Workshop

„Animal models for lead selection against vector borne parasitic diseases within the One Health concept"

8th of September 2023
(online event)

Chairs: Guy Caljon and Mirco Bundschuh (WG3 leaders)

Time (CET)	Topic/Presenter
14:00-14:05	Welcome Guy Caljon (University of Antwerp, Belgium)
14:05-14:15	Update on OneHealth*drugs* COST Action Maria Paola Costi (University of Modena and Reggio Emilia, Italy)
14:15-14:35	"The 3Rs Principle in the field of toxicology" Birgit Mertens & Mieke Van Mulders (Sciensano, Belgium)
14:35-14:55	"The use and utility of the ARRIVE Guidelines 2.0 for reporting animal research" Stephen Turnock (National Centre for the Replacement, Refinement and Reduction of Animals in Research, UK)
14:55-15:10	"Imaging infections by vector-borne protozoan parasites in mice for lead selection" Joana Tavares (Universidade do Porto, Portugal)
15:10-15:25	"What *Leishmania* reporter strains could do for us" Sarah Hendrickx (University of Antwerp, Belgium)
15:25-15:40	"Using different strains to improve lead selection for Leishmaniasis" Nuno Santarem (Universidade do Porto, Portugal)
15:40-16:00	"Clinglobal, research solutions to the global animal health community" Maxime Madder, Alec Evans and German Graff (Clinglobal, Mauritius)
16:00-16:20	"The use of *in silico* models to evaluate ecotoxicity of pharmaceutical compounds" Emilio Benfenati (Istituto di Ricerche Farmacologiche Mario Negri, Italy)
16:20-16:40	"What can we learn from model organisms; the paradigm of the nematode *Caenorhabditis elegans* and the proteasome" Niki Chondrogianni (Institute of Chemical Biology, Greece)
16:40-16:55	"Flavonolignans from *Silybum marianum* inhibit viability of model flatworm larvae *in vitro* via interference with antioxidant and phase II detoxifying enzymes" Gabriela Hrčková (Slovak Academy of Sciences, Slovakia)
16:55-17:00	Closing remarks Guy Caljon

Abstract 1

The 3Rs Principle in the field of toxicology

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Although initially introduced in the early 1960s, the 3Rs principle (Refinement, Reduction & Replacement) is still considered one of the fundamentals for high-quality science. In Europe, scientists are even legally obliged to adhere to the 3Rs principle as it is anchored in the **European Directive 2010/63** concerning the protection of animals used for scientific purposes. According to this legislation, animals may only be used for scientific or educational purposes if no alternative is available. These 'alternative methods' include different types of technologies such as, for example, the use of complex cell and tissue cultures (*in vitro*) and computer models (*in silico*). If an animal experiment does prove necessary, the minimum number of animals that will provide a scientifically reliable result should be used. Finally, scientists should refine their animal experiments to avoid or reduce as much as possible the discomfort caused to laboratory animals, for example, by administering drugs. Over the last years, a fourth R for "**Responsibility**" has been introduced, referring to the "Culture of Care" philosophy in laboratories to which each scientist should contribute. According to this philosophy, scientists and lab technicians are committed to working in an ethical and correct way, obtaining as much scientific progress as possible while minimizing animal suffering. Until today, replacement remains the most challenging R. In toxicology, success stories have been reported for local effects such as eye irritation, whereas no methods are available to replace more complex toxicological endpoints. Nevertheless, many efforts integrating innovative technologies and human-derived materials are ongoing to stimulate the transition towards more human-relevant next-generation toxicity testing.

Abstract 2

The use and utility of the ARRIVE Guidelines 2.0 for reporting animal research

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Transparent reporting of research methods and findings is an essential component of reproducibility. In 2020, the NC3Rs released an updated version of the ARRIVE guidelines (ARRIVE 2.0) for the reporting of *in vivo* research [1]. This second iteration of the guidelines aims to help researchers improve the rigour and transparency of the scientific process by identifying the essential items needed for good reporting of animal research in manuscripts and provides explanation and elaboration of these requirements. These items encompass key aspects such as sample size determination, randomisation, blinding and statistical analysis, which are fundamental to generating reliable and reproducible findings. By adhering to these guidelines, researchers enhance the standardisation of experimental protocols and promote the credibility of their findings, facilitating comparisons across studies and reducing the risk of biased interpretations. The benefits of scientific research involving the use of animals (knowledge) are underpinned by accurate and open dissemination of this information.

References

[1] Percie du Sert N, *et al*. (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18(7): e3000410. https://doi.org/10.1371/journal.pbio.3000410

Abstract 3

Imaging infections by vector-borne protozoan parasites in mice for lead selection

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Vector-borne protozoan parasites such as *Plasmodium* spp., *Leishmania* spp., *Trypanosoma brucei* and *Trypanosoma cruzi* are responsible for malaria, leishmaniasis, human African trypanosomiasis and Chagas disease respectively [1]. Testing the efficacy of promising chemical entities in animals is an important component of the drug discovery process. The possibility to directly image parasites in living animals using whole-body bioluminescence imaging (BLI), is minimally invasive and thus highly relevant to meaningful longitudinal studies and compliance with the three Rs (Replace, Reduce, Refine) on animal usage. BLI typically uses transgenic parasites expressing the firefly luciferase or, alternatively, a red-shifted version of this reporter for ultrabright bioluminescence with less tissue attenuation in living subjects [2]. We have been using transgenic *Plasmodium* spp., *L.* infantum, *T. brucei and T. cruzi* expressing luciferase to investigate fundamental aspects related to parasite dissemination, infectivity, and growth [3-4] but also in drug and vaccine testing in mice [5-8]. These models are particularly relevant for determining parasite burden in internal organs without the need to euthanize infected animals as traditional methods that despite being very sensitive, require organ collection. We will present and discuss the contribution and potential of these animal models for lead selection in the drug discovery process.

References

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Abstract 4

What *Leishmania* reporter strains could do for us

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Leishmaniasis is a neglected tropical disease affecting the poorest populations worldwide. Although leishmaniasis is an ancient disease dating back to prehistorical times, a lot of gaps still exist in our knowledge of vector transmission, disease onset, immunology and treatment. The development of new animal models would not only facilitate the search for novel chemotherapeutics, but could also help to broaden our general understanding of disease dynamics in the mammalian host.
To allow longitudinal follow-up of infection in view of the 3R concept, our group developed double reporter strains, expressing both the red-shifted firefly luciferase gene *PpyRE9* and the red fluorescent *dsRed* gene. This approach not only allows the evaluation of *in vivo* infection dynamics in the same animal over time via bioluminescent imaging using a range of injectable substrates [1], but can also be used to assess drug treatment dynamics [2], post-treatment relapse, parasite dissemination upon natural transmission and might even support the search for hidden parasite niches [3]. The addition of the fluorescent reporter gene simultaneously allows the acquisition of immunological information in specific target organs via flow cytometry and fluorescence-activated cell sorting.
As the role of the vector is increasingly understood to extend beyond that of a 'flying syringe' and both parasite and vector influence the dynamics and severity of the infection, the reporter strains have been also adapted to the in-house *Lutzomyia longipalpis* sand fly model. This enables the study of infections initiated by natural transmission, which is proven to be a valuable asset when evaluating vaccine candidates for example. So far, natural transmission models have already been developed for *L. major* and *L. tropica* in mice, reproducing the clinical pathology associated with cutaneous leishmaniasis.

References

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Abstract 5

Using different strains to improve lead selection for Leishmaniasis

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Leishmaniasis is a vector-borne disease caused by protozoan parasites of the genus *Leishmania*. Leishmaniasis constitutes both a human and veterinary concern with millions at risk and several thousand fatalities every year[1]. The search for improved drugs has been a long-lasting unmet medical need. Well-characterized experimental animal infection models have been considered the gold-standard for lead selection, with parasites used in such models being considered a proxy for *Leishmania* spp behavior. However, such a principle may not be an adequate assumption, with consequences for drug development. In zoonotic species like *L. infantum* both animals and humans are exposed to long, often ineffective, treatment regimens, the perfect environment for the acquisition of naturally-induced drug resistance. Genetic recombination while in the vector also contributes to a perfect scenario for parasites dissemination with altered drug susceptibility. To address this issue, we set up an assay to evaluate the drug susceptibility landscape to Miltefosine, Antimony, Pentamidine, Paromomycin, and Amphotericin B using promastigotes from over 20 *L. infantum* recent clinical isolates from Portugal. Using a population approach for "normal EC50-range" determination for each drug, we were able to identify several isolates with significantly altered EC50s. Then we selected a panel of representative strains to evaluate their susceptibility to compounds ongoing anti-*Leishmania* drug development in our team. For one of the molecules, the obtained EC50 was increased with a similar pattern to Miltefosine susceptibility. The EC50 was distinct from our strain used for in vitro susceptibility testing. Thus, this molecule might present less potential to advance for subsequent in vivo animal testing than what was initially apparent. Overall, the data generated demonstrates a non-negligible risk for drug development efforts from circulating strains that are exposed to known anti-leishmanial drugs. The predictable impact on *in vivo* models is still to addressed but should be considered in drug development.

References

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Abstract 6

Clinglobal, research solutions to the global animal health community

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Clinglobal is part of a group of contract research organisations born in South Africa with the foundation of Clinvet in 1999 and solely dedicated to the animal health industry. Since then, the ever-expanding Clinglobal group of companies (Clinglobal, Clinvet, Clinomics, Clindata and Rtilab) has provided extensive research solutions to the global animal health community. Positioned between the academic world and the pharmaceutical industry, Clinglobal is an ideal partner for assisting academic organisations in translating ideas and discoveries into products.

By providing contract based services, or partnering in projects, our unique business model provides flexible solutions to the academic community and the animal health industry. Through the provision of *in vitro* and small animal screening models, the group's clinical sites in South Africa, Morocco, New York (USA) and South Dakota (USA) facilitate lead selection. Downstream, we support product registration projects by performing studies according to GCP and/or GLP standards in target animals and in field studies. Clinomics' biotechnology platform supports these projects by providing biological solutions on molecular, serological, biochemical and clinical pathology diagnostics and bespoke molecular and antibody-based assay development.

With a large biobank of recently collected vectors (ticks, sand flies, fleas, tsetse flies, mosquitoes, and more), and their most commonly associated vector-borne pathogens, we specialise in conducting studies with vectors and associated disease animal model studies, including several zoonoses.

The Clinglobal group adheres to the 3R principle by developing *in vitro* models and alternatives to terminal studies. We are familiar with and abide by the ARRIVE, WAAVP, and VICH guidelines in the conduct of safety and efficacy studies.

Different models and opportunities to support the development of new drugs in the One Health vector-borne disease field will be presented in more detail during the session.

Abstract 7

The use of in silico models to evaluate ecotoxicity of pharmaceutical compounds

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The use of in silico models for ecotoxicological and environmental properties is quite common, but the available models often fail in the case of pharmaceutical substances. Indeed, the existing models are typically based on collections of industrial chemicals. The difficulties for pharmaceuticals is related to the poor availability of experimental data for structures which are more complex than industrial chemicals.
Within the PREMIER project we are addressing this difficulty. The strategy is to take advantage of local information using the read-across approach. Then, in silico predictions can be combined with the evaluation based on read-across.
The new read-across strategy has been implemented within a software program, called VERA. The similarity between the target compound and the similar ones is calculated using several components: a structural similarity – using the VEGA algorithm, a similarity based on representative molecular groups, and a similarity based on the structural alerts related to the adverse effect. The key driver is the information on the adverse effect to start grouping on similar substances. These are then clustered in different families depending on the molecular groups. Different methodologies to integrate the results are applied, depending on the availability of the data for each kind of similarity.
The results of the VERA program can be integrated with those from the in silico models within VEGA, within a program called SWAN. The reliability of each result is taken into account to integrate the values.

Abstract 8

What can we learn from model organisms; the paradigm of the nematode *Caenorhabditis elegans* and the proteasome

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Aging is a physiological inevitable process that represents a high-risk factor for the progression of age-related diseases. It is regulated by environmental, genetic and epigenetic factors. Several molecular pathways deteriorate with aging or age-related proteinopathies, including the proteasome system. Proteasomes are constituents of the proteostasis network responsible for the regulated proteolysis of normal and abnormal proteins. Using the replicative senescence model of human primary fibroblasts and the nematode *Caenorhabditis elegans*, we were able to induce proteasome activation either through genetic means (overexpression of the β5/*pbs-5* proteasome subunit)[1,2] or through natural or bio-inspired chemical compounds[3-4,unpublished results]. Proteasome activation promoted lifespan extension at both cellular and organismal level while this extension was also accompanied by healthspan improvement. The latter has attracted increasing interest over the last few years since the importance of maintaining the well-being for longer, thus reducing frailty, has been widely recognized. With regard to the progression of the Alzheimer's disease (AD) phenotype, enhanced proteasome function conferred lower paralysis rate in various AD nematode models accompanied by decreased Aβ deposits, thus ultimately decelerating the progression of the disease phenotype. More recently, we were able to reveal the cell non-autonomous regulation of proteasome function in *C. elegans* [2], thus pinpointing the importance of tissue-specific proteasome activation. In total, our results demonstrate the pivotal role of the proteasome in the progression of aging and AD. Moreover, they also reveal the importance of identification of compounds acting as proteasome activators that might be used for healthspan maintenance as well as in preventive strategies against aging and proteinopathies, such as AD. In this seminar, the proteasome system will be presented and the animal model *C. elegans* will be discussed as it is a valuable model for drug identification, including toxicity testing that helped us revealing the effects of proteasome activation in aging.

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Abstract 9

Flavonolignans from *Silybum marianum* inhibit viability of model flatworm larvae in vitro via interference with antioxidant and phase II detoxifying enzymes

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Silymarin, the extract from fruits of Milk thistle (*Silybum marianum*), contains several flavonolignans, among them silybin and silychristin are the most abundant. From silybin 2,3-dehydrosilybin was prepared. Zoonotic diseases caused by genus Echinococcus and Taenia are rank among the most neglected parasitic diseases causing high morbidity in humans and animals. Mortality rate is high in untreated cases due to capability of the asexual proliferation and cysts growth. Larval stage, tetrathyridium, of cestode *Mesocestoides vogae* (syn. *M. corti*) has the ability to proliferate asexually and is considered as a good experimental model for pharmacological studies. In this study we investigated the in vitro effects of three natural flavonolignans—silybin (SB), 2,3-dehydrosilybin (DHSB) and silychristin (SCH) on M. vogae larvae at concentrations of 5 and 50 µM under standard cultivation conditions for 24 and 72h. Viability of larvae was significantly reduced at higher compound´s concentrations in order SB<SCH<DHSB corresponding to the changes in metabolic activity (MTT test) and uptake of nutrients (Neutral red uptake). Antioxidant enzymes (superoxid dismutase -SOD, glutathion peroxidase -GPx and phase II detoxifying enzymes (glutathion transferase - GST) represent essential parasite defence system against host´s immunity and drugs, using glutathion as the potent redox regulatory molecule. In comparison with control larvae, concentration- and time-dependent elevation of SOD and its metabolic product H_2O_2 was associated with decrease of GPx and mostly glutathion indicating that all three flavonolignans reduced activity of complex II anaerobic energy generating enzymatic system (quinol fumarate reductase) present in flatworm mitochondria. By contrast, GST activity was significantly reduced only by DHSB at higher concentration. In conclusion, SB, SCH and DHSB showed larvicidal activity on parasites and interfered with antioxidant defence system probably resulting in alleviation of mitochondrial functions. DHSB exhibited the highest larvicidal effect associated with the reduction of both antioxidant and detoxifying systems.

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