3Rs for ONE Science. Alternative Methods: from complexity to predictivity

Lake Como School of Advanced Studies, June 8-9, 2022

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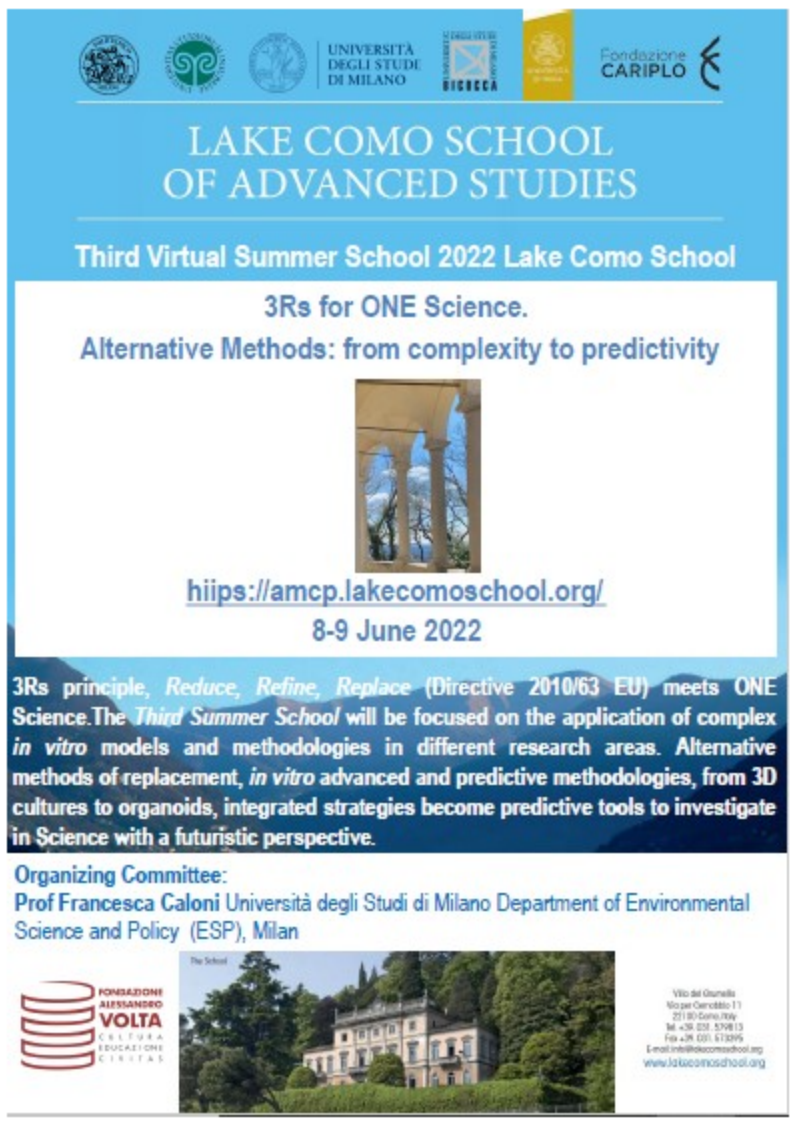

3Rs principle, Reduce, Refine, Replace (Directive 2010 63 EU) meets ONE Science

The Third Summer School will be focused on the application of complex in vitro models and methodologies in different research areas.

Alternative methods of replacement, *in vitro* advanced and predictive methodologies, from 3D cultures to organoids, integrated strategies become predictive tools to investigate in Science with a futuristic perspective.

3Rs for ONE Science. Alternative Methods: from complexity to predictivity

Lake Como School of Advanced Studies, June 8-9, 2022

Organizing Committee

Prof Francesca Caloni
Università degli Studi di Milano Department of Environmental Science and Policy (ESP), Milan

Speakers

Leonora BUZANSKA

Present position:

2019 – present: Professor, Director of Mossakowski Medical Research Institute, Polish Academy of Sciences (MMRI PAS), 5 Pawinskiego Str, 02-106 Warsaw, Poland, buzanska@imdik.pan.pl

2015 – present: Professor, Head of Department of Stem Cell Bioengineering, Mossakowski Medical Research Centre (MMRI PAS).

Previous Employment:

2010 – 2014 associate professor, Head of Stem Cell Bioengineering Laboratory in NeuroRepair Dept., MMRC PAS

2005 – 2009 senior research scientist in Institute of Health and Consumer Protection, Joint Research Center, the Research Directorate General of the European Commission., Ispra, Italy

2007 – 2009 associate professor, NeuroRepair Dept, MMRC PAS

1996 – 2007 assistance professor, NeuroRepair Dept, MMRC PAS

1981 – 1996 assistant, assistance professor, Warsaw University, Department of Biology

Degrees and Titles

2014 – Professor of Medical Sciences, nomination by the President of the Republic of Poland

2007 – D.Sc. (habilitation) in medical sciences, MMRC, PAS, Warsaw, Poland

1990 – Ph.D. in cell and molecular biology, Department of Biology, Warsaw University

1981 – M.Sc. in biology, Dept Biol, Warsaw University, Poland

Professional Memberships

Committee of Neurobiology, PAS; Executive Board of MMRI PAS; Inter Soc for Stem Cell Res (ISSCR), Int Soc Fed of Europ Neurosci (FENS), Tissue Engineering and Regenerative Medicine International Society (TERMIS), Europ Soc of Tox In Vitro (ESTIV – Executive Board), Polish Neurosci Soc, Polish Society for Cell Biol (Executive Board), Polish Biochem Soc, Warsaw Sci Soc (Executive Board),

Scientific Interests and Expertise

Developmental neurobiology, developmental neurotoxicity, human neural stem cells, brain organoids.

Download the abstract (PDF file): BUZANSKA

Francesca CALONI (School Director)

Full Professor, Department of Environmental Science and Policy (ESP), Università degli Studi di Milano

2005-2011 Board member of the Italian Association of in vitro Toxicology, Celltox

2008-2016 Board Member of the European Society of in vitro Toxicology, ESTIV

2009-Appointment as EFSA expert in the FEEDAP Panel on Feed Additives and Contaminants (from 06/2009 to 01/2010)

2012-present Associate Editor of Frontiers in Pharmacology, Frontiers in Predictive Toxicology

2012- Expert in Coordinating Group of the Center for Alternative Methods, Laboratory Animal Welfare and Care

2013-present Board Member of IPAM, Italian Platform on Alternative Methods

2013-present Member of the 'Advisory Board of the Center for Alternatives to Animal Testing-EU (CAAT-EU)

2015-2018 President of the Italian Association of in Vitro Toxicology, Celltox

2016-2017 Member of the Steering Committee for the European Platform for Laboratory Animals Science

2016-present Associate Editor of Frontiers in Veterinary Science, Open access

2015-2021_Member of the Ethical Commitee University of Milan

2018-present Past President of the Italian Association of in Vitro Toxicology, Celltox

Pubblications: https://www.scopus.com/authid/detail.uri?authorId=6603565370

Arno Christian GUTLEB, Distinguished Professor, PhD, ERT

Dr. Arno Gutleb is Group Leader Environmental Health at the Luxembourg Institute of Science and Technology (LIST). The group is developing in vitro assays with a focus on the alveolar region of the lung and inflammation and respiratory sensitization as the main endpoints.

He graduated from the University of Veterinary Medicine, Vienna, Austria and holds a PhD (2006) in Environmental Sciences from Wageningen University, The Netherlands. He has a habilitation from the University Lorraine, France (2018) and is a European Registered Toxicologist (ERT) (since 2002).
He is Distinguished Professor at the University Iuliu Hatieganu, Cluj, Romania and Visiting Professor at the Universidad Andrés Bello, Santiago de Chile. He is a member of the Network for Preliminary Assessment of Regulatory Relevance (PARERE) of the European Union Reference Laboratory for Alternatives to Animal Testing (ECVAM). Since 2020 he is Co-chair of the US-EU NanoEHS CoR.

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Helena KANDAROVA

Dr Helena Kandarova, ERT, is a Senior Scientist at the Institute of Pharmacology and Toxicology, Centre of Experimental Medicine, Bratislava. She is also a Lecturer at the Institute of Biochemistry and Microbiology at the Faculty of Chemical and Food Technology (FCHPT) in Bratislava.

From 2007 – 2018, Dr Kandarova held the position of Senior Scientist and General Acting Manager for EU at MatTek Corporation, USA. She established and built up MatTek In Vitro Life Science Laboratories in Europe and led the company in the position of Executive Director between 2009 – 2018. Dr Kandarova has been a board member in several toxicology-oriented organisations (ESTIV, ASCCT, SETOX, EUROTOX – communication subcommittee) and a member of the international expert pannels (as e.g. EPAA-MG, OECD expert groups). She is a founding member and chair of the Slovak National Platform for 3Rs. In 2020 she became president of the European Society for Toxicology in Vitro.

Dr Kandarova has been involved in many international projects aiming to validate 3D reconstructed human tissue models for topical toxicity and phototoxicity testing of chemicals, cosmetics, pesticides, and medical devices. She is a co-author for several internationally implemented protocols adopted into the OECD and ISO 10993 guidelines.

She published 60 papers, 9 book chapters (H-index 20, over 1200 citations) and obtained several scientific awards related to the research aiming into the development and validation of alternative methods, amongst which the most prestigious is the 2021 Doerenkamp-Zbinden Foundation Award.

More at www.helenakandarova.com

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Laura CERIOTTI

Laura Ceriotti got the degree in Biological Science at the University of Insubria (Varese, Italy) in 1998. From 1998 to she 2003 worked at the Institute of Microtechnology of the University of Neuchâtel in Switzerland where she obtained the title of PhD in Science with a thesis on the development of integrated microsystems for the analysis of proteins and nucleic acids. From 2004 to 2008 she worked at the Nanotechnology Unit of the Institute for Health and Consumer Protection of the JRC in Ispra (Italy). Here, she was involved, in collaboration with ECVAM, in the development and application of real-time impedance-based methods for toxicity studies and functionalization of surfaces for cell patterning.

Between 2012-2014 she attended the Master in Regulatory Affairs at the University of Pavia, with a final thesis on the impact of alternative methods in the regulatory framework of different industrial sectors (cosmetic, pharmaceutical, medical devices, biocides, agrochemicals, food). Since 2013 she collaborates for regulatory and scientific aspects with VitroScreen, a CRO dealing with in vitro preclinical regulatory and research studies. Since 2015 she covers at VitroScreen the role of Regulatory Specialist and Archivist and support Dr Meloni in the promotion of in vitro methods in the preclinical assessment of medicines, MD, cosmetics, and food components and dissemination of alternative approaches participating to Summer Schools, Seminars and Workshops organized by the University of Milan, associations like AFI, Celltox and SITOX.

She is author and coauthor of more than 20 international peer reviewed papers and she has participated to international and European conferences.

Questions

- The knowledge of the skin sensitization Adverse Outcome Pathway (AOP) has prompted the development and better organization of non animal methods (*in silico*, *in chemico*, *in vitro*) addressing specific KEs. The first initiating event (KE1) of the skin sensitization AOP is:

- Chemical penetration into the skin
- Keratinocytes activation
- **Covalent binding to skin proteins**
- Activation and proliferation of specific T-cells

- The human Cell line Activation test (h-CLAT) can be used to address the activation of dendritic cells (KE3). In this assay

- the expression of cell surface markers (i.e. CD86 and CD54) in the human monocytic leukaemia cell line THP-1 is quantified by qRT-PCR
- **the expression of cell surface markers (i.e. CD86 and CD54) in the human monocytic leukaemia cell line THP-1 is quantified by flow cytometry**
- test chemicals with Log Kow greater than 3.5 tend to produce false positive results
- the results can be used alone to assess sensitizing potency

- The Defined Approaches currently described in OECD guideline 497 can be used for hazard identification to discriminate between skin sensitisers and non-sensitisers and for potency sub-categorisation. They include:
- Only *in vitro* methods
- Only *in silico* predictions
- ***In vitro*, *in silico* and *in chemico* methods**
- *In vivo* and *in vitro* methods
- The limitation of DAs derives from the limitations of the individual non animal methods data. The predictions obtained from DAs
- are always conclusive and can be used on their own to provide hazard and/or potency prediction
- if inconclusive, can be used only for potency sub-categorization
- require an expert judgment to conclude on skin sensitization potential
- **can be either conclusive or inconclusive predictions**
- According to ECHA (European Chemical Agency), the OECD guideline 497 can be used to meet REACH information requirements. Thus, under REACH

- **all available information on the chemical including existing *in vitro*, *in vivo*, historical human data, data from valid (Q)SARs and data from structurally related substances (read-across approach) should be evaluated before conducting any testing on skin sensitization**
- a corrosivity and irritation test should be performed before conducting any testing on skin sensitization
- skin sensitization potential of chemicals should be assessed first *in vivo* and then using *in vitro* methods
- skin sensitization potential of chemicals should be assessed *in vivo* when *in vitro* methods give negative results

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Marisa MELONI

During 15 years Marisa Meloni, PharmaD and PhD in Drug delivery and Cutaneous Biophysic from the University Renée Descartes Paris V, has been in charge for the research and development of *in vitro* strategies and the implemenation of Alternatives to animal testing within the pharmaceutical and cosmetic industries.

She has been for more than 15 years contract professor for formulation technology and safety assessment of topical products at Milano, Salerno and Padova Universities.

In 2001, she founded VitroScreen, a CRO concentrated on *in vitro* technologies with the mission to introduce high quality, relevant and predictive *in vitro* models in life sciences for regulatory, pre-clinical and R&D needs.

At VitroScreen she has developped "mechanism based experimental models" for active and finished product testing, that focus on the identification of the most relevant biological models and predictive signatures for a wide type of skin targets. This approach better mimics the *in vivo* skin response because it takes into account the dynamic of the biological response.

In the last years, VitroScreen provides customized organotypic scaffold free microtissue models and colonized 3D tissue models to offer new predictive and relevant biological models for pre-clinical and mechanicistic studies.

EXPERIENCES

- **1981 -1985:** CNR (Centro Nazionale Ricerche) contract for research on discovery new botanical anti-inflammatory actives (Sassari University)(I)
- **1986-1990:** Lecturer on Pharmacie galénique at Dep. Pharmacie galénique (Prof.MC Poelman and Chaumeil), Paris V (F)
- **1986-1990**: Teacher at ISIPCA (Versailles –F)
  •**1992-1993:** R & D manager Laboratori GUIEU (Paris-Milan)
- **1993- 2001:** R&D Manager at Diana de Silva cosmétiques SpA and Responsible of the In vitro testing Laboratory
- **1994-2001**: Contract Professor of Cosmetic Science and Technology at Milan and Salerno Universities
- **2002-2006**: Contract Professor of Safety assessment at Padova University
  •**Present position**: CEO and GLP Testing Facilities Director at VitroScreen s.r.l

MEMBERSHIP

- Member of the UNIPRO technical committee (responsible for WGs product's evaluation) (1993-2013)
- Member of the COLIPA Project Team Sun Issues (since 1994-2013)
- Partecipation of the ECVAM expert meeting for Eye irritation (2005)
- EU Commission – DG ENTREPRISE, team member for the guidelines on "Safety evaluation of finished cosmetic products considering a ban on animal testing" (1999-2000)
- Member of IPAM and CELLTOX (President 2011-2014)
- Member of PARERE (Network for Preliminary Analysis of Regulatory Relevance) as Italian expert and coordinator of the Cosmetic sector (since July 2016)

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Giulia RANALDI

Giulia Ranaldi graduated in Biological Sciences at the University La Sapienza of Rome and obtained her PhD in Experimental, Environmental and Occupational Toxicology at the University of Messina. She is a Researcher at the Food and Nutrition Research Center of the Italian Council for Agricultural Research and Economics (CREA-AN) in Rome, Italy. Her research activity is mainly focused on the development of in vitro models for intestinal absorption and toxicity studies and on the characterization of specific mechanisms of trans-epithelial passage of nutrient, bioactives and xenobiotic. Her work is also aimed to the comprehension of the mechanisms involved in the potential protective effects of food bioactives on intestinal mucosa.

Download the abstract (PDF file): RANALDI

Hassan RASHIDI

UCL Great Ormond Street Institute of Child Health, University College London, London, UK

"Human pluripotent stem cell-derived hepatocyte-like cells as a tool to predict drug-induced liver toxicity"

The adverse drug reaction (ADR) is the main reason for the high attrition rate of promising drug candidates in the post-marketing stages [1-2]. Therefore, animal safety tests have been used to reduce the attrition rate. However, they are often poor predictors for human toxicity events due to interspecies differences in liver-specific functions [3].

The use of primary human hepatocytes (PHHs) is considered the gold standard *in vitro* model for drug toxicity testing [4]. However, their scarcity and transient *ex vivo* phenotype have limited their application in pre-clinical drug development studies [5]. Consequently, a variety of alternative 2-dimensional (2D)-models have been developed, including platforms based on hepatoma cell lines [6,7] and human pluripotent stem cell (hPSC)-derived hepatocyte-like cells [8]. Although these models have allowed for the *in vitro* analysis of several mechanisms of drug-induced liver injury (DILI), such as oxidative and endoplasmic reticulum stress and mitochondrial toxicity, 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. Here, a number of recently developed tools will be discussed.

- Waring, M.J., et al., Nat Rev Drug Discov, 2015. 14(7): p. 475-86.
- Lauschke, V.M., L. Milani, and M. Ingelman-Sundberg, AAPS J, 2017. 20(1): p. 4.
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- Gomez-Lechon, M.J., et al., Expert Opin Drug Metab Toxicol, 2014. 10(11): p. 1553- 68.
- Lauschke, V.M., et al., Hepatology, 2016. 64(5): p. 1743-1756.
- O'Brien, P.J., et al., Arch Toxicol, 2006. 80(9): p. 580-604.
- Tolosa, L., et al., Toxicol Sci, 2012. 127(1): p. 187-98.
- Sirenko, O., et al., Assay Drug Dev Technol, 2014. 12(1): p. 43-54.

Download the abstract (PDF file): Hassan RASHIDI

Doris WILFLINGSEDER

studied zoology at the Leopold-Franzens-University in Innsbruck, Austria. During her thesis at the Division of Physiology, Medical University Innsbruck, and her first postdoc years at the Division of Hygiene and Medical Microbiology, Medical University Innsbruck, she focused on cell biology and immunology and worked on signal mechanisms in human primary cell models upon stimulation with fungal or viral pathogens. She continued these analyses using dendritic cells (DC) and differentially opsonized HIV-1 during her stay in collaboration with Paul Kellam at the Division of Infection and Immunity, University College London. Her research group at the Institute of Hygiene and Medical Microbiology of the Medical University Innsbruck is interested in modulation of immune cell function by pathogens in relevant human 3D cell culture microenvironments containing epithelial borders. To address these issues they use molecular biologic, imaging and immunologic approaches and primary cell models – where possible – or appropriate cell lines for e.g. gene editing approaches.

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Program

8 June 2022

9:30 Registration of Participants

10:30 **3Rs for ONE Science. Alternative Methods: from complexity to predictivity**

Francesca Caloni- Università degli Studi di Milano Department of Environmental Science and Policy, Milan

11:30 **Everything you ever wanted to know about successful validation but were afraid to ask – The secrets revealed**

Helena Kandarova – Institute of Biochemistry and Microbiology Faculty of Chemical and Food Engineering, Slovak Technical University / Centre of Experimental Medicine, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences

12:30-14:00 Lunch

14:00 **Eye hazard classification: the road to discriminate between UN GHS category 1 and 2**

Marisa Meloni, *Laura Ceriotti*– Vitroscreen, Milan_

15:00 **Necessary or unnecessary complexity of *in vitro* lung models?**

Arno Gutleb – Luxembourg Institute of Science and Technology (LIST), Luxembourg

18:00 Virtual Welcome Reception

9 June 2022

9:30 **Human pluripotent stem cell-derived hepatocyte-like cells as a tool to predict drug-induced hepatotoxicity.**

Hassan Rashidi – Great Ormond Street Institute of Child Health, University College London, London

10:30 **Application of Confocal Laser Scanning Microscopy in intestinal in vitro studies.**

Giulia Ranaldi – Food and Nutrition Research Centre – Council for Agricultural Research and Economics,CREA-AN, Rome

11:30 ***In vitro* disease models – from complexity to therapy** *Doris Wilflingseder* – Institute for Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck

12:30-14:00 Lunch

14:00 Therapeutic and in vitro testing potential of brain organoids

Leonora Buzanska – Mossakowski Medical Research Institute, Polish Academy of Sciences, Department of Stem Cell Bioengineering, Warsaw

15:00 Open Debate with the Participants

16:00 Conclusions