Abstract Book

→ WORKSHOP

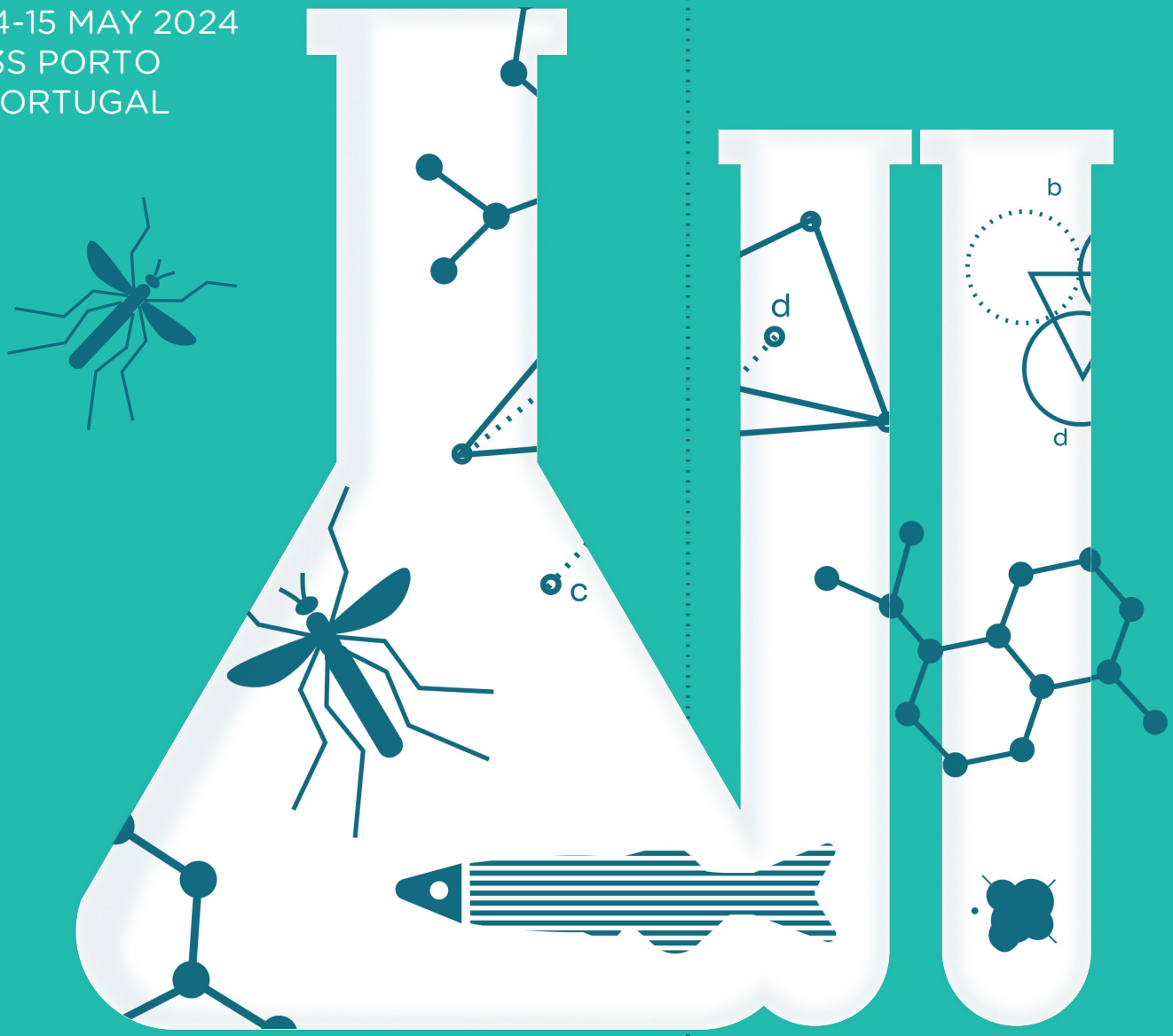
Environmentally friendly systems to replace classical animal models in drug testing for vector-borne diseases

14-15 MAY 2024
i3S PORTO
PORTUGAL

15 MAY 2024
i3S PORTO
PORTUGAL

→ MINI-WORKSHOP

Young Innovators



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Venue

The meeting will be held at i3S – Instituto de Investigação e Inovação em Saúde, University of Porto.

Rua Alfredo Allen, 208; 4200-135 Porto, Portugal
Tel: +351 226 074 900 | E-mail: events@i3s.up.pt
GPS coordinates: 41º 10' 30.008'' N, 8º 36' 12.488'' W.

A. Arriving at Porto by airplane

Directions from Francisco Sá Carneiro Airport (Porto) to i3S by subway:
You can take the **line E (direction: Estádio do Dragão)** at the Airport station. **Destination station: Trindade**; at this station you have to **change to line D (direction: Hospital São João). Final destination station: Pólo Universitário**
Average time: 45 minutes | Single ticket price: 2,15 € | For more information: https://en.metrodoporto.pt/

B. Arriving at Porto by train

Metro or bus are available from Campanhã or São Bento train station. However, the metro is the easiest way to reach i3S.

From Campanhã train station:

• Metro: **All lines are possible (A, B, C, E and F) | Destination Station: Trindade** and then **change to line D - yellow (destination Hospital São João)** – and leave at **station: Pólo Universitário**
Average time: 25 minutes |
Single ticket price: 1,30 € | For more information: https://en.metrodoporto.pt/

From São Bento train station:

• Metro: **Line D - yellow | Destination Station: Hospital São João**; leave at **station: Pólo Universitário** (no connections – direct line)
Average time: 15 minutes |
Single ticket price: 1,30 € | For more information: https://en.metrodoporto.pt

C. Arriving at Porto by car

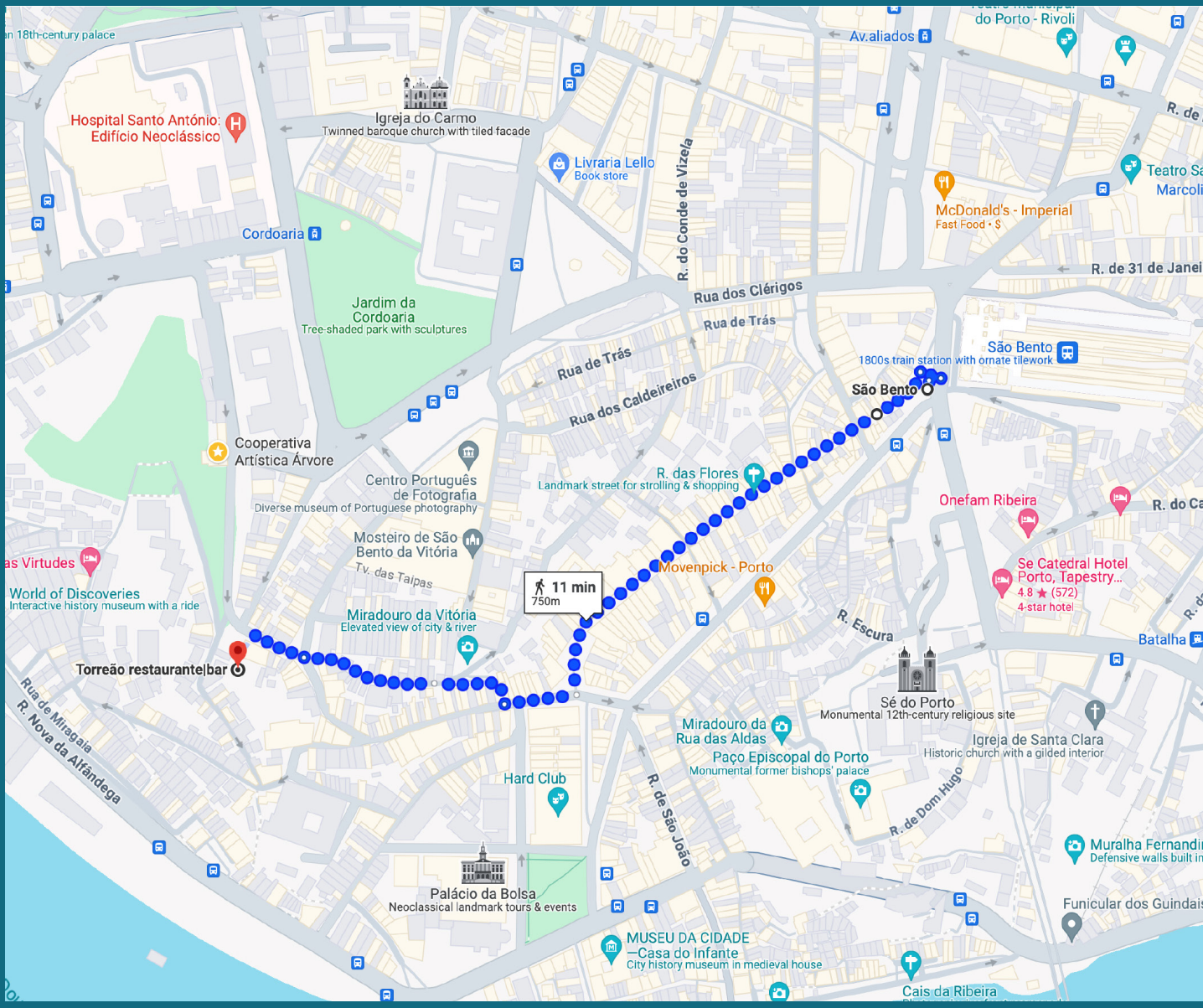
The i3S Institute - GPS coordinates are: 41º 10' 30.008'' N, 8º 36' 12.488'' W.
Please note that i3S does not offer parking space.

Social program

Dinner at Torreão (cost 35€)
R. das Virtudes 37, 4050-630 Porto

Participants are responsible for their own transportation from i3S to the dinner venue.

Directions from i3S to the restaurant:
Metro: Line D - yellow (destination Santo Ovídio) - leave at station: São bento (no connections – direct line)
It is a 10-minute walk from São Bento Station until Torreão:



Information

Registration
The registration desk will be open from 12:45 until 13:00 on 14th May 2024.

Badges
For identification and security purposes, participants must wear their name badges when in the venue. The distribution of badges will be done during registration in alphabetical order by first name.

Food restrictions
Participants with food restrictions should ask the catering enterprise for their meals.

Oral Communications
Invited talks will have a duration of 20 minutes followed by up to 5- minute discussion and the keynote lecture will have a duration of 45 minutes followed by up to 10-minute discussion.
Oral communication will have a duration of 12 minutes followed by up to 3 minute-discussion.
Participants with talks must bring the presentation in a USB flash drive. Speakers presenting in the morning should hand in their presentations in the auditorium before their session starts. Those having their presentations in the afternoon sessions, should hand in their presentations during the lunch break.

Poster Communications
Posters will be available online. Participants will also the opportunity to presentation the poster in the form of a single slide and limit it to two minutes. Participants must bring the presentation in a USB flash drive.

Internet Access
Wireless Internet is available for free.
Network: i3S_Temp | Password: Password2015

Program

DAY 1 | 14 MAY

13h00 Refreshment

14h00 Welcome Session

Joana Tavares, Maria Paola Costi

14H15 SCIENTIFIC SESSIONS: INVITED SPEAKERS

Chairs: Guy Caljon and Paul Selzer

Recent developments in the human-animal relationship - reviewing the 3Rs concepts in animal experimentation.

Stephanie Krämer, Justus-Liebig-Universität Giessen, Germany (virtual presentation)

Capabilities and limitations of zebrafish embryos in drug discovery toxicology for VBD

Prof. Steven Van Cruchten, University of Antwerp, Belgium

Bottom-up approaches for recreating perfusable microvascular 3D Networks *in vitro*

Cristina Barrias, i3S, University of Porto, Portugal

The Chick Chorioallantoic Membrane (CAM): An alternative beyond traditional animal models in drug testing

Marta Teixeira Pinto, i3S, University of Porto, Portugal

16h00 Coffee break and **networking**

16H30 SCIENTIFIC SESSIONS: INVITED SPEAKERS

Chairs: José Alunda and Nuno Santarém

Computer-Based Modelling in the field of 3R Animal Protection

Peter Jedlicka, Faculty of Medicine Justus-Liebig-University and NeuroScience Center Clinical Neuroanatomy, Germany (virtual presentation)

Profile-quantitative structure-activity relationship (pQSAR) platform

Vicki Feher, Novartis Institute for Tropical Diseases, CA, USA (virtual presentation)

17:30 KEYNOTE LECTURE

Chair: Joana Tavares

Drug Discovery for parasitic diseases: powered by technology, enabled by pharmacology, informed by clinical sciences.

Srinivasa Rao, Novartis Institute for Tropical Diseases, CA, USA (virtual presentation)

18:30 GETTING TOGETHER NETWORKING

20H00 DINNER

DAY 2 | 15 MAY

09H30 i3S SCIENTIFIC PLATFORM VISITS

Zebrafish – **Joana Marques**

The Chick Embryo Model – **Marta Teixeira Pinto**

10H30 POSTER PITCHES SESSION

Chair: Harry-De-Koning

11h00 Coffee break and poster discussion

11H30 SELECTED SPEAKERS FROM ABSTRACTS SUBMISSION

Chairs: Margarida Duarte and Ana Pinto

Simple, quick, low-cost assays to evaluate anti-parasite hit compounds before embarking on *in vivo* experimentation

Harry De Koning, University of Glasgow, United Kingdom

Application of the CAM model to the study of disseminating and non-disseminating *Leishmania* infections

Filipa Dias, i3S, University of Porto, Portugal

Incorporating host sanctuary and parasitic quiescence in animal-friendly antileishmanial drug screening assays

Caljon Guy, University of Antwerp, Belgium

Advancing drug discovery against malaria: advantages of using a non-classical mouse model for efficacy studies

Joana Tavares, i3S, University of Porto, Portugal

Machine Learning-Based Predictive Models for Assessing Toxicity Towards Model Organisms: A Sophisticated Approach

Johander Inacio Dos Ramos Azuaje, University of Ljubljana, Slovenia (virtual presentation)

13h00 Refreshment

14H00 ECOTOXICOLOGY

Chairs: Maria Paola Costi and Anabela Cordeiro-da-Silva

Enhancing ecotoxicology and drug testing: Towards healthier medicines and healthier habitats

Eli Thoré, Swedish University of Agricultural Sciences in Umeå, Sweden

Quantifying wildlife behavioural responses: a new approach to chemical regulation and possibilities for implementation during drug development

Michael Bertram, Swedish University of Agricultural Sciences in Umeå, Sweden (virtual presentation).

14h50 Closing

Ana Tomás

15H00 YOUNG INNOVATORS ORGANIZATION MINI-WORKSHOP

Welcome session

Elisa Uliassi

15H10 GREEN AND SUSTAINABLE PRACTICES IN SCIENTIFIC RESEARCH

Chairs: Elisa Uliassi and Eli Thoré

Green Lab Initiative: reducing the carbon footprint of the i3S since 2020.

Daniela Sousa, i3S, University of Porto, Portugal

Sustainable chemistry for greening the synthesis of active pharmaceutical ingredients (API)

Dario Corbisiero, University of Bologna, Italy (virtual presentation).

16h00 Coffee break

16H30 CAREER DEVELOPMENT IN RESEARCH

Chairs: Omer Dilek and Clara Lima

16h30 Responsible conduct in research

Susana Magalhães, i3S, University of Porto, Portugal

17h00 Roadmap to Career Development: Crafting your path in Academia and Industry

Paula Perez, i3S, University of Porto, Portugal

17h30 Closing

Clara Lima

Invited speakers

01

Recent developments in the human-animal relationship - reviewing the 3Rs concepts in animal experimentation

Stephanie Krämer

02

Capabilities and limitations of zebrafish embryos in drug discovery toxicology for VBD

Steven Van Cruchten

03

Bottom-up approaches for recreating perfusable microvascular 3D Networks *in vitro*

Orge ID, Rita Reis, André Maia, Silva MB, Bidarra SJ, **Barrias CC**

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The Chick Chorioallantoic Membrane (CAM): An alternative beyond traditional animal models in drug testing

Marta Teixeira Pinto

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Computer-Based Modelling in the field of 3R Animal Protection

Peter Jedlicka

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Profile-quantitative structure-activity relationship (pQSAR) platform

Vicki Feher

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Drug Discovery for parasitic diseases: powered by technology, enabled by pharmacology, informed by clinical sciences.

Srinivasa Rao

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Quantifying wildlife behavioural responses: a new approach to chemical regulation and possibilities for implementation during drug development

Michael Bertram

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Enhancing ecotoxicology and drug testing: Towards healthier medicines and healthier habitats

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Green Lab Initiative: reducing the carbon footprint of the i3S since 2020

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Sustainable chemistry for greening the synthesis of active pharmaceutical ingredients (API)

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Responsible conduct in research

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Roadmap to Career Development: Crafting your path in Academia and Industry

Paula Perez

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01

Recent developments in the human-animal relationship - reviewing the 3Rs concepts in animal experimentation

Stephanie Krämer

Justus-Liebig-University Giessen, Professorship for Laboratory Animals Science and Animal Welfare/ ICAR3R – Interdisciplinary Center for 3Rs in Animal Research, Giessen, Germany;
stephanie.kraemer@vetmed.uni-giessen.de

Animal experimentation - do we (still) need this?

The question of today is the question of the point in time from which action is taken and judgements are made, looking back and looking ahead (Mathias Mayer).

Almost no other sentence describes the dynamics of dealing with animal experiments so accurately. The use of animals in order to gain scientific knowledge has always been the subject of highly controversial debate. A large number of biological, physiological and pathological phenomena in human and veterinary medicine have been explained on the basis of animal experiments. However, with the introduction of the 3Rs concept as the gold standard in biomedical research, the question arises as to whether the current use of laboratory animals is still indispensable in terms of replacing animal experiments with alternative methods.

In their essay "*The Principle of Humane Experimental Technique*", William Russell and Rex Burch first described the 3Rs principle in 1959 with the aim of avoiding animal experiments (Replacement), reducing the number of animals used in animal experiments (Reduction) and limiting their exposure in experiments to the indispensable level (Refinement). This principle was incorporated into European law in 2010 with European Directive 2010/63/EU.

Against the backdrop of the OneHealth approach, which pursues the protection of humans, animals and nature in the broadest sense, the debate on animal testing is entering a new dimension. The question of indispensability is put to the test here. The extent to which animal experiments are or are not essential to fulfil these requirements will be part of the talk and analysed against the complex challenges of the OneHealth concept.

02

Capabilities and limitations of zebrafish embryos in drug discovery toxicology for VBD

Steven Van Cruchten

CoPeD lab, University of Antwerp

Zebrafish embryos have emerged as a powerful tool in drug discovery toxicology as they exhibit a diverse repertoire of biological processes and possess fully integrated vertebrate organ systems. As such, a much broader range of phenotypes can be assayed in zebrafish embryos than in cultured cells. In addition, emerging automated technologies allow relatively unbiased capture of a substantial proportion of the complete phenotypic repertoire in a medium to high throughput manner. This presentation will focus on the advantages but also limitations of the zebrafish embryo in drug discovery toxicology for VBD, using some concrete examples.

03

Bottom-Up Approaches for Recreating Perfusable Microvascular 3D Networks *In Vitro*

Orge ID[1,3], Rita Reis[1], André Maia[1], Silva MB[1], Bidarra SJ[1,2], **Barrias CC**[1,2,3]

[1]i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; [2]INEB-Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; [3]ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal;

The establishment of a functional vasculature is pivotal for successful tissue development and regeneration but remains a major challenge in the field of tissue engineering. To address this challenge, our group has been focusing on the development of bottom-up tissue engineering approaches to promote tissue vascularization. Our strategy is based on the use of specialized pre-vascularized microtissues that serve as building blocks to initiate the self-assembly of functional microvascular beds surrounded by stromal tissue. These microtissues, comprised of endothelial progenitor cells and organ-specific fibroblasts, demonstrate high angiogenic potential when embedded in fibrin hydrogels. The immature angiogenic sprouts gradually mature into stable capillaries, enveloped by a basement membrane, and undergo anastomosis to form robust 3D networks.

We have demonstrated that these microvessel networks can become perfused both "on-chip," as illustrated using a biomimetic fibrin-based vessel-on-chip (VoC), and *in vivo*, as evidenced by the chick embryo chorioallantoic membrane assay (CAM). By integrating pre-vascularized microtissues with organoids in the VoC, we have shown that the generated microvessels progressively reach and surround the organoids, forming capillary beds extending throughout the bulk hydrogel. This facilitates the vascularization of multiple organoids and the concurrent formation of stromal tissue around them, highlighting the remarkable potential of vascular units (VUs) as building blocks for engineering microvascular networks. Such approaches have versatile applications, ranging from regenerative medicine to the development of 3D *in vitro* models.

ACKNOWLEDGEMENTS

EndoSWITCH project (PTDC/BTMORG/5154/2020), funded by the FCT-Fundação para a Ciência e a Tecnologia.

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04

The Chick Chorioallantoic Membrane (CAM): An alternative beyond traditional animal models in drug testing

Marta Teixeira Pinto

Head of i3S *in vivo* CAM assays Scientific Platform. i3S - Institute of Investigation and Innovation in Health, 4200-135 Porto, Portugal; mtpinto@i3s.up.pt

Ethical considerations, cost, and time constraints underscore the urgency of developing alternatives to rodent *in vivo* models for assessing drug candidates. Among these alternatives, the chicken embryo Chorioallantoic Membrane (CAM) emerges as a particularly promising option, widely adopted for preclinical testing across diverse fields.

One of the key advantages of this model is its alignment with the 3Rs principles for animal experimentation, as advocated by regulatory bodies. By providing predictive *in vivo* data that correlate with outcomes observed in rodent models, the CAM model significantly reduces the reliance on traditional animal models.

CAM assays, particularly in angiogenesis studies, offer a robust platform for investigating blood vessel formation and its regulators. Additionally, owing to its early developmental stage and immuno-incompetence, the embryo readily accepts cancer cells from various sources without triggering rejection. The CAM's nutrient-rich vasculature fosters the spontaneous proliferation of human cells, making it invaluable for studying cancer biology, including cancer-induced angiogenesis, tumor progression, invasion, metastasis, and the dynamics of cancer stem cells. Moreover, it serves as an effective tool for assessing the efficacy of anti-cancer drugs and drug delivery systems [1,2].

Beyond oncology, the CAM model finds validation in diverse applications. It enables the assessment of compound toxicity, evaluation of wound healing processes and potential agents, vaccine development, study of antimicrobial agents and microbial infections, and assessment of biomaterial compatibility with living tissues.

These varied applications underscore the versatility of the chick chorioallantoic assay in biomedical research, offering a compelling alternative or complement to traditional animal models across a spectrum of experiments.

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05

Computer-Based Modelling in the field of 3R Animal Protection

Peter Jedlicka[1,2,3]

[1]Translational Neuroscience Network Giessen, Germany; [2]Computer-Based Modelling in the field of 3R Animal Protection, ICAR3R, Faculty of Medicine, Justus-Liebig-University, Giessen, Germany; Peter.Jedlicka@informatik.med.uni-giessen.de

Qualitative, cartoon-based understanding of physiological processes is not sufficient and needs to be taken to a deeper, quantitative (computational) level. Two types of computational models, namely statistical & mechanistic models, can be used to predict subcellular, cellular and supracellular phenomena *in silico*. This talk will mainly focus on mechanistic models. A simulation of a large number of parameter combinations may lead to a reduction in the number of necessary experiments. However, experimental data from *in vitro* and *in vivo* experiments are necessary to adjust the parameters of computer models. Relatively recently established, so called population-based compartmental modeling will be presented as a promising tool to predict and partially replace pharmacological/genetical perturbations. Examples from cardiac and neuronal physiology will be described to show how population-based computational modeling enables studies of the functional impact of intercellular ion channel variability (Britton *et al*. PNAS 2013, Schneider *et al*. PLoS Comput Biol 2023). In addition, morphological modeling (Cuntz *et al*. PLoS Comput Biol 2010) will be mentioned as a useful complementary approach to traditional compartmental modeling of electrophysiological data in neurobiology. In combination, morphological and compartmental modeling facilitates generalization of computational predictions to any morphology and supports the search for universal principles valid across different species and cell types (Beining *et al*. eLife 2017; Cuntz *et al*. Neuron 2021; Mittag *et al*. J Physiol 2023). In line with the principles of the 3Rs (Replacement, Reduction and Refinement), computational models are excellent tools to support sharing of data and resources by using available experimental datasets and by distributing the source code of new models in online databases.

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ACKNOWLEDGMENT

This work was supported by the Bundesministerium für Bildung und Forschung (BMBF No. 579 031L0229) and by the Deutsche Forschungsgemeinschaft (DFG No. 467764793, JE 528/10-1).

06

Application of the machine-learning method, Profile-QSAR, to Malaria and Chagas Disease

Victoria Feher[1] & Eric Martin[1]

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The promise of AI in drug discovery and safety is that *in silico* predictions can reduce large scale empirical processes such as chemical synthesis followed by exhaustive testing in *in vitro* and *in vivo* assays.

At Novartis, an *in silico* method, profileQSAR, leverages our proprietary database and machine learning methodology to provide predictivity for 72% of our assays with a median correlation comparable to a 4-concentration IC50 assay (r^2=54%). Every month, we retrain the 13,000 pQSAR models, recalculating and storing the 66 billion predicted pIC_{50}s for the 5.5 million Novartis compounds across the 13,000 assays. Over the years, profileQSAR has been used in a number of ways to minimize biochemical and phenotypic screening toward identification of promising chemical matter, prediction of off-target liabilities for hits or lead series and mechanism-of-action predictions for phenotypic screens.

This presentation will describe the pQSAR method and provide examples of it's implementation for several Global Health programs in malaria and Chagas disease.

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07

Drug Discovery for parasitic diseases: powered by technology, enabled by pharmacology, informed by clinical sciences

Srinivasa Rao

Novartis Institute for Tropical Diseases, CA, USA

Parasitic diseases such as malaria, leishmaniasis, Chagas disease have significantly affected millions of people living across different continents. Although, there has been significant advances in development of novel drugs, there is a constant need to find novel therapies for these diseases due to resistance issues or non-optimal efficacy and safety. Innovative therapeutics are also needed to achieve disease control and elimination targets for neglected parasitic diseases. Extraordinary advances in drug discovery technologies have occurred over the past decades, along with accumulation of scientific knowledge and experience in pharmacological and clinical sciences that are transforming many aspects of drug research and development across disciplines. In this presentation, we reflect on how these advances have propelled drug discovery for parasitic infections, focusing on malaria, kinetoplastid diseases and cryptosporidiosis. We also discuss challenges and research priorities to accelerate discovery and development of urgently needed novel anti-parasitic drugs.

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08

Quantifying wildlife behavioural responses: a new approach to chemical regulation and possibilities for implementation during drug development

Bertram, M.G.[1,2,3,*]

[1]Department of Wildlife, Fish, and Environmental Studies, Swedish University of Agricultural Sciences, Umeå, Sweden; [2]Department of Zoology, Stockholm University, Stockholm, Sweden; [3]School of Biological Sciences, Monash University, Melbourne, Australia; *michael.bertram@slu.se

Behavioural analysis has been attracting significant attention as a broad indicator of sub-lethal toxicity, and has secured a place as an important subdiscipline in ecotoxicology. One of the most notable characteristics of behavioural research, compared to other established approaches in sub-lethal ecotoxicology (e.g. reproductive and developmental bioassays), is the wide range of study designs being used and the diversity of endpoints considered. At the same time, environmental hazard and risk assessment, which underpins regulatory decisions to protect the environment from potentially harmful chemicals, often recommends that ecotoxicological data be produced following accepted and validated test guidelines. These guidelines typically do not address behavioural changes, meaning that these, often sensitive, effects are not represented in hazard and risk assessments. In this talk, I will discuss new frontiers in quantifying wildlife behavioural responses to chemical pollution. I will also present our newly developed tool, the EthoCRED evaluation method, for assessing the relevance and reliability of behavioural ecotoxicity data, which considers the unique requirements and challenges encountered in this field. This method, and accompanying reporting recommendations, are designed to serve as an extension of the 'Criteria for Reporting and Evaluating Ecotoxicity Data (CRED)' project. As such, EthoCRED can both accommodate the wide array of experimental design approaches seen in behavioural ecotoxicology, and is able to be readily implemented into regulatory frameworks in different jurisdictions to allow better integration of knowledge gained from behavioural testing into environmental protection. Furthermore, through our reporting recommendations, we aim to improve the reporting of behavioural studies in the peer-reviewed literature, and thereby increase their usefulness in chemicals regulation. I will also highlight possibilities for implementing behavioural toxicity testing during the drug design process, and how this can be facilitated using the EthoCRED method.

ACKNOWLEDGEMENTS

The Author acknowledges financial support from the Swedish Research Council Formas (2020-02293), the Kempe Foundations (SMK-1954 and SMK21-0069), and the Marie-Claire Cronstedt Foundation.

09

Enhancing ecotoxicology and drug testing: Towards healthier medicines and healthier habitats

Eli S.J. Thoré[1,2,3]

[1]Swedish University of Agricultural Sciences, Sweden; [2]TRANSfarm KU Leuven, Belgium; [3]Stockholm University, Sweden; eli.thore@slu.se

The escalating consumption of pharmaceuticals is resulting in their inadvertent release into the environment, posing a significant threat to wildlife and ecosystems. This threat is especially pronounced in freshwater habitats, which already face an acute biodiversity crisis. Integrating ecotoxicological assessments into the drug development process may hold the key to sustainable solutions, aiming to produce pharmaceuticals that are not only efficacious but also environmentally benign.

Current ecotoxicological tests are primarily designed to assess the toxicity of pesticides and heavy metals, but often fall short in adequately capturing the ecological impacts of pharmaceutical pollutants. These tests mainly focus on acute exposure effects and traditional endpoints such as mortality, growth, and reproduction, thereby overlooking other biological yet ecologically significant responses to chronic, low-dose exposures typical of pharmaceutical pollutants.

As the field of ecotoxicology transitions away from animal testing methods and toward alternative approaches, it becomes crucial to address the inherent limitations of existing testing paradigms. By incorporating these insights into alternative methods, we not only have the chance to replace animal testing but also to ensure the relevance and reliability of these alternative approaches. This holistic approach is essential for the development of pharmaceuticals that effectively safeguard the health of humans, animals, and the ecosystems they inhabit.

ACKNOWLEDGEMENTS

I thank all my collaborators from KU Leuven and Swedish University of Agricultural Sciences for their support. Furthermore, I thank Research Foundation - Flanders and Kempe Foundations for their financial support.

10

GreenLab Initiative – On the Path for Sustainable Research

Ana Carolina Monteiro[1], **Daniela M. Sousa**[1]

[1]i3S Green Lab Initiative, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal (e-mail: greenlab@i3s.up.pt)

In 2020, a group of researchers at i3S founded the i3S GreenLab Initiative to boost a more environmentally sustainable Institute.

i3S GreenLab Initiative has been promoting a diversity of activities to reduce i3S carbon footprint by promoting energy saving, lab waste reduction, and social waste reduction, such as 1) developing a platform for sharing surplus laboratory reagents; 2) increasing the ultra-freezers' temperature and promoting a bimonthly cleaning of filters; 3) replacing plastic/paper cups by coffee mugs; 5) promoting the re-usage of Styrofoam boxes and cooler blocks by external entities; 6) implementing a survey on Green Mobility, and 7) supporting the i3S signed commitment to *Pacto do Porto para o Clima*.

i3S was the first Institute in Portugal with LEAF-certified laboratories. By the end of 2023, eleven research groups and one scientific platform were certified, and it is expected that in 2024 we will reach 70% of certification in the institute.

At the i3S Animal facility, a system to replace the disposable personal protective equipment with reusable ones was implemented. In 2022, this initiative contributed to a reduction of 919 kg in disposable gowns and 69 kg in disposable shoe covers, which resulted in a 50% reduction in equipment costs (compared to 2019).

Throughout the year, the i3S GreenLab Initiative also engaged in social initiatives in partnership with external entities. During the Christmas season, we collaborated with E-cycle to gather electronic waste, which was then converted into money to be donated to social institutions and families in need. Additionally, we worked with *Papel por Alimentos* throughout the year, exchanging collected paper and cardboard for food, which was then donated to *Banco Alimentar Contra a Fome*. Together, these initiatives allowed to raise awareness among researchers and promote the adoption of a more sustainable lifestyle within the Institute.

11

Sustainable chemistry for greening the synthesis of active pharmaceutical ingredients (API)

Corbisiero D.,[1] Ferrazzano L.[1]

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Discovering new drugs and pharmaceutical compounds entails a lengthy, costly, and laborintensive endeavor, prompting pharmaceutical companies to invest billions of euros/dollars annually. However, the synthetic procedures for generating active pharmaceutical ingredients (APIs), particularly those originating from academic research, often prove unfeasible on an industrial scale due to their reliance on stoichiometric quantities of reagents, deployment of protective group strategies, excessive utilization of organic solvents, prolonged heating periods, and the formation of potentially hazardous reactive intermediates.[1] Pharmaceutical firms are keenly interested in exploring innovative research avenues to establish efficient and sustainable manufacturing pathways for the synthesis of high-value chemicals and medications.[2] Presently, in addition to conceptualizing and identifying novel APIs, medicinal chemists must prioritize considerations such as eco-friendliness, scalability, and overall sustainability in synthetic processes.

Implementing methodologies that offer environmentally friendly synthetic alternatives—such as enzyme utilization, organocatalysis, benign solvent deployment, milder reaction conditions, or multicomponent strategies to circumvent intermediate isolation—will yield substantial enhancements in the manufacturing and advancement of 21st century medicines.[3] Pharmaceutical industries are actively engaged in the pursuit of innovative approaches to produce APIs with minimal environmental impact and maximal efficiency simultaneously. For this reason, the development of new sustainable synthetic processes is a priorioty expecially in the manufacturing of those pharmaceutical entities belonging to growing market segments, like peptides, oligonucleotides, and small molecules. Thus, we started an exploration of the essential methodologies required to attain these molecular classes through sustainable processes, emphasizing the utilization of green metrics for their objective assessment.[4]

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12
Responsible Conduct in Research

Susana Magalhães

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The field of responsible research is multilayered, with ethical, moral and procedural dimensions, that can only be adequately addressed if each research group has time and space to listen to its members and *think*, not only *about* ethics & integrity as stated in the norms, but also *think with* ethics & integrity as applied to daily life. The increase of regulation on research integrity, which is positive in itself, can lead to the erosion of ethics, shifting the focus from agency and behavior to procedures. Focusing exclusively on the procedural dimension of good science (integrity) without considering its ethical dimension poses the risk of disregarding the relational basis of researchers' daily work: how they interact with peers, with supervisors/mentors/leaders, with (potential) participants in their research and with society. Integrity in research can only be meaningful if it addresses what really matters to each individual researcher. Authorship and the ethics of publishing, supervision, conflicts of interest, data management and the ethics of AI in research are some of the key issues of responsible research that will be discussed in this presentation.

13

Roadmap to Career Development: Crafting your path in Academia and Industry

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The individual career journey is a dynamic process marked by choices, opportunities, and challenges. From the earliest stages of research to master's, PhDs, and postdocs, understanding the essence of career development is noteworthy. In this session, we delve into the intricate interplay between individual aspirations, contextual factors, and broader trends, fostering a deeper understanding of the complexities inherent in career decision-making and progression.

We will discuss practical strategies for career exploration and planning to the distinctive landscapes of academia and industry, spotlighting the divergent pathways and essential competencies for each domain. Ultimately, this session serves as an encouragement of guidance, offering some theoretical frameworks and practical tools to navigate the complexities of career development with some more confidence and clarity.

Oral Communications

01

Simple, quick, low-cost assays to evaluate anti-parasite hit compounds before embarking on *in vivo* experimentation

Harry P. de Koning

02

Application of the CAM model to the study of disseminating and non-disseminating *Leishmania* infections

Dias, F., Pinto, M.T., Leite M., Oliveira, M.J., Duarte, M., Tomás, A.M.

03

Incorporating host sanctuary and parasitic quiescence in animal-friendly antileishmanial drug screening assays

Nicolaes Y., Dirkx L., Maes L., **Caljon G**

04

Advancing drug discovery against malaria: advantages of using a non-classical mouse model for efficacy studies

Sofia Meirinho, André Gomes, Joana Ribeiro, Alessandro Greco, Oliver Keminer, Sheraz Gul, Maria Alice Carvalho, Pedro Ferreira, Cordeiro-da-Silva & **Joana Tavares**

05

Machine Learning-Based Predictive Models for Assessing Toxicity Towards Model Organisms: A Sophisticated Approach

Johander I. Azuaje, Črtomir Podlipnik

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01

Simple, quick, low-cost assays to evaluate anti-parasite hit compounds before embarking on *in vivo* experimentation

Harry P. de Koning

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There are good reasons to minimize the use of laboratory animals, ethical considerations, costs, duration of the experiment and expensive facilities among them. It is therefore sensible to have a set of simple, inexpensive and quick assays to evaluate the suitability of the hits and to narrow down the number of possible candidates, before starting *in vivo* work. We typically start with a standardized screening on a panel of parasite species and strains using a resazurin-based assay.[1] The species chosen strategically for the eventual application (e.g. *Trypanosoma evansi* for surra; *L. major* for cutaneous leishmaniasis) and additional tests use strains resistant to the key drugs currently in use and to which replacement drugs cannot be cross-resistant. A resazurin assay is also used to test the hits on a panel of mammalian cell lines.[2] These tests give an early ranking based on EC_{50} value, cross-resistance profile and selectivity index. Growth curves in the presence of 2x and 5x EC_{50} concentration show how quickly the drug acts on cultured cells (rapidly cidal, cytostatic, slow acting etc) and placing the parasites in fresh medium after a set period shows whether the effects are reproducible. For rapidly acting cells much more information on the rate of cell death cell at various concentrations can be obtained using the propidium iodide exclusion test, monitoring its fluorescence in real time; this increases proportionally to cell death.[3] This assay lends itself very well to the co-incubation with a series of potential inhibitors/antagonists of the test compound's action (e.g. uptake inhibitors; depolarisation of mitochondrial membrane) in a 96-well format. Possible effects on cell division can be quantified by flow cytometry or fluorescence microscopy after nucleic acid staining.[4] Effects on respiration, ATP levels and the production of reactive radicals are easily assessed using commercial kits.

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02

Application of the CAM model to the study of disseminating and non-disseminating *Leishmania* infections

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Dissecting the molecular mechanisms that enable intracellular parasites to disseminate within their hosts is essential to understand the infectious process and resulting pathologies, especially when considering the development of novel therapeutic strategies for those diseases. Here, we aim to implement a new infection model to explore the mechanisms behind *Leishmania* infection and dissemination, the chick embryo chorioallantoic membrane (CAM), a model traditionally used in cancer research. Towards this goal, we pre-stained murine bone marrow derived macrophages with PKH67 dye before infecting with *Leishmania infantum* or *Leishmania* major promastigotes expressing a fluorescent dTomato protein. Naïve and *Leishmania* infected macrophages were detached from the culture plates and grafted onto the CAM of 10-day old embryos. On day 13 of embryonic development the eggs were chemically fixed, and the CAMs were retrieved to be i) photographed for analysis of angiogenesis, and ii) processed for confocal imaging of the entire height of the tissue, to evaluate cell invasion. Preliminary data suggest that *L. infantum* infected cells lead to higher angiogenesis compared to naïve or *L. major* infected cells, but further experiments are required to determine if these observations have statistical significance. Furthermore, our data indicated that *L. major* infected macrophages tended to penetrate/invade the CAM to a lesser degree than naive and *L. infantum*-infected cells. Upon optimization, this protocol is expected to allow us to i) investigate migration of infected cells in a complex *in vivo* model, ii) and study the dissemination of *Leishmania*. The CAM model might additionally provide a new *in vivo* model to screen for effective new treatment options against leishmaniasis.

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03

Incorporating host sanctuary and parasitic quiescence in animal-friendly antileishmanial drug screening assays

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For a plethora of infectious diseases, host sanctuary and pathogen quiescence are increasingly understood to underlie post-treatment relapse. Our recent research has formally linked parasite survival in stem cells in the bone marrow to treatment failure in visceral leishmaniasis [1]. These cells were identified as a sanctuary niche where parasites can survive drug treatment by transitioning through a quiescent state, serving as a source of systemic parasite spread and relapse. However, the current R&D pipeline does not capture this risk of relapse, as the underlying mechanisms are only recently being uncovered. Moreover, no effective prognostic tests exist to predict the treatment response in patients.

To complement the existing conventional animal models, we set out to establish a novel assay system for antileishmanial drug discovery that captures host cell sanctuary and parasitic quiescence. For this purpose, an *ex vivo* culture system has been adopted to expand stem cells isolated from the bone marrow.
These cells have been characterized for infection with *Leishmania* parasites and drug efficacy experiments. Ultimately, this allows for the development of an animal-friendly drug screening platform incorporating host sanctuary and parasite quiescence to improve antileishmanial lead selection and reduce the risk of relapse.
In addition, it offers opportunities for reverse genetics approaches to study the host-parasite interaction.

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04

Advancing drug discovery against malaria: advantages of using a non-classical mouse model for efficacy studies

Sofia Meirinho[1], André Gomes[2], Joana Ribeiro[3], Alessandro Greco[4], Oliver Keminer[5,6], Sheraz Gul[5,6], Maria Alice Carvalho[2], Pedro Ferreira[1], Cordeiro-da-Silva[3,7] & **Joana Tavares**[3]

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Malaria is a parasitic disease that affects millions of people worldwide. The most common and dangerous form of malaria is caused by *Plasmodium falciparum*, which is responsible for most cases and deaths. Unfortunately, the current drug treatments, including the most effective artemisinin-combination therapy (ACT), are becoming less effective due to the emergence of drug resistance [1]. Therefore, there is an urgent need for new antimalarials that are active against drug resistant *P. falciparum*. To contribute to drug discovery against *P. falciparum*, we have been investigating the antimalarial activity of pyrimido [5,4-d] pyrimidine-based (PP) molecules.
We used structure-based *in vitro* studies to modify the core scaffold while considering their activity against parasites and their toxicity to human cells. Molecules were tested on blood-stage forms of *P. falciparum,* including both susceptible and multidrug-resistant strains, named 3D7 and Dd2 respectively. Some of these molecules, showed activity against both strains, with IC_{50} values below 100 nM, and a selective index ranging from 100 to 1000-fold [2]. We also conducted early ADME-Toxicity *in vitro* studies to assess compounds' safety. The results obtained were encouraging, which supports further development of the safest and most active molecules for *in vivo* efficacy studies. Traditionally, drug efficacy studies *in vivo* use rodent malaria models. However, *Plasmodium berghei* or other rodent-infecting species are not human pathogens. The development of the humanized mice model NODscidIL2Rγcnull (NSG) engrafted with human erythrocytes renders mice susceptible to infection with *P. falciparum* strain 3D70087/N9 [3]. This model, known as the PfalcHuMouse model, has been critical in advancing numerous candidate molecules for clinical trials. In this presentation, we will also explore the advantages of utilizing the PfalcHuMouse non-classical humanized mice model to complete antimalarial preclinical studies.

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05

Machine Learning-Based Predictive Models for Assessing Toxicity Towards Model Organisms: A Sophisticated Approach

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Predictive models based on machine learning offer a sophisticated approach to determining toxicity towards model organisms across various methodologies. The study and assessment of compound toxicity constitute a burgeoning field of research, driven by the necessity to evaluate the environmental impact of numerous industrial products. While *in vivo* tests traditionally serve as the primary method for this evaluation, the process entails numerous steps. These include selecting representative organisms, controlling experimental conditions, employing hazardous reagents, and enduring lengthy data collection periods to yield reliable results. In the contemporary landscape, machine learning has emerged as a sophisticated tool across diverse domains, facilitating advancements in pharmaceutical and industrial processes. Predictive models represent a novel avenue for elucidating various aspects of new and unexplored compounds.

Multiple algorithms can be harnessed to develop these models, according to the complexity of the problem at hand, thereby yielding more accurate and precise results. Organisms such as *Daphnia magna* and *Danio rerio* (zebrafish) serve as models for toxicity determination, adhering to specific testing criteria, and sufficient data can be utilized to train models and generate novel insights. This study focuses on the development of machine learning models for compound classification based on toxicity, assessing their effects on model organisms, and enabling the determination of pLC50 or pEC50 values. Therefore, techniques such as random forest, multivariate linear regression, neural networks, and support vector machines, along with various molecular descriptors to encode pertinent features related to toxicity, represents a comprehensive approach. This methodology offers versatility, enabling toxicity assessment for existing compounds, those commonly found in products, as well as pharmaceuticals and industrial compounds in developmental stages, thereby aiding in the identification and mitigation of environmentally harmful substances.

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Posters

01

Updates and achievements in OneHelthdrugs

Maria Paola Costi, Anabela Cordeiro da Silva

02

Leishmanicidal activity of α-bisabolol, the main sesquiterpene in chamomile essential oil: An innovative approaches to evaluate the effectiveness and safety of drug therapie

S. Hajaji, **H. Akkaria**

03

Identification of a new chemotype as pteridine reductase 1 (PTR1) inhibitors with trypanocidal potential

Elisa Uliassi, Annachiara Gandini, Cecilia Pozzi, Chiara Borsari, Sheraz Gul, Carolina B. Moraes, Nuno Santarem, Anabela Cordeiro-da-Silva, Maria Paola Costi, Maria Laura Bolognesi

04

A SAR study in 4,8-disubstituted pyrimido[5,4-d]pyrimidines with antileishmanial activity

André Lopes, Sofia Teixeira, Nuno Santarém, Alessandro Greco, Oliver Keminer, Sheraz Gul, Anabela Cordeiro-da-Silva, Maria Alice Carvalho

05

Brugia Pahangi: Drug Screening On Adult Worms And Microfilaria *In Vivo*

Erjona Abazaj, Fatgzim Latifi, Oltiana Petri, Mizan Saidin, Noor Rain Abdullah

06

Genotyping of the Duffy Domain among Patients Suspected of Malaria Infection in the Dominican Republic

Claudia Federo, Francisco Cuevas, Margarida Gonçalves, Bruno Freitas, Isaac Miguel, Nuno Osório, Modesto Cruz, Maria Isabel Veiga

07

In vivo toxicity, redox-modulating capacity and intestinal permeability of novel aroylhydrazone derivatives with high *in vitro* antimycobacterial activity

Simeon Dimitrov, Violina Angelova, Rumyana Simeonova, Milka Mileva, **Violeta Valcheva**

08

Eco-Friendly Methods To Take The Place Of Traditional Animal Models In VBD Medication Testing

Omer Gurkan Dilek

09

What are alternatives to animal testing? What are the best options?

Doloresa Mullaliu

01

Updates and achievements in OneHelthdrugs

Maria Paola Costi[1] & Anabela Cordeiro da Silva[2]

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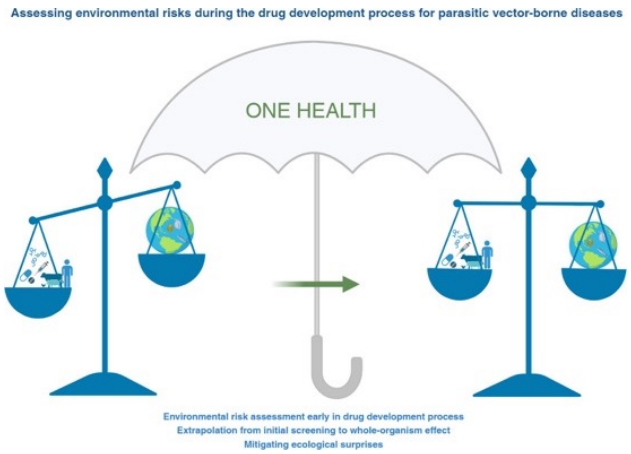
Nearly 75% of emerging human infections worldwide originate from animals. Current drugs for both human and animal (H&A) vector-borne diseases (VBD) are in short supply and have limited efficacy, potential toxicity, and limited resources. Emerging environmental issues in pharmaceutical use/manufacturing are increasing attention in this area. Collaboration and research between different expertise involving human and animal medicines therefore essential to define how new drugs can be developed using a more sustainable approach. All the drug discovery process can be developed including the greener principles. *OneHealthdrugs* [1], a COST Action, is dedicated to the development of innovative strategies for the supply of drugs with a more environmentally friendly profile and low environmental impact [2]. The new drugs aim to combat H&A VBD while maintaining the principles of an optimal profile for both organisms, improving the quality of the drugs and associated delivery technologies. R&D experts from various fields, including chemical/biological/human/veterinary and earth sciences, are working together to propose guidelines and improve existing drug discovery tools in the field of NID discovery. The platform includes preclinical drug discovery, animal studies and drug delivery. Approaches such as compound and target database generation, bioinformatics and omics studies, medicinal chemistry strategies and nanotechnology tools will be implemented.

We expect the results of *OneHealthdrugs* to have a significant impact both in Europe and in disease-endemic countries. The latest advances in the compound database, including the greener principles derived properties [3] and the drug targets database managed by adopting reduced off-target species filtered with appropriate software, as well as the sustainable drug profiles in H&A health represent the most recent advance in the project.

We area also developing standard operative procedure for more sustainable animal testing.



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COST Action CA21111: One Health Drugs against Parasitic Vector-Borne Diseases in Europe and Beyond (OneHealthDrugs).

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02

Leishmanicidal activity of α-bisabolol, the main sesquiterpene in chamomile essential oil: An innovative approaches to evaluate the effectiveness and safety of drug therapie

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According to the World Health Organization, leishmaniasis is considered as a major neglected tropical disease causing an enormous impact on global public health. Available treatments were complicated due to the high resistance, toxicity, and high cost. Therefore, the search for novel sources of anti-leishmania agents is an urgent need. In the present study, an *in vitro* evaluation of the leishmanicidal activity of the essential oil of Tunisian chamomile (Matricaria recutita L.) was carried out. Chamomile essential oil exhibits a good activity on promastigotes forms of *L. amazonensis* and *L. infantum* with a low inhibitory concentration at 50% (IC_{50}) (10.8 ± 1.4 and 10.4 ± 0.6 µg/mL, respectively). Bio-guided fractionation was developed and led to the identification of (-)-α-bisabolol as the most active molecule with low IC_{50} (16.0 ± 1.2 and 9.5 ± 0.1 µg/mL for *L. amazonensis* and *L. infantum*, respectively).

This isolated sesquiterpene alcohol was studied for its activity on amastigotes forms (IC_{50} = 5.9 ± 1.2 and 4.8 ± 1.3 µg/mL, respectively) and its cytotoxicity (selectivity indexes (SI) were 5.4 and 6.6, respectively). The obtained results showed that (-)-α-bisabolol was able to activate a programmed cell death process in the promastigote stage of the parasite (1). It causes phosphatidylserine externalization and membrane damage. Moreover, it decreases the mitochondrial membrane potential and total ATP levels. These results highlight the potential use of (-)-α-bisabolol against both *L. amazonensis* and *L. infantum*, and further studies should be undertaken to establish it as novel leishmanicidal therapeutic agents.

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03

Identification of a new chemotype as pteridine reductase 1 (PTR1) inhibitors with trypanocidal potential

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Trypanosomatids are among the most critical parasites for public health due to their impact on human and animal health. *Trypanosoma* and *Leishmania* parasites cause serious diseases for which there are no effective treatments [1]. A 320-membered proprietary library was screened against pteridine reductase 1 (PTR1) from *Trypanosoma* and *Leishmania* species, as a validated trypanosomatid drug target [2]. NMT-TY0177, a thioxanthine derivative, has been identified as a hit compound. Target-based approaches, including x-ray crystallography with *Trypanosoma brucei* (Tb)PTR1, guided the design and optimization of **1–22**. Once synthesized, whole-cell phenotypic assays against T. *brucei* and *cruzi*, and *L. infantum* have been performed to assess the trypanocidal potential of **1–22**. Selectivity of **1–22** has been evaluated against mammalian cells (A549 and THP1) along with their early ADME-tox properties in terms of hERG and cytochrome P450 (CYP) inhibition, and mitochondrial viability. Compounds **10** and **12** emerged as the most promising PTR1 inhibitors with good trypanocidal activity and safety profile. Additionally, combination studies of the newly developed PTR1 inhibitors with methotrexate, a competitive inhibitor of dihydrofolate reductase, have been caried out to evaluate whether the synergistic inhibition of the trypanosomatid folate pathway in *T. brucei* might provide a more effective treatment.

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04

A SAR study in 4,8-disubstituted pyrimido[5,4-*d*]pyrimidines with antileishmanial activity

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Leishmaniasis, a neglected tropical disease, caused by vector-borne protozoa[1] has become a huge threat to health in third-world countries. Current treatment relies on chemotherapy; however, the available treatment options have low efficacy and serious side effects. Furthermore, the increase in drug resistance parasites has become a growing concern.[2] The search for new treatment options is a priority, and recently, pyrimidopyrimidine-based compounds have been described as new promising compounds with activity against *Leishmania* parasites[3].

In this work, a set of new derivatives was synthesized and *in vitro* evaluated against *L. infantum* promastigotes and intramacrophage amastigotes. The cytotoxicity *in vitro* was determined using the THP1 cell line, and early ADME-Tox was also carried out, *in vitro* and *in silico*, for selected compounds. The combined *in vitro* results allowed the identification of two compounds as the hits to develop as antileishmanial. The synthesis and biological results will be presented.

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05

Brugia pahangi: drug screening on adult worms and microfilaria *in vivo*

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Introduction: Lymphatic filariasis caused by nematodes *Wuchereria bancrofti* and *Brugia spp* are among the most important tropical diseases. Diethylcarbamazine (DEC) being one of the drugs used in the Global Programme for the Elimination of Lymphatic Filariasis. It is an annual drug *and* it continues to play an important role in the treatment of *Brugia spp*.

Aim: The study aimed to determine the antifilaria activity of the standard antifilaria (*B. pahangi*) drug diethylcarbamazine (DEC) *in vivo* study.

Methods: Mongolian gerbils (*Meriones unguiculatus*) were the animal hosts for this experimental study design. Four gerbils were obtained from the Animal Resources Unit, Institute for Medical Research. The L3 harvested were counted and inoculated into clean Gerbils. A hundred fifty L3 were used for each inoculation in a gerbil by intra-peritoneal injection. The infection was for the production of Adult worms and microfilaria. The gerbils divided into 2 groups (control group and test substance use) were ready for the experiment after 90 days of infection. The DEC dose required for each gerbil was 1.5mg/kg BW. Calculations of drugs were based on the gerbil weight and the amount required for 1.5mg/kg BW, and the DEC was diluted in 2ml distilled water. The route of administration of drugs was orally by intubation needle (gauge), and gerbils were sacrificed after seven days for the collection of worm mf and adult.

Results: The activity was determined by motility and MTT assay against the mf and adult stages. The finding showed that DEC does not affect the motility and viability of the two filaria stages tested, the microfilaria and the adult worm at 1.5 mg/kg BW given orally at a single dose. The effectivity of DEC towards adult worms was almost useless after all adult worms were shown to be alive.

Conclusion: In this study, only four infected gerbils were used. DEC at 1.5mg/kg BW as a single dose was not effective for microfilaria. Maybe the application of a second dose at a later time could give the desired effect of the treatment of this parasite in gerbils. Continuation of the experiment using a higher number of gerbils and the application of another study design scheme in the future is recommended to see the desired effects of DEC as a drug.

Limitation: The number of gerbils that were infected with *B. pahangi* was limited to have a bigger study number in each group.

Keywords: Gerbils, filariasis, *B. pahangi*, DEC, experimental study

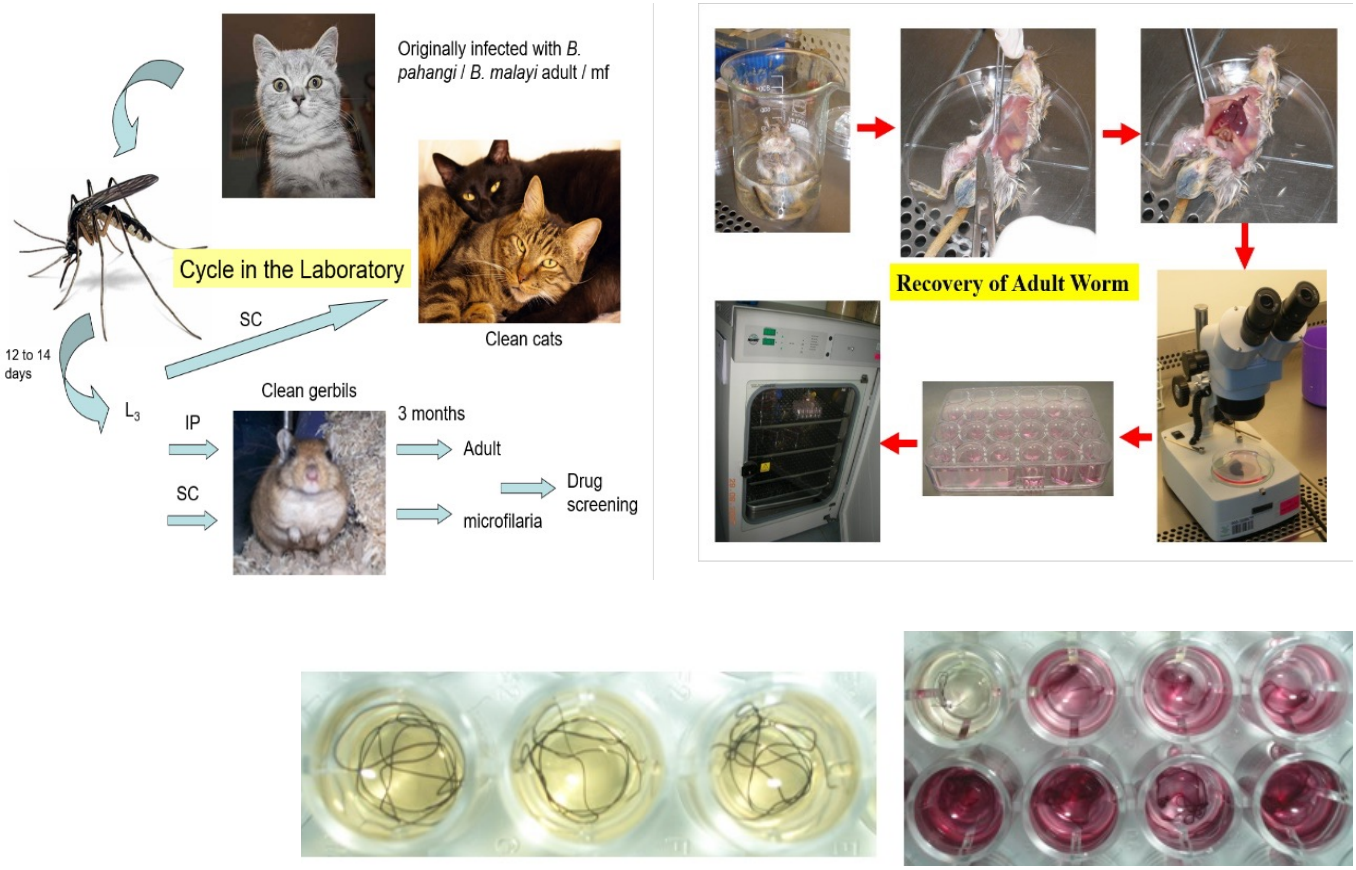

Figure 1. Image during the study project

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06

Genotyping of the Duffy Domain among Patients Suspected of Malaria Infection in the Dominican Republic

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The Duffy blood group system has drawn significant attention in erythrocytic invasion by malaria parasite *Plasmodium vivax*, the most widespread form of human malaria. A T-33C polymorphism in the gene encoding for the Duffy glycoprotein, *fy*, results in a Duffy null Fy(a-b-) phenotype, which is predominant in populations of African ancestry and is believed to confer protection against *P. vivax* infection. Dominican Republic (DR), and to an extent the entire island of "La Hispaniola", account mainly malaria cases due to *P. falciparum* infection, opposing neighboring Latin-American countries in which *P. vivax* infection predominant. Understanding the prevalence of the Duffy null phenotype in DR, which boasts a high degree of African ancestry, may provide an answer to it's low *P. vivax* infection prevalence.

This study focused on screening the *fy* gene of a sample pool of individuals from DR. DNA was extracted from immunochromatographic columns used for rapid malaria tests, which had initially tested negative. Real-time polymerase chain reaction (PCR) was employed to genotype all samples regarding the Duffy blood group.

The genotyping analysis unveiled that 59% of the samples tested Duffy negative/null while the remaining 41% tested Duffy positive.
These allele frequencies within DR population offer crucial data for comprehending genetic diversity, ancestry, and the level of protection against *Plasmodium vivax* infection in the region.

This research sheds light on the frequency of genotyping for the rs2814778 mutation within the DR population. These findings contribute to our existing knowledge regarding the Duffy blood group system, population genetics, and potential associations with enhanced protection against *P. vivax*. Further investigations may elucidate the functional significance of these genotypes and their implications in health outcomes and pharmacogenomics.

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07

In vivo toxicity, redox-modulating capacity and intestinal permeability of novel aroylhydrazone derivatives with high *in vitro* antimycobacterial activity

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Despite significant progress in the development of new drugs against tuberculosis, many therapies and preventive measures do not lead to the expected favorable health results for various reasons. This opens the way to identify novel, structurally diverse compounds, whose structure–activity–toxicity relationships must be thoroughly elucidated prior to further development. We present the aroylhydrazone compounds (3a,b,c) about their: (i) acute and subacute toxicity in mice; (ii) redox-modulating capacity by *in vivo* and *in vitro* investigations; (iii) pathomorphological observation in differentiated tissue specimens; (iv) intestinal permeability; and (v) *in vitro* antimycobacterial activity. They were characterized by 1H-NMR, 13C NMR and HRMS spectroscopic data. The minimum inhibitory concentration (MIC) was determined using the broth microdilution assay against *M. tuberculosis* H37Rv. The screening identified 3a (MIC=0.0730 µM, cytotoxicity - HEK-293T IC50 = 256.7 µM, SI=3516), 3b (MIC=0.3969 µM, cytotoxicity - HEK-293T IC50 = 785 µM, SI=1978.83) and 3c (MIC=0.4412 µM, cytotoxicity - HEK-293T IC50 = 279.5 µM, SI=633.49) as a new promising hit compounds against *M. tuberculosis* H37Rv. According to the Hodge and Sterner toxicity scale, 3a,b,c are classified as slightly toxic with an LD50 > 2000 mg/kg for both oral and intraperitoneal administration. Changes in behavior, and amounts of food and water intake were not observed during 14 days oral administration at two doses of 1/10 and 1/20 of the LD50. The histological examination proved that the tissue findings do not show toxic changes. Liver findings showed isolated changes without a pathological organ profile. The in vitro antioxidant assays confirmed the results found *ex vivo*. High GIT permeability at all tested pH values was possess for 3a and 3b. These compounds display promising antitubercular drug-like properties and can be used for further investigation.

08

Eco-Friendly Methods To Take The Place Of Traditional Animal Models In VBD Medication Testing

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Replacing classical animal models in drug testing for vector-borne diseases (VBDs) with environmentally friendly systems is a progressive approach that aligns with the principles of animal welfare and sustainability. Here are some environmentally friendly alternatives: *In vitro* Models, utilizing cell cultures and tissue cultures can provide valuable insights into the efficacy and toxicity of drugs without the need for animal testing. These models can mimic the complex interactions between pathogens and host cells, allowing for the screening of potential drug candidates. Organ-on-Chip Technology; This innovative technology involves microfluidic devices that replicate the structure and function of human organs. Organ-on-chip models can simulate the physiological conditions of the human body more accurately than traditional animal models, offering a more relevant platform for drug testing. Computational Modeling and Simulation; Advances in computational biology and bioinformatics have enabled the development of sophisticated models that can predict the behavior of drugs in the human body. These models integrate data from various sources, such as genomics, proteomics, and epidemiology, to simulate the effects of drugs on VBDs. 3D Bioprinting, 3D bioprinting allows the fabrication of complex tissue structures using bioinks composed of living cells. This technology can be used to create customized tissue models for studying VBDs and testing drug candidates in a more physiologically relevant environment. Humanized Animal Models; Rather than using traditional animal models, researchers can develop humanized animal models by introducing human cells or tissues into non-human organisms. These models can provide insights into the human-specific aspects of VBDs and drug responses while reducing the need for large-scale animal testing. Epidemiological Studies; Observational studies of human populations affected by VBDs can provide valuable data on disease progression, treatment outcomes, and drug efficacy. By analyzing real-world data, researchers can identify trends and patterns that inform drug development efforts.

Keywords: Vector, animal model, drug.

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09

What are alternatives to animal testing? What are the best options?

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Introduction:

Using alternatives to animal testing in clinical research when suitable does not put patients at risk or delay medical progress. Instead, non-animal testing methods such as human cell- and tissue-based testing, human volunteer testing, and computational and mathematical models can be more accurate, cost-effective, and quicker than traditional animal models.

Methods:

1) Human Cell- and Tissue-based testing

Miniature cellular and tissue models, such as organs-on-a-chip and 3D bioprinting, use human cells to mimic organ functions and structures to screen treatments and test drugs. This allows researchers to simplify a system, limiting the number of variables. Instead of animals, these human-based models can be used to study biological and disease processes and drug metabolism.

2) Organs-on-a-Chip

Microfluidic organs-on-a-chip are small, clear, flexible polymer devices that comprise human cells and push fluid through tiny channels to imitate blood flow. In 2010, a team at Harvard University's Wyss Institute developed the first successful human-cell chip. The first of its kind, the lung-on-a-chip, carried out basic lung functions, like respiration. And now, researchers have expanded upon this concept by successfully creating chips that mimic the liver, stomach, intestine, brain, and skin, among others.

IMPORTANCE: Because roughly 30% of medications fail in human clinical trials due to toxicity — despite pre-clinical data using animal and cell models — tissue chips function as new human cell-based approaches that help researchers accurately determine how effective a therapeutic candidate would be in clinical studies.

By eliminating toxic or ineffective drugs earlier in development, drug manufacturers can save valuable time and money. These chips also could teach scientists a great deal about disease progression, leading to better prevention, diagnosis, and treatment approaches.

Because many industry experts recognize the widespread benefits of human-specific chips, this method is becoming popular in drug discovery and development.

Organs-on-a-chip technology allows scientists to easily replicate human tissue and organ functions to assess the safety and efficacy of new drugs. For instance, the Liver-Chip, a liver-on-a-chip device, can detect drug-induced liver injury missed by animal testing models.

3) Tissue Bioprinting

Three-dimensional (3D) tissue bioprinting is a revolutionary scientific advancement in drug discovery and development that uses new assay models to predict drug impacts on humans better. These tissue models mimic characteristics of live human tissues and are developed on microplates to test the toxicity and efficacy of small molecules or other therapeutics. By leveraging tissue engineering, stem cell research, disease biology, and in situ detection devices for tissue characterization and drug development, 3D tissue bioprinting produces disease-relevant

tissue models that can reduce the predictability gap between the results from current 2D cell-based assays and the results from testing in humans.

4) Human Volunteer Testing

Thanks to numerous technological advances, new and sophisticated scanning devices and recording methods can now be used to study human volunteers safely. For example, advancements in brain imaging techniques allow researchers to see inside the brain to monitor the progression and treatment of certain brain diseases. Researchers use these approaches to better understand diseases by comparing their results with the results of healthy volunteers.

In other research areas, such as nutrition, substance use, and pain management, consenting humans can help replace animal testing models. Compared to animal subjects, human volunteers provide a significant advantage by having the ability to speak with researchers and offer additional information during the study. As opposed to animal testing, human volunteers that donate healthy and compromised tissues via surgery provide a more appropriate way of studying human biology and disease. For example, skin and eye models made from reconstituted human skin and other tissues have been developed to replace rabbit irritation tests. By donating tissue, alive and deceased donors increase the number of human samples available for research and reduce the number of animal subjects needed. In the past, post-mortem brain tissue has provided important breakthroughs in understanding brain regeneration and the effects of multiple sclerosis and Parkinson's disease.

5) Computational And Mathematical Models

Does IA play a role In further correlation?

With the growing capabilities of computers and computer programs, the ability to model certain aspects of the human body has become easier than ever.

Current computer models of the heart, lungs, kidneys, skin, and digestive and musculoskeletal systems have been developed to conduct virtual experiments based on existing mathematical data and information. Additionally, data mining tools assist researchers in making predictions about one substance based on existing data from similar substances. Many alternatives to animal testing methods aim to overcome translational barriers toward developing urgently needed treatments for unmet medical needs. As a result, using non-animal models for research could save the lives of more humans and animals, time, and money. And without sacrificing quality and safety, alternatives to animal testing could improve the quality of society while improving health outcomes.

Results:

Overall, I advocate a switch from viewing AI as a substitute to seeing AI as a complement to our research work. In other words, we should consider viewing AI as a teammate or collaborator (as it becomes smarter than a mere tool) in the support of human work. This paradigm in AI is referred to as collaborative intelligence, where humans and AI join forces to solve problems. Thus, we can think about how researchers can work synergistically with AI, instead of considering it as a threat to our existence. To what degree and for which research tasks may, or may not, AI substitute for, or at least complement, researchers?

COST ACTION 2111: OneHealthdrugs
One Health drugs against parasitic vector borne diseases in Europe and beyond

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