



ESNATS Publishable Summary Year 1



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1. Publishable summary



The aim of the ESNATS project is to develop a novel toxicity test platform based on embryonic stem cells (ESCs), in particular human ESC (hESCs), to streamline the drug development R&D process and evaluation of drug toxicity in clinical studies, reduce related costs and thus to not only increase the safety of patients but also to reduce the number of animals due to earlier detection of adverse effects.

ESNATS addresses current shortcomings in toxicity testing:

- A major part of safety testing takes place late in the research and development cycle, implying protracted experimentation involving high numbers of animals and generating significant costs.
- Some *in vitro* assays rely on cells lines of malignant origin or primary cells that are hard to standardise and limited in terms of quantity, homogeneity and genetic diversity.
- Existing assay systems based on primary animal and human cell lines do not reliably represent the physiological situation of cells in native tissue.

To reach the project goals, a battery of toxicity tests is being developed using ESC lines subjected to standardised culture and differentiation protocols. Tests will cover ESCs in several stages of development as well as differentiated derivatives, including gamete and neuronal lineages, complemented with systems for hepatic metabolism. Genomics approaches will be used to determine predictive toxicoproteomics and –toxicogenomics signatures. The individual tests will be integrated into an "all-in-one" testing strategy. To ensure practical usage in the pharmaceutical industry, concepts for automated ESC culture will be developed and the test systems will be scaled up. In a later stage of the project, the predictivity, quality and reproducibility of the test strategy will be evaluated in a "proof of concept" study.

ESNATS is divided into four main research areas, each one representing a sub-project (SP). These SPs are complemented by central work packages (cWPs) which cover transversal scientific aspects of the project (see Figure 1).

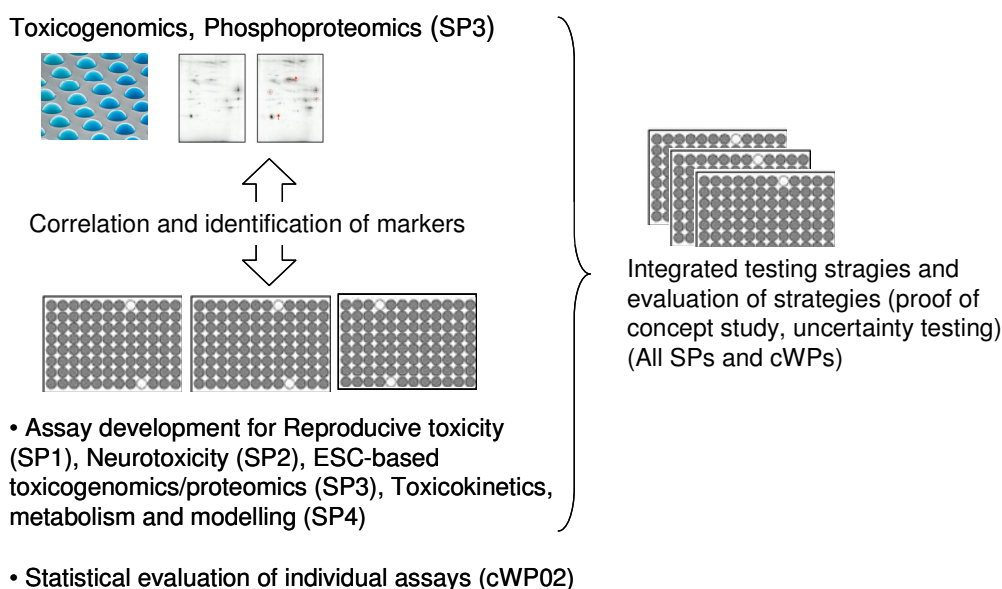


Figure 1 ESNATS main research areas

ESNATS objectives will be achieved in a five-year multidisciplinary collaboration of leading European researchers in alternative testing, toxicology, ESC research, genomics, modelling, and automation. The consortium also includes representatives from regulatory bodies, the pharmaceutical industry and ethical advisors to provide guidance to ensure rapid applicability of the developed test systems.

In the first project year, the ESNATS partners initiated the set up of the *in vitro* toxicology test systems for reproductive toxicity and neurotoxicity. Cell culture systems were developed for the use of embryonic stem cells in reproductive, developmental and neural toxicity testing, including the differentiation of stem cells into a wide range of cells and tissues, such as various neural cell types and engineered neural tissues, for cardiomyogenesis and for mouse ES cell-derived spermatogenesis. SOPs for two- and three-dimensional neural differentiation were established.

Standard methods for RNA sample generation and processing were developed as well as quality control protocols for hESC cultures to ensure reliability and comparability of results. A database for online management of samples and associated data was developed. The first samples for toxicogenomics and –proteomics profiling have been processed and signatures for individual experiments were identified.

Culture conditions of primary mouse and human hepatocytes have been optimised in order to be able to integrate them as metabolising systems into the testing strategies with ESC derived cells. Work has started on PBPK modelling.

A list of test substances was drafted by the sub-projects and a first recommendation of selected test substances was made by the Steering Committee. Industrial and regulatory specifications for assay development were provided.

The user requirements specification (URS) for equipment to automate standard protocols was drafted and passaging methods were identified and evaluated for their applicability to automation.

Training courses were carried out by the ESNATS partners on phosphoproteomics, microarray expression analysis and ethical aspects. A workshop on statistics was organised together with the Predict-IV project.

The ESNATS public website was set up and the ESNATS flyer was edited and distributed at the first public event, organised by UNEW in April 2009.

In the next year, the ESNATS project will finalise the cellular *in vitro* systems including the *in vitro* gametogenesis model systems, the *in vitro* trophoblast model, the *in vitro* teratogenicity model systems (for cardiac and neuronal tissues), the *in vitro* microtissues, the two-dimensional mixed neural cultures and the three-dimensional human engineered neural tissues. Using these test systems, the collection of toxicogenomics and –proteomics signatures will be ramped up. The production of stem-cell derived hepatocyte-like cells will be optimised and the metabolic profile of these cells will be assessed with regard to their possible integration into the ESNATS toxicity tests. The first summer school will be held in 2009. Lectures, courses and fruitful discussions are expected to further enhance collaboration within the project.

ESNATS partners

The ESNATS consortium is composed of the following organisations:

- Universität zu Köln – Universitätsklinikum (UKK)
- Commission of the European Communities – Directorate General Joint Research Centre JRC (JRC)
- University of Newcastle upon Tyne (UNEW)
- Université de Genève (UNIGE)
- Forschungsgesellschaft für Arbeitsphysiologie und Arbeitsschutz e.V. (IFADO)
- European Consensus Platform on 3R Alternatives to Animal Experimentation (ecopa)
- The Automation Partnership (Cambridge) Limited (TAP)
- OÜ Quretec (QURE)
- ProteoSys AG (PSY)
- Université de Liège (Ulg)
- Cellartis AB (Cellartis)
- Cell Cure Neurosciences Ltd. (CELL CURE)
- Universität Konstanz (UKN)
- National Biological Standards Board (NIBSC)
- Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek – TNO (TNO)
- The University of Edinburgh (UEDIN)

- Vrije Universiteit Brussel (VUB)
- Technische Universität München Klinikum Rechts der Isar (TUM)
- ARTTIC (ARTTIC)
- The School of Pharmacy, University of London (ULSOP)
- N.V. Organon (Org)
- Läkemedelsverket / Medical Products Agency (MPA)
- H. Lundbeck A/S (Lundbeck)
- In Vitro Testing Industrial Platform (IVTIP)
- Bundesinstitut für Risikobewertung (BfR)
- Edinethics Ltd (Edinethics)
- Gottfried Wilhelm Leibniz Universität Hannover (LUH)
- F. Hoffmann-La Roche, Ltd. (Roche)
- Avantea srl (Avantea)

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