



Minutes of the NESCI ESNATS CONFERENCE ON STEM CELL-BASED TOXICOLOGY AND DRUG SCREENING held on 22/04/2009 in Newcastle, England

DATE: 05/06/2009
AUTHOR: S. Snykers, VUB

1. Meeting references

Venue: NorthEast England Stem Cell Institute (NESCI), University of Newcastle
International Centre for Life
Bioscience Centre
Newcastle upon Tyne
NE1 4EP

Participants: See attendance list in annex to these minutes

Agenda: The agenda is listed below!

2. Abstract

The pharmaceutical industry spends over £3.4 billion per year on research and development (R&D) of new medicines. A key objective for the industry is a reduction in inefficiencies and high cost associated with taking compounds through to late stage development – many drugs fail due to an unacceptable safety profile. Availability of validated human cell-based in vitro toxicity screens may facilitate earlier attrition of compounds with unacceptable safety profiles, and therefore, also reduce the use of animals. Whilst the potential of in vitro toxicity tests using cell lines is promising, practical results are most unsatisfactory. Past attempts have involved human primary cell lines and ESC of mice. The main problem is that their predictive value is very low. In contrast, the exceptional value of hESC is well recognised. They offer the possibility to standardise and compare toxicological results across different tissue specific cell types developed from the same undifferentiated hESCs and the

possibility to analyse the in vitro development from undifferentiated to differentiated cells and evaluating the corresponding toxicological issues. The stem cells are an important new tool for developing unique, in vitro model systems to test drugs and chemicals and a potential to predict or anticipate toxicity in humans. This conference provides an overview of the applications and recent advances of embryonic stem cell technology in the area of toxicology.

3. Agenda

- 9.00 Registration and posters
- 9.30 **Introduction to Conference**, (*Prof. Karim Nayernia*)
Welcoming Speech , (*Prof. Chris Day, Pro-Vice-Chancellor, Faculty of Medical Sciences*)
- 9.45 **Introduction to NESCI**, (*Prof. Michael Whitaker, NESCI Co-director*)
- 10.00 **Introduction to ESNATS**, (*Prof. Juergen Hescheler, ESNATS Coordinator*)
- 10.15 **Challenges and opportunities in the use of stem cells in early drug discovery**
(*Dr. Philip Wright, Chief Executive SC4SM*)
- 10.50 **Application of embryonic stem cell model for drug discovery and development**
(*Prof. Agapios Sachinidis, University of Cologne*)
- 11.25 **Automation in stem cell-based Toxicology**
(*Dr. Rosemary Drake, The Automation Partnership*)
- 12.00 **Stem Cells in Embryo Toxicology**
(*Dr. Paul DeSousa, University of Edinburgh*)
- 12.35 Lunch Break
- 13.30 **Stem Cells in Female Reproductive Toxicology**
(*Dr Giovanni Lazzari, Laboratorio di Tecnologie della Riproduzione*)
- 14.05 **Stem Cells in Male Reproductive Toxicology**
(*Prof, Karim Nayernia, University of Newcastle*)
- 14.40 **Industrial Applications of human Embryonic stem cells in toxicology**
(*Dr. Raimund Strehl, Cellartis*)
- 15.15 **Stem Cell-based Model of Neurotoxicity**
(*Prof. Karl-Heinz Krause, Geneva University Hospital*)
- 16.525 **Human Neural Stem Cell Approaches in Neurotoxicology**
(*Dr. Chris Morris, University of Newcastle*)
- 17.00 **Stem Cells in Toxicology Testing: The legal & Regulatory Landscape**
(*Dr. Sebastien Sethe, NESCI*)

4. Minutes



Prof. Karim Nayernia, partner of the ESNATS consortium and organiser of the NESCI-ESNATS conference opened the scientific meeting. The welcoming speech was given by *Prof. Chris Day*, Pro-Vice-Chancellor of the Faculty of Medical Sciences at the NESCI, followed by a short introduction to NESCI by *Prof. Michael Whitaker*, NESCI Co-director. Prof. Whitaker underlined that the **NorthEast England Stem Cell Institute (NESCI)** is a collaboration between Durham and Newcastle Universities, the Newcastle Hospitals NHS Foundation Trust and other partners, including the Centre for Life in Newcastle. It particularly combines research on embryonic stem cells with research into somatic and umbilical cord blood stem cells. One of their primary aims is the development of new stem cell treatments and providing research tools for drug discovery. To do this, NESCI has a broad range of collaborations with academic, clinical and commercial institutes.



The coordinator of the European Community's Seventh Framework Programme ESNATS¹ *Prof. Jürgen Hescheler* (Institute of Neurophysiology, University of Cologne) next introduced the consortium. **ESNATS** stands for **Embryonic Stem cell-based Novel Alternative Testing Strategies** (official website: <http://www.esnats.eu/>) and brings together leading European researchers in alternative testing, toxicology, embryonic stem cell research, genomics, modelling, and automation. The consortium also includes representatives from regulatory bodies, the pharmaceutical industry and ethical advisors to provide guidance and to ensure rapid applicability of the developed test systems.

ESNATS aims at developing a novel toxicity test platform based on embryonic stem cells (ESCs), in particular human ESC (hESCs) to accelerate drug development, to increase patient safety, reduce related R&D costs and propose a powerful alternative to animal tests (3Rs principle). To reach these goals, ESNATS will develop a battery of toxicity tests using hESC lines subjected to standardised culture and differentiation protocols. The different tests cover reproductive toxicity, neurotoxicity, metabolism and toxicokinetics, and will finally be integrated into an "all-in-one" test system. Culture of the required hESC lines will be automated and scaled-up to enable future industrial use of the developed toxicity tests.

Prof. Jürgen Hescheler stressed that the success of ESNATS highly depends on a good cooperation between the different consortium members.



Stem Cells for Safer Medicines The actual scientific programme was opened by *Dr. Philip Wright*, Chief Executive of SC4SM (Stem Cells for Safer Medicines; <http://www.sc4sm.org/>). This public-private organisation aims at the creation a bank of stem cells, open protocols and standardised systems in stem cell technology that will enable consistent differentiation of stem cells into stable homogenous populations of particular cell types, with physiologically relevant phenotypes suitable for

¹ The ESNATS project has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° HEALTH-F5-2008-201619.

toxicology testing in high throughput platforms. In his talk, Dr. Wright focused on the '**Challenges and opportunities in the use of stem cells in early drug discovery**', and particularly as a tool to reduce the attrition of drugs during clinical trials'. In fact the failure of potential drug candidates due to safety reasons remains stubbornly high despite an array of new technologies applied in early predictive toxicology and improved knowledge on the relevance of animal models in pre-clinical research. A 10% reduction in attrition, however, could lead to substantive reduction in the average cost of drug development for new medicines reaching the market by around 7%. ABPI members had previously identified the key reasons for such failure and the objective of the research programme is to facilitate the development of priority screens with real utility, focusing on hepatotoxicity and cardiotoxicity. The key challenges are: validity of differentiated cells; reproducibility; scalability; predictability. Prof Wright emphasized that the success depends upon the ability to integrate diverse platform technologies and the navigation of a complex intellectual property environment. For example, the development of a bank of compounds with known relevant toxicity and creation of a bank of stem cells that represent key genotypes involved in drug metabolism could provide real added value and help facilitate a reduction in animal use and improve the productivity of drug development.



A nice example of the actual use of **ESC as a model for drug discovery and development** was outlined by *Prof. Agapios Sachinidis*, member of the ESNATS consortium and Professor at the Institute of Neurophysiology, University of Cologne (<http://www.uni-koeln.de/med-fak/physiologie/np/sachinidis.htm>). He particularly discussed the use of human ESC as a model to evaluate toxicity of cytarabine and thalidomide. More specifically, increasing concentrations of these compounds resulted in increased cytotoxicity, with cytarabine being more toxic than thalidomide (IC₁₀ of cytarabine = 1nM; IC₁₀ of thalidomide is 10µM). Most differences in transcripts, assessed by means of microarray analysis, were observed at the level of nervous system developmental genes with respect to hES exposure to cytarabine; In contrast, hES exposure to thalidomide unravelled differences in genes involved in the developmental and metabolic processes. In particular, several mesodermal and endodermal genes were downregulated. Prof Sachinidis concluded that these first trials confirm the successful contribution of ESC models in drug development.



A critical aspect of the industrial use of **hES derived toxicity tests** is **automation**. *Dr. Rosemary Drake*, ESNATS member and Chief Scientific Officer of the Automation Partnership (Cambridge) Limited (TAP, www.automationpartnership.com) elaborated on this topic. The Automation Partnership (TAP) is a major provider of automated equipment for the pharmaceutical and biotechnology industries. This organisation is particularly specialised in supplying solutions that industrialise complex laboratory processes, such as those involved in genomics, drug discovery and high throughput biology. Their automated mammalian cell culture systems are used for therapeutic protein production, to provide cells for R&D and high throughput screening, and for cell-based regenerative medicine therapies, including autologous therapies. Dr. Rosemary Drake underlined the industrial need for high-throughput procedures. To date, most stem cell technologies, including strategies to control and manipulate the cellular microenvironment of undifferentiated stem cells and their differentiated progeny, are optimised on laboratory scale. To be relevant to the

pharmaceutical industry, miniaturisation and scaling up towards industrial needs are obligatory. Therefore, within the ESNATS project, TAP will contribute to (i) the development of methods for automating stem cell culture and (ii) the scale up of stem cell production and cell banking. TAP will bring expertise in: process analysis, process optimisation for scale up, automation of mammalian cell culture, design and development of automated equipment (hardware and software) for aseptic processing, automated liquid handling.



The morning session was closed by *Prof. Paul De Sousa*, member of the ESNATS consortium, leader of the Roslin Institute group and Senior Research Fellow at the University of Edinburgh (UEDIN; www.scrm.ed.ac.uk). He focused on the use of **Human Embryonic Stem cells (hESCs) in Embryo Toxicology**, specifically hESC derived trophoblast as an alternative to the current use of mouse embryos to assess toxic effects on reproduction at the time of implantation. It has now well established by a number of groups including Prof. De Sousa's that hESC can be stimulated to differentiate into trophoblast simply by addition of specific growth factors to culture media. This is simpler than in the case of mouse ESC wherein gene expression must be artificially modified. hESC derived trophoblast express a number of definitive factors which define these cells including lineage defining transcription factors (i.e. Cdx2) and hormones (human chorionic gonadotropin- β). The latter is secreted to quantifiable levels which can be readily monitored in culture media as a toxicological assay. When grown in suspension under standardised conditions, hESC derived trophoblast also form hollow vesicles whose diameter can provide a quantifiable measure of trophoblast cellular function. Taken together, these assays would permit the kind of throughput necessary for toxicological screening purposes.



Dr Giovanna Lazzari, member of the ESNATS consortium and senior research scientist at Avantea and the Laboratorio di Tecnologie della Riproduzione (<http://www.avantea.it/index.php?lang=en>) mainly discussed the **use of embryonic stem cells in female reproductive toxicology**. As project leader at Avantea, she is involved in ReProTect (LSHB-CT-2004-503257, www.reprotect.eu), an Integrated Project of the EU (IP) funded within the 6th Framework Programme and aimed at the development of alternative tests in reproductive and developmental toxicology. The main goal of this EU project is to pre-validate and validate the most promising available *in vitro* models based on gametes, embryos and embryonic stem cells for reproductive and developmental toxicity testing. It has been estimated that the detection of reproductive/developmental toxicants under the Registration, Evaluation and Authorisation of CHemicals (REACH) will have the highest impact on the animal use for regulatory safety testing. An average testing of 2893 chemicals for reproductive and 2135 chemicals for developmental toxicity testing has been estimated. By following the guidelines for regulatory safety testing, millions of animals will be required only for the detection of reproductive/developmental toxicants. A promising *in vitro* model to screen for developmental toxicity is the Embryonic Stem Cell Test (EST) model. Yet, current experience with the EST indicates that the prediction model require further study before implementation of EST in testing strategies can be considered. Recently, the Bovine Oocytes model has been shown to be useful to study processes of oocyte maturation and oocyte fertilization. More specifically, evaluation of the results of 15 tested chemicals, on the basis of the mechanism of action of the chemicals and of the specific biological events taking place during oocyte maturation and

fertilization, demonstrates the suitability of this model for detecting relevant toxicological mechanisms. Dr. Lazzari underlined that these tests may represent reliable building blocks for the construction of an in vitro testing strategy for reproductive toxicology (Lazzari et al., 2008 Toxicology and Applied Pharmacology 233, 360-370).



With the next talk, we remained in the field of reproductive toxicology. More specifically, *Prof. Karim Nayernia*, ESNATS member and organiser of the NESCI-ESNATS conference (NESCI, The University of Newcastle; <http://www.ncl.ac.uk/ihg/staff/profile/karim.nayernia>) discussed **the use of stem cells in male reproductive toxicology**. He particularly focused on the establishment of in vitro gametogenesis systems based on embryonic stem cells for germ cell toxicity screening. Prof. Nayernia has been working with germ cells and germline stem cells for over 18 years. Worldwide, he is the first scientist who was able to produce living animals from embryonic stem cell derived male gametes. To date, scientists have observed several facts indicating the effect of environmental factors on male fertility like: declining semen quality, increasing incidence of hypospadias and cryptorchidism, increasing incidence in testicular cancer in Northern European countries including Estonia and Finland. Prof. Nayernia emphasized that the development of an in vitro spermatogenesis system might thus be a very useful tool for the establishment of novel screening strategies. Particularly, as it could lead to a reduced attrition of promising drug candidates during late stage drug development. His group has established a promoter-based in vitro spermatogenesis system. This system is based on the expression of the fluorescent gene for each construct is driven by a germ cell specific promoter; therefore each marker permits the isolation of cells at a specific stage during ESC differentiation to germ cell lineage. Cells expressing these markers in vivo include undifferentiated spermatogonia cells, meiotic germ cells and postmeiotic (spermatids). Prof. Nayernia stressed that the establishment of ES-derived germ-like cells provides an in-vitro cell-based model that could allow access to mammalian spermatogenesis and an accessible system for toxicological testing.



In a next talk, *Dr. Raimund Strehl*, ESNATS member and Principal scientist and laboratory director of Cellartis (www.cellartis.com; raimund.strehl@cellartis.com) outlined the scope of their company with particular focus on the **Industrial Applications of human Embryonic stem cells in toxicology**. The expertise of Cellartis lays in the applications of hESC technologies, including development, production and provision of hESC based products and technologies for the industry and the research community. This young biotech company (founded in 2001) is specialised in the derivation, culture and in vitro application of hESC and performs R&D towards the scale up of hESC culture and development of a hESC-based early human developmental toxicity assay. *Dr. Raimund Strehl* emphasized that hESC and their differentiated progeny in fact provide unique new opportunities for application in drug discovery and toxicology. For this reason, Cellartis has been developing the basis for the industrial application of hESC. The focus areas are derivation of functional human hepatocytes and cardiomyocytes from hESC, bulk up of cell production to industrial scale as well as applications in hepatotoxicity, cardiotoxicity and developmental toxicity testing. In this context, Cellartis has successfully developed functional hepatocytes, expressing phase I and phase II biotransformation enzymes. In addition, human ESC-derived cardiomyocytes have been produced for research application. Basically, the main goal of Cellartis is to help the industry in the development of better and safer drugs by providing relevant human cell models and assays.



A highly important aspect of the ESNATS project is the delivery of innovative and reliable **neurotoxicity** assays. The Pathology Department of the University of Geneva (UNIGE) is particularly specialised in this field. During the next talk, *Prof. Karl-Heinz Krause*, member of the ESNATS consortium and Professor at UNIGE (Karl-Heinz.Krause@medecine.unige.ch) addressed (i) the development of a highly efficient approach to differentiate hESC into neurons; (ii) the characterisation of the neuronal differentiation to understand basic mechanisms of differentiation and to advance towards cell therapy of central nervous system diseases; (iii) the analysis of the effect of neurotoxic compounds on minibrains (the so-called ENTs or engineered neural tissues), and (iv) the development of innovative neurotoxicity assays. His lab has developed a very simple high-throughput assay to assess neuronal differentiation. More specifically, genetically engineered ESC, based on a dual promoter construct, are cultured in the presence of phenazopyridine. This compound induces/synchronizes the early steps of neuronal differentiation and enhances cell survival, ultimately leading to a homogeneous population of neurons. The neurotoxic effects of compounds, exposed to these neurons, can be read out upon 5 days of culture using a luciferase assay. With these amazing results, Prof. Karl-Heinz Krause confirms the applicability of pluripotent stem cells as a tool for the assessment of neurotoxicity of compounds.



Most *in vitro* neurotoxicology studies rely on the use of stable cell lines derived from neuroectodermal, neuroendocrine or glial tumours of both man and animals, and primary cultures derived usually from embryonic rodent brain. Tumour-derived cell lines could provide an almost indefinite supply of cells for research; yet in contrast to the adult CNS, they display rapid growth rates and therefore do not closely reflect the *in vivo* situation. In addition, tumour-derived cell lines are difficult to differentiate into stable cultures. Primary cultures, on the other hand, provide a useful supply of stable, often non-dividing cells but must be continuously produced in order to provide the necessary amounts of tissue for research and testing. In addition, they are of non-human origin. To overcome these limitations *Dr. Chris Morris* and colleagues (The Medical Toxicology Centre, Wolfson Unit, University of Newcastle) have derived human Neural Precursor Cell (hNPC) lines as an alternative source. The preliminary evaluation for the **suitability of these human embryonic neural precursor stem cells in vitro neurotoxicological approaches** was presented during his lecturer. Dr. Morris stressed that hNPC lines could be maintained for long-term (> 12 months) in serum free defined media as “neurospheres”. Moreover, hNPC can be successfully regrown from liquid nitrogen storage providing a long term supply. Successful differentiation into neurones, astrocytes, and oligodendrocytes is accomplished by simple growth factor withdrawal and serum addition. By exposure to additional differentiation factors these cells can be directed to differentiate into specific cell types (e.g. dopaminergic neurones). Once differentiated, the cell lines remain stable and show minimal cell division over several weeks. They express neuronal (e.g. Gad67) and glial (GFAP) antigens, and are killed by a range of known neurotoxins (e.g. kainic acid). These preliminary data seem very promising. *Dr. Chris Morris* suggested that these hNPCs may provide a potential suitable cell system to complement existing *in vitro* models used in neurotoxicology.



The NESCI-ESNATS meeting was closed by Dr. *Sebastien Sethe*, the Legal & Regulatory Officer at NESCI (University of Newcastle; legal@nesci.ac.uk). In a comprehensive and interesting way, he managed to guide the public through the different aspects of '**The legal & Regulatory Landscape implicated in the use of Stem Cells as a tool for Toxicology Testing**'. For more information, please visit www.nc3rs.org.uk.