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## Meeting Report

# Giving Meaning to Alternative Methods to Animal Testing

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The 3<sup>rd</sup> edition of the advanced theoretical-training course “Giving meaning to alternative methods to animal testing” was held in Genoa on July 6-7, 2017. This course, chaired by Prof. Anna Maria Bassi (LARF-DIMES, University of Genoa, Italy) with the valuable support of the LARF Research Team, provided an update on new *in vitro* approaches. The welcome addresses were given by Prof. Marco Frascio, Prof. Adriana Voci, as Coordinators of Medicine and Surgery, and Biological Sciences degrees, respectively, of the University of Genoa, Italy, and by Dr Giuliano Grignaschi, head of the Animal Care Unit – Mario Negri Institute, Milano, Italy. In their welcome speech, all stressed the relevance of new *in vitro* technologies to improve the application of *in vitro* methods in toxicological research. The theoretical modules included talks by specialists from companies engaged in the field of advanced *in vitro* technologies, who offered participants the possibility to try out their technologies in the training modules.

The ethical issues associated with animal-based research were the topic of contributions by Prof. **Rosagemma Ciliberti**, **Ilaria Baldelli** (DISSAL, University of Genoa, Italy) and

**Susanna Penco** (LARF-DIMES, University of Genoa, Italy). Their joint input focused on clinical evidence of the failure of animal-based models to predict effectiveness and safety of chemical agents in drug research. Dr **Eleanore Irvine** (Biogelx Inc., Scotland, UK) introduced new 2D/3D culture methods using self-assembling hydrogels made from di- or tri-peptide components that can be used as coating for 2D cultures and for embedding cells in 3D models. The stiffness of the gel can be regulated depending on a cell culture’s requirements by changing the ratio of powder to water and the hydrogels can be functionalized with proteins and growth factors. Dr **Silvia Letasiova** (MatTek In Vitro Life Science Laboratories, Bratislava, Slovakia) introduced an *in vitro* alternative to the animal-based Draize Eye Irritation test. EpiOcular™ is a 3D reconstruction of human cornea developed by culturing normal, human-derived epidermal keratinocytes to form a stratified, squamous epithelium, which can be used for ocular irritation assessment of cosmetic, personal care and household products. Dr **Vladimir Mazurov** (acCELLerate GmbH, Hamburg, Germany) presented the acCELLerate *in*

*vitro* services, which include cytotoxicity assay kits containing Frozen Instant Cells (THP-1, HepG2, Caco2, HaCaT, Jurkat, 3T3, etc). The cells are cryopreserved at a highly functional state and are ready-to-use without prior cultivation, passaging or counting. Cell handling, seeding protocols and incubation times are optimized to achieve a robust reproducibility and reliability of bioassays. Dr **Costanza Rovida** (CAAT-Europe) gave an overview from development of alternative methods to EURL-ECVAM validation processes and implementation of regulatory tests. Good Cell Culture Practice is a prerequisite to assure the quality and the traceability of all materials and methods used and to establish and maintain adequate measures to protect individuals and the environment from potential hazards, to provide relevant and adequate education and training for all personnel, and thus to promote high quality work and safety. Dr **Susanna Alloisio** (ETT spa, Genoa, Italy), introduced the “High Throughput Electrophysiology Assay” for functional neurotoxicity prediction. This method is based on the microelectrode array (MEA), a sensitive and high information content system allowing long-term recordings that can be used to evaluate the neurotoxic potential of agrochemicals, chemicals, drugs, “smart” drugs and biotoxins. Dr **Massimo Di Donato** (Tebu-bio, Milan, Italy) offered an overview of the use of human primary cells and models for cosmetic and metabolic assays. He then focused on recombinant CYP preparations like bacosomes, bacterial membranes containing cytochrome P450s co-expressed with NADPH-CYP reductase, which have a higher specific activity than liver microsomes and can be used for the production of high quantities of metabolites as drug candidates. Dr Di Donato also spoke about synthetic matrixes for coating plates, specialized plates that enable cells to auto-regulate oxygen and CO<sub>2</sub> levels in culture media, and a cellular invasion assay. Dr **Paola Miranda** (BioSPA, Milano, Italy) presented a 3-D hydrogel system (Cellends Hydrogel Technology, Germany) based on maleimide-functionalized polymer and a thiol-functionalized crosslinker. These two components can be functionalized for cell recovery with dextran or Cd-link to allow enzymatic matrix degradation. The gelling speed can be controlled by adjusting the pH or by modifying maleimide-functionalized polymer/thiol-functionalized crosslinker concentration. Dr

**Tommaso Sbrana** and Dr **Daniele Cei** (IVTech, Massarosa, Lucca, Italy) introduced the 3D dynamic millifluidic models Live box 1 (single flow bioreactor) and Live box 2 (double flow bioreactor). The bioreactors ensure a balanced, continuous medium flow to cultured cells via a peristaltic pump. Prof. **Anna Maria Bassi** (LARF-DIMES, University of Genoa, Italy), explained the importance of predictive toxicology to protect human health. The use of animal models only is unable to achieve this goal, because animal models often do not mimic human diseases sufficiently (e.g., Alzheimer or autism models). Therefore, the number of laboratories involved in the research and development of non-animal methods is growing. She referred to promising approaches such as stem cells and induced pluripotent stem cells (iPSCs), which can be differentiated into several human cell types, and new bioinformatics and mathematical tools, which can be integrated into multidisciplinary approaches.

In the training modules the experts offered several hands-on experiences of the presented technologies to each participant with the assistance of the LARF team (Chiara Scanarotti, Stefania Vernazza and Sara Tirendi). The evaluation questionnaires revealed satisfaction of all participants, who requested the scheduling of other advanced courses. This positive feedback highlights how the promotion and organization of training activities can promote the interest in and future use of non-animal models.

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