasome system (UPS) also influence parasite susceptibility. Artemisinin induces protein damage that is managed by the UPS, making it an important drug target. Additionally, proteasome inhibitors synergize with artemisinin. Previously, we demonstrated that the 738K mutation in the rpn2 gene, a proteasome subunit, confers resistance to dihydroartemisinin (DHA) by stabilizing proteasome activity during drug exposure. We now aim to understand how this mutation modulates antimalarial response in *P. falciparum* parasites in the context of a k13-resistant genetic background. Interestingly, we found that the resistance phenotype of rpn2738K was masked by the k13580Y mutation in DHA susceptibility assays. Additionally, we observed a pleiotropic effect of both mutations leading to resistance to other antimalarial drugs, highlighting their broader implications in parasite drug resistance. Despite the increased survival of k13-mutant parasites, functional assays revealed no direct alterations in *P. falciparum* proteasome activity, but rather differences in the generation and/or degradation of proteins, confirming that k13 acts upstream of the proteasome. Altogether, our findings provide new insights into the role of the UPS in antimalarial action and resistance in Plasmodium spp.

III.8 Recombinant antigens for rapid *Pneumocystis jirovecii* detection

Sibo, Y[1], Salsoso, R[2], **Cardoso, FMH[1]**

1 Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa
2 IBiS, Sevilha, Espanha

Pneumocystis jirovecii is a major cause of pneumonia in immunocompromised patients, and rapid point-of-care diagnostics remain limited. This work reports the production of two relevant antigens, Kexin (Kex) and the Major Surface Glycoprotein (MSG), and their preliminary evaluation as capture elements for rapid immunochromatographic detection. Codon-optimized Kex and MSG were expressed in E. coli BL21(DE3) and purified by nickel-affinity chromatography. Final yields reached 5–8 mg/L (Kex) and 10–12 mg/L (MSG). Proteins were covalently coupled to aminated agarose polybeads using glutaraldehyde. Feasibility was tested using dot beads assays on nitrocellulose membranes with different pore sizes, comparing (i) anti-Kex or anti-MSG antibodies spotted on membrane versus (ii) Kex- or MSG-conjugated beads as capture spots. Bead-based capture generated clear, specific signals using *Pneumocystis*-positive sera, with minimal background in

negatives. These results support advancing Kex- and MSG-coated beads toward optimized lateral-flow prototypes for low-cost, rapid Pneumocystis diagnostics.

III.9 Single-cell transcriptomics analysis of cell cycle and life cycle gene expression dynamics during in vitro growth of two *Leishmania* species

Catarina A. Marques[1], Ross F. Laidlaw[1], Felix Warren[1,2], Gabriel L.A. da Silva[1], Matthew Young[1], Kathryn Crouch[1], Richard Burchmore[1], Thomas D. Otto[1], Martin Llewellyn[2], Richard McCulloch[1*] and Emma M. Briggs[3*]

1 Centre for Parasitology, School of Infection and Immunity, University of Glasgow
2 School of Biodiversity, One Health and Veterinary Medicine, University of Glasgow
3 Biosciences Institute, Newcastle University

The intricacies of the *Leishmania* cell cycle are still poorly understood. Akin to other closely related organisms such as *Trypanosoma brucei* (where the cell cycle has been studied to a larger extent), the processes that make up the cell cycle – from the molecular machinery involved, to the timing of expression and their regulation/control – diverge considerably from those described in model eukaryotes like yeast and human. Using single-cell transcriptomics on unsynchronised cell populations of cultured *Leishmania major* and *Leishmania mexicana* replicative stages (promastigotes), we have computationally reconstructed these parasites' cell cycles and identified hundreds of genes whose transcription is cell cycle-dynamic. Comparison with T. brucei data further revealed core conserved processes between the various species as well as diverging ones. Additionally, the resulting data also allowed for the analysis of different life cycle forms present in the two in vitro grown *Leishmania* species for comparison with published in vivo data and to understand the interlink between cell cycle and the different replicative/non-replicative life cycle stage forms of the parasite. Overall, this work offers insight into *Leishmania* biology and provides groundwork for further functional genomic studies as well as potential targets for future drug development.

III.10 Unveiling the impact of gut microbiota on vaccination against malaria

Diana Moita[1], Andreia Mósca[1], Miguel Prudêncio[1,2]

1 Gulbenkian Institute for Molecular Medicine
2 Faculdade de Medicina, Universidade de Lisboa

Whole-sporozoite (WSpz) malaria vaccines are among the most promising strategies to achieve durable and sterilising protection against *Plasmodium* infection. However, their efficacy varies substantially across populations, suggesting that host-related factors critically influence vaccine-induced immunity. Emerging evidence identifies the gut microbiota as a key modulator of vaccine responses. Yet its role in shaping immunity to the liver stage of *Plasmodium*, the primary target of WSpz vaccines, remains unexplored. Here, we present the rationale and experimental framework of a newly-funded project aimed at determining whether and how gut microbiota composition influences immune priming and protective efficacy induced by WSpz malaria vaccination. Using a rodent *P. berghei* model, we will compare vaccine-induced immune responses and protection in mice with intact microbiota and in animals subjected to antibiotic-mediated microbiota depletion. In parallel, we will assess whether targeted microbiota modulation through probiotic supplementation enhances WSpz vaccine efficacy. Vaccine-induced immunity and protection will be examined by combining multiparameter immune profiling with sporozoite challenge, enabling integrated analysis of T cell activation, cytokine production, antibody responses, and infection outcomes. This work addresses a previously unexplored layer of host regulation of WSpz vaccine-induced immunity, focusing on how gut microbiota composition shapes immune priming to *Plasmodium* pre-erythrocytic stages.

Session IV: Parasites in the Global World: Ecology, Evolution and One Health

Exploring parasites within ecological, evolutionary, and One Health frameworks to understand transmission dynamics and global health challenges.

IV.1 Strengthening national capacity in medical entomology: establishment of an *Aedes* mosquito insectary at the Gulbenkian Institute for Molecular Medicine (GIMM)

Gonçalo Seixas[1], Tiago Melo[2], Rita Fernandes[3], Eva Dias[1], Joana Ricardo[1], Nuno C. Santos[1,3]
1 Gulbenkian Institute for Molecular Medicine
2 Laboratório de Farmacognosia, Universidade de Brasília/UnB
3 Faculdade de Medicina, Universidade de Lisboa

The ongoing expansion of invasive mosquito vectors in Portugal and Southern Europe poses increasing challenges for public health, highlighting the need for sustained national capacity in medical entomology and vector-borne disease research. In this context, a dedicated Aedes insectary was established at GIMM as a strategic infrastructure supporting experimental and translational research on mosquito biology and vector control.The insectary operates under controlled environmental conditions and currently maintains laboratory colonies of Aedes albopictus established from field collections. As part of the infrastructure implementation and quality assurance process, baseline molecular and genetic characterization was conducted. Species identity and population origin were confirmed using cytochrome c oxidase subunit I (COI) barcoding. Insecticide resistance-associated markers were assessed through screening of knockdown resistance (kdr) mutations in the voltage-gated sodium channel gene, and colonies were screened for the presence of Wolbachia using conventional PCR.These baseline data ensure traceability, reproducibility, and long-term reliability of downstream experimental work. Beyond colony maintenance, this insectary constitutes a national research platform enabling future studies on vector competence, host–symbiont–pathogen interactions, and the evaluation of vector control strategies. Planned expansion to additional mosquito species will further strengthen national preparedness for mosquito-borne disease research and surveillance in Portugal.

IV.2 Understanding the role of secreted protein with an altered thrombospondin repeat (SPATR) in malaria sporozoite infectivity

Filipa Neves[1,2], David Mendes Costa[1,2,3], Mónica Sá[1,2,3], Joana Ribeiro[1,2], Ana Rafaela Teixeira[1,2,3], Adriana F. Gonçalves[4], Sylvain Golba[5], Helena Sefiane-Djemaoune[5], Pauline Formaglio[6], José António Manso[1,7], Anabela Cordeiro da Silva[1,2], Pedro E. Ferreira[4], Blandine Franke-Fayard[8], Chris J. Janse[8], Rogerio Amino[6] and Joana Tavares[1,9]

1 i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto
2 FFUP – Faculdade de Farmácia, Universidade do Porto
3 IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto
4 ICVS/3Bs – Life and Health Sciences Research Institute/Biomaterials, Biodegradables and Biomimetics Assoc; ate Laboratory, Universidade do Minho
5 Center for Production and Infection of Anopheles, Institut Pasteur
6 Unit of Malaria Infection and Immunity, Institut Pasteur
7 Molecular Biology Department, University of Cantabria
8 Leiden Malaria Research Group, Leiden University Medical Center
9 ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto

Malaria sporozoites, the parasite stage transmitted by mosquitoes, represent a bottleneck in the *Plasmodium* life cycle. Understanding the molecular basis of sporozoite infectivity is essential for improving preventive strategies. SPATR is upregulated in sporozoites of several Plasmodium species. It contains a predicted signal peptide and two adhesive domains. Despite lacking a GPI-anchor or transmembrane domain, it has been found at the sporozoite surface. Herein, we use the *P. berghei* rodent malaria model to elucidate SPATR function in sporozoites. Stage-specific downregulation of SPATR, using 'promoter-swap', led to sporozoites with impaired

salivary gland invasion, decreased infectivity in mice, and compromised gliding motility. The latter is likely due to defective TRAP shedding. The KD-phenotype was reverted by genetic complementation with full spatr, but not by variants lacking its adhesive domains. Using SPATR-tagged lines, U-ExM and immunofluorescence, we found that SPATR specifically accumulates at the focal adhesion points in sporozoites. Co-immunoprecipitation studies did not reveal specific partners, indicating SPATR is present in specific vesicles owing to its punctate distribution. Lastly, SPATR 3D-structure prediction revealed a disordered N-terminal region, which we found refractory to genetic manipulation. We are now investigating its role in protein trafficking using both the *P. berghei* sporozoites and *P. falciparum* blood-stages.

IV.3 Trait based assessment of the invasion potential of disease vector mosquitoes - which species are likely to spread next?

Rebecca Pabst[1*], Carla A. Sousa[1], César Capinha[2,3]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Centre of Geographical Studies, Institute of Geography and Spatial Planning, University of Lisbon
3 Associate Laboratory TERRA, Lisboa

Mosquito-transmitted parasites and pathogens pose a growing global health threat, largely driven by the human-mediated spread of vector species beyond their native ranges. The geographic expansion of these vectors is critically reshaping disease transmission patterns, facilitating the establishment of diseases such as dengue, chikungunya, and Zika in previously unaffected regions. Anticipating which mosquito species are most likely to invade and establish new populations is therefore central to proactive surveillance and the prevention of vector-borne diseases.
We present a trait-based framework to quantify the invasion potential of 184 mosquito species associated with human parasites and pathogens. We compiled 26 ecological, life-history, and macroecological traits and linked them to global records of introduction and establishment using random forest models. Our results show that species native to Asia and Australia, adapted to human-made breeding sites, and tolerant of climatic extremes are consistently more likely to be introduced and to establish in non-native regions. Based on this trait profile, we identify 24 species with no known invasion history, but which are likely to spread in the future, 17 of which also exhibit a high probability of establishment. Our framework provides a quantitative early-warning tool to support targeted biosecurity actions and to mitigate the future emergence and establishment of mosquito-borne diseases.

IV.4 Prevalence of triple, quadruple, quintuple, and sextuple mutant Plasmodium falciparum parasites resistant to sulfadoxine-pyrimethamine in Guinea-Bissau

Vasco Nandubé[1], Andréa Luciana Soares da Silva[1,2], Mariana Andrade Ramos Pinto[1], Ronise Silva[1,3], Amabelia Rodrigues[3], Ana Paula Arez[1,4], Márcia M. Medeiros[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Laboratório de Genética de Doenças Complexas, Instituto de Ciências Biológicas, Universidade Federal do Pará
3 Bandim Health Project, Apartado 861, 1004 Bissau Codex
4 Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra,

The prevalence of resistance markers to sulfadoxine-pyrimethamine in *Plasmodium falciparum* is essential to advise chemoprevention in children under two. Further data on the prevalence of these markers is required to safely recommend chemoprevention in pregnancy and different age ranges in childhood. This study aimed to determine the prevalence of sulfadoxine–pyrimethamine resistance markers in *P. falciparum* in Guinea-Bissau. DNA extracted from fingerprick whole blood samples collected on filter paper from *P. falciparum*-infected

individuals across the country in 2017 was subjected to endpoint PCR targeting the *P. falciparum* dihydrofolate reductase (pfdhfr) and dihydropteroate synthase (pfdhps) coding genes. Amplicons were sequenced using the Sanger method on the ABI 3730x platform. Sixty-nine samples (92.0 %) carried the triple-mutant haplotype associated with fully pyrimethamine resistance at codons N51I, C59R, and S108N in the pfdhfr gene. Three samples (4.0 %) harbored two SNPs only, two with mutations at codons C59R and S108N and one at codons N51I and S108N. One sample (1.3%) carried only the mutation at codon S108N, and another showed no mutations (1.3%). Evaluation of sulfadoxine- associated resistance markers is ongoing. The results highlight widespread pyrimethamine resistance and reinforces the need for continuous molecular surveillance to inform safe malaria chemoprevention strategies.

IV.5 Bacterial modulation of malaria transmission: insights from Pseudomonas–mosquito interactions

Maria Carvalho[1,2]; Margarida Marques[1,2]; Luís Bogaio[1,2]; Sandra N. Pinto[2]; Henrique Silveira[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Institute for Bioengineering and Biosciences (iBB), Instituto Superior Técnico, Universidade de Lisboa

Malaria transmission depends on complex interactions between *Plasmodium* parasites, *Anopheles* mosquitoes and the mosquito midgut microbiota. Increasing evidence indicates that resident bacteria can modulate vector susceptibility, yet the mechanisms underlying this refractoriness remain incompletely understood. Among midgut-associated taxa, *Pseudomonas spp*. have been implicated in interference with parasite development. To directly evaluate the transmission-blocking potential of the mosquito microbiota, this study characterized the biofilm formation and extracellular vesicles (EV) production of *Pseudomonas* mendocina Ag_C1, a strain isolated from *Anopheles* midguts. Biofilm formation was characterised by confocal microscopy, revealing dense and heterogeneous structures. EVs were isolated from biofilm supernatants and quantified by nanoparticle tracking analysis, with later-stage biofilms yielding higher particle numbers. Functional assays showed that EVs enhanced early bacterial adhesion and promoted biofilm development in *P. mendocina* and other Anopheles midgut bacterial isolates. In vivo, colonisation of *Anopheles stephensi* with *P. mendocina* resulted in a significant reduction in Plasmodium berghei oocyst burdens. Ongoing work aims to further dissect the contribution of biofilm-associated factors to this transmission-blocking phenotype. These findings highlight bacterial biofilms as key modulators of vector refractoriness and support *P. mendocina* as a promising candidate for microbiota-based malaria control strategies.

IV.6 From genome sequencing to promoter analysis: advancing genetic tools for *Babesia ovis*

Gisele Albuquerque[1], Susana Ribeiro[1], Guilherme Borges Dias[2], Hannes Yngve [3], Monica Mazuz[4], Ana Domingos[1,5], **Sandra Antunes[1,5]**

1 Instituto de Higiene e Medicina Tropical – Universidade NOVA de Lisboa
2 National Bioinformatics Infrastructure Sweden, Uppsala University
3 Uppsala Genome Center, Science for Life Laboratory, Dept. of Immunology, Genetics and Pathology, Uppsala University
4 Division of Parasitology, Kimron Veterinary Institute
5 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Babesia ovis is a tick-borne apicomplexan parasite responsible for ovine babesiosis, a disease of major veterinary and economic relevance. Genomic resources are essential to support functional studies and the development of genetic tools. Here, we report the sequencing, assembly and annotation of the *B. ovis* Israeli strain genome and its application to the evaluation of promoter activity. High-quality DNA was sequenced using the PacBio Sequel IIe HiFi platform, generating 28.84 Gbp of data. The final decontaminated assembly comprised nine contigs, with

an estimated genome size of 8.58 Mb, an N50 of 2.41 Mb and 96% completeness based on BUSCO. A total of 3,549 predicted genes were identified, 85.6% with assigned putative functions. Based on this genomic resource, promoter regions from five *B. ovis* genes (actin, ef-1α, hsp70, tpx-1 and calmodulin) were identified and cloned upstream of a luciferase reporter. Transient transfection assays were performed and luciferase expression was monitored between 24 and 72 hours post-nucleofection. A heterologous *Babesia ovata* ef-1α promoter was used as a positive control, while a promoterless construct served as negative control. Significant differences among constructs were detected at 24 hours, with only the heterologous promoter showing significantly higher activity. The *B. ovis* promoters did not display significant activity under the tested conditions.

Poster Session A

A1. Selective uptake of external 2,3-diphosphoglycerate and metabolic secretion profile in Plasmodium falciparum *in vitro* cultures

Ana Balau[1], Emmanuela Rodrigues[1], Alexandra Maria Moita Antunes[2], Ana Paula Arez[1,3]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Centro de Química Estrutural (CQE), Instituto Superior Técnico (IST), University of Lisbon
3 Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra

The emergence of resistance to current antimalarial therapies underscores the need for host-targeted strategies that impair Plasmodium falciparum survival by modulating host metabolism. Although the parasite possesses most glycolytic enzymes, it lacks bisphosphoglycerate mutase, a key enzyme of the Rapoport–Luebering shunt. Consequently, P. falciparum infection may alter host red blood cell (RBC) metabolism, leading to increased ATP and decreased 2,3-diphosphoglycerate (2,3-DPG) levels in infected RBC (iRBC). In contrast and unexpectedly, neighbouring non-infected RBC (niRBC) in in vitro cultures display reduced ATP and elevated 2,3-DPG levels compared with RBC not exposed to infection or infected cells. Notably, supplementation of cultures with 2,3-DPG significantly impaired parasite development without major effects on host cells.This study aims to determine whether 2,3-DPG is preferentially taken up by iRBC and to characterize metabolites released into the medium of infected cultures. Uptake of exogenous 2,3-DPG was assessed using isotope-labelled 2,3-DPG, and secreted metabolites were analysed by LC–MS.Preliminary results indicate differential 2,3-DPG uptake between iRBC and niRBC, altered extracellular metabolite profiles, and a significant acidification of infected culture media. These findings contribute to a better understanding of how this host-specific metabolite affects parasite development.

A2. Expanding beauvericin's antileishmanial activity against Old World Leishmania spp.

Oliveira-Filho V.A.[1], Epifanio, L.C.S. [2]; Berlinck. R.G.S.[2]; Cortes S.[3]; Miguel, D.C.[1]

1 Universidade Estadual de Campinas
2 Universidade de São Paulo
3 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Leishmaniasis is a group of neglected tropical diseases caused by protozoa of the genus *Leishmania*, with clinical manifestations ranging from cutaneous lesions to potentially fatal visceral form. Current therapies are limited by efficacy and toxicity, rising the need for new anti-Leishmania agents. Beauvericin, a fungal metabolite, recently shown activity against New World *Leishmania spp*. in vitro and in vivo. In this study, we provide a preliminary assessment of beauvericin's activity against *L. infantum*, *L. tropica*, *L. donovani*, and *L. major*. Promastigote viability assays yielded EC50 values of approximately 1.3–3 μM across all species tested, indicating broad-spectrum activity against parasites associated with both cutaneous and visceral leishmaniasis. Cytotoxicity assays in THP-1 and U937 cells indicated low initial toxicity, with a time-dependent increase; nevertheless, after 48 h of incubation, beauvericin maintained a selectivity index >9. Ongoing work includes evaluating its efficacy against intracellular amastigotes, characterizing promastigote morphological changes by microscopy, and studies to elucidate its mode of action. Collectively, these findings support beauvericin as a promising candidate for the development of novel therapeutic strategies against leishmaniasis.

A3. Decoding trypanosome interactions with the host vasculature

Jane Dias[1], Sandra Trindade[1], Sophie Marcus-Wade[1] and Luisa M. Figueiredo[1]

[1]Gulbenkian Institute for Molecular Medicine

Trypanosoma brucei (*T. brucei*) is a unicellular, extracellular parasite responsible for human and animal African trypanosomiasis, a neglected tropical disease that is invariably fatal without treatment. In the mammalian host, *T. brucei* colonizes solid tissues such as the brain and adipose tissue, which poses significant challenges for diagnosis, contributes to disease pathology, and hinders eradication efforts. Despite its clinical relevance, the mechanisms by which T. brucei interacts with and crosses blood vessel barriers remain poorly understood. This project aims to identify parasite-intrinsic factors that facilitate transmigration and characterize the host vascular responses during infection. To achieve this, we employ a multidisciplinary approach integrating genetic manipulation of the parasite—using CRISPR/Cas9 and/or RNA interference (RNAi) to generate loss-of-function mutant lines—with in vitro migration assays and high-resolution imaging techniques. By combining molecular genetics, cellular biology, and advanced microscopy, this study seeks to uncover key determinants of vascular traversal by *T. brucei*, thereby providing new insights into tissue tropism and host–parasite interactions. Understanding these processes will improve our knowledge of trypanosome biology and may inform future strategies for disease control and treatment.

A4. Deciphering Mitochondrial Complex III in *Plasmodium falciparum*

Vitória Baptista -1,2, Julian C. Rayner – 2, Lilach Sheiner -1

1 Glasgow Centre for Parasitology, University of Glasgow, United Kingdom
2 Cambridge Institute for Medical Research, University of Cambridge, United Kingdom

Apicomplexan parasites, including *Plasmodium falciparum* and *Toxoplasma gondii*, cause diseases with major global health impact. *P. falciparum*, the causative agent of malaria, accounts for hundreds of thousands of deaths annually, mainly among children under five. Escalating resistance to antimalarial therapies has intensified the search for new drug targets. The mitochondrial electron transport chain (mETC), particularly Complex III (CIII), is a validated target, as demonstrated by the efficacy of the antimalarial atovaquone. Yet, despite its clinical relevance, the structural organisation and full subunit composition of *P. falciparum* CIII remain poorly understood. To understand how CIII might be selectively targeted in apicomplexans, our group identified both conserved and parasite-specific subunits in *T. gondii* and revealed how atovaquone binds within a unique parasite-specific pocket. Similar divergence has been suggested in *P. falciparum* CIII, but its molecular architecture is still unresolved. We have shown that the mammalian-homologous CIII subunits are essential for Plasmodium survival and transmission. We now focus on defining the roles of *P. falciparum*-specific CIII subunits in complex formation, parasite viability and mitochondrial function. This work will illuminate key aspects of *P. falciparum* mitochondrial biology and support the development of new targeted therapies to improve malaria treatment and global parasite control.

A5. Understanding how *T. brucei* induces lipolysis in adipocytes

David Valente Ferreira[1], Henrique Machado[1], Abdulbasit Amin[1] e Luisa Figueiredo[1]

1 Gulbenkian Institute for Molecular Medicine

Trypanosoma brucei infection leads to extreme weight loss, mainly due to the reduction of fat mass. While the immune response contributes to this wasting, a significant part of the fat loss is independent of immune activity and is associated with parasite accumulation in the adipose tissue. Using an in vitro adipocyte *T. brucei* co-culture system, we found that both parasites and parasite-conditioned media are able to induce a strong lipolytic response, comparable to that triggered by classical lipolytic drugs. This effect is driven by secreted small parasite molecules (<3 kDa), non-protein, and not transported via extracellular vesicles. Interestingly, although *T. brucei* induces adipocyte lipolysis to the same level as Forskolin (a standard lipolytic drug), the activity is only detected after 24hr while drugs are much faster (10min). These observations suggest that the mechanism of parasite-induced lipolysis is different from the fast cAMP-PKA dependent mechanism triggered by Forskolin. My PhD focuses on identifying these parasite-derived molecules and understanding the signaling pathways activated in adipocytes.

A6. Dynamic forces shaping the Anopheles mosquito microbial community across its lifespan

Luis Constantino[1,2], Henrique Silveira[3], Leonilde M. Moreira[1,2]

1 iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, University of Lisbon
2 i4HB – Institute for Health and Bioeconomy at Instituto Superior Técnico, University of Lisbon
3 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Mosquito microbiota plays a fundamental role in development, behaviour, physiology, and vectorial competence, varying significantly among species, developmental stages, and environment. However, the microbial profiles associated with different life cycle stages of the emerging malaria vector *Anopheles stephensi* remain poorly studied. In this research, 16S rRNA gene amplicon sequencing (V3-V4 regions) was used to explore the dynamics of microbial communities throughout development. Diversity analyses revealed significant variation in microbial composition among life cycle stages (egg, larva, pupa, and adult). L4 larval and pupal stages exhibited greater bacterial richness compared to adults, eggs and early stages. Taxonomic analysis identified the phylum Proteobacteria and class Gammaproteobacteria as dominant. Predominant bacterial genera were *Enterobacter*, *Elizabethkingia*, *Flectobacillus*, *Perlucidibaca*, *Methylocystis*, and Acinetobacter. Notably, *Flectobacillus* was the most abundant taxon in pooled adults, while Perlucidibaca dominated individual adults. Pupae and L4 larvae were dominated by *Flectobacillus*, L1-L3 larvae by *Flectobacillus* and *Reyranella*, and eggs by Enterobacter and *Methylobacterium*. Results demonstrate significant microbiota shifts between aquatic and terrestrial stages, with no significant differences between sexes.

A7. Navigating the Surface: Deciphering Divergent Trafficking Pathways in *P. falciparum*

Gwendolin Fuchs[1,2], Stephanie Nofal[1,2], David Jones[2], Hugo Belda[1,2], Henrique Ramos[1], Moritz Treeck[1,2]

1 Cell Biology of Host-Pathogen Interactions, Gulbenkian Institute for Molecular Medicine
2 Signaling in Apicomplexan Parasites Laboratory, The Francis Crick Institute

Plasmodium falciparum exports various proteins to the surface of the infected red blood cell to mediate cytoadherence and immune evasion; two central aspects of malaria pathogenesis. Among these surface proteins are members of the erythrocyte membrane protein 1 (EMP1) and the repetitive interspersed (RIFIN) protein families. To traffick these proteins to the surface, *P. falciparum* establishes Golgi-like trafficking hubs within the host cell cytosol, known as Maurer's Clefts. However, whether Maurer's Clefts actually sort cargo similar to the Golgi and distinguish between different classes of surface proteins is not known. Here, we dissect the trafficking pathways of EMP1 and RIFINs using proximity labelling to identify pathway-specific interactomes. Conditional deletion of candidate trafficking genes at major sites of protein export and surface translocation reveal pathway-specific requirements for EMP1 and RIFIN trafficking. This supports a critical role for the Maurer's Clefts in cargo sorting, reinforcing their functional analogy to the Golgi. We are currently investigating which domains hold information for the trafficking trajectory and whether the RIFIN trafficking route represents a conserved trafficking mechanism across *Plasmodium* species compared to EMP1, which relies on numerous *P. falciparum*- specific proteins. These results shine a new light on the function of Maurer's Clefts and the evolution of protein trafficking pathways beyond the parasite boundaries.

A8. Assessment of Soil Contamination by *Toxocara spp.* in the City of Praia, Santiago Island – Cape Verde: A Pilot Study

V Veiga; P Ferreira ; **Manuela Calado**[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Toxocariasis is one of the most common parasitic zoonoses worldwide. It is transmitted through contact with contaminated soil or sand in public areas, which may be contaminated by the faeces of stray or non-dewormed domestic animals. Human infection is caused by nematodes of the genus *Toxocara*, which can result in cutaneous or visceral larva migrans. The aim of this study was to assess the prevalence of Toxocara spp. eggs in soil and faecal samples collected from urban parks and public gardens in Praia, Cape Verde. A total of 160 samples were analyzed, including 20 faecal samples and 140 soil samples. In the parasitological examination of the 20 fecal samples, 16 were negative and 4 were positive for *Toxocara spp*. eggs. Of the soil samples, 134 were negative and six were positive. Molecular diagnosis showed higher sensitivity as it detected three additional soil samples and two faecal samples positive for *Toxocara canis* that had not been identified by parasitological methods. Based on these preliminary results, it is possible to confirm the presence of contamination in public spaces frequented by stray animals. This highlights the need to implement control measures to prevent contamination of these widely used public places.

A9. To be or not to be (cleared) - How single cell heterogeneity shapes *Toxoplasma* infection outcome

Christian Gnann[1,2], Catarina Rosa[1], Manuel Tanqueiro[1], Gabriel G Martins[1], Moritz Treeck[1]

1 Gulbenkian Institute for Molecular Medicine
2 Stockholm University, Department of Molecular Biosciences, The Wenner-Gren Institute

Toxoplasma gondii (*T. gondii*) is an obligate intracellular apicomplexan parasite capable of infecting virtually any nucleated cell in warm-blooded hosts. In cell culture, only a subset of cells becomes infected, and among those, only some successfully clear the parasite. We hypothesize that heterogeneity between single host cells determines these divergent infection outcomes. Using high-throughput immunofluorescence imaging and advanced computational image analysis, we profile the expression and subcellular distribution of 40 cellular markers spanning different metabolic, signalling, innate immune defense, cell cycle pathways, and organelles, all contributing to the heterogenous host cell state. Our preliminary data recapitulate known infection-induced host cell remodelling, including cell cycle arrest and metabolic shifts, and provide novel insights into which cell states are more susceptible to infection. In the upcoming months, we will profile all markers in the same cells using 4i multiplexed imaging. This approach will define the molecular basis that determines whether a cell is resistant to infection or has an increased clearance potential. The results will likely translate to infections with other intracellular pathogens and highlight the broader relevance of accounting for cellular heterogeneity across the entire field of infection biology.

A10. Exploring the kinetics of *T. congolense* sequestration

Madalena Rodolfo[1], Jorge de-Carvalho[2], José Lourenço[1], Sara Silva Pereira[1]

1 Católica Biomedical Research Centre, Católica Medical School, Universidade Católica Portuguesa
2 ITQB-NOVA

Trypanosoma congolense is a major cause of animal African trypanosomiasis, a disease of livestock. The resulting losses in productivity are estimated at ~USD$4.5 billion annually. *T. congolense* colonises the blood of infected mammals and sequestration, or its attachment to the inner walls of the blood vessels, is a key virulence strategy that directly affects disease progression and outcome. In theory, disturbing sequestration could abrogate disease. However, little is known about the mechanism of sequestration, including its determinants and kinetics. In this project, we aim to decode the kinetics of sequestration under distinct microenvironments. To achieve this, we use 2D microfluidic channels under a constant, physiologically relevant flow conditions that replicate laminar flow in venules, seeded with a monolayer of endothelial cells of different organotypic origins. We determine parasite fate after sequestration and assess detachment and reattachment rate and probabilities at both individual and population levels. Subsequently, we will investigate the role of cell-cycle in sequestration kinetics by inducing cell cycle arrest before and after kinetics assessment. This work builds a quantitative foundation for understanding, and ultimately targeting, the vascular niche that enables *T. congolense* persistence and its associated pathology.

A11. Evaluation of *Strongyloides venezuelensis* antigens in immunodiagnosis of strongiloidosis

Ereso Undinho João[1]; Fabiana Martins de Paula[2]; Silvana Belo[1] & Pedro Manuel Ferreira[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Laboratório de Investigação Médica (LIM-06) Hospital das Clinicas and Instituto de Medicina Tropical de São Paulo, Faculdade de Medicina, Universidade de São Paulo, Brasil

Human Strongyloidiasis is a chronic parasitic disease, neglected and cosmopolitan, caused by *Strongyloides stercoralis*, affecting between 300 to 600 million individuals, mainly immunocompromised patients, namely those submitted to corticosteroid therapies. Autoinfection can evolve into the Hyperinfection/Dissemination Syndrome, a severe and frequently fatal condition. Three *Strongyloides venezuelensis* antigens (L3CTAB, STL, and STL-W), obtained by distinct extraction methods, were tested in ELISA technique with a panel of 75 serum samples, covering individuals proven to be infected by *S. stercoralis*, non-infected individuals, suspected individuals and individuals infected by other parasitic diseases (cross-reactivity evaluation). The results were compared and validated by an Anti-Strongyloides ELISA (IgG) Kit EUROIMMUN.Based on ROC Curve, The STLW antigen presented the best performance, with a Sensitivity of 77.78% and a Specificity of 81.36%. The L3CTAB antigen showed a Sensitivity of 77.75% and a Specificity of 72.88%, while the STL Ag demonstrated a Sensitivity of 66.67% and a Specificity of 76.27%. The limited Sensitivity was related with the insufficient number of confirmed positive samples. However, *S. venezuelensis* antigens showed immunogenic potential. Despite these statistical limitations, the work validates the use of heterologous *S. venezuelensis* antigens as a promising and economically viable basis for the continuous development of an in-house ELISA.

A12. Beyond the Usual Suspects: Evidence of *Colpodella* in an Avifauna-Diverse Site and Taxonomic Ambiguity in *Rhipicephalus* Ticks

João Lucas[1,], Leonardo Moerbeck[1,2], Sandra Antunes[1,2], Ana Domingos[1,2]

1 Instituto de Higiene e Medicina Tropical – Universidade NOVA de Lisboa (IHMT-NOVA)
2 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Ticks and their associated microorganisms form complex ecological systems with implications for human and animal health. This study surveyed questing tick communities at three ecologically distinct sites in the district of Santarém, Portugal, using integrated morphological and molecular approaches. Tick assemblages were overwhelmingly dominated by Rhipicephalus sanguineus sensu lato across all environments. Comparative morphological examination and phylogenetic analyses based on mitochondrial 16S rRNA and coxI markers did not support the separation of *Rhipicephalus hibericus* and *R. sanguineus*, corroborating previous evidence that these taxa cannot be reliably distinguished using classical morphological characters or commonly applied molecular markers. Molecular screening detected medically relevant bacteria, including *Rickettsia spp.*; however, the most noteworthy finding was the detection of Colpodella-like sequences in ticks collected exclusively at a single rural site characterized by high avifaunal diversity and ecological complexity. Traditionally considered free-living protists, *Colpodella spp.* have increasingly been reported in ticks and, more rarely, in human clinical samples, raising questions regarding their ecological role and potential pathogenicity. The site-specific occurrence observed here suggests that local ecological drivers—potentially involving bird–tick interactions—may influence *Colpodella* circulation. These results emphasize the value of integrative taxonomy and exploratory molecular surveillance in revealing overlooked components of tick-associated microbial communities.

A13. Resistance profiling of *Plasmodium falciparum* infections in Mozambique using custom dual indexing and Illumina next-generation amplicon sequencing

Daniela Matias[1], Celso Chaves[1,2], Clemente da Silva[3], Nordino Chivale[4], Crizolgo Salvador[4], Paulo Arnaldo[4], Sonia Enosse[5], Fátima Nogueira[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Faculdade de Ciências de Saúde, Universidade Lúrio, Campus Universitário de Marrere
3 Goodbye Malaria, Parque Industrial de Beluluane, Maputo, Mozambique.
4 Instituto Nacional de Saúde (INS), Av. Eduardo Mondlane Nº 1008
5 Malaria Consortium, Av. Lucas Elias Kumato, Nº 118. Bairro da Sommershield, Maputo, Mozambique.

The burden of malaria remains high in endemic countries, especially in sub-Saharan Africa, where the species Plasmodium falciparum is the most common cause of malaria. The spread of P. falciparum resistance against antimalarials threatens their efficiency and the success of control strategies in many malaria-endemic countries. Mozambique, a country located on the eastern coast of Southern Africa, accounted for ≈9,256,000 malaria cases in 2023, ranking 5th worldwide in burden of disease caused by malaria. Malaria is endemic throughout Mozambique, with regions ranging from hyperendemic to hypoendemic seasonal transmission. Using samples collected from seven Mozambican provinces in 2021, our lab has developed a protocol and analysis pipeline for the study of molecular markers associated with antimalarial resistance, with the use of dual-indexing and amplicon sequencing technology (Illumina MiSeq). It allows for the analysis of multiple samples at once, at a reduced price, and returns multiple reads for each sample. We have focused on pfcrt, pfk13 and pfmdr1 polymorphisms associated with resistance to Mozambique's main antimalarial therapies: artesunate-amodiaquine, artemether-lumefantrine and dihydroartemisinin-piperaquine. Knowledge of the prevalence of these molecular markers associated with antimalarial resistance is essential for the success of Mozambique's malaria control strategies.

A14. mosquitoWEB: Citizens detection of *Aedes allbopictus* presence.

Maria Teresa Novo[1], Luis Filipe Lopes[1,2], Carla A. Sousa[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 cE3c - Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal.

mosquitoWEB is a Citizen Science project whose main objective is the detection and tracking of the presence of invasive mosquito species in Portugal. The first record of Aedes albopictus occurred in 2017 (Penafiel/Porto). New submissions were made in 2020 (Loulé/Faro). In 2021 and 2022, occurrences of this species were also recorded only in the Algarve region (Olhão, Tavira, Faro, and Loulé in 2021, and Olhão and Tavira in 2022). In 2023, through mosquitoWEB submissions, this species was detected for the first time in Lisbon (Lisbon and Oeiras), increasing the number of municipalities with submissions in the Algarve. In 2024 and 2025, the trend of higher citizen participation from the Algarve continued, but with expansion and new findings in the northern and central regions of the country (Porto, Braga, Aveiro, Coimbra).

A15. Zinc-based regulation of the *Trypanosoma brucei* ZIP3 transporter.

Teresa Leão[1,2,3], Anna Trenaman[3], Michele Tinti[3], Gustavo Bravo Ruiz[3], Idálio J. Viegas[4], Luisa M. Figueiredo[4], Margarida Duarte[1], Ana Maria Tomás[1,2,*] and David Horn[3,*]

1 i3S – Universidade do Porto
2 ICBAS - Universidade do Porto
3 Faculty of Life Sciences, University of Dundee
4 Gulbenkian Institute for Molecular Medicine

Transition metals such as zinc are essential yet toxic to trypanosomatids, requiring tight regulation of metal uptake in response to host-imposed fluctuations. We show that transcript levels of the Trypanosoma brucei ZIP transporter TbZIP3 are upregulated under zinc limitation induced by the chelator TPEN and return to basal levels upon zinc supplementation. In contrast, treatment with an iron chelator (DFO) or addition of Fe2+ or Fe3+ do not alter TbZIP3 expression, indicating zinc-specific regulation. To investigate the underlying regulatory mechanisms, we generated a reporter strain in which neomycin resistance is controlled by the TbZIP 3′UTR. This reporter responds appropriately to zinc limitation and excess and was used in a genome-scale RNAi (RIT-seq) screen, where increased fitness under neomycin selection identifies negative regulators of TbZIP3 expression. TbZIP3 genes were prominent hits themselves, consistent with reporter activation under zinc-limiting conditions caused by TbZIP3 knockdown. Importantly, we also identified a nuclear RNA-binding protein containing zinc knuckle motifs and a putative nuclease domain, which we propose acts as a conserved mediator of trypanosomatid zinc-responsive gene regulation.

A16. Copy number variation of plasmepsin2 and multidrug resistance-1 genes in *Plasmodium falciparum* in Luanda before and after ACTs implementation

Ana Dias[1], Débora Serrano[2], Teresa Baptista Fernandes[1], Luís Varandas[2], Cláudia Conceição[2], Fátima Nogueira[2]

1 Laboratório de Microbiologia Clínica e Biologia Molecular, Unidade Local de Saúde de Lisboa Ocidental
2 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Artemisinin partial resistance has emerged in Africa and is feared to spread across the continent, potentially compromising artemisinin-based combination treatment (ACT). Of interest, piperaquine resistance is associated with plasmepsin2 (pfpmp2) copy number variation (CNVs), while CNVs in the multidrug efflux pump, multidrug resistance-1 (pfmdr1), increase resistance to amodiaquine and lumefantrine. This study aimed to evaluate the CNV of pfpm2 and pfmdr1 in Plasmodium falciparum isolates from Luanda, Angola, before and after the introduction of ACTs as first-line treatment. Blood samples were collected from patients with uncomplicated malaria during two distinct periods: pre-ACT (2003-2004) and post-ACT implementation (2018-2023). Copy number estimates were determined using a SYBR green-based quantitative PCR assay. Among the analyzed samples, multiple copies were observed in 4.4% for pfpm2 and 17.1% for pfmdr1. The prevalence of pfpm2 was 6.1% in 2003–2004 and 1.5% in 2018–2023, and pfmdr1 was 23.5% and 7.4% in the same periods. The prevalence of increased CNV in both pfmdr1 and pfpm2 was lower in the post-ACT period compared to pre-ACT. This reduction may be indicative of changes in antimalarial drug use and selective pressure following ACT introduction. This study supports the continued need for molecular surveillance, ideally countrywide, to improve the detection of ACT resistance before it becomes widespread.

A17. Nanopore Profiling of Polyadenylation in *T. brucei*

Michał Małecki[1], Christoph Wenzl[1], Luisa M Figueiredo[1]

1 Gulbenkian Institute for Molecular Medicine

The mRNA poly(A) tail is a key determinant of cytoplasmic mRNA stability. In *Trypanosoma brucei*, where gene expression is controlled almost entirely at the post-transcriptional level, differential mRNA decay rates are major drivers of transcript abundance. To investigate polyadenylation dynamics in this organism, we leveraged Oxford Nanopore Technologies to perform genome-wide profiling of poly(A) tail length and composition.Using Splice Leader–anchored cDNA Nanopore sequencing, we developed an approach to monitor mRNA deadenylation in vivo and to detect terminal non-templated nucleotides at single-molecule resolution. In parallel, we employed Nanopore direct RNA sequencing to identify non-adenosine residues within *Trypanosoma* poly(A) tails. Together, these complementary methods enabled comprehensive characterisation of poly(A) tail behaviour across the transcriptome.Our analyses indicate that deadenylation rate is a key regulator of mRNA stability in T. brucei. Notably, Variant Surface Glycoprotein (VSG) mRNA—essential for parasite virulence and representing ~10% of total cellular mRNA—exhibits exceptionally high stability. Examination of VSG polyadenylation dynamics reveals complex regulatory features that, we propose, contribute to its remarkable stability.

A18. Design of a Broad-Spectrum Antiviral Strategy Against Flaviviruses

Eva Dias[1], Nelly Silva[1,2], Ivo C. Martins[1,2], Rita Fernandes[2], Gonçalo Seixas[1], Nuno C. Santos[1,2]

1 Gulbenkian Institute for Molecular Medicine
2 Faculdade de Medicina, Universidade de Lisboa

Flaviviruses such as dengue virus (DENV) and Zika virus (ZIKV) remain major global health threats, and their continued expansion into new geographic regions underscores the urgent need for innovative antiviral strategies. In this study, we designed and evaluated two antiviral molecules developed in our laboratory. Compound pep14-23 was previously developed and demonstrated antiviral activity against ZIKV and is now being tested for other flaviviruses. In addition, we are now evaluating a newly developed molecule based on a different strategy.Vero 81 cells were infected with ZIKV and viral replication was quantified by plaque assay. DENV replication was assessed by indirect immunofluorescence. In ZIKV-infected cells, pep14-23 produced only a modest reduction in viral replication, whereas the new molecule induced strong inhibition both at 12 and 24 h incubations. In DENV-infected cells, pep14-23 showed no significant effect, while the new molecule achieved near-complete suppression of viral replication, with no detectable DENV from 3 to 24 h.Overall, our findings identify pep14-23 and the newly developed molecule as promising broad-spectrum antiviral candidates against flaviviruses, offering compelling insights into their translational potential and paving the way for their future application as innovative tools for antiviral therapy and vector control.

A19. It takes two to tango: novel insights into phospho-STAT6 signaling in *T. gondii* infected cells

Ana Matias[1,2], Simon Butterworth[2], Franziska Hildebrandt[1], Gwendolin Fuchs[1,2], Francesca Torelli[1,2], Moritz Treeck[1,2]

1 Gulbenkian Institute for Molecular Medicine
2 The Francis Crick Institute

To survive within the host cell, Toxoplasma secretes proteins from its specialised secretory organelles, rhoptries and dense granules, that interfere with host cell immune functions. The *Toxoplasma* rhoptry kinase ROP16 phosphorylates STAT6 (P-STAT6), a host transcriptional factor. This leads to STAT6 activation and ultimately induces an M2-like polarisation of infected macrophages, which renders the infected cell as anti-inflammatory. We have previously shown that SOS1 knockout (KO) parasites promote host transcriptional changes similar to ROP16-KO infected cells. SOS1-KO parasites are unable to sustain the P-STAT6 signal 24 h post-infection (hpi), revealing that, although SOS1 is not required to initially phosphorylate STAT6, it is necessary to sustain it. However, the mechanism behind it remains unknown. Transmission electron microscopy reveals that SOS1 deletion does not disrupt rhoptry morphology. Transcriptomic analyses of infected cells shows that SOS1-KO parasites do not induce a distinct host response beyond that of ROP16. We are currently testing three hypotheses: a) SOS1 interferes with the canonical host STAT6 activation pathway; b) SOS1 regulates the levels of ROP16 secreted into the host cell cytosol; c) SOS1 modifies ROP16 increasing its kinase activity. Understanding how SOS1 contributes to sustained STAT6 activation will provide critical insights into *Toxoplasma*'s immune evasion strategies.

A20. Lipid-dependent heme detoxification in *Plasmodium falciparum*: Localization and functional studies

Ruth Esho[1], Vitória Baptista[1,2,3,4], Nuno Osorio[1,2] , Angel Vizoso-Vázquez[5], Maria Isabel Veiga[1,2]

1 Life and Health Sciences Research Institute (ICVS), University of Minho
2 ICVS/3B's—PT Government Associate Laboratory
3 University of Cambridge Institute for Medical Research
4 University of Glasgow Centre for Parasitology
5 Facultade de Ciencias, Universidade da Coruña

Malaria remains a major cause of morbidity and mortality worldwide. During the intra-erythrocytic stage, *P. falciparum* detoxifies free heme by converting it into insoluble hemozoin crystals. This process, though not fully understood, is essential for parasite survival and is exploited as a target by several frontline antimalarial drugs. Evidence suggests that lipids may facilitate hemozoin formation. Perilipins, which coat lipid droplets and regulate lipid metabolism, could play a role in this process. These proteins have never been characterized in *Plasmodium*, and their potential involvement in heme detoxification remains unexplored. We hypothesize that perilipin-like proteins contribute directly to hemozoin formation and aim to clarify their function in P. falciparum biology. We explored several *P. falciparum* proteins with perilipin-like signatures candidates by generating tagged, conditional knockdown lines for functional studies using the selection link integration technique. Due to unsuccessful integration into the parasite genome we are using a different approach that have shown greater success in our laboratory – CRISPR/cas9 system. Using CRISPR we will generate tagged *Plasmodium falciparum* strains. We will perform localization and functional studies. Validating the presence and function of perilipins will reveal novel molecular targets for antimalarial drug development, potentially contributing to new strategies to combat drug-resistant malaria.

A21. Pulmonary infection by *Leishmania infantum*: implications for treatment efficacy

Ricardo Monteiro[1,2], Carolina Faria[1,3], Joana Rodrigues[1], Nuno Santarém[1,2], Anabela Cordeiro-da-Silva[1,2], Joana Tavares[1,4,*]

1 Host-Parasite Interactions Group, i3S - Institute for Research and Innovation in Health, University of Porto
2 Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal.
3 Department of Chemical and Biological Engineering, Faculty of Engineering, University of Porto
4 Department of Molecular Biology, ICBAS - School of Medicine and Biomedical Sciences, University of Porto

Leishmania parasites, transmitted by the bite of infected female phlebotomine sandflies, contribute annually to a substantial disease burden. In the Mediterranean basin, *Leishmania infantum* is the causative agent of visceral leishmaniasis (VL), the most severe form of the disease, fatal if left untreated. Following dissemination from the bite site, parasites preferentially infect the liver, spleen, and bone marrow. Using a mouse model that exhibits some clinical features of VL, such as hepatosplenomegaly, and combined with live imaging, we found that early after blood dissemination the lungs contain approximately 90% of the total parasites' bioluminescent signal among multiple organs, even after transcardiac perfusion. Quantification of parasite burdens using the sensitive method of organ limiting dilution showed that parasites are rapidly cleared from the lungs, with some persisting for several weeks, regardless of the infection route. These findings led us to evaluate the efficacy of conventional antileishmanial treatments at eliminating lung-residing parasites. So far, our results indicate that not all reference drugs are effective at eliminating lung parasites. The involvement of the respiratory system in VL is supported by autopsy findings and by more recent studies showing that VL patients frequently present with cough, dyspnea, and abnormal spirometry. Altogether, this work contributes to unravel an unconventional tissue niche of *L. infantum* and supports the need to study the mechanisms that control lung infection by this parasite.

A22. Multistage Steroid Derivatives as Next-Generation Antimalarials

Beatriz Velosa[1,2,3,4], Carla Calçada[1,2], Vitória Baptista[1,5], Joana Sousa[1,2], Carina Calixto[1,2], Nuno Osório[1,2], Liliana Mâncio-Silva[4], Maria Manuel Silva[3], Maria Isabel Veiga[1,2]

1 Life and Health Sciences Research Institute (ICVS), University of Minho
2 ICVS/3B's—PT Government Associate Laboratory
3 Faculty of Pharmacy, University of Coimbra (FF-UC)
4 Pasteur Institute Paris (IPP)
5 University of Cambridge

In 2024, global malaria cases rose to 282 million, a crisis exacerbated by emerging artemisinin resistance across Africa. To address the urgent need for new treatments, this study explores rationally modified steroids as antiplasmodial agents. We synthesized novel derivatives and evaluated their efficacy against Plasmodium falciparum blood stages. Two lead compounds, CC1 and CC12, emerged with high potency and minimal cytotoxicity. Mechanistic analysis revealed these compounds induce lethal oxidative stress by disrupting the parasite's natural heme detoxification. Significantly, their activity remains effective regardless of mutations in key resistance transporters (PfCRT and PfMDR1), suggesting they can bypass common resistance mechanisms. Additionally, they demonstrated synergistic effects when combined with standard antimalarials. Collectively, these results identify rationally modified steroid scaffolds as a promising chemical space for antimalarial discovery, providing a solid foundation for further medicinal chemistry optimisation towards next-generation therapies.

A23. Nature's Toxins as Tools: Antimalarial Activity of Snake Venom Fractions and Venom-Derived Peptides

Inês Morais[1,2]; Sofia Cortes[1]; Paula Dantas Oliveira Moreira[3]; Milena Daniela Souza Silva[4]; Fernanda Calheta Vieira Portaro[5]; Sonia Aparecida de Andrade Chudzinski[6]; Paula Gomes[2]; Fátima Nogueira[1,2]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal
3 Development and Innovation Laboratory, Butantan Institute, São Paulo, Brazil
4 Laboratory of Cellular Immunology Applied to Health, Oswaldo Cruz Foundation, FIOCRUZ Rondônia, Porto Velho-RO, Brazil
5 Laboratory of Structure and Function of Biomolecules, Butantan Institute, São Paulo, Brazil
6 Escola Superior do Instituto Butantan, Butantan Institute, São Paulo, Brazil

Snake venoms (SVs) contain diverse bioactive molecules, including phospholipases A2, metalloproteinases, and serine proteases, many of which display therapeutic potential. This work aims to screen crude SVs and their fractions for (i) antiplasmodial action against erythrocytic stages of *P. falciparum*, (ii) anti-*Leishmania* activity, and (iii) cytotoxic and hemolytic effects. In parallel, peptides from scorpion venoms are being screened for antiplasmodial activity. A panel of eight samples was screened against the *P. falciparum* 3D7HT-GFP clone. Fractions showing >70% inhibition were selected for further determination of IC50. Cytotoxicity was assessed in V79 fibroblasts and THP-1 monocytes, and hemolysis was evaluated using erythrocytes. Fraction P1 and the Pool inhibited parasite growth by >70% and their IC50 profiles suggest an effect on egress and/or reinvasion. In turn, only the crude venom was active against the Leishmania clones tested. No fraction caused significant hemolysis (<10%). P1 and the Pool exhibited low cytotoxicity in V79 cells, showing favorable selectivity (SI>10), whereas only the crude venom was highly toxic to THP-1 cells. These findings identify SVs fractions with selective antiplasmodial activity, supporting their potential as leads for antimalarial drug development. Future work includes testing additional fractions, characterizing molecular components, and investigating putative mechanisms of action.

A24. *Galleria mellonella*: an invertebrate model with potential for *Leishmania* research

E. Gaspar[1], M. Andrade[1], J. Marques[1], S. Santos Costa[1], S. Cortes[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Leishmaniasis is a vector-borne disease caused by Leishmania spp. parasites. Drug development studies with *Leishmania spp.* rely heavily on in vitro and in vivo models. *Galleria mellonella* (wax moth) is an invertebrate insect model that has been established in recent years as an alternative system for studying various aspects of infectious diseases. This model is relatively inexpensive and easy to maintain, and its short life cycle and large number of individuals support the refinement and optimisation of experimental techniques and procedures. This study aimed to optimise culture conditions for *G. mellonella* hemocytes and to evaluate their potential as an in vitro model for *Leishmania spp*. *G. mellonella* hemocyte cultures were established in Grace's medium + 10% FBS, L-cysteine, and a drug cocktail. Hemocyte cytotoxicity in response to standard drugs was evaluated, as well as infection with *Leishmania donovani* promastigotes. Successful hemocyte cultures were achieved using the drug cocktail, and infection of *G. mellonella* hemocytes by *L. donovani* promastigotes was observed, with amastigotes detected inside hemocytes. These results validate the proposed infection assay protocol. Future work includes assessing hemocytte preservation and testing other *Leishmania* species.

Poster Session B

B1. MHV68 reshapes the hepatic microenvironment and reduces *Plasmodium* liver infection

Andreia F. Mósca[1], Beatriz Câncio[1], Viriato M'bana[2], J. Pedro Simas[3], Miguel Prudêncio[1]

1 Gulbenkian Institute for Molecular Medicine
2 University of Massachusetts UMass Chan Medical School
3 Católica Biomedical Research, Católica Medical School, Universidade Católica Portuguesa

Malaria, caused by Plasmodium parasites and transmitted by Anopheles mosquitoes, remains a major health burden in Africa. This region also shows high prevalence of gammaherpesviruses such as Epstein-Barr virus (EBV), which is linked to endemic Burkitt lymphoma, the most frequent pediatric cancer in Africa. Despite their overlapping distribution, the co-infection with these pathogens remains poorly defined. Although the liver is central for both EBV-related manifestations and the clinically silent but essential liver stage of Plasmodium development, the impact of gammaherpesviruses on subsequent Plasmodium liver-stage infection is largely unexplored. Using murine gammaherpesvirus-68 (MHV68) as a surrogate for EBV, we show that MHV68 is hepatotropic in mice and that a prior infection with this virus reduces the liver burden of a subsequent Plasmodium berghei infection, indicating viral modulation of the hepatic microenvironment. Defining how MHV68 reshapes liver immunity may reveal mechanisms by which viral infections alter susceptibility to malaria. We aim to determine how MHV68 hepatotropism alters liver function and immune responses, and how these changes influence subsequent Plasmodium infection by assessing viral persistence, shifts in immune cell populations, and their impact on malaria outcomes using in vitro and in vivo approaches. This work addresses a major gap in viral-parasitic co-infection research with implications for disease management in endemic areas.

B2. Unravelling the role of exported effectors on host cell remodelling during *Plasmodium falciparum* gametocyte infection

Stephanie D. Nofal[1], Hugo Belda[1], Gwendolin Fuchs[1], Franziska Hildebrandt[1], Moritz Treeck[1]

1 Cell Biology of Host-Pathogen Interactions Lab, Gulbenkian Institute for Molecular Medicine

Of the six *Plasmodium* species that cause malaria in humans, the majority of fatalities are caused by *Plasmodium falciparum*. Central to this species' increased virulence is its ability to remodel its host cell. This is mediated by effector proteins that the parasite exports into the erythrocyte in which it resides. While most of our understanding on host cell remodelling comes from the asexual stages, there is now mounting evidence of significant remodelling of the host cell by gametocytes, the sexual stages responsible for disease transmission. Prior to being taken up by mosquitos, immature gametocytes sequester to the bone marrow where they can infect nucleated erythrocyte precursors and interact with other stem cells, leading to changes in erythroid maturation, cytokine secretion and angiogenesis; ultimately leading to remodelling of the bone marrow macro-environment. However, the mechanisms underlying these remodelling events remain unknown. I will give an overview of the approaches I am using to decipher how gametocytes remodel their macro-environment, allowing us to expand our understanding of how this complex parasite subverts its host.

B3. Disentangling the Roles of pfmdr1 Copy Number Variations and SNPs in *Plasmodium falciparum* Antimalarial Resistance

Maria Eduarda Pereira Mascarenhas[1,2,3], Diego Paradinha Pacheco[2,3], Beatriz Velosa da Fonseca[2,3], Bruno Freitas[2,3], Vitória Baptista[2,3], Nuno Sampaio Osório[2,3], Taís Nóbrega de Sousa[1], Maria Isabel Veiga[2,3]

1 Grupo de Biologia Molecular e Imunologia da Malária, Instituto René Rachou, Fundação Oswaldo Cruz (FIOCRUZ)
2 Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho
3 ICVS/3B's─PT Government Associate Laboratory

Malaria remains a major global health challenge, with increasing resistance threatening the efficacy of artemisinin-based combination therapies (ACTs). The *Plasmodium falciparum* multidrug resistance protein 1 (pfmdr1) plays a central role in modulating parasite susceptibility to several antimalarial drugs through single-nucleotide polymorphisms (SNPs) and copy number variations (CNVs). While the contribution of specific SNPs has been explored, the impact of CNVs compared to SNPs remains insufficiently understood. This project aims to investigate the relative contribution of pfmdr1 CNVs and SNPs to antimalarial drug resistance using a targeted genetic engineering approach. Recombinant P. falciparum lines derived from the Dd2 strain, which harbors multiple copies of pfmdr1, will be generated to selectively reduce gene copy number across distinct SNP backgrounds. Zinc-finger nucleases are employed to induce double-strand breaks at the 5' region of pfmdr1, enabling gene truncation through homology-directed repair and the incorporation of a GFP reporter to facilitate identification of recombinant parasites. By establishing parasite lines with controlled genetic backgrounds, this study seeks to disentangle the individual and combined effects of pfmdr1 CNVs and SNPs on antimalarial drug susceptibility. The results are expected to advance the understanding of PfMDR1-mediated resistance mechanisms and contribute to the validation of molecular markers for resistance surveillance.

B4. Quinazoline Derivatives as Potential Anti-malarial Drugs: a Drug Design Approach

Margarida Leonardo[1], Karla Menezes[2,3], Inês Morais[1,4], Carolina Vieira[5], Sara Silva Pereira[5], Igor Nascimento[2,3], Ricardo Moura[2,3], Rui Moreira[6], Fátima Nogueira[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Postgraduate Program in Technological Development and Innovation in Pharmaceuticals, Federal University of Paraíba, João Pessoa, PB, Brazil;
3 Drug Development and Synthesis Laboratory, Department of Pharmacy, State University of Paraíba, Campina Grande, PB, Brazi
4 LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal
5 Católica Biomedical Research Centre, Católica Medical School, Universidade Católica Portuguesa
6 Faculdade de Farmácia da Universidade de Lisboa

Malaria is a major public and global health concern, with 282 million cases and 610 thousand deaths in 2024, accounting for an increase of 12 thousand deaths compared to 2023. New antimalarial drug discovery remains a challenge, with the rise of resistance of anti-malarial drugs in *Plasmodium spp.*, together with climate changes that benefit the spread of *Anopheles spp.* mosquito.
The study of structure-activity relationship (SAR) in quinazolines remains interesting due to their wide range of pharmacological activities. Thirty-two quinazoline derivatives were synthetized and screened in vitro for antimalarial activity against the chloroquine-sensitive *Plasmodium falciparum* 3D7-GFP strain. Compounds with at least 70% of growth inhibition were considered active and were selected for estimation of their half-maximal inhibitory concentration (IC_{50}) against *P. falciparum* 3D7-GFP, as well as their cytotoxicity (CC_{50}) against the human monocytic THP-1 cell line.

Four compounds (IN-27, IN-28, IN-30 and IN-33) showed the best antimalarial activity, with IC_{50} values of 1.70-4.36 μM, CC_{50} of 10.22-17.49 μM, corresponding to selectivity indexes (CC_{50}/IC_{50}) above 10. Lipophilic substituents and a CF3 groups showed better results, providing guidance for further optimization, focused on solubility and selectivity.

B5. Evaluation of environmental DNA passive samplers for detection of trematodes and snail intermediate hosts

E. Barros[1]; S. Cunha[1]; M.T. Bispo; F.M.H. Cardoso; P. M. Ferreira; S. Belo; M. Calado; **I. L. Maurício**

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Trematodes are responsible for several neglected tropical and zoonotic diseases of major public health importance, affecting both humans and other animals. Environmental DNA (eDNA) surveillance in water offers a cost and effort-effective alternative to conventional field surveys, including for their freshwater snail intermediate hosts. Passive eDNA samplers (PEDS) allow long-term sampling over single-time sampling. We evaluated the performance of low-cost PEDS, based on adsorbent resins and filter membranes, to detect trematode parasites and their snail hosts in water. Selected materials for eDNA capture, were field deployment in schistosomiasis-endemic areas of Sofala Province, Mozambique, and biofilm was compared to water sampling in Alqueva, Portugal. Molecular detection by PCR targeted Schistosoma spp. and planorbid snails., as well as *Trichobillharzia franki* and *Ampullaceana balthica*. The evaluated PEDS showed consistent DNA recovery and detection. Detection was consistent with expected results in terms of seasonality and presence of parasites and snails. The results demonstrate that PEDS are an effective, robust, and scalable tool for integrated monitoring of trematodes and snail intermediate hosts, with clear potential to complement or replace conventional water filtration and malacological surveys in resource-limited settings.

B6. The impact of *Plasmodium falciparum* infection on host glycolysis: a focus on Rapoport-Luebering shunt

Emmanuela Rodrigues, Ana Balau, Gustavo Capatti, Ana Paula Arez.

1 Institute of Hygiene and Tropical Medicine (IHMT), NOVA University of Lisbon

Malaria remains a major global health burden, particularly in sub-Saharan Africa, where *Plasmodium falciparum* is responsible for most infections. The emergence of resistance to antimalarial drugs highlights the need for alternative strategies, including disruption of parasite development through modulation of host metabolic pathways such as glycolysis.
Red blood cell (RBC) pyruvate kinase deficiency (PKD), which increases levels of 2,3-diphosphoglycerate (2,3-DPG) produced via the Rapoport–Luebering shunt by bisphosphoglycerate mutase (BPGM), has been associated with protection against severe malaria. Because *P. falciparum* lacks BPGM, it cannot metabolize 2,3-DPG, potentially rendering the parasite vulnerable to elevated concentrations of this host-specific metabolite. Infected normal RBC (iRBC) exhibit higher ATP and lower 2,3-DPG levels than non-infected RBC (niRBC), reflecting increased parasite pyruvate kinase activity without compensatory 2,3-DPG production.
This study evaluates Rapoport–Luebering shunt activity in iRBC and niRBC from *P. falciparum* in vitro cultures using normal RBC and RBC with an induced PKD phenotype. ATP, 2,3-DPG, and BPGM levels were quantified using commercial assays. Preliminary results reveal significant alterations in ATP and 2,3-DPG levels in both iRBC and neighbouring niRBC. BPGM amount was controlled and remains stable across all samples, as expected. These results suggest active parasite-driven impact on host glycolytic metabolism.

B7. Preliminary antiplasmodial activity of new quinoline derivatives

Karla Menezes,[1,2], Misael Cavalcanti[2,3], João Vieira[2] , Verônica Nascimento[2], Inês Morais[4,5], Fátima Nogueira[4,5], Ricardo Moura[1,2]

1 Postgraduate Program in Technological Development and Innovation in Pharmaceuticals, Federal University of Paraíba, João Pessoa, PB, Brazil;
2 Drug Development and Synthesis Laboratory, Department of Pharmacy, State University of Paraíba, Campina Grande, PB, Brazil
3 Postgraduate Program in Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil
4 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
5 LAQV-REQUIMTE, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

Malaria is a parasitic disease caused by Plasmodium spp. and transmitted by the female Anopheles spp. mosquito. Drugs such as chloroquine and mepacrine can be used to treat malaria, but they have limitations related to resistance and toxicity. Thus, the aim of this work was to develop new antiplasmodial drug candidates from the privileged structures of chloroquine and mepacrine. To this end, 9 quinoline and acridine compounds were designed and synthesized, and subsequently subjected to molecular docking in different targets to evaluate their potential mechanisms of action. The compounds were then evaluated in vitro by screening with the 3D7HT-GFP strain of P. falciparum (sensitive to chloroquine and mefloquine), followed by the determination of IC50 in 3D7HT-GFP with the best compounds. As a result, 3 compounds obtained favorable IC50 values and were tested in the Dd2 strain of P. falciparum (multidrug-resistant) to evaluate cross-resistance. Docking results have indicated potential multitarget activity against P. vivax and P. falciparum, which is a positive aspect of the work. Further cytotoxicity studies will be conducted with the compounds to ensure their safety. If the results are positive, the compounds described here can be compared with commercially available compounds.

B8. Decoding mammal blood-parasite interaction in *Trypanosoma cruzi* infection

Armanda Rodrigues[1], Vânia Vieira[1], Juliana Weber[1], Micheli Ferla[1], Cláudia Moreno[1], Joana Palma-Marques[1], Maria de Aires Pereira[1,2,3], Graça Alexandre-Pires[4,5], Isabel Pereira da Fonseca[4,5], Gabriela Santos-Gomes[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Instituto Politécnico de Viseu, Escola Superior Agrária de Viseu
3 CERNAS-IPV Research Centre, Instituto Politécnico de Viseu
4 CIISA - Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon
5 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS)

Chagas disease (ChD) is a life-threatening neglected tropical disease caused by *Trypanosoma cruzi*, which can infect wild and domestic mammals, acting as reservoirs, sustaining its transmission cycle. Understanding parasite–host interactions is essential for developing effective One Health control strategies. This study aims to explore the immune response of peripheral blood cells from non-classical hosts (sheep) to *T. cruzi*. A whole blood (WB) culture model was established using peripheral blood of healthy sheep and exposed to *T. cruzi* epimastigotes or stimulated with soluble parasite antigen or parasite extracellular vesicles (EVs). The exposition to parasites revealed a transient increase in major histocompatibility complex (MHC) class II antigen presenting cells and the presence of EVs increased T CD8+ cytotoxic cells. It also originated a mixed immune response with generation of pro-inflammatory interleukin (IL)-1β, IL-12p40 and Interferon (IFN)-γ, balanced by an induction in IL-10 and transforming growth factor (TGF)-β. Altogether, the use of in vitro WB model proved useful to analyse the

first contact between parasite and mammal blood, detailing the immunopathological process of ChD in non-classical models.

B9. Repositioning of antimalarial drugs in ovine babesiosis: in vitro evaluation

Eduarda Machado[1], Raquel Soares[2], Jéssica Malaquias[3], Maristela Peckle[1], Gustavo Cassiano[4], Sandra Antunes[3,4], Ana Domingos[3,4].

1 Department of Animal Parasitology, Veterinary Institute, Federal Rural University of Rio de Janeiro
2 NOVA Medical School - Universidade NOVA de Lisboa
3 Instituto de Higiene e Medicina Tropical - Universidade NOVA de Lisboa
4 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa

Babesiosis is a veterinary-relevant hemoparasitic disease caused by protozoa of the genus *Babesia*, with significant impacts on animal production. *Babesia o*vis, the agent of ovine babesiosis, is primarily transmitted by *Rhipicephalus bursa* and can cause severe clinical conditions, high mortality, and economic losses. Disease control includes antiparasitic drugs, attenuated vaccines, and vector management; however, limitations such as toxicity, residues in animal products, and incomplete parasite clearance highlight the need for new therapeutic strategies. Due to the evolutionary closeness between *Babesia spp.* and *Plasmodium* species, antimalarial compounds have been investigated as potential therapeutic alternatives. Fluorescence-based assays using SYBR Green I enable rapid and reproducible evaluation of in vitro parasite growth inhibition. In this study, a culture and fluorescence-reading protocol for *B. ovis* was optimized, with a 96-hour experimental period, daily medium replacement, and 10% hematocrit, ensuring linearity between fluorescence and parasitemia and a Z′ factor suitable for high-throughput screening. Compounds selected in initial screenings were analyzed in detail. Preliminary assays with the antimalarial LDT-146 determined the 50% inhibitory concentration (IC_{50}) and assessed parasite viability, showing strong inhibition of B. ovis. These results indicate that antimalarials can be repurposed as therapeutic options and validate the fluorescence-based protocol as a robust tool for drug screening in *B. ovis*.

B10. N-glycosylation is required for *T. congolense* cytoadhesion

Marta Tiago[1], Leonor Loira[1], Eduardo Esteves[1], Bill Wickstead[2], Catarina Gadelha[2], Luisa Figueiredo[3], Sara Silva Pereira[1]

1 Católica Biomedical Research Centre, Católica Medical School, Universidade Católica Portuguesa
2 School of Life Sciences, University of Nottingham
3 Gulbenkian Institute for Molecular Medicine

Trypanosoma congolense is an extracellular parasite that causes nagana, a parasitic disease of livestock in sub-Saharan Africa and a potential zoonotic source of Human African Sleeping Sickness. Nagana challenges agricultural development and animal welfare, leading to productivity losses of USD 1.2 billion/year. Our previous findings showed that *T. congolense* cytoadhesion determines disease severity and parasite proliferation, suggesting that targeting this host-parasite interaction might mitigate disease. Yet, the molecular mediators of cytoadhesion remain unknown. In vivo direct RNAi fragment sequencing (DRiF-Seq) revealed genes, whose silencing, reduced cytoadhesion capacity in a competition context. One of these genes encodes a UDP-glycosyltransferase (UGT) predicted to be involved in N-glycosylation. To assess the individual impact of UGT in cytoadhesion, we engineered a *T. congolense* UGT knockout cell line and quantified its cytoadhesion ability under static and flow conditions. Genetic ablation of UGT drastically reduced attachment to bovine endothelial cells. Our work suggests that N-glycosylation is essential for *T. congolense* cytoadhesion to the mammalian host and provides further evidence of the importance of cytoadhesion for parasite proliferation.

B11. *In vitro* antileishmanial activity of snake venom fractions

Paula Dantas Oliveira Moreira[1]; Milena Daniela Souza Silva[2]; Sónia Aparecida de Andrade Chudzinski[1]; Fernanda Calheta Vieira Portaro[4]; Juliana Pavan Zuliani[2]; Fátima Nogueira[3]; Sofia Cortes[3]

1 Development and Innovation Laboratory - LDI, Butantan Institute
2 Laboratory of Cellular Immunology Applied to Health, Oswaldo Cruz Foundation, FIOCRUZ Rondônia
3 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
4 Laboratory of Structure and Function of Biomolecules, Butantan Institute

Leishmaniasis, considered as neglected tropical diseases (NTDs), urgently needs more effective, safe, and accessible treatments. In this context, it becomes relevant to prospect for new therapeutic strategies. This study aims to screen natural and synthetic compounds derived from venoms in order to identify leishmanicidal prototypes. Combinations of toxins from snakes *Bothrops jararaca* and *Bothrops atrox* were used, and the antiparasitic activity was evaluated against an epidemiologically relevant panel of *Leishmania spp.* species: *L. infantum*, *L. donovani*, *L. major*, and *L. tropica*. Cytotoxicity was evaluated in monocytic human cell lines (THP-1 and U937) The experimental approach aims to identify compounds with anti-*Leishmania* activity associated with low cell toxicity, allowing the selection of selective candidates that can serve as prototypes for the development of new therapies.

B12. Poly(A) polymerases and the dynamic regulation of VSG mRNA in *Trypanosoma brucei*

Christoph Wenzl[1], Sheryll Ashu[2], Michal Malecki[1], Luísa Figueiredo[1]

1 Gulbenkian Institute for Molecular Medicine
2 Technische Universität Berlin

The unicellular parasite *Trypanosoma brucei* evades host immune responses by periodically switching its Variant Surface Glycoprotein (VSG). Both VSG mRNA and protein are produced in exceptionally high amounts, enabled by RNA polymerase I–driven transcription and an unusually long mRNA half-life—about ten times that of typical transcripts. We recently found that VSG mRNAs possess poly(A) tails roughly twice as long as those of other mRNAs, suggesting that tail elongation may underlie their remarkable stability. However, how this elongation is achieved and why it occurs specifically for VSG transcripts remain unknown. To address this, we investigated two conserved sequence motifs in the VSG 3′ untranslated region. Using reporter cell lines and nanopore sequencing, we show that a 16-mer motif is essential for both mRNA stabilization and poly(A) tail elongation, while a 9-mer motif mediates VSG transcript destabilization during parasite differentiation. We further identified six candidate enzymes that could catalyze VSG-specific poly(A) tail elongation. Ongoing knockdown experiments combined with poly(A) tail length analysis aim to pinpoint the responsible enzyme and elucidate the underlying molecular mechanism. Our study will advance understanding of selective RNA stabilization in *T. brucei* and may have broader biomedical implications, for example in the context of mRNA therapeutics.

B13. Isothermal Nucleic Acid Amplification with Recombinase Polymerase for Rapid Detection of Drug Resistance in *Plasmodium* Parasites: A Feasible Point-of-Care Technology

Mariana Pinto[1], Vasso Skouridou[1,2], Ana Tavares[1], Vasco Nandubé[1], Andrea Luciana Soares da Silva[1,3], Ronise Silva[1,4], Amabelia Rodrigues[4], Miguel Viveiros[1], Ana Paula Arez[1,5], Ciara K. O'Sullivan[1,2] and Márcia M. Medeiros[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Departament d'Enginyeria Química, Universitat Rovira i Virgili
3 Laboratório de Genética de Doenças Complexas, Instituto de Ciências Biológicas, Universidade Federal do Pará
4 Bandim Health Project, Apartado 861, 1004 Bissau Codex
5 Research Centre for Anthropology and Health (CIAS), Department of Life Sciences, University of Coimbra

The emergence of *Plasmodium falciparum* parasites resistant to antimalarial drugs compromises treatment and chemoprevention strategies. Sulfadoxine–pyrimethamine is widely used in chemoprevention programs during pregnancy and early childhood. Prevalence cut-offs for fully and super resistant *P. falciparum* parasites to sulfadoxine/pyrimethamine advise for safe chemoprevention in children under two, preventing resistance-driven disease exacerbation. More data are necessary for safe chemoprevention recommendations to be extended to other target groups. Point-of-care (PoC) devices could enhance genomic epidemiology of drug-resistant parasites in endemic countries. Despite this, these tools remain largely focused on detecting parasites rather than profiling resistance to them.We developed a solution-phase recombinase polymerase amplification (RPA)–based isothermal assay for the detection of single nucleotide polymorphisms (SNPs) associated with pyrimethamine resistance (N51I, C59R, and S108N). The assay was evaluated using synthetic DNA templates and genomic DNA extracted from P. falciparum clones carrying the target SNPs, demonstrating high sensitivity at low parasitaemia levels, strong specificity, and no cross-reactivity with other Plasmodium species. Validation using field-collected *P. falciparum* isolates from Guinea-Bissau is currently underway. These findings support the potential of a solution-phase RPA PoC platform for resistance surveillance, contributing to evidence-based safe chemoprevention recommendations and improved malaria control in endemic settings.

B14. Characterization of host-parasite interactions in the bovine blood-brain barrier in the context of *Trypanosoma congolense* infection

Maria Zorrinho-Almeida[1], Marta Tiago[1], Jorge de-Carvalho[2], Maria Bernabeu[2], Sara Silva Pereira[1]

1 Católica Biomedical Research Centre, Católica Medical School, Universidade Católica Portuguesa
2 ITQB-NOVA
3 EMBL Barcelona

African Animal Trypanosomiasis (AAT) is caused mostly by *Trypanosoma congolense*, a protozoan parasite transmitted by the tsetse fly. AAT is a major parasitic disease of livestock in sub-saharan Africa, and it can be chronic or acute. Acute disease is characterized by severe neuropathology, resulting in Acute Cerebral Trypanosomiasis (ACT), where animals succumb rapidly of neuroimmunopathology. ACT is driven by a poorly characterized complex interaction between the parasite, the vascular endothelium and the immune response. Decoding the mechanisms behind ACT pathogenesis is therefore essential to mitigate disease. Here, we combine parasitology, computational biology, and bioengineering to disentangle the role of parasite attachment, endothelial activation and immune infiltration in ACT. We are developing a 3D model of bovine blood-brain-barrier (BBB), which will allow us to study parasite-endothelial cell interactions during BBB dysfunction and assess the parasite-specific immune response in the brain. Our work will contribute to a better understanding of ACT pathogenesis, ultimately leading to novel intervention strategies to mitigate disease.

B15. Parasitic infections in transplanted and other severely immunocompromised patients

Jaime Nina[1]

1 UEI Clínica – IHMT

Transplanted patients should underwent through a heavy regime of immune suppression with the aim of reducing the risk of organ rejection. However, this immunosuppressed state leaves the patient at the risk of severe infections, with a wide range of agents. Some of these agents are new infections, but most are old infections, with which the patient had lived in an asymptomatic state of "arms-race" equilibrium between the infection and the immune system. This equilibrium was kept for years, sometime decades. The suppression of the immune system of the transplantation regime changes these equilibria, transforming asymptomatic carriers into severely sick patients, with uncontrolled infections, sometimes fatal ones. Other than transplantation, several other treatments with the end result of heavy immunosuppression may also expose the infection vulnerability of these very sick patients. In this work, I do an overview of parasitic infections than have caused unpleasant surprises, and sometimes death, in nosocomial immunosuppressed patients.

B16. From monkeys to men - *Toxoplasma gondii* as a model to study the evolution of host-pathogen interactions

Franziska Hildebrandt[1], Stamatios Alan Tahas[2], Nuno Carmo[1], Jens Hedelund Madsen[2], Moritz Treeck[1]

1 Gulbenkian Institute for Molecular Medicine
2 Copenhagen Zoo, Roskildevej 32, 2000 Frederiksberg, Denmark

Toxoplasma gondii is a highly successful intracellular parasite capable of infecting all warm-blooded animals. Upon host cell infection, the parasite modulates the host response, using ~250 effector proteins. The majority of these proteins remains uncharacterized, but some proteins have different effects in mouse and human infections. For example, we previously showed that parasites missing GRA57 are restricted more and ubiquitinated less in human cells. Given the wide host-range, we hypothesize that additional effector proteins may act in a host-specific manner. We extracted primary skin cells of different mammals, including primates. We aim to identify transcendent and species-specific effector proteins by investigating their effects on parasite restriction and ubiquitination across primary cells using cell biology, and microscopy-based methods. Our first results indicate that compared to humans; primary cells of Baboons (*Cercopithecidae*), a close human relative, and Cotton top tamarins (*Platyrrhini*), a more distant human relative, interact differently with GRA57 deletion mutant parasites. These preliminary results support our hypothesis that there is a larger number of undiscovered host-specific effector proteins. Identifying species transcendent and specific effector proteins will elucidate on the evolution of host-parasite interactions, and can assist conservation efforts in host species vulnerable to deadly Toxoplasmosis.

B17. Assessment of the biological activities and phytochemical profile of the root of Vernonia britteniana

Maria dos Anjos Valente[1,2], Pedro Ferreira[3], Telmo Nunes[4], Mavilde Pires[2], Silvana Belo[3], Olga Silva[1]

1 Research Institute for Medicines (IMED), Faculty of Pharmacy, Universidade de Lisboa
2 Instituto de Investigação Veterinária, Avenida Nuno Álvares, Bairro Santo António, Huambo, Angola
3 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
4 Laboratório de Microscopia da Faculdade de Ciências da Universidade de Lisboa

Medicinal plants remain an important source of therapeutic agents due to their rich phytochemical composition and diverse biological activities. *Vernonia britteniana*, a plant traditionally used in folk medicine, has attracted scientific interest for its potential health benefits. This study aimed to assess the phytochemical profile and evaluate selected biological activities of the root of *Vernonia britteniana*. A total of 29 g of roots were collected in the district of Huambo, Angola. Identification was performed from the Faculty of Sciences, University of Lisbon, under the number LISC 131027. The collected material was air-dried at room temperature. The root material was dried, powdered, and extracted using suitable solvents, followed by qualitative phytochemical screening. Biological activity assays were conducted to evaluate the potential of ovicidal and cercaricidal activities of the root extract. The results demonstrated no cercaricidal activity at an extract concentration of 125 µg/mL. However, LC/UV-DAD and LC/UV/MS profiling of *V. britteniana* root extract revealed the presence of saponin compounds. Overall, the *V. britteniana* root extract exhibited cercaricidal and ovicidal activities at other tested concentrations.

B18. Exploring the role of Major Surface Protease A in *T. brucei* adipose tissue colonization

Sophie Marcus-Wade[1], Jane Pires Dias[1], Luisa M. Figueiredo[1]

1 Gulbenkian Institute for Molecular Medicine

During *Trypanosoma brucei* infection, a substantial fraction of parasites localize to adipose tissue (AT). This raises the question, how does *T. brucei* exit the vasculature and establish infection in AT? To test this, our laboratory in collaboration with Gadelha et al. performed a surfeome-wide RNAi screen in vivo comparing the fitness of parasites in blood versus AT. A notable hit was Major Surface Protease A (MSP-A). MSP-A knockdown caused the strongest fitness defect in AT on day 3 post-infection, with diminishing effects on days 6 and 15. This pattern suggests MSP-A may be important in vascular escape or early AT colonization, rather than long-term persistence. These findings highlight MSP-A as a candidate factor mediating *T. brucei* entry into adipose tissue. *T. brucei* expresses two forms of MSP-A: a unique gene and a paralogous set. The original screen targeted only one of the paralogous MSP-As. My preliminary goal is to knock down the unique MSP-A, which was excluded from the initial screen, to test whether it plays a similar role in establishing AT infection and to clarify MSP-A's overall contribution to adipose tissue residency.

B19. Identification of novel dual-stage antiplasmodial hits using high-throughput phenotypic screening

Maria Manuela da Silva[1]; Sofia Benfeito[2,3]; Fernando Cagide[2]; Daniel Chavarria[4]; Sara Mendes[1]; Ana Paula Arez[1]; Pedro Cravo[1]; Antônio Pombinho[5*]; Fernanda Borges[2,3*]; Gustavo Capatti Cassiano[1*]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 MedInUP, Center for Drug Discovery and Innovative Medicines, Department of Biomedicine, Pharmacology and Therapeutics Unit, Faculty of Medicine, University of Porto
3 Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto
4 CIQUP-IMS− Chemistry Research Center of the University of Porto, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto
5 i3S - Institute for Research and Innovation in Health, University of Porto

Malaria is a life-threatening disease caused by *Plasmodium* parasites and transmitted by *Anopheles* mosquitoes. In 2024, the WHO reported around 282 million cases and 610 000 deaths, mostly in children under the age of five. A major challenge, is the emergence of resistance to current antimalarial drugs, including artemisinin-based combination therapies. Therefore, there is an urgent need to identify new drugs with novel mechanisms of action. In this project, we aimed to perform a high-throughput phenotypic screening, against the asexual blood stage of *Plasmodium falciparum*, using a SYBR-Green fluorescence-based assay. Methods: We screened 283 synthetic compounds from the PT-OpenScreen library, tested at 2 µM in 384-well plates. Parasite growth inhibition was measured after 72 hours. Active compounds, were further tested to determine their IC50 values against both the drug-sensitive 3D7 strain and the multidrug-resistant Dd2 strain.We also evaluated hemolysis, cytotoxicity in HepG2 cells, and gametocytocidal ctivity. Results: From the initial screen, 30 compounds showed over 70% inhibition without significant toxicity in HepG2 cells. Ten of these, were selected for dose-response assays, showing IC50 values ranging from 0.045 to above 20 µM. Importantly, no cross-resistance was observed against the Dd2 strain, and no hemolytic effect was detected at 50 µM. Additionally, six compounds demonstrated gametocytocidal activity, effectively killing the transmissible stages at 10 µM. Conclusion: Overall, our study identified promising new antiplasmodial candidates with selective activity and good safety profiles, supporting their potential for future optimization and development.

B20. Tick-borne pathogens harbored by hard ticks and their microbiota in mainland Portugal

Leonardo Moerbeck[1,2*], João Lucas[1*], Lianet Abuin-Denis[3], Gonçalo Seixas[1,2], Rita Velez[1,2], Ana Sofia Santos[4,5], Alejandro Cabezas- Cruz[6], Ana Domingos[1,2], Sandra Antunes[1,2]

1 Instituto de Higiene e Medicina Tropical – Universidade NOVA de LisboaLisboa, Portugal
2 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
3 Animal Biotechnology Department, Center for Genetic Engineering and Biotechnology, Cuba
4 Centro de Estudos de Vetores e Doenças Infeciosas Dr. Francisco Cambournac, Instituto Nacional de Saúde Doutor Ricardo Jorge (CEVDI-INSA)
5 Instituto de Saúde Ambiental, Faculdade de Medicina, Universidade de Lisboa
6 ANSES, INRAE, Ecole Nationale Vétérinaire d'Alfort, UMR BIPAR, Laboratoire de Santé Animale,
* Shared co-first

Ticks are hematophagous ectoparasites that harbor complex microbial communities, including symbionts, commensals, and pathogens, which influence tick biology and pathogen transmission. This study has a twofold aim: first, to investigate the prevalence of tick-borne pathogens (TBPs) in questing ticks from mainland Portugal; and second, to assess the impact of *Babesia ovis* infection on Rhipicephalus bursa tissues and the influence of geographic origin on the microbiota of *Ixodes ricinus*. Questing ticks were collected between 2019 and 2021 across multiple ecological regions and screened for TBPs using molecular methods. In parallel, bacterial

microbiota profiling was performed by Illumina MiSeq sequencing of the 16S rRNA gene (V3–V4) in field-collected I. ricinus females and laboratory-reared R. bursa females, uninfected and experimentally infected with *B. ovis*. Several TBPs were detected, including *Babesia bigemina* and *Rickettsia slovaca* infecting *I. ricinus*. Bacteriome analysis, conducted using DADA2-based pipeline with SILVA taxonomic assignment, showed that uninfected salivary glands were dominated by 4 families, whereas infected salivary glands displayed increased heterogeneity. Uninfected midguts exhibited higher relative abundances of 3 bacterial families, while infected midguts showed increased *Comamonadaceae* and nearly undetectable *Coxiellaceae*. These findings provide updated insights into TBP circulation, and enhance understanding of tick–pathogen–microbiota interactions.

B21. Genomic and phenotypic characterization of bacteria isolated from *Anopheles* mosquitoes midgut microbiota

Margarida Marques[1], Vânia Pobre[2], Sofia G. Seabra[1], Sofia Santos Costa[1], Sandra N. Pinto[3], Henrique Silveira[1]

1 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
2 Instituto de Tecnologia Química e Biológica António Xavier, ITQB
3 iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico

Malaria is the most prevalent mosquito-borne disease and available control strategies are becoming limited. An alternative malaria control approach involves the *Anopheles* midgut microbiota manipulation, to reduce transmission. The genomic and phenotypic characterization of *Anopheles* microbiota is crucial for understanding bacterial mechanisms that could be explored in this context, as biofilm formation. This study emphasizes the value of whole genome sequencing (WGS), combined with in vitro assays, to evaluate biofilm formation of potential candidates. WGS was performed for *Pseudomonas mendocina* Ag_C1 and Serratia marcescens As_A1-C1, bacteria isolated from *Anopheles* midguts. Sequencing was performed using Illumina paired-end technology, with de novo assembly using Unicycler and annotation with Prokka. In vitro biofilms were characterized by microbiological and biophysical tools, including confocal microscopy. In vitro, we observed that S. marcescens As_A1-C1 formed poorly structured biofilms. In contrast, *P. mendocina* Ag_C1 produced highly thick, heterogenous biofilms*. P. mendocina* carried genes for flagella, type IV pili, and the alg operon, supporting the formation of mucoid biofilms. *S. marcescens* carried genes for motility, matrix production but also genes linked to reduced biofilm formation (bssS, tabA) and lacked quorum-sensing swr genes. Overall, these findings support *P. mendocina* Ag_C1 as a promising candidate for microbiota-based malaria control strategies.

B22. Venoms against malaria: in search of the next generation of drugs.

Milena Daniela Souza Silva[1]; Paula Dantas Oliveira Moreira[2]; Inês Morais[3]; Sonia Aparecida de Andrade Chudzinski[2]; Fernanda Calheta Vieira Portaro[4]; Juliana Pavan Zuliani[1]; Sofia Cortes[3]; Fátima Nogueira[3]

1 Laboratory of Cellular Immunology Applied to Health, Oswaldo Cruz Foundation, FIOCRUZ Rondônia
2 Development and Innovation Laboratory - LDI, Butantan Institute
3 Global Health and Tropical Medicine, GHTM, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa
4 Laboratory of Structure and Function of Biomolecules, Butantan Institute

The emergence and spread of antimalarial drug resistance threatens global advances in malaria control, reinforcing the need to identify agents with new mechanisms of action. This study proposes to perform a pharmacological screening based on pools and fractions of toxins derived from the venoms of the snakes *Botrhops jararaca* and *Bothrops atrox*, including combinations with potential synergistic effect, as well as synthetic peptides derived from the scorpion *Tityus serrulatus*. The initial focus is on the evaluation of antiplasmodic activity against *Plasmodium falciparum* (clone 3D7HT-GFP), with direct comparison to reference drugs. Cytotoxicity is evaluated in monocytic human cell lines (THP-1 and U937), allowing preliminary analysis of selectivity. In this sense, the present study aims to identify bioactive pools and fractions relevant to antimalarial activity associated with a favorable safety profile, contributing to the prospection of new therapeutic prototypes capable of circumventing resistance in malaria.

B23. Development of a computer-assisted QSAR model for predicting antiparasitic activity

Manuel Londa Vueba[1,2]

1 Instituto Superior Politécnico Privado do Kilamba
2 Instituto de Ciencias da Saude-UAN-Angola

The development of new antiparasitic agents is a significant challenge, especially due to the increase in resistance to available drugs and the high costs and long timeframes associated with experimental drug development. In this context, computer-assisted Quantitative Structure–Activity Relationship (QSAR) models have proven to be efficient tools for predicting the biological activity of chemical compounds. This work describes the development of a QSAR model aimed at predicting antiparasitic activity, using molecular descriptors obtained by computational methods and statistical and/or machine learning techniques. Initially, a database containing compounds with known antiparasitic activity was constructed. Next, the relevant molecular descriptors were calculated and selected to reduce redundancies and improve the robustness of the model. The QSAR model was then trained and validated using internal and external validation methods to evaluate its predictive performance. The results obtained indicate that the model developed has a good capacity to correlate the structural properties of compounds with their antiparasitic activity, demonstrating its potential as an auxiliary tool in the virtual screening of new drug candidates. Thus, the use of computer-assisted QSAR models can contribute significantly to accelerating the process of discovering new antiparasitic agents, reducing costs and the need for initial experimental testing.

List of Participants

Name	Email Address	Affiliation
Adriana Gonçalves	id10896@alunos.uminho.pt	ICVS
Alexandre Costa	alex.c.costa@hotmail.com	UFRRJ
Ana Afonso	anajuliaafonso74@gmail.com	Instituto Politecnico de Santarém
Ana Armada	aarmada@ihmt.unl.pt	IHMT
Ana Balau	a21001549@ihmt.unl.pt	IHMT
Ana Dias	apardalpiresdias@gmail.com	ULSLO
Ana Domingos	adomingos@ihmt.unl.pt	IHMT
Ana Fernandes	ritamfernandes323@gmail.com	FMUL
Ana Manuel Figueiredo	anamfigueiredo@ua.pt	UA
Ana Margarida Vigário	anavig@staff.uma.pt	Universidade da Madeira / GIMM
Ana Matias	ana.matias@gimm.pt	GIMM
Ana Mineiro	ana.mineiro@cm-alenquer.pt	Câmara Municipal Alenquer
Ana Paula Arez	aparez@ihmt.unl.pt	IHMT
Ana Tomás	atomas@ibmc.up.pt	i3S / ICBAS
Anabela Vilares	anabela.vilares@insa.min-saude.pt	INSA
Andréa L. Soares da Silva	andrea.silva@ihmt.unl.pt	IHMT
Andreia Mósca	andreia.mosca@gimm.pt	GIMM
Andreia Wendt	afrrla@hotmail.com	SwissTPH/Charles University/GHTM
Ângelo Ferreira Chora	angelo.chora@gimm.pt	GIMM
Arlete Troco	a21002527@ihmt.unl.pt	IHMT
Armanda Rodrigues	armanda.rodrigues@ihmt.unl.pt	IHMT
Augusta Maurício	mauricioaugusta5@gmail.com	unknown
Bárbara Teixeira	barbara.teixeira@gimm.pt	GIMM
Beatriz Fonseca	Beatrizvelosafonseca@gmail.com	ICVS
Bishara Marzook	bishara.marzook@crick.ac.uk	GIMM / The Francis Crick Institute
Bruno Freitas	id11853@uminho.pt	ICVS
Carla Oliveira	carla.oliveira@gimm.pt	GIMM
Carolina Albino	carolina.albino@gimm.pt	GIMM
Carolina Faria	carolina.faria@i3s.up.pt	i3S
Carolina Vieira	carovieira@ucp.pt	CBR
Catarina de A. Marques	catarina.dealmeidamarques@glasgow.ac.uk	University of Glasgow
Catarina Ribeiro	catarina.ribeiro@soquimica.pt	Soquímica
Catarina Rosa	catarina.rosa@gimm.pt	GIMM
Christian Gnann	christian.gnann@gimm.pt	GIMM
Christoph Wenzl	christoph.wenzl@gimm.pt	GIMM
Daniela Matias	dmmatias@fc.ul.pt	IHMT
Danielle Cosac	cristinacosac@gmail.com	IHMT
David Anaguano	david.anaguano@gimm.pt	GIMM
David Ferreira	david.ferreira@gimm.pt	GIMM
David Ribas	david.ribas@nzytech.com	NZYtech
Davy Sapatini	davy.sapatini@unesp.br	UNESP

Diana Moita	diana.moita@gimm.pt	GIMM
Dyonatan Silva	df_servicos99@hotmail.com	UFRN
Eduarda Machado	eduardamachadovet@gmail.com	UFRRJ
Elena Maria Christ	elena.christ@gimm.pt	GIMM
Ema Fundanga	emarufinabae@gmail.com	unknown
Emmanuela Rodrigues	a21002176@ihmt.unl.pt	IHMT
Ereso João	a21002191@ihmt.unl.pt	IHMT
Eunice Kaizeler	eunice.kaizeler@hotmail.com	IHMT
Eva Dias	eva.dias@gimm.pt	GIMM
Eyob Addise Workneh	eyob.workneh@gimm.pt	GIMM
Fátima Nogueira	FNogueira@ihmt.unl.pt	IHMT
Fernando Cardoso	fcardoso@ihmt.unl.pt	IHMT
Filipa Neves	fneves@i3s.up.pt	i3S
Franziska Hildebrandt	franziska.hildebrandt@gimm.pt	GIMM
Geovano Costa	a21001992@ihmt.unl.pt	IHMT
Giada Susannini	a21002692@ihmt.unl.pt	IHMT
Gonçalo Alves	a21002067@ihmt.unl.pt	IHMT
Gonçalo Seixas	goncalo.seixas@gimm.pt	GIMM
Gwendolin Fuchs	gwendolin.fuchs@gimm.pt	GIMM
Helena Nunes Cabaço	helena.cabaco@gimm.pt	GIMM
Helga Waap	helga.waap@iniav.pt	INIAV
Henrique Ramos	henrique.ramos@gimm.pt	GIMM
Henrique Silveira	hsilveira@ihmt.unl.pt	IHMT
Hugo Belda	hugo.belda@gimm.pt	GIMM
Idalina Ferreira	idalina.ferreira@insa.min-saude.pt	INSA
Inês Marques	inescatarinamarques@gmail.com	ULSAS
Inês Morais	ines.morais@ihmt.unl.pt	IHMT
Isabel Maurício	isabel.mauricio@ihmt.unl.pt	IHMT
Isabel Moules	isabel.moules@gmail.com	Hospital Santo Espírito de Angra do Heroísmo
Isabel Pereira da Fonseca	Ifonseca@fmv.ulisboa.pt	FMV-UL
Jacinto Gomes	jacinto.gomes@ipportalegre.pt	Escola Superior de Biociências de Elvas
Jaiganesh Jagadeesh	jaiganesh.jagadeesh@gimm.pt	GIMM
Jaime Nina	jnina@ihmt.unl.pt	UEI Clínica - IHMT
Jane Dias	jane.dias@gimm.pt	GIMM
Jane Munday	jane.munday@glasgow.ac.uk	University of Glasgow
Joana Costa	joana.s.costa@gimm.pt	GIMM
Joana Marques	joana.a.marques@ihmt.unl.pt	IHMT
Joana Pinho Barbosa	jbarbosa@i3s.up.pt	i3S
Joana Ribeiro	jcribeiro@i3s.up.pt	I3s
Joana Ricardo	joana.ricardo@gimm.pt	GIMM
Joana Tavares	jtavares@i3s.up.pt	I3s
João Lozano	jlozano@fmv.ulisboa.pt	FMV-UL
João Lucas	joaovlucas@live.com	IHMT
Jônathan D. R. Chagas	jonathanchagas@ufrrj.br	UFRRJ / IHMT
Jorge Pedrosa	jpedrosa@med.uminho.pt	ICVS
José Cristóvão	jcristovao@ihmt.unl.pt	IHMT

José Lourenço	jolourenco@ucp.pt	CBR
Juliana Inês Weber	juinesweber@gmail.com	IHMT
Karla Menezes	menezeskarla5@gmail.com	UFPB / UEPB
Kyle Cunningham	kyle.cunningham@research.fchampalimaud.org	Champalimaud Foundation
Lara Likar	lara.likar@gimm.pt	GIMM
Leonardo Moerbeck	moerbeck.leonardo@gmail.com	IHMT
Leonor Loira	leonorcloira@gmail.com	CBR
Liliana Silva	liliana.silva@iniav.pt	INIAV
Luis Constantino	luisbogaio@gmail.com	Instituto Superior de Lisboa, UL
Luis Madeira de Carvalho	madeiradecarvalho@fmv.ulisboa.pt	FMV-UL
Luisa Figueiredo	luisa.figueiredo@gimm.pt	GIMM
M. Isabel Veiga	mariaveiga@med.uminho.pt	ICVS
Mª dos Anjos S. Q. Valente	valentemaria@edu.ulisboa.pt	FFUL
Madalena Rodolfo	mrodolfo@ucp.pt	CBR
Manuel Londa Vueba	vuebamanuel@gmail.com	ISPP-Kilamba, Luanda
Manuela Calado	mcalado@ihmt.unl.pt	IHMT
Márcia Medeiros	marcia.medeiros@ihmt.unl.pt	IHMT
Margarida Barroso	mmbarroso@ucp.pt	CBR
Margarida Duarte	mduarte@ibmc.up.pt	I3s
Margarida Leonardo	mc.leonardo@campus.fct.unl.pt	IHMT
Margarida Marques	a21002435@ihmt.unl.pt	IHMT
Maria Carvalho	mariadcarvalho@ihmt.unl.pt	IHMT
Maria João Gargaté	m.joao.gargate@insa.min-saude.pt	INSA
Maria Mascarenhas	maria.mascarenhas@aluno.fiocruz.br	Instituto René Rachou, Brazil / UM
Maria Rebelo	mariarebelo@medicina.ulisboa.pt	FMUL
Maria Silva	a21001276@ihmt.unl.pt	IHMT
Maria Teresa Bispo	mtbispo@ihmt.unl.pt	IHMT
Maria Teresa Novo	tenovo@ihmt.unl.pt	IHMT
Maria Zorrinho-Almeida	s-mczalmeida@ucp.pt	CBR
Mariana Louro	marianacl@fmv.ulisboa.pt	FMV-UL / INIAV
Mariana Pinto	a21002042@ihmt.unl.pt	IHMT
Marta Monteiro	mnmonteiro@fmv.ulisboa.pt	FMV-UL
Marta Tiago	mtiago@ucp.pt	CBR
Marta Varandas	martacvarandas@gmail.com	FMV-UL
Michal Malecki	michal.malecki@gimm.pt	GIMM
Micheli Ferla	micheliferla@gmail.com	IHMT
Miguel Miranda	migsmiranda@outlook.pt	Trofa Saude
Miguel Patricio	miguel.patricio@research.fchampalimaud.org	Champalimaud Foundation
Miguel Prudencio	miguel.prudencio@gimm.pt	GIMM
Miguel Sousa	jm.gs@ua.pt	UA
Milena D. Souza Silva	milena.silva@ihmt.unl.pt	IHMT
Mónica Franco	monica.franco@gimm.pt	GIMM
Mónica Galocha	monica.galocha@gimm.pt	GIMM
Moritz Treeck	moritz.treeck@gimm.pt	GIMM
Nuno Santarém	santarem@i3s.up.pt	I3s
Nuno Santos	nuno.santos@gimm.pt	GIMM / FMUL

Patrícia Silva	patricia.silva@soquimica.pt	Soquímica
Paula Moreira	paula.moreira@ihmt.unl.pt	IHMT
Pedro Ferreira	pedroferreira@ihmt.unl.pt	IHMT
Pedro Ferreira	pedroferreira@med.uminho.pt	ICVS
Rafael Rocha	rafael.amorim.rocha@gmail.com	IHMT
Raquel Soares	raquel.cruz.soares@gmail.com	IHMT
Raquel Ventura	raquel.ventura@gimm.pt	GIMM
Rebecca Pabst	a21002014@ihmt.unl.pt	IHMT
Renata Matinhos	renata.matinhos@gimm.pt	GIMM
Ricardo Agrícola	ricardoagricola.equinevet@gmail.com	FMV-UL
Ricardo Monteiro	ricardopsm@i3s.up.pt	i3S
Ricardo Silvestre	ricardosilvestre@med.uminho.pt	ICVS
Richard McCulloch	richard.mcculloch@glasgow.ac.uk	University of Glasgow
Rita Velez	rvelez@fmv.ulisboa.pt	FMV-UL
Roberto Kuyanga	a21002249@ihmt.unl.pt	IHMT
Ruth Esho	meuamigo709@gmail.com	ICVS
Sahar Tehrani	sahar.tehrani@research.fchampalimaud.org	Champalimaud Foundation
Sandra Antunes	santunes@ihmt.unl.pt	IHMT
Sandra Santos	sandracerdeira@gmail.com	unknown
Sandra Trindade	sandra.trindade@gimm.pt	GIMM
Sara Mahomed	mahomed.saram@gmail.com	GiMM
Sara Silva Pereira	sarapereira@ucp.pt	CBR
Sheryll Ashu	sheryll.ashu@gmail.com	GIMM
Silvana Belo	SilvanaBelo@ihmt.unl.pt	IHMT
Simão Nogueira	simao.nogueira@research.fchampalimaud.org	Champalimaud Foundation
Sofia Cortes	scortes@ihmt.unl.pt	IHMT
Sofia Ramos	sofia.ramos@i3s.up.pt	i3S
Sophie Marcus-Wade	sophie.marcus-wade@gimm.pt	GIMM
Stephanie Nofal	stephanie.nofal@gimm.pt	GIMM
Susana Martins	susana.martins@insa.min-saude.pt	INSA
Susana Morgado	scmorgado@fmv.ulisboa.pt	FMV-UL
Tânia Reis	tania.reis@insa.min-saude.pt	INSA
Teresa Leão	teresa.leao@i3s.up.pt	i3S
Tiago Gonçalves	tiagoorgoncalves@gmail.com	ICVS
Tiago Melo	a21002273@ihmt.unl.pt	IHMT
Vasco Nandubé	a21002379@ihmt.unl.pt	IHMT
Virlânio A. de Oliveira-Filho	alvesvirlanio@gmail.com	IHMT / UNICAMP
Vitória Baptista	vitoria.dacunhabaptista@glasgow.ac.uk	University of Glasgow