Meeting Report

Third Virtual Summer School 3Rs for ONE Science: Alternative Methods: From Complexity to Predictivity

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The international *Third Virtual Summer School*[1] was focused on the application and predictivity of complex methods and approaches in different disciplines of science. The event, held on June 8-9, 2022 and chaired by Francesca Caloni, Università degli Studi di Milano, was attended by young researchers from all over the world.

Francesca Caloni, opened the summer school, emphasizing that new approach methodologies (NAMs) are useful predictive and investigative tools for a multifaceted science with a global perspective.

Helena Kandarova, CEM & FChFT Bratislava, gave a lecture entitled "Everything you ever wanted to know about successful validation of alternative methods but were afraid to ask – The secrets revealed". The goal of any validation trial is to show that a method or model is relevant, reproducible, and predictive, ultimately leading to its regulatory acceptance. In the field of NAMs, it also has the ethical aspect of replacing the use of experimental animals. The presentation summarized critical aspects of the validation process and illustrated some practical examples of prospective and retrospective validation trials. Tips and tricks for designing a successful validation study were provided.

Laura Ceriotti, in collaboration with **Marisa Meloni**, Vitro-Screen, Milan, Italy, gave a lecture entitled "Eye hazard classification: the road to discriminate between UN GHS category 1 and 2". According to the Globally Harmonized System of Classification and Labelling of Chemicals (UN GHS), test chemicals can be classified as inducing serious eye damage (UN GHS Category 1), inducing eye irritation (UN GHS Category 2), and not classified for eye irritation or serious eye damage (UN GHS No Category). Considerable progress has been made towards replacement of the *in vivo* Draize eye test, and several non-animal test methods have been validated. Due to the complexity of the eye responses towards chemical exposure, it is generally accepted that no single *in vitro* test method is sufficient, but a combination of several alternative test methods within integrated approaches to testing and assessment (IATA) may be able to fully replace the Draize eye test. Up to now, such methods or testing strategies allow to directly identify Cat. 1 and/or No Category, while Cat. 2 is indirectly identified through a testing strategy. Recently the SkinEthic™ HCE test method was developed for the evaluation of liquids and solids using a time-to-toxicity approach based on a multiple time exposure. This is the first test method submitted for regulatory acceptance to OECD for discriminating on its own the three UN GHS categories; it was adopted as OECD TG 492B in June 2022. The potential to directly identify eye irritants is important for the cosmetic industry as well as for the pharmaceutical, chemical, and pesticide sectors and will improve and speed up their safety assessment.

Arno C. Gutleb, Environmental Research and Innovation (ERIN) Department, Luxembourg Institute of Science and Technology, presented "Necessary or unnecessary complexity of *in vitro* lung models?" The classical approach for cell culture is that *in vitro* models are based on single cell types cultured submerged in medium. In recent years, complex models using multiple cell types cultured at the air-liquid interphase have added a new dimension of complexity to *in vitro* models. Such complex models need careful characterization of the properties of the cell types, especially when culture conditions change such as in co-culture. The similarities and discrepancies among 3D-*in vitro* models, human tissue and animal models used in the past need to be understood. Overall, models should be as simple as possible and as complex as necessary to mimic physiological responses.

Hassan Rashidi, NIHR Great Ormond Street Hospital Biomedical Research Centre, UCL Great Ormond Street Institute of Child Health, University College London, presented a lecture entitled "Human pluripotent stem cell-derived hepatocyte-like cells as a tool to predict drug-induced liver toxicity". The use of primary human hepatocytes (PHHs) is considered the gold standard *in vitro* model for drug toxicity testing to predict drug-induced liver toxicity (DILI) (Gomez-Lechon et al., 2014). However, their scarcity and transient *ex vivo* phenotype limit their use (Lauschke et al., 2016). A variety of alternative 2-dimensional (2D) models have been developed, but none has been able to predict DILI more accurately. Recent advances in the generation of 3D liver organoids and the development of microfluidic platforms, including liver-on-chip and human-on-chip, have opened new avenues to develop more sophisticated *in vitro* platforms to predict DILI more accurately. Recently developed tools were discussed.

Giulia Ranaldi, Food and Nutrition Research Centre, Council for Agricultural Research and Economics, CREA-AN, Rome gave a presentation entitled "Application of Confocal Laser Scan-

[1] https://amcp.lakecomoschool.org/

ning Microscopy in intestinal *in vitro* studies". Confocal laser scanning microscopy allows viewing the ultrastructural organization of cells using fluorochromes. To explain employment, advantages and limits of confocal microscopy techniques, confocal fluorescent experiments performed on the human intestinal Caco-2 cell line challenged with inflammatory stimuli were shown.

The lecture presented by **Doris Wilflingseder**, Medical University of Innsbruck, was entitled "*In vitro* disease models – from complexity to therapy". Emerging infectious diseases, such as COVID-19, or resistant pathogens indicate the need to speed up research on repurposing already approved drugs or testing novel innovative compounds. Since effective drugs or vaccines must induce both humoral and cellular responses against pathogenic challenges, novel alternative human approaches are needed, and improved methods for delivery must be tested. Rapid developments in high-content screening as well as organotypic cultures provide groundbreaking new tools to study pathogen transfer at entry sites or to test novel vaccination strategies or unconventional drugs. Optimized intelligent human barrier models can be combined with infection-relevant immune cells and humoral components to characterize and hinder overshooting host responses, pathogen entry, and initial transmission steps within a 3D system. These human systems offer improved power to test delivery methods, adjuvants, repurposing of drugs or novel vaccination approaches in high throughput.

Leonora Buzanska, Department of Stem Cell Bioengineering, Mossakowski Medical Research Institute Polish Academy of Sciences, Warsaw, Poland, presented "Therapeutic and *in vitro* testing potential of brain organoids". The ability of pluripotent stem cells to self-organize under 3D *in vitro* culture conditions into highly structured tissue patterns opened the era of "brain organoids". This technological advancement, based on human induced pluripotent stem cells (hiPSC) obtained by reprogramming of somatic cells, enables researchers to model human neurodevelopment and neuropathology *in vitro*. While the brain organoid system can model early neurodevelopment and its pathology well, it has anatomical and functional limitations to study later developmental stages due to the lack of correct neuronal network connectivity and vascularization. Much work has now addressed these limitations by developing new protocols to generate replicas of multiple brain regions incorporating vascular-like structures and microglia (Cakir and Park, 2022) or by regulatory control of the system through bioengineering approaches such as gene editing and optogenetic technology as well as by upgrading the physiological relevance of the system with microfluidic "organ-on-chip" devices. Further developments include new technologies for unbiased, integrated organoid analysis and functional readouts based on automated high-throughput platforms. Such developments will maximize the benefit of organoid systems and their applications beyond basic research especially for drug testing and toxicological purposes (Renner et al., 2021).

After the presentations, the participants were invited to express their opinions and thoughts on the topics of the summer school via a questionnaire.

References

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Acknowledegments

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