Program

13th European Congress of Toxicologic Pathology

in collaboration with the

British Society of Toxicological Pathology

From gene to drugs: an insider's view on cell pathology

© visit Surrey

22nd – 25th September 2015
in Surrey, United Kingdom

European Society of Toxicologic Pathology
estp

BSTP

www.eurotoxpath.org

www.bstp.org.uk

MEETING YOUR SCIENTIFIC AND BUSINESS NEEDS

Contact:
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- Pharmacodynamics
- Pharmacokinetics
- Environmental Toxicology
- Analytical Chemistry
- Regulatory Affairs
- Literature searches
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RESPONSIVENESS

- Direct access to Study Directors and Management
- 24-hour or less response
- Study issues reported to Sponsor within 24 hours (troubleshooting)
- >90% on-time delivery of draft reports

ADAPTABILITY – SPEED

- Experiments can very often be started as soon as possible (size of facility)
- Short period of time between co
- Completion of experiment and draft report
- 1 to 4 weeks between draft report and final report
- Interim reports
- Short-time period for proposal generation (less than 1 week)
- Flexibility: Willingness to customize processes, protocols and report templates
- Non-bureaucratic

SCIENTIFIC EXPERTISE

- More than 50 years of Expertise
- Low turn-over in workforce
- Most technicians are with LPT for more than 10 years
- Toxicologists, Study Directors and Histopathologists working in their fields for more than 10 years

SCIENTIFIC EXPERTISE
GENERAL FEATURES - QUALITY

- No expert report ever rejected by the authorities
- No study ever rejected by the authorities
- Passed all FDA inspections on GLP
- Passed all German GLP inspections

SCIENTIFIC EXPERTISE – GMP CONFIRMATION

- Since 2000 Regular GMP-Authority inspections
- Since 2009 FDA: Inspection of Botulinum Assay
  Result: FDA approved
- Since 2010 GMP-Certificate according to § 14 (4) AMG by the local German GMP-Authority for biological potency assays

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estp Pathology

Welcome

Dear colleagues and friends,

The European Society of Toxicologic Pathology and the British Society of Toxicological Pathology are pleased to welcome you to the 13th EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY to be held jointly with the 30th ANNUAL MEETING of the BRITISH SOCIETY OF TOXICOLOGICAL PATHOLOGY, in Guildford, Surrey.

We hope this will be an exciting opportunity for members of both societies and their colleagues, together with other interested scientists from Europe and beyond, to share their expertise, challenges and vision of Toxicologic Pathology on the topic of "From gene to drugs: an insider's view on cell pathology".

The joint Scientific Committee has prepared an exciting scientific program with invited international expert speakers and opportunities for interactions on additional relevant topics of interest to both toxicologic pathologists and preclinical safety scientists (including genomics, informatics, phenotyping, gene therapy, models of disease and toxicology, clinical translation, regulatory viewpoints and human pathology). Participants from Europe and overseas will include pathologists and scientists from industry, universities and other research and governmental institutes, and trainees in the field.

Our aim is to offer a mix of plenary lectures, interactive presentations, poster sessions, a trade exhibition and plenty of opportunities for networking both formally and informally with colleagues and friends.

Guildford, the County and University town of Surrey, England is located 27 miles (43 km) southwest of central London and 50 min from the historical port of Portsmouth and the coast. It's therefore a great base from which to explore the south of England and with central London's Waterloo station only 40 min away, a direct train link to Gatwick or a 45 min journey to Heathrow, it is easily accessible. The University of Surrey is close to the centre in a modern and leafy campus and opens its new School of Veterinary Medicine buildings this year.

As is usual for ESTP congresses, the social program will include a welcome reception and congress dinner.

For all these reasons, we are pleased to welcome you in Surrey!



Vanessa Schumacher, Franck Chanut and Aude Roulois
On Behalf of the Scientific Organizing Committee

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General Information

Scientific Organizing Committee


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IRCCS San Raffaele
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Stephanie Klein,
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Vanessa Schumacher,
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Cheryl Scudamore,
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Roberto La Ragione,
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Shirley Price,
University of Surrey

General Information

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Julia Gerhold,
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Roberto La Ragione,
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General Information

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Congress Venue

Vet School Main Building
Daphne Jackson Road
University of Surrey
Guildford
GU2 7AL
UK

Registration Desk

The desk will be located at the ground floor in the foyer. All the congress documents can be picked up from the registration desk. An identification badge must be worn to enter all the congress sessions and events.

Registration is possible during the whole congress.

Opening hours of registration desk:
22nd September 2015 07:00 am – 05:00 pm
23rd September 2015 08:00 am – 06:00 pm
24th September 2015 08:00 am – 05:00 pm
25th September 2015 08:00 am – 12:00 pm

Registration for the IATP satellite meeting:
22nd September 2015 07:00 am – 08:00 am

Speaker Information

Video projector and PC are available for presentations. Please give your presentations to the front desk before your session. Please use CD-ROM, USB stick or comparable format. The use of your own PC is not recommended.

General Information

Poster Presentation

Posters will be exhibited during the entire Congress. Poster sessions are scheduled during coffee and lunch breaks.

Authors are kindly requested to be at their posters during the poster sessions during the breaks on Wednesday to answer potential questions.

The poster boards are kindly provided by

ESTP Interactive session

Interactive sessions on different cases of toxicologic pathology are organized on Thursday and Friday afternoon. Images and/or slide scans from the cases are available on the ESTP website for review prior to the session.

Abstract Publication

Presentations given at this congress will be published on the ESTP website in pdf-format.

Awards

The congress program will also include the following awards

- The Chirukandath Gopinath Lecture Award
  sponsored by the British Society of Toxicological Pathology (BSTP)
- The Edgar Hartley Kettle Lecture Award
  sponsored by the Royal College of Pathologists
- The Joachim Kohn Lecture Award
  sponsored by the Royal College of Pathologists
- The ESTP Publication Award
  sponsored by Novartis
- The Award for the Best Poster
  sponsored by the French Society of Toxicologic Pathology (SFPT)
- The Charles Capen Trainee Award
  sponsored by the International Academy of Toxicologic Pathology (IATP)
- The IFSTP Trainee Award
  sponsored by the International Federation of Societies of Toxicologic Pathology (IFSTP)

The award ceremony is scheduled for Thursday 24th of September during the Congress Dinner, with the exception of the Lecture Awards, which will be presented prior to the respective talks.

General Information

Industry Exhibition

As in previous years, an exhibition featuring Pharmaceutical and Product Companies, Technical Equipment Companies and Medical Publishers will be held at the conference. Entrance is free to those registered to the Conference and registered accompanying persons.

The exhibition will open on Tuesday, September 22, at 07.30 pm and will then follow the same schedule as the conference.

The exhibition will close after the afternoon coffee break of Thursday September 24th.

The industry exhibition provides information about the newest technologies and developments available within our scientific area. The ESTP and BSTP value the support provided by exhibitors and believes that the on-site discussion and exchange of experience between exhibitors and the congress participants is of invaluable importance and benefit.

Please visit the booths of our exhibitors!

Exhibition Quiz

There will be an exhibition quiz and you will receive details of how to participate when you register for the meeting. The prize will be an eReader which will be presented on Thursday before the afternoon coffee break.

The prize is kindly sponsored by

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General Information

Additional Meetings

Committee for Scientific Standards F2F Meeting
Tuesday, September 22 12:45 – 01:15 pm – the room will be announced on the info board

BSTP Annual General Assembly
Tuesday, September 22 06:00 – 07:00 pm in the main conference room.

ESTP 2016 Scientific Organizing Committee F2F Meeting
Wednesday, September 23 07:30 – 08:30 am – the room will be announced on the info board

ESTP Executive Committee board F2F meeting
Wednesday, September 23 12:45 – 02:00 pm – the room will be announced on the info board

RoeLee Meeting
Wednesday, September 23 03:30 – 05:30 pm – the room will be announced on the info board

ESTP Annual General Assembly
Wednesday, September 23 06:00 – 08:00 pm in the main conference room.

STP, BSTP, ESTP webinar Meeting
Thursday, September 24 10:45 – 11:15 am – the room will be announced on the info board

STP Presidents Meeting
Thursday, September 24 01:00 – 02:00 pm – the room will be announced on the info board

INHAND Meeting
Thursday, September 24 01:00 – 02:00 pm – the room will be announced on the info board

Other additional Meetings
Other additional Meetings will be announced on the information board next to the registration desk in the meeting venue.

General Information

Refreshments

Coffee, tea, soft drinks and pastries will be served during the coffee breaks.
Lunch is provided during the lunch breaks on:
Wednesday, September 23
Thursday, September 24

Social Events

Welcome Reception – Tuesday 22nd September, 07:30 – 09:30 pm

On the evening of Tuesday 22nd September we would like to invite you to join us at the Welcome Reception.

The congres venue is in walking distance to the Holiday Inn Guildford Hotel. If you stay in the city of Guildford it is easy to reach by public transfer. There are several busses going from the city centre directly to the congress venue.
The Reception will take place in the congress venue where you will have the opportunity to meet colleagues and friends, to chat and prepare yourself for the following days of the conference.

Join us!

Conference Dinner – Thursday 24th September, 06:30 pm

The Conference dinner will take place in the beautiful "Loseley Park".

We invite you to come with us on a journey into "Old England", enjoy a drink in the rose garden and take a look into the Great Hall of Loseley House, where you'll join royalty and nobility from ages past. The surroundings are truly sumptuous, the atmosphere unique and the sense of occasion it brings incomparable.

The dinner itself will be held in "Tithe Barn" that once stored tithes – tax in the form of grain paid by medieval farmers – and which now forms a fabulous and spacious venue.

A shuttle service will be provided.

Language

The official language of the congress is English. No simultaneous translation will be provided.

Internet Access

Free Wifi is available in the whole congress area.

General Information

Messages

There is a message board close to the congress registration desk.

Congress Bags

Congress bags can be picked up at the registration desk. They are kindly sponsored by TPL Path Labs

Safety and Security

Please, wear your name badge while in the congress area (access will be denied otherwise). Remove your name badge when leaving the congress area.

The name badges are kindly sponsored by GlaxoSmithKline

The lanyards are kindly sponsored by Insten Information Solutions For L

In case of emergency, please follow directions from the congress staff and chair persons.

Emergency calls

999 is theemergency number for the United Kingdom, but calls are also accepted on the European Union emergency number, 112. Calls are free to these numbers.

Currency

The currency throughout the UK is the Pound (£), divided into 100 pence.
Bank ATMs, which are often known in the UK as Cashpoints, cash machines or informally as 'holes in the wall', are widely available. Traveller's cheques can be exchanged at most banks.
Be aware: some non-bank ATMs (often found at petrol/gas stations and convenience stores) may charge a fixed fee (around £1.75) for withdrawing money, on top of what your home bank may charge. There will always be an on screen warning if you will be charged...

Visa, MasterCard, Maestro and American Express are accepted by most shops and restaurants, although American Express maymay not be accepted by all retailers.

General Information

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Congress Program

Tuesday	Wednesday	Thursday	Friday
7.00 am Opening of Registration	Session 2a: Gene therapy: Where are we now	Session 4a: Animal models	Session 6a: Innovative tools and techniques
IATP satellite meeting separate registration required	8.45 – 9.45 am Keynote Lecture: Gene therapy overview *Luigi Naldini*	8.45 – 9.45 am Keynote Lecture: Translational considerations in drug development using animal models of neuromuscular disease *Dominic Wells*	8.45 – 9.45 am Pathology 2.0 session: Fundamentals of Histopathologic Image Analysis *Jennifer Cann*
8:00 – 8:40 am Overview of Ultrasound Imaging with Correlation to Gross or Histopathology *Kathy Gabrielson*			
8:40 – 9:30 am In Vivo Optical Imaging: Toxicology and Beyond *Vyacheslav Kalchenko*	9.45 – 10.45 am Gene therapy and regulation, viewpoints from the regulators and the GLP accredited facility *Maria Cristina Galli / Patrizia Cristofori*	9.45 – 10.45 am Immune variations among the strain and sources of inbred mice *Cory Brayton*	9.45 – 10.45 am Automated ISH, ISH/IHC double stain and ISH image analysis *Jürgen Funk*
9:30 – 10:10 am Multimodal Molecular Histology in Toxicologic Pathology Investigations *David Bonnel*			
10:10 – 10:30 am Tea break	10.45 – 11.15 am Tea break	10.45 – 11.15 am Tea break	10.45 – 11.15 am Tea break
10:30 – 11:10 am The Utility of Compact MRI for Assessment of Phenotypes and Therapeutic Efficacy *Yael Schiffenbauer*	Session 2b: Gene therapy: examples of application	Session 4b: Animal models	Session 6b: Innovative tools and techniques
11:10 – 11:50 am Nuclear Imaging in Cancer Studies with Correlation to Gross and Histopathology *Kathy Gabrielson*	11.15 am – 12.00 pm Common histopathology lesions in mice models used in preclinical toxicity studies performed to support human clinical studies using allogeneic haematopoietic stem cell transplantation. *Franck Chanut*	11.15 am – 12.00 pm Translation Safety: From clinical to preclinical and back *Dominique Brees*	11.15 am – 12.15 pm Data visualisation workshop: Data Visualization – Visual Communication *Kuno Wuersch*
	12.00 – 12.45 pm Modeling the clinical behavior of genetically targeted T cells in immunodeficient mice *Attilio Bondanaza*	12.00 – 1.00 pm Translational aspects of neurologic models *Caroline Zeiss*	12.15 – 12.45 pm Interactive case presentations
12.45 – 1.15 pm Welcome, Introduction and Exhibitors presentations	12.45 – 2.00 pm Lunch	1.00 – 2.00 pm Lunch	12.45 – 1.00 pm Concluding remarks
Session 1a: Cell pathology	Session 3a: Stem cells and regenerative medicine	Session 5a: Clinical pathology	
1.15 – 2.15 pm Keynote Lecture: Overview of Cell Death Modalities *Susan Elmore*	2.00 – 3.00 pm Safety testing for advanced/cell based therapies *Dominique Brees*	2.00 – 3.00 pm Biomarkers of Drug-Induced Vascular Injury: A Search for the Holy Grail? *Calvert Louden*	
2.15 – 3.15 pm Keynote Lecture: Autophagy and pharmacological manipulation in neurodegeneration *David Rubinsztein*	3.00 – 3.45 pm General Toxicity Testing of Stem Cell-derived Products *Julia Baker*	3.00 – 3.30 pm Clinical pathology: Renal biomarker update *Jarig Darbes*	
3.15 – 3.45 pm Tea break	3.45 – 4.15 pm Tea break	3.30 – 4.00 pm Tea break	
Session 1b: Cell pathology	Session 3b: Stem cells and regenerative medicine	Session 5b: "Clinical pathology"	
3.45 – 5.15 pm INHAND lymphoid system multiple presenters	4.15 – 5.00 pm Use of Pluripotent stem cells – "reprogramming, differentiation and application in Toxicology" *Kyle Kolaja*	4.00 – 5.00 pm HESI genomics symposium/ workshop *Philippe Couttet*	
5.15 – 6.00 pm Harnessing the immune system to treat cancer *Viia Valge-Archer*	5.00 – 5.45 pm Development of cell therapies, the risk-based approach *Guilia Leoni*	5.00 – 5.30 pm Interactive case presentations	
6.00 – 7.00 pm BSTP AGM	6.00 – 8.00 pm ESTP AGM		
7.30 – 9.30 pm Welcome reception		6.30 pm Conference Dinner at Loseley Park	

IATP Satellite Symposium – Program & Abstracts

International Academy of Toxicologic Pathology (IATP)
Applications of In Vivo and Ex Vivo Multimodality Imaging in Toxicologic Pathology
Satellite Symposium at the ESTP/BSTP Congress
University of Guildford, UK
September 22, 2015

8:00 – 8:40: Overview of Ultrasound Imaging with Correlation to Gross or Histopathology

Kathy Gabrielson, Johns Hopkins University, Maryland

The presentation on ultrasound will emphasize practical examples from cardiovascular toxicity and pharmacological efficacy investigations, cancer xenograft studies and monitoring fetal development with correlation to gross and histopathology. Ultrasound can serve as an important addition to toxicologic pathology by noninvasively and nondestructively providing 2 or 3-dimentional digital data sets, quantitative morphological details, cardiac function data, blood flow and vascular bed assessment. This safe and easy to use tool eventually leads to a more comprehensive assessment of toxicological effects and disease progression with serial imaging assessments, in contrast to end of study conventional histopathology.

8:40 – 9:30: In Vivo Optical Imaging: Toxicology and Beyond

Vyacheslav Kalchenko, Weizmann Institute of Science, Israel

This presentation will provide an overview of optical imaging techniques from micron to whole organism levels stressing the benefits and limitations of optical imaging modalities for preclinical applications and animal toxicology studies. In addition to whole body fluorescence and bioluminescence imaging, opto-acoustic imaging, fluorescence molecular tomography and dynamic contrast imaging will be explained with relevant examples for the toxicologic pathologist. Examples of 2D, 3D, and 4D optical images and anatomical and functional images will be presented along with approaches used in image data collection, analysis and interpretation.

9:30 – 10:10: Multimodal Molecular Histology in Toxicologic Pathology Investigations

David Bonnel, Imabiotech, France

Toxicity studies need investigations to identify the cause of atypical findings, and more particularly if this is related to drug or its metabolites. Multimaging™ is a new advanced label free approach based on the combination of Mass Spectrometry Imaging (MSI) and classical histology techniques. It provides a quantitative molecular distribution of all detected molecules directly on tissue sections. This multimodal molecular histology approach supports drug safety studies by following targeted molecules such as drugs and their related metabolites (targeted analysis) or by identifying new toxicity markers (untargeted analysis). The presentation will emphasize examples of this approach for toxicologic pathology investigations.

10:10 – 10:30: Break

IATP Satellite Symposium – Program & Abstracts

10:30 – 11:10: The Utility of Compact MRI for Assessment of Phenotypes and Therapeutic Efficacy

Yael Schiffenbauer, Aspect Imaging and Abraham Nyska, Israel

The presentation dealing with compact MRI will emphasize, through practical examples from pharmacological efficacy investigations, target organ toxicity, and carcinogenicity studies, how this technology can serve as an important adjunct to toxicologic pathology by nondestructively providing 3-dimentional (3-D) digital data sets, detailed morphological insights, and quantitative information. This safe and easy to use tool eventually leads to a more comprehensive assessment of toxicological effects and disease progression, in contrast to the limited number of 2-dimentional (2-D) tissue slices afforded by conventional histopathology.

11:10 – 11:50: Nuclear Imaging in Cancer Studies with Correlation to Gross and Histopathology

Kathy Gabrielson, Johns Hopkins University, Maryland

This presentation on nuclear imaging will emphasize examples of molecular imaging different types of cancers, with comparisons to gross, histopathology and optical imaging. In the cases reviewed, an imaging plasmid is injected into the mouse and is activated in cancer cells due to a cancer specific promoter driving thymidine kinase followed by SPECT imaging. Examples of plasmid delivery with luciferase will be presented for comparison overviewing the advantages and disadvantages of these two imaging modalities in molecular imaging cancer cells. Conventional histopathology is used to validate the images in this novel method that is still in development in animal models.

Speaker Abstracts

S01: Overview of Cell Death Modalities

Susan A. Elmore, MS, DVM, DACVP, DABT, FIATP
NTP Pathologist and Staff Scientist,
National Institute of Environmental Health Sciences Cellular and Molecular Pathology Branch
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The field of cell death research continues to move forward at an alarming speed. As such, it is challenging to keep apprised of all the new developments unless one is active in the field. This talk will give an overview of the most well known forms of cell death: apoptosis, necrosis, autophagy, necroptosis, pyroptosis, eryptosis, and anoikis. The definition, mechanisms, causes, important features and morphology will be discussed. Although **apoptosis** and **necrosis** are the two most well known forms of cell death, there is still confusion about terminology. The mechanisms and morphology differ and special techniques can be used to confirm apoptosis if needed. Despite our knowledge of the mechanisms of apoptosis, it is still referred to as "single cell necrosis" in the literature. However, this misnomer is slowly falling out of favor and the INHAND documents will provide needed guidance for toxicologic pathologists and others in the field. **Autophagy** is a genetically regulated and evolutionarily conserved pathway for the degradation of subcellular components whereas autophagic cell death is considered a form of programmed cell death. But there is an issue about whether autophagic activity in dying cells is the cause of cell death or an attempt to prevent it. This controversy will be discussed. **Necroptosis** is a form of cell death that is specific to vertebrates and is considered to be a programmed form of necrosis. It does not involve caspase activation, is RIP-mediated, immunogenic in nature, and acts as an alternative "fail safe" cell death pathway in cases where cells are unable to undergo apoptosis, as with some viral infections. **Pyroptosis** is an inherently inflammatory caspase-1 dependent form of cell death that is associated with antimicrobial responses during inflammation. Because pyroptosis is caspase-mediated, it was not initially distinguished from apoptosis. But the mechanism, characteristics and outcome differ from apoptosis. Morphologically it is indistinguishable from necrosis. **Eryptosis** is the form of cell death that erythrocytes undergo when they experience survival-threatening injury prior to senescence. It is similar to apoptosis of nucleated cells in that it involves coordinated suicidal death that leads to the removal of defective or injured cells without rupture of the cell membrane. But erythrocytes lack nuclei and mitochondria, which actively participate in apoptosis. Two benefits of eryptosis are the clearance of defective erythrocytes prior to hemolysis and the clearance of infected erythrocytes. **Anoikis** is the final form of cell death to be discussed. It involves apoptosis that is induced by the lack of correct cell/extracellular matrix attachment (ECM). It is considered the mechanism by which cells *in vivo* use ECM-derived signals to maintain tissue integrity. This form of cell death has implications in tumor metastasis in that the cells must be able to survive in inappropriate locations.

References

1. Elmore SA (2007) Apoptosis: A review of programmed cell death. *Toxicol Pathol*. 35:495-516
2. Linkermann A and Green DR (2014) Necroptosis. *The New England Journal of Medicine*. 370: 455-465
3. Lang F et al. (2012) Physiology and pathophysiology of eryptosis. *Transfus Med Hemother*. 39: 308-314
4. Ryter et al. (2014) The impact of autophagy on cell death modalities. *International Journal of Cell Biology*. Article ID 502676. http://dx.doi.org/10.1155/2014/502676
5. Bergsbaken et al. (2009) Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol*. 7(2): 99-109
6. Goldstein P and Kroemer G (2006) Cell death by necrosis: towards a molecular definition. Trends in biochemical sciences. 32: 37-43
7. Gilmore AP (2005) Anoikis. *Cell Death and Differentiation*. 12: 1473-1477

Speaker Abstracts

S02: Autophagy and pharmacological manipulation in neurodegeneration

David C Rubinsztein
Professor of Molecular Neurogenetics,
Cambridge Institute for Medical Research,
Wellcome Trust/MRC Building,
Cambridge Biomedical Campus,
Hills Road, Cambridge,
CB2 oXY, U.K.

Intracellular protein aggregation is a feature of many late-onset neurodegenerative diseases, including Parkinson's disease, tauopathies, and polyglutamine expansion diseases like Huntington's disease (HD). Many of these mutant proteins, like that causing HD, cause disease via toxic gain-of-function mechanisms. Therefore, the factors regulating their clearance are crucial for understanding disease pathogenesis and for developing rational therapeutic strategies.

The two major intracellular protein degradation pathways are the ubiquitin-proteasome system and (macro) autophagy. Autophagy is initiated by double-membrane structures which engulf portions of cytoplasm. The resulting autophagosomes ultimately fuse with lysosomes, where their contents are degraded.

I will briefly describe the basic biology of autophagy before outlining its roles in neurodegeneration. We have shown that the autophagy inducer rapamycin reduced the levels of mutant huntingtin protein and attenuated its toxicity in cells, and in Drosophila and mouse HD models. We have extended the range of intracellular proteinopathy substrates that are cleared by autophagy to other related neurodegenerative disease targets and have provided proof-of-principle in cells, Drosophila and mice. In order to induce autophagy long-term, we have been striving to identify safer alternatives to the mTOR inhibitor rapamycin. To this end, we have been trying to discover novel components of the autophagy machinery and new signaling pathways and drugs that impact on autophagy. While autophagy induction is protective in models of various neurodegenerative diseases, certain other conditions, including lysosomal storage disorders, are associated with compromised autophagy. I will review these data and then describe how impaired autophagy compromises cellular processes, including the ubiquitin-proteasome system.

Speaker Abstracts

S03: Harnessing the immune system to treat cancer: Preclinical assessment of immunobiology and combinatorial activity

Viia Valge-Archer,
Principal Scienntist
MedImmune Ltd.,
Cambridge
UK

Evasion of the immune system is one of the hallmarks of cancer and immune mediated therapies for cancer (IMT-C), such as anti-CTLA-4 and anti-PD-1/PD-L1 antibodies, are showing significant promise in the treatment of solid tumors. However, although these treatments can show significant overall benefit, a subset of patients fails to respond. It is believed that activity in these patients is limited by a lack of immune priming or by immunosuppression. Combination with standard of care therapies (such as chemo- and radiotherapy), molecular targeted therapies and or other IMT-Cs, has the potential to overcome these hurdles to response and maximize patient benefit. A preclinical approach to examining the effects of both immune monotherapy as well as combination with the above modalities to develop a greater understanding of how these may affect an anti-tumour immune response will be presented.

Speaker Abstracts

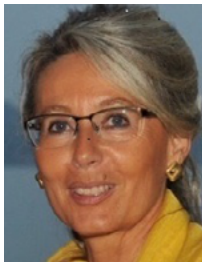
S04: Gene therapy Overview

Luigi Naldini
Professor of Cell and Tissue Biology and Professor of Gene and Cell Therapy
"Vita Salute San Raffaele" University School of Medicine
Milan, Italy

Speaker Abstracts

S05: Gene therapy and regulation, viewpoints from the regulators and the GLP-accredited facility

Maria Cristina Galli[1],
Patrizia Cristofori[2]



[1] Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Roma, Italy*
[2] GSK David Jack Centre for R&D, Park Road, Ware, Hertfordshire, SG12 0DP, United Kingdom

Several gene therapy clinical trials using gene-modified hematopoietic stem cells have shown encouraging signs of therapeutic efficacy in multiple disease areas. This therapeutic strategy is applied to an increasing number of diseases and rigorous studies in appropriate non-clinical models are needed to assess the risk/benefit ratio at translational level and fulfill the requirements for future market approval. Studies supporting Gene Therapy Medicinal Products (GTMPs) development are not standard and have to be designed on a case-by-case basis to properly address safety and provide the scientific basis for conducting clinical trials. The fact that GTMP trials are often sponsored by academia, charities and small companies with limited financial resources or regulatory experience further complicates this process.
Due to the novelty and complexity of GTMPs, areas of non-compliance should be identified and their significance evaluated. In the evaluation process it is worth working closely with Regulators, who themselves should be aware of GTMP specificities with respect to chemical and biological drugs.
This presentation will discuss the experience of HSR-TIGET Test Facilities, recently certified to perform Gene Therapy studies according to GLP OECD principles, as well as the regulatory perspective on this activity.
TIGET is the Telethon Institute for Gene Therapy and is a joint venture between the Telethon Foundation, a charity funding medical research, and Ospedale San Raffaele (OSR), a private hospital in Milan, Italy. In 2010 TIGET started an Alliance with GlaxoSmithKline to promote the development of gene therapy for the treatment of genetic diseases.
This presentation will present examples from the application of GLP principles to biodistribution, toxicity and tumorigenicity studies performed to support the regulatory submission of gene therapy medicinal products.

* The views expressed in this abstract are author's personal views, and may not be understood or quoted as being made on behalf of Italian Competent Authority.

Speaker Abstracts

So6: Common histopathology lesions in mice models used in preclinical toxicity studies performed to support human clinical studies using allogeneic hematopoietic stem cell transplantation.

Franck Chanut [1],
Francesca Sanvito[2–3],
Patrizia Cristofori[1]

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Rare diseases are defined as life-threatening or chronically debilitating conditions that affect few people. Depending on the definition used (US or EU); there are approximately 5-7000 rare diseases. Gene therapy is a promising approach to treat some of these rare diseases.
Preclinical toxicity studies performed to support the human clinical studies with allogeneic hematopoietic stem cell transplantation (HSCT) use a specific study design. They are complex and the use of mouse models of human diseases makes interpretation of the results difficult. In addition, they rely on the use of conditioning agents that must induce sufficient myeloablation to allow engraftment of HSCT and must be sufficiently immunosuppressive to overcome graft rejection of the donor stem cells by the recipient immune system.
We will first explain the preclinical study designs, the inherent risks of gene therapy and how these can be addressed.
We will then be presenting the major toxicity of two conditioning agents (Busulfan and Radiation Therapy) commonly used in preclinical toxicity studies performed to support human clinical studies using allogeneic HSCT.
We will conclude by discussing the lymphoproliferative disorders induced by the conditioning regimen (Busulfan).

All animal studies were ethically reviewed and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and GSK's Policy on the Care, Welfare and Treatment of Animals.

Speaker Abstracts

S07: Modeling the clinical behavior of genetically targeted T cells in immunodeficient mice

Dr. Attilio Bondanaza
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Speaker Abstracts

So8: Safety testing for advanced/cell based therapies

Dominique Brees
Novartis Institutes for BioMedical Research. Basel, Switzerland

The presentation will provide a summary/review of clinical safety aspects of engineered TCR and CAR-based therapies. TCR based therapies are engineered TCR-expressing T cells directed to target peptides in the context of MHC molecules. A key safety issue is cross-reaction to other peptide/MHC combinations and therefore cytotoxic reactions in unrelated tissues. In addition, due to instable expression of MHC genes, tumor cells might be less vulnerable to the attack of engineered TCR-expressing T cells than healthy, non-targeted tissue cells. A review of the current literature will be presented. CAR-based therapies also carry some similar liabilities in terms of tissue cross-reactivity with some targets expressed both in healthy and disease tissue, consequently creating a challenging clinical risk assessment. Further safety concerns of CAR-based therapies arise from cytokine release syndrome (CRS); for example destruction of tumor cells may lead to tumor lysis syndrome (TLS). The CRS potential of a CAR-based therapy is usually difficult to predict by non-clinical approaches due to the lack of relevant preclinical animal models which mimic the high individual variability in clinical response. In general, human T cell therapy products are not cross-reactive to animals, hence requiring the use of homologous animal model or immunocompromised mouse model. The former has the disadvantage that the clinical product cannot be assessed. In the latter, the efficacy in tumor killing can be evaluated but the animals do not have a complete immune response.

In conclusion, there is a definitive need for sound target selection and validation of appropriate non-clinical models to evaluate the safety of novel T cell therapies in the future.

Speaker Abstracts

S09: General Toxicity Testing of Stem Cell-derived Products

Julia Baker
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Cell-derived therapeutic products rely on the introduction of cells into a tissue to effect a cure. This branch of Regenerative Medicine frequently focuses on degenerative or genetically-based disorders, and may or may not include gene therapy. Strictly used, the term encompasses not only the use of stem cells, but also the transplantation of bone marrow and other differentiated cell types, with or without a "medical device" scaffold or capsule.

Studies involving cellular products are unique in the field of toxicology because they involve not one, but two living systems – the test system and the test article itself. This creates significant challenges for the investigator who must ensure that the test article retains its viability both before and during the administration procedure. This talk will address some of the problems associated with the in-life phase, and will also consider the development of a rigorous but affordable pathology protocol and the interpretation of pathology findings.

Speaker Abstracts

S10: Use of pluripotent stem cells- "reprogramming, differentiation and application in toxicology"

Kyle Kolaja

Speaker Abstracts

S11: Development of cell therapies, the risk-based approach

Guilia Leoni
Catapult,
UK's centre for the acceleration of the translation of cell therapies,
Nonclinical Safety and Immunotherapy Strategy at the Cell Therapy

Unlike the nonclinical pathway for small molecule and biologic-based therapies, the requirements for a cellular therapy product can seem unclear. The basic aim for any nonclinical program is to determine the efficacy and safety of the product; however, with a cellular therapy a key challenge can be determining how to do this. Although cellular therapies can share some of the same principal characteristics, it is recognized by the regulatory agencies that cellular therapies are not a homogeneous class of products. In addition, the level of scientific knowledge and clinical experience of a given cellular therapy is highly variable. A cellular therapy product safety depends on many factors including the type of cell therapy, the differentiation status and proliferation capacity of the cells, the route of administration, the intended clinical location, long term survival of the product and/or engraftment, the need for repeated administration, the disease to be treated and the age of the population. Given the product specific attributes of most cellular therapies, a case-by-case risk-based approach can be taken when designing the nonclinical testing programs.

Speaker Abstracts

S12: Translational considerations in drug development using animal models of neuromuscular disease.

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Animal models play a key role in understanding the pathophysiology of diseases and in developing treatments for such diseases. However the translational success of many studies has been disappointing. Careful consideration of the nature of the animal models and the assessment methods for evaluation of the effects of treatments may help to explain at least part of this poor translational success and provides guidance for more predictive studies in the future. Examples will be provided in the context of two models of neuromuscular diseases, the mdx mouse model of Duchenne muscular dystrophy and the transgenic SOD1 G93A model of amyotrophic lateral sclerosis. The mdx mouse has a much less severe phenotype than the human condition and a different life history. However, it recapitulates some elements of the human condition, in particular biochemistry, physiology and early histopathology and thus those elements should be the focus for translational studies (Godfrey et al., 2015).

The use of standard operating procedures is also important to allow comparisons between labs (TREAT-NMD SOPs). Likewise, the SOD1 G93A mouse is a useful model if used correctly and well considered guidelines are available for best experimental practice that helps to eliminate the false positives that have contributed to unsuccessful clinical trials (Scott et al., 2008). Common problems across these and other animal models include use of poorly controlled outcome measures, poor statistical power, inappropriate dosing, non-translatable routes of delivery, biologically meaningless statistical differences and a lack of replication by independent laboratories. Examples will be used to illustrate the above points and a standardised protocol for testing drugs in the mdx mouse will be proposed. Reviewing drug-development plans via the Treat-NMD Advisory Committee on Therapeutics has revealed many of these animal model problems are common (Heslop et al., 2015)

References:

Godfrey C, Muses S, McClorey G, Wells KE, Coursindel T, Terry RL, Betts C, Hammond S, O'Donovan E, Hildyard J, El Andaloussi S, Gait M J, Wood, MJ, Wells DJ (2015) How much dystrophin is enough: the physiological consequences of different levels of dystrophin in the mdx mouse. Hum Mol Genet. May 1. pii: ddv155. [Epub ahead of print]

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Scott S, Kranz JE, Cole J, Lincecum JM, Thompson K, Kelly N, Bostrom A, Theodoss J, Al-Nakhala BM, Vieira FG, Ramasubbu J, Heywood JA. (2008) Design, power, and interpretation of studies in the standard murine model of ALS. Amyotroph Lateral Scler. 9(1):4-15.

Treat-NMD SOPs: http://www.treat-nmd.eu/research/preclinical/dmd-sops/

Speaker Abstracts

S13: Immune variations among the strain and sources of inbred mice

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Laboratory mice are widely used in research. Variation in responses to infections among mice has been recognised at least since the early 1900's. Although many common strains are considered to be 'immune sufficient' or 'immune competent', genetic contributors to immune and disease phenotypes are increasingly well characterized, and variation in phenotypes should be expected. But, 'the mouse model' is blamed for its limitations; for confounding, disparate or otherwise problematic research outcomes; and for poor reproducibility and poor predictivity of translational studies. Inbred mice are isogenic and homozygous at all loci, and are remarkable models for various aspects of immune responses. Examples of even a few immune relevant genotypes predict divergent immune and disease phenotypes, and illustrate that mouse strains should be assessed critically for their suitability to answer a research question. Increasingly accessible genotype and phenotype data may lead to the selection of several strains to address a specific question, and also should raise serious concerns regarding the use of genetically mixed mice with undefined contributions from different backgrounds. Non genetic factors such as diet, housing and the microbiome also influence immune and disease phenotypes, and should be considered in experimental design. Improved reporting that includes adequate and accurate communication of mouse and strain information, husbandry and experimental design, could also improve the reproducibility and predictivity of translational studies involving mice.

Speaker Abstracts

S14: Translation safety: From clinical to preclinical and back

Dominique Brees, Michael Kammueller, Daniel Stiehl
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Drug safety is a continuum whereby preclinical and clinical data feed each other into an iterative loop to better understand clinical signals and the predictive values of preclinical safety species.

Most commonly, the approaches have been unidirectional; mechanistic safety focused on understanding the relevance of preclinical findings on clinical outcome and on the development of monitorable biomarkers of such events. Whilst this approach is successful, clinical safety signals are still observed in the absence of any predictable signals from the preclinical work. Mining clinical database and understanding such clinical signals, their relevance to the drug treatment and/or to the disease state of the patient, can directly inform us on the development of bespoken preclinical models to recapitulate such events, enable the development of individual based biomarkers, or allow us to compare the safety profile of drugs.

Speaker Abstracts

S15: Translational Aspects of Neurologic Models

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Most neurologic diseases in humans remain stubbornly resistant to therapeutic intervention. The same is not true for mice. Why is this case? In this lecture, we explore the application of rodent models to the understanding and treatment of human neurologic diseases. Classic models will be used to illustrate the roles of systems biology and the reductionist method in the study of differently complex systems.

Speaker Abstracts

S16: Biomarkers of Drug-Induced Vascular Injury: A Search for the Holy Grail?

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Vascular damage is relatively common in rats, dogs and humans as a spontaneous natural disease or as a result of toxic injury following administration of a diverse range of pharmacologically active agents including small molecules and biotherapeutics that include oligonucleotides, peptides and antibodies. The causes of vascular injury are complex and some agents targeting endothelial and/or smooth muscle cells are known to cause acute drug-induced vascular injury (DIVI). However, other agents such as oligonucleotides that do not target endothelial (EC) or smooth muscle cells (SMC) can cause vascular injury when administered at high doses. Generally, the resulting pathology is due to effects on endothelial cell and or smooth muscle cells with secondary inflammation and/or immune mediated effects. Evidence from morphologic pathology-based studies combined with molecular investigations suggested that EC and or SMC perturbation with subsequent damage may be an early and obligatory step in the development of vascular injury. In humans and animals, the absence of a specific and reliable biomarker of EC/SMC perturbation, activation, and/or damage represents an unmet need in the pre-clinical as well as in the clinical settings. Because vascular injury is an occult pathology rarely associated with adverse clinical signs in animals, there is a strong interest in evaluating blood (cells, plasma and/or serum) to identify and characterize potential circulating biomarkers derived from EC/SMC which, when activated, release circulating molecules e.g. proteins that reflect structural alteration and/or systemic functional disturbances. Such a molecule would bridge this biomarker deficit/gap, particularly if it is vascular specific and released during periods of endothelial cell activation, perturbation and/or injury. However, efforts to identify biomarkers consistent with DIVI have not been successful, largely attributed to the complex nature of the vascular responses. Because of the uncertain translation of DIVI in preclinical studies to humans and of the lack of robust biomarkers, preclinical DIVI findings can cause considerable delays in, or even halt development of promising new drugs. This presentation will focus on the strengths and weaknesses of potential DIVI biomarkers and on points to consider when DIVI is encountered in a pre-clinical development toxicology studies.

Speaker Abstracts

S17: Clinical Pathology: Renal Biomarker update

Dr Jarig DARBES, DVM
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Ideal kidney biomarkers in people and laboratory animals would be sensitive and specific, predicting kidney toxicity before significant tissue damage occurs and without interference from potential effects on other organ systems. There has been a long-standing perception that traditional serum-biochemical renal function biomarkers like blood urea nitrogen (BUN) and serum creatinine (SCr) would benefit from additional biomarkers that would add both sensitivity and specificity to the *in vivo* assessment of toxic kidney effects. There are a steadily growing number of novel kidney function biomarkers aiming to fulfill that need. The relative performance of 12 urinary kidney biomarkers in the rat was evaluated in 22 studies using 12 exclusive kidney toxicants (glomerular or tubular) and 10 non-kidney toxicants with toxicities limited to non-renal tissues. The 12 urinary biomarkers evaluated included Kim-1, clusterin, osteopontin, osteoactivin, albumin, lipocalin-2, GST-α, β2-microglobulin, cystatin C, retinol binding protein 4, total protein, and N-acetyl-β-D-glucosaminidase (NGAL). Sensitivity was assessed by comparison of the urinary biomarker levels with the standardised scores from the renal histopathology assessment in studies with specific nephrotoxicants. Specificity was assessed by measurement of the urinary biomarker levels in studies with compounds eliciting non-renal target organ toxicities (and without histopathological renal findings). To determine the relative performance of the urinary biomarkers, the results of sensitivity studies alone and sensitivity and specificity studies combined, were computed and receiver operator characteristic (ROC) curves generated for each urinary biomarker and for BUN and SCr. Kim-1, clusterin, and albumin showed the highest overall performance for detecting drug-induced renal tubular injury in the rat in a sensitive and specific manner, whereas albumin showed the highest performance in detecting drug-induced glomerular injury. Our results suggest that among the urinary biomarkers tested, Kim-1, clusterin, and albumin are best suited for routine *in vivo* monitoring of tubular and glomerular injury in the rat. It is of note that, whereas most of the evaluated urinary biomarkers were more sensitive in detecting kidney toxicity compared with BUN and SCr, all of them demonstrated some lack of specificity, most notably NGAL and osteopontin, emphasizing the need for caution when interpreting urinary biomarker increases in rat samples from studies with compounds of unknown target organ toxicity. The rat data are further discussed in light of additional data available from non-rodents (dogs and non-human primates).

Speaker Abstracts

S18: HESI genomics symposium/workshop

*Philippe Couttet**
on behalf of the Hesi genomics committee and projects team members.

**Novartis Institutes for Biomedical Research,*
Preclinical Safety/ Discovery and Investigative Safety, Basel, Switzerland

The mission of HESI's Genomics Committee is to advance the scientific basis for the development and application of genomic methodologies to mechanism-based risk assessment, to address scientific issues relating to the use of these new technologies as a means for understanding toxic response and mechanisms and to provide a scientific forum for a consensus-based approach to interpreting and applying these data. In this forum, we will cover the HESI Genomics microRNA best practices (1), microRNA atlas (2), and FFPE sequencing (3) projects.

1. To facilitate biomarker discovery and assessments, the microRNA best practices working group has evaluated the reproducibility of absolute quantitation of injury-related microRNA in serum, plasma and urine across and within sites. Multi-site analysis was performed using quantitative RT-PCR on a focused set of microRNAs in biofluids of rats treated with a dose of isoprenalin that induced heart injury. Novel data analysis procedures are proposed and assay parameters identified that affect data reproducibility.

2. In order to discover previously unidentified rat miRNAs and maximize the use of circulating microRNAs as biomarkers of drug-induced tissue injury in general, the microRNA Atlas working group has generated a rat microRNA Atlas from 23 different tissues by using a deep sequencing approach. Data and our analysis approaches with the goal of identifying tissue-specific and/or tissue-enriched microRNAs will be discussed.

3. Archived FFPE samples provide a major source of animal and human tissues that could, in principle, allow retrospective mechanistic analyses through toxicogenomic profiling. However, these samples have been considered unsuitable for molecular analyses because formalin fixation leads to RNA degradation and protein-RNA crosslinks. Since RNA sequencing works with short fragments, it was hypothesized that it would be amenable to FFPE tissues, The FFPE sequencing working group tested and established several protocols for toxicogenomic profiling using FFPE liver samples obtained from a rat study with furan. Transcriptomic profiles were compared to those obtained from fresh frozen liver tissue.

In this forum, data from these 3 activities will be presented and potential impacts will be discussed.

Speaker Abstracts

S19: Fundamentals of Histopathologic Image Analysis

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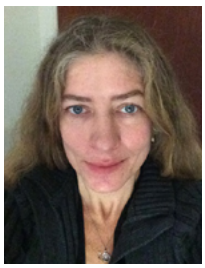

This talk will focus on the basic principles of quantitative histopathologic image analysis from the perspective of the research pathologist. Topics covered will include practical approaches to quantitation; reproducibility, objectivity, and accuracy; the effects of pre-analytical variables on quantitation; study design; and special software considerations.

Case examples will be used to demonstrate key concepts.

Speaker Abstracts

S20: Automated ISH, ISH/IHC double stain and ISH image analysis

Jürgen Funk
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Immunohistochemistry (IHC) and in situ hybridization (ISH) are currently the most commonly used methods for the detection of proteins and nucleic acids in tissue sections and both methods can complement each other. While IHC is established in most laboratories, ISH is regarded as a time-consuming, labor-intensive and technically complex method. Depending on the type of probe used (oligonucleotide or riboprobe) the optimization of this method can be difficult.

A significant improvement in mRNA ISH methodology has been achieved recently. RNAscope, a novel mRNA ISH method, has improved both sensitivity and specificity and also has the capability for full automation on conventional immunostainers. A novel target probe design strategy (a double-Z design) and a hybridization-mediated signal amplification scheme similar to the branched DNA (bDNA) method have been developed. The target probes are unmodified short oligonucleotides and can be uniformly designed for the whole transcriptome and manufactured within a short timeframe. Another advantage is that the ISH method described here works well, with very little or no modification, when different target probe sets and cell/tissue types are used. The bDNA ISH method does not require repeated cycling through elevated temperatures. Because repeated incubation at high temperatures can damage delicate cell morphology, avoiding high-temperature incubations is important for applications in which preservation of intricate cell morphology is important, e. g. automated image analysis. The assay is fully automated, highly reproducible and consistent from run to run, providing more accurate and reliable data.

With this method we were able to add critical and decision-relevant data to several projects in drug discovery and development. We have established ISH to complement IHC in target assessment/ validation and target tissue profiling in different species including animal disease models and humans, e.g. alpha-synuclein (Snca), vascular endothelial growth factor receptor 1 (Flt1/VEGFR1), discoidin domain receptor family member 1 (Ddr1) and cannabinoid receptor 2 (Cnr2). Another application of ISH was for the characterization of transgenic mouse models, e.g. Fc fragment of IgG, receptor, transporter, alpha (FCGRT). Furthermore, ISH was used for mechanistic investigations of critical histopathology findings from toxicology studies, e.g. glucagon-like peptide 1 receptor (Glp1r), hepcidin antimicrobial peptide (Hamp). Due to high specificity and sensitivity with very low background staining, consistency among slides and well preserved tissue morphology, we also successfully applied automated image analysis on full slide scans from ISH (e.g. Hamp). In addition, we were able to establish an ISH/IHC double stain to show co-localization, e.g. growth differentiation factor 15 (Gdf15) with CD68 IHC, endothelial-specific receptor tyrosine kinase (Tek) ISH with CD31 IHC.

In summary, we have established the automated ISH as a supplementary automated tool in drug discovery and toxicology where IHC fails (no antibodies suitable for IHC, i.e. for mouse, rat and minipig, or high unspecific background staining in IHC), to complement IHC for confirmation of staining results or for double staining with IHC. The automated ISH method for sensitive detection of mRNA target sequences overcomes many of the challenges facing ISH techniques today. It helps us to add critical data at all stages of drug discovery and development, enabling early decision making.

Speaker Abstracts

S21: Data Visualization – Visual Communication

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Today in preclinical development and safety testing, new and more refined techniques often combined with more frequent observations result in a continuously growing number of data points. For the first time, the SEND initiative allows to share this data more easily and more rapidly between laboratories, sponsors, and health authorities. It is becoming an important core competence of today´s scientific community to be able to investigate these large data sets efficiently and to communicate significant results appropriately to stakeholders. Software solutions assist in accessing and visualizing the data and to unravel the information behind it. Powerful data visualization across multiple parameters should be a multi-disciplinary area of work involving not only biostatisticians and IT specialists but also the scientist.

Visualization of data already existed in prehistoric times in the form of petrographs describing hunting scenes or wild animals. Later examples include a Babylonian city map (Catalhöyük, 6000 B.C.) and the much more elaborate geographic maps used in subsequent centuries for navigation purposes. Even more time elapsed until time-series plots evolved and were used in scientific publications. In the late 1700s, Johann Heinrich Lambert (1728-1777), a Swiss-Alsacian scientist and mathematician applied a time-series plot to describe the variation of soil temperature in relation to the depth under the surface. Even better known is William Playfair (1759-1823); a Scottish engineer and political economist who is thought to have been the inventor of line graphs, bar charts, pie charts and circle graphs *(Michael Friendly, 2008)*. Great and important data visualizations followed, exemplified by John Snow (1813-1858) mapping fatal cholera cases in London in relation to water pumps in 1854 and by Charles Joseph Minard (1781-1870) visualizing the loss of men during Napoleon´s military campaign to Russia in 1864. In the 18th and 19th centuries, collecting data on economic trade and characteristics of people (census) began to be gathered more frequently. Governments recognized the importance of data to respond to new needs and to make decisions based on predictions.

In the 20th century statistical graphs were often viewed with suspicion. A common perception was that graphs showed trivial information to an amateurish audience. Information graphics were also used for angled news communication or for propagandistic purposes. Then in the late 1960s, John Tukey made statistical graphs respectable again by creating new designs (i.e. box plots) and using them effectively to analyze complex data. Edward Tufte, an influential thought leader in the field of data analysis and visualization, has set the standard for data visualization in his book "The Visual Display of Quantitative Information" (2001). He describes graphical excellence as giving the viewer the greatest number of ideas in the shortest time with the least ink in the smallest space. Furthermore, he describes graphical excellence as being almost always multivariate. On the other hand, he warns of graphical pitfalls such as using two-dimensional visualizations for one-dimensional data (i.e. pie charts) and describes superfluous graphical elements as "chartjunk".

Today, powerful computers and software are at the center of turning large data sets into impressive visualizations and allow unraveling new information. The famous TED talk by Hans Rosling (The best stats you´ve ever seen, 2006) is a well-known example sourcing global economic and health data from publically available sources such as the UN or WHO. It is the same WHO that puts pressure on the pharmaceutical industry to disclose all clinical trial data. Public data sharing has also become the new standard for the EMA. Since 2014, summaries of clinical trials are being published after a drug is approved and additional data can be retrieved upon request. Besides transparency and increased trust, more efficient pharmaceutical research is the potential gain of this paradigm shift.

INHAND Abstracts

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CASE TITLE:	Update on INHAND
ABSTRACT:	The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions) has been operational since 2005. Great progress has been made with 9 rodent organ systems published to date. INHAND representatives serve in advisory role for the FDA SEND initiative. FDA has indicated a preference to utilize microscopic pathology terminology developed by INHAND as these terms will be published in a peer-reviewed journal.

INHAND Abstracts

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CASE TITLE:	Challenges in diagnostic terminology for the lymphoid and hematopoietic organs
ABSTRACT:	Recirculating lymphocytes, complex vascular and lymphatic arrangements, compartmentalization and stromal meshworks are some of the unique features that make nomenclature of the lymphoid and hematopoietic organs challenging. Our current understanding of the structure and function of these organs raises questions about how best to apply standard diagnostic terms such as atrophy and hyperplasia in describing changes in cellularity. The INHAND Lymphoid and Hematopoietic Organ Working Group has developed a two-tiered system of nomenclature for the bone marrow, thymus, lymph node, spleen and MALT to give pathologists the flexibility to tailor terminology to the needs of their studies. Conventional terminology can be used for changes that are best described in broad generalities, such as those seen in chronic carcinogenicity studies. Enhanced terminology can be used in short term studies, such as early investigative studies where detailed descriptive terminology may be more useful. In the forthcoming INHAND document for the lymphoid and hematopoietic organs, enhanced terminology will be coupled with conventional terminology for most lesions.

INHAND Abstracts

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CASE TITLE:	Enhanced Histopathology, Case 1
ABSTRACT:	Enhanced Histopathology is a tool that the pathologist can use to assist in the identification of immunomodulatory drugs and chemicals. It allows for the more precise evaluation of cellular changes within lymphoid organs and can provide insight into the putative target cell population and mechanism of action. Potential changes in cell production and cell death as well as cellular trafficking and recirculation can be assessed. Compartments within each lymphoid organ are evaluated individually and semi-quantitative descriptive terminology is used.
Label on histoslides	n/a
ANIMAL(S):	
Species, breed	B6C3F1 mice
Sex	Male
Age	4 weeks
Study type	4 week gavage
Treatment	2',3'-dideoxycytidine (DDC) and 3'azido-3'deoxythymidine (AZT)
Clinical findings	n/a
Organ(s)	Bone Marrow
Gross finding(s)	n/a
Staining	H&E
LITERATURE:	Elmore, SA. Enhanced histopathology of the bone marrow (2006). *Toxicologic Pathology* 34, 666-86 Elmore, SA. Enhanced Histopathology Evaluation of the Lymphoid Organs (2010). *Immunotoxicity Testing: Methods in Molecular Biology*, vol. 598. RR Dietert (ed.). Humana Press. 323-340 Elmore, SA. Histopathology of the Immune System: A Review and Update (2012). *Toxicologic Pathology*, 40: 148-156
WHAT'S YOUR DIAGNOSIS ?	

INHAND Abstracts

CASE TITLE:	Enhanced Histopathology, Case 2
ABSTRACT:	n/a
Label on histoslides	n/a
ANIMAL(S):	
Species, breed	F344/N rat
Sex	Male
Age	3 months old
Study type	Subchronic gavage
Treatment	N,N-dimethyl-p-toluidine
Clinical findings	n/a
Organ(s)	Spleen
Gross finding(s)	n/a
Staining	H&E
LITERATURE:	Elmore, SA (2006). Enhanced histopathology of the spleen. *Toxicologic Pathology* 34, 648-55.
WHAT'S YOUR DIAGNOSIS ?	

INHAND Abstracts

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CASE TITLE: 1	Thymus lesion in a CD-1 mouse
ABSTRACT:	This case is part of the presentation of the INHAND nomenclature of the immune system, non-proliferative lesions of the thymus.
Label on histoslides	N/A
ANIMAL(S):	mouse
Species, breed	CD-1
Sex	female
Age	18 months
Study type	carcinogenicity study
Treatment	control
Clinical findings	none
Organ(s)	thymus
Gross finding(s)	gross enlargement
Staining	H&E
LITERATURE:	Bradley A, Mukaratirwa S, Petersen-Jones M: Incidences and range of spontaneous findings in the lymphoid and haemopoietic system of control Charles River CD-1 mice (Crl: CD-1(ICR) BR) used in chronic toxicity studies. Toxicol. Pathol 40: 375 - 381; 2012 Pearse, G: Histopathology of the thymus. Toxicol. Pathol 34: 515 - 547; 2006. Ward, J.M, Regh JE, Morse HC III. Differentiation of rodent immune and hematopoietic System reactive lesions from Neoplasias. Toxicol. Pathol 40: 425 - 434; 2012
WHAT'S YOUR DIAGNOSIS ?	

INHAND Abstracts

CASE TITLE: 2	Thymus lesion in a Wistar rat
ABSTRACT:	This case is part of the presentation of the INHAND nomenclature of the immune system, non-proliferative lesions of the thymus.
ANIMAL(S):	rat
Species, breed	Wistar
Sex	female
Age	about 10 weeks
Study type	28 day gavage study
Treatment	20 mg/kg CSA
Organ(s)	Thymus
Gross finding(s)	decreased thymic size
Staining	H&E
LITERATURE:	Elmore, SA (2006) Enhanced histopathology of the thymus. Toxicol.Pathol. 34: 656 - 665. Elmore, SA (2012) Enhanced Histopathology of the Immune System. A Review and Update. Toxicol.Pathol. 40: 148 -156. Schulte A, Althoff J, Ewe S, Richter-Reichhelm HB; BGVV Group Investigators (2002). Two immunotoxicity ring studies according to OECD TG 407-comparison of data on cyclosporin A and hexachlorobenzene. Regul Toxicol Pharmacol. 36: 12 - 21
WHAT'S YOUR DIAGNOSIS ?	

INHAND Abstracts

IHo5: Kuper, Frieke; TNO, Utrechtseweg 48, 3704 HE Zeist, the Netherlands,
Tel. Number: +31 88 866 17 36
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CASE TITLE: 1	Lesions of MALT in rodents
ABSTRACT:	This case is part of the presentation of the INHAND nomenclature of the Immune System, Proliferative lesions of MALT
Label on histoslides	N/A
ANIMAL(S):	
Species, breed	Rat
Sex	male
Age	Approx. 12 weeks
Study type	28-day oral study
Treatment	Hexachlorobenzene
Clinical findings	N/A
Organ(s)	Peyer's patches
Gross finding(s)	Enlarged Peyer's patches
Staining	H&E
LITERATURE:	INHAND nomenclature; Richter-Reichhelm et al. 1995, Regul Toxicol Pharmacol 22:54-56; Kuper et al. 2000, Toxicol Pathol 28:454-466
ADDITIONAL COMMENTS:	The case illustrates challenges related to harmonization of the diagnoses, how to connect with the Enhanced Histopathology, and to what extent a diagnosis can be based on H&E-stained sections only. How to diagnose the prominent HEVs? Are they more prominent because of hypertrophy of the endothelium or because of hyperplasia? Are prominent HEVs always accompanied by increased lymphocyte cellularity or increased size of compartment and are they thus always a sign of activation of the Peyer's patches?
WHAT'S YOUR DIAGNOSIS ?	

INHAND Abstracts

CASE TITLE: 2	Lesions of MALT in rodents
ABSTRACT:	This case is part of the presentation of the INHAND nomenclature of the Immune System, Non-proliferative lesions of MALT. The case may contribute to a discussion on the usefulness of compartments in the diagnosis.
Label on histoslides	N/A
ANIMAL(S):	
Species, breed	Rat
Sex	
Age	
Study type	
Treatment	Untreated control
Clinical findings	
Organ(s)	Peyer's patches
Gross finding(s)	
Staining	H&E
LITERATURE:	Background information has been published in several papers. This particular lesion is not reported in the open literature.
WHAT'S YOUR DIAGNOSIS ?	

INHAND Abstracts

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Tel.Number: +49-61726865897,
E-mail: johannes.harleman@resenius-kabi.com

CASE TITLE: 1	Lymphoma in the rat
ABSTRACT:	The new INHAND tumor nomenclature will be presented including this case study.
Label on histoslides	N/A
ANIMAL(S):	Rat
Species, breed	Wistar
Sex	Female
Age	about 2 year
Study type	Oral carcinogenicity study
Treatment	o mg/kg
Clinical findings	
Organ(s)	Lymph nodes
Gross finding(s)	Increase size of lymph nodes and spleen
Staining	H&E
LITERATURE:	INHAND and Rita nomenclature (see under goreni and ESTP website
WHAT'S YOUR DIAGNOSIS ?	

INHAND Abstracts

CASE TITLE: 2	Myeloid leukemia in the rat
ABSTRACT:	The new INHAND tumor nomenclature will be presented including this case study.
Label on histoslides	N/A
ANIMAL(S):	Rat
Species, breed	Wistar
Sex	Female
Age	about 2 year
Study type	Oral carcinogenicity study
Treatment	o mg/kg
Clinical findings	
Organ(s)	Liver
Gross finding(s)	Increase size of lymph nodes, liver and spleen
Staining	H&E
LITERATURE:	INHAND and Rita nomenclature (see under goreni and ESTP website
WHAT'S YOUR DIAGNOSIS ?	

Case Presentations

CP01: Interactive Slide Session Pathology Cases

ESTP Case 1: Esther Sutter, Novartis Pharma, Basel, Switzerland

CASE TITLE:	A Microscopic Finding in a Mammalian Species Discussed on the Background of the Branchial Apparatus Development
ABSTRACT:	During embryonic development the branchial apparatus gives rise to a variety of structures in the face, neck and mediastinum. The branchial region consists of the three germ layers and forms into distinct arches, clefts and pouches. They further differentiate during embryo-fetal development into numerous tissues and organs, such as skeletal muscles, bone and cartilage as well as the thymus, parathyroid or ultimobranchial bodies. This case report describes an incidental microscopic finding in the mediastinum of a mammalian species. Its varied morphological appearance by H&E made the tissue of origin difficult to discern and this case will be discussed on the background of the branchial apparatus development in an interactive session.
Label on histoslides	No slides provided, pictures only: ESTP Case 1
ANIMAL(S):	Mammalian species
Species, breed	Will be presented during session
Sex	Female
Age	Several weeks
Study type	Investigative
Treatment	None
Clinical findings	None
Organ(s)	Will be presented during session
Gross finding(s)	None
Staining	HE
WHAT'S YOUR DIAGNOSIS ?	

Case Presentations

CP02: Interactive Slide Session Pathology Cases
ESTP Case 2: Pierreluigi Fant, WIL Research, Lyon, France

CASE TITLE:	Disseminated microgranulomas in purpose-bred cynomolgus monkeys from a 4-week toxicological study
ABSTRACT:	A 4-week toxicity study in cynomolgus monkeys was conducted for the evaluation of a peptidic drug candidate administered daily by subcutaneous injection. There were 5 groups receiving the vehicle or ascending doses of the test item. At histopathological examination, multiple small granulomatous foci were observed in the connective/adipose tissue surrounding many organs including the heart, thymus, thyroids and salivary glands, kidneys, pancreas, GI tract, mesenteric lymph node, epididymides and adrenals in 23 out of the 30 monkeys, including controls. The granulomas were generally observed in up to 10 organs in each animal. They were composed of macrophages, multinucleated giant cells and a few neutrophils and eosinophils. Granular, non-polarizing material, generally eosinophilic and occasionally radiate, was observed in the cytoplasm of the giant cells. Special stains of representative lesions did not reveal any infectious agent within the lesions.
Label on histoslides	No slides provided, pictures only: ESTP Case 2
ANIMAL(S):	Non human primate
Species, breed	Cynomolgus monkeys (Macaca fascicularis)
Sex	Both genders
Age	Age at initiation of treatment: 2 to 3 years
Study type	4-week subcutaneous toxicity study
Treatment	Daily subcutaneous administration of peptide in vehicle (5.5 % m/v mannitol in 20mM acetate buffer pH 5.0)
Clinical findings	None
Organ(s)	Fat tissue surrounding several organs
Gross finding(s)	None
Staining	HE, Ziehl-Nielsen, PAS, Gram
WHAT'S YOUR DIAGNOSIS?	

Case Presentations

CP03: Interactive Slide Session Pathology Cases
ESTP Case 3: Peter Maslej, CiToxLAB, Vezprem, Hungary

CASE TITLE:	Liver cords changes in Wistar rat
ABSTRACT:	The change in the hepatocytes and altered staining characteristics can be challenging to observe and grade. The lesion presented in this case was associated with dramatic weight loss in the liver. The features of the lesion and clinical findings will be discussed in an interactive session with pictures from the lesion.
Label on histoslides	No slides provided, pictures only: ESTP Case 3
ANIMAL(S):	Rat
Species, breed	Wistar
Sex	Male
Age	Young adults
Study type	Reproduction/Developmental Toxicity Screening Test by Dietary Administration
Treatment	–
Clinical findings	Keep reserved until presentation
Organ(s)	Liver
Gross finding(s)	Keep reserved until presentation
Staining	HE
WHAT'S YOUR DIAGNOSIS ?	

Case Presentations

CP04: Interactive Slide Session Pathology Cases
ESTP Case 4: Pierre Maliver, F. Hoffmann-La Roche Ltd., Basel, Switzerland

CASE TITLE:	Investigation in new liver and skeletal muscle biomarkers with a reference compound: Clofibrate
ABSTRACT:	The objective of the study was to investigate new liver and skeletal muscle biomarkers in a daily oral gavage study in the rat with Clofibrate. In addition to the classical liver and skeletal muscle biomarkers, CK18 cleaved form, HMGB-1, Arginase-1, GLDH were analysed as new liver biomarkers and Fabp3, Myl3 as new skeletal muscle biomarkers. Histopathological examination revealed clofibrate-induced hepatocellular hypertrophy as well as induced type-1 myofiber degeneration. Fabp3 and Myl3 levels confirmed the skeletal muscle degeneration, with Fabp3 being more specific for type 1 myofiber and also more sensitive than Myl3 or Creatine Kinase. CK18, GLDH and Arginase-1 results confirmed the absence of liver toxicity at the low dose (200 mg/kg/day), despite increased ALT/AST. Increases in CK18, GLDH and Arginase-1 at ≥ 400 mg/kg/day suggested some liver toxicity at these doses. In conclusion the use of these new biomarkers helped in differentiating liver from skeletal muscle toxicity, when used in combination with the classical biomarkers.
Label on histoslides	No slides provided, pictures only: ESTP Case 4
ANIMAL(S):	Rat
Species, breed	Wistar
Sex	Male
Age	12 Weeks
Study type	8 Day oral gavage study
Treatment	Clofibrate at 200/400/750 mg/kg/day
Clinical findings	Rats at 750 mg/kg/day dosed for 2 days and one rat at 400 mg/kg/day were sacrificed for humane reasons on days 3 or 4 (loss of body weight, hypoactivity).
Organ(s)	–
Gross finding(s)	–
Staining	HE
WHAT'S YOUR DIAGNOSIS?	

Case Presentations

CP05: Interactive Slide Session Pathology Cases

ESTP Case 5: Stephanie Klein, DSM, Innovative Medicines, AstraZeneca, UK

CASE TITLE:	Unusual pneumonia in 2 beagle toxicology studies
ABSTRACT:	Unusual pneumonia found in 2 beagle toxicology studies which was not typical for a spontaneous lesion or a treatment-related effect. The lesions resembled those described in pet dogs associated with pathogenic extraintestinal E.coli. PCR was performed on FFPE lung blocks from the 2 studies and confirmed the presence of pathogenic extraintestinal E.coli. Therefore these results allowed the progression of both compounds as the pneumonias observed in treated animals were confirmed to be spontaneous in nature
Label on histoslides	3M, 3002, 13. 4M 4002, 31.
ANIMAL(S):	Dog
Species, breed	Marshall beagle dogs
Sex	Males
Age	Young adults
Study type	1 month oral gavage
Treatment	–
Clinical findings	Respiratory distress
Organ(s)	Lungs
Gross finding(s)	Hemorrhagic-necrotizing pneumonia
Staining	HE, Gram
WHAT'S YOUR DIAGNOSIS ?	

Poster Abstracts

P01: Early renal changes in a mouse model of radiation nephropathy

G. Pellegrini[1], S. Wunderlin Giuliani[1], K. Siwowska[2], C. Müller[2] and A. Kipar[1]

[1] Laboratory for Animal Model Pathology, Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich;
[2] Center for Radiopharmaceutical Sciences ETH-PSI-USZ, Paul Scherrer Institute, Switzerland

Radiation toxicity of the urinary tract is a clinically relevant entity in patients who have received targeted radionuclide therapy and often presents as a delayed, though abrupt reaction after a latency period of at least 6-12 months. Accordingly, in rodent preclinical models, the earliest, generally subtle, morphological changes in the kidneys are detected only several weeks or months after exposure to the radioactive dose, especially in mice, which are known to be relatively resistant to radiation-induced renal injury. We present here the first results of our investigation into the pathophysiological aspects of early stage radiation injury and suggest a morphological work up that can be useful for the early detection of radiation nephropathy in mice and the comparison of different radiotherapies, without undertaking expensive, long term studies.
In our studies, we used folate-based radiopharmaceuticals coupled with radionuclides (lutetium-177 and yttrium-90), which have a promising potential for targeted tumour therapy, but also accumulate in the kidneys, due to folate receptor expression in the proximal tubule cells. They were administered i.v. in different quantities (5, 10 and 20 MBq) to athymic nude mice. Animals were euthanised at 13 and 90 days post treatment (dpt). A histological, immunohistological and ultrastructural examination of the kidneys, aiming at the detection of early nephrotoxic changes, was undertaken. The results were retrospectively compared to those obtained from earlier studies in mice euthanised at 9 months post lutetium-177 exposure.
Whilst no histological abnormality was detected in the HE-stained kidney sections of mice euthanised at 14 dpt, we observed a prominent dose-related increase in the number of cells expressing phosphorylated H2A-x, a marker of DNA damage. The positive cells were tubular epithelial and glomerular cells, several of which were identified as endothelial cells, based on their co-expression of factor VIII-related antigen. Moderate numbers of cleaved caspase 3-positive apoptotic tubular epithelial and glomerular endothelial cells were observed in mice that had been exposed to high radiation doses. In selected animals there was evidence of mesangial cell activation, indicated by expression of α-SMA. At 90 dpt, kidneys exhibited subtle histological changes (nuclear enlargement and tubulolysis) and only a few cells expressing H2A-x and cleaved caspase 3. Severe, "classical" radiation nephropathy, dominated by cortical scarring, thrombotic microangiopathy, mesangiolysis and tubular changes was detected in the mice examined at 9 months post radionuclide exposure.
Our study showed that selected morphological changes can be identified in the kidneys of nude mice already at an early stage after application of radiopharmaceuticals. These are mainly represented by evidence of DNA damage in endothelial cells and mesangial activation. These changes likely represent the first steps in the pathogenesis of radiation nephropathy, which then only manifests several months later, as a complex injury involving glomeruli, tubules, interstitium and blood vessels. Further studies are needed to fully characterise the early stages of radiation nephropathy and identify novel biomarkers that can indicate the onset of renal radiation injury long before manifestation of the disease.

Poster Abstracts

P02: Immunohistochemical techniques in guinea pig FFPE tissues. The use of tuberculosis infection model to label immune cells

A. Watkins, D. Grainger, F. Ryrie, E. Rayner, A. Rawkins, S. Di Palma, R.M. La Ragione, F.J. Salguero

Dept. of Pathology and Infectious Diseases. School of Veterinary Medicine. University of Surrey

Guinea pigs are used in numerous studies as models of infection and toxicity. However, immunohistochemistry (IHC) techniques on guinea pig derived tissues have proven to be challenging due to both the lack of available primary antibodies raised against guinea pig epitopes and the poor cross reactivity of many antibodies raised against other species (including humans). In this study, we have used a guinea pig infection model of Mycobacterium tuberculosis. The hallmark lesions of tuberculosis is the granuloma, and the fact that guinea pigs develop granulomas similarly to those observed in humans, makes it a good surrogate model for this disease.

The granuloma is formed as a local immune reaction within tissues and is characterized by the presence of numerous macrophages, neutrophils and lymphocyte subsets. Here, we used a variety of primary antibodies raised against guinea pig cell markers (CD3) or human markers (CD3, MAC387, CD79, iNOS and IDO) to standardize protocols for immunohistochemistry and localize specific cells expressing the markers of interest.

We used a variety of epitope demasking techniques (high temperature antigen retrieval with different buffers and enzymatic digestion) and the avidin-biotin peroxidase technique (Pierce). All the IHC runs included isotype control and OMIT slides. These studies successfully standardized protocols to label CD3+ T lymphocytes (with both an anti guinea pig and an anti-human antibody), myeloid cells using the MAC387 antibody and the immunomodulatory enzyme indolamine 2,3-dioxygenase (IDO). The development of the above protocols will enhance pathological interpretation of tissues derived from studies conducted in the guinea pig.

Poster Abstracts

P03: In vitro organ culture (IVOC), an alternative to In vivo studies

R.M. La Ragione, F.J. Salguero, S. Di Palma

Dept. of Pathology and Infectious Diseases. School of Veterinary Medicine. University of Surrey; Royal Surrey County Hospital, Guildford, Surrey

The principles of 3Rs in animal research (Replacement, Reduction & Refinement) were developed over 50 years ago and have been embedded in the international legislation regulating the use of animals in research. In the recent years, there has been great progress in the replacement of animals in fundamental research and the pharmaceutical industry. To reach this aim, many alternative in vitro models have been developed to avoid or minimize the use of animals in research.
Two such models developed in our laboratory are 3D cell culture and In Vitro Organ Culture (IVOC). In the studies presented here we developed novel porcine and human 3D cell culture models using the Rotating Wall Vessel System (RWVS). Moreover, porcine, bovine, chicken and ovine intestinal in vitro organ culture (IVOC) models were developed by using cell crown (CC) technology (CCIVOC) (Scaffdex).
To date the models have been used to study host microbe interactions with a number of pathogens including E. coli, Salmonella, Clostridia, Brachyspira, Yersinia and viruses. The models have also been used to assess the efficacy of pre and probiotics and to assess the toxicity of a number of compounds.

Poster Abstracts

P04: The effect of fasting and refeeding on liver and biomarker levels in laboratory mice

Saulol Hamid NF [1,2], Williams D[3], Antoine D[1], Benson C[1], Webb H[1], Kipar A[1,2,4]

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[4] Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich

Food availability affects the basal metabolism and therefore the function of the liver as the major metabolic organ. Fasting of mice changes several physiological parameters and moreover, these changes are depending on a variety of environmental factors. In experimental settings, fasting is primarily used as a way to ensure uniform drug absorption and reduce the variability in basal blood glucose levels. Both is helpful in toxicology tests and pharmacokinetics studies in general and helps to reduce the number of animals per dosing group to achieve statistical significance. Since mice are primarily nocturnal and consume two-thirds of their total food during the night, the effect of food deprivation is likely greatest during the dark phase (scotophase), the time where the animals are most active. Previous studies have shown that fasting can lead to a reduction of basal metabolic rates, which can persist after food re-introduction, even when the body weight is returning to a normal range.

To gather reliable baseline data that would allow better interpretation of the results from acute drug induced liver injury studies and to understand the effects of fasting on hepatocyte function, we undertook a 24 hour time course study to assess the hepatic GSH content, serum ALT levels, and the morphological features of the liver in male CD-1 and C57BL/6J mice that had been consistently fed ad libitum, or had been fasted for 16 or 24 hours and then refed for different time spans thereafter.

After fasting, all mice had lost a proportion of body weight, the extent of which was variable and related to the time of day when the animals were first deprived of food, ranging between 5% (onset of fasting between 3 and 6 am) and almost 12% (onset during daytime or in early evening). Serum ALT levels were highly variable in both fed and fasted mice, without any significant effect of fasting. GSH levels were consistently higher in C57Bl6 mice and followed a circadian rhythm (highest in the morning). Fasting reduced the levels to evening levels in fed mice, but refeeding led to overshooting GSH levels for several hours. Upon completion of the fasting period, livers appeared devoid of glycogen; however, this fully restituted rapidly upon refeeding, within 1 hour (C57BL/6J mice) and 4 hours (CD-1 mice).

It has been shown that body weight and food intake can vary substantially between mouse strains. However, this appears not to affect the average weight loss that is seen after fasting. Instead, our study highlights again the relevance of the time of day for the degree of weight loss that is seen as a consequence of fasting. This is important to consider when starting an experiment with fasted mice, in particular since mice that have already lost more than 10% of their body weight will reach the endpoint of a permitted experiment after a relatively moderate additional weight loss due to treatment. Our results confirm the effect of fasting on hepatic GSH levels, but also highlight the additional effect of refeeding. All these factors, together with the known circadian variation of a range of markers, can affect the outcome of acute hepatotoxicity studies in mice.

Poster Abstracts

P05: Toxicity in modern times: Ipilimumab-induced perforating colitis.

S. Di Palma, F.J Salguero, I. Fry, R.M. La Ragione

Dept. of Histopathology. Royal Surrey County Hospital, Guidlford, Surrey, U.K.; Dept. of Pathology and Infectious Diseases. School of Veterinary Medicine. University of Surrey, Guildford, U.K.

Ipilimumab, a monoclonal antibody anti-CTLA-4 (cytotoxic T-lymphocyte-associated antigen 4), is an approved treatment modality for metastatic melanoma which has improved survival rates. One of the most common side effects associated with ipilimumab is diarrhoea and colitis. The cause of the ipilmumab colitis is believed to be an immune-related adverse event usually controlled by using systemic steroid therapy. In rare occasion colitis is complicated by perforation and thus requires urgent surgical resection of colon. A review of the literature has revealed numerous cases of ipilimumab-induced colitis, but only in a minority of them were included a full description of histological findings.
Here we report a case of perforating colitis in a patient treated with ipilimumab for metastatic melanoma. The patient required urgent subtotal colectomy after perforation due to the severity of his ipilimumab-induced colitis. The essential histologic findings assessed on the colectomy specimen showed fissuring ulcers with evidence of perforation, dilated crypts, pseudopolyps and increased inflammatory cells infiltrate in lamina propria not associated with an increased number of intraepithelial lymphocytes.
This study illustrates that the medical profession needs to become more aware that modern treatments such as humanised monoclonal antibody like ipilimumab are associate with severe toxicity.

Poster Abstracts

P06: Prednisolone enhances Candida albicans-induced mucosal proliferation and chronic inflammation in alloxan-induced diabetic rats

Yui TERAYAMA, Yusuke NAKAJIMA, Yuto HUKATSU, Shiori YOSHIDA, Tsuyoshi MORIO, Tetsuro MATSUURA, Isao NARAMA, Kiyokazu OZAKI

Laboratory of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University

Introduction: Chronic oral and esophageal infections with Candida albicans (C. albicans) in human are sometimes associated with mucosal epithelial hyperplasia rarely progressing to carcinoma. Previously, we reported that alloxan-induced diabetic rats have proliferative lesions of the squamous epithelium with chronic inflammation and C. albicans infection in the forestomach, and that some advanced to squamous cell carcinoma (SCC), but the carcinogenic rate is low in this model. In the present study, we investigate whether immunosuppression may accelerate early-onset of C. albicans infection and the subsequent proliferative and inflammatory changes in alloxan-induced diabetic rats.

Methods: Female WBN/Kob rats were divided into 3 groups; alloxan-induced diabetic rats (A group), alloxan-induced diabetic rats treated with prednisolone (10mg/kg) once every 2 weeks (AP group), and non-diabetic rats treated with prednisolone (10mg/kg) every 2 weeks (P group). They were sacrificed at 30 weeks after initial treatment of prednisolone, and the proliferative and inflammatory changes were analyzed at the upper alimentary tract using histopathological and immunohistochemical (CD68, CD163, CD204, CD3, CD45RA and myeloperoxidase) techniques.

Results: Squamous cell hyperplasia and lymphoplasmacytic infiltration (chronic inflammation) in the submucosa and lamina propria were observed in almost all rats of the AP and A groups, with highest severity of the lesions in the AP group. In addition, squamous cell carcinoma was detected in one rat of AP group. Mucosal surface of squamous cell hyperplasia was infiltrated by C. albicans, neutrophils and macrophages in both AP and A groups.
Discussion: These findings strongly suggest that prednisolone may enhance proliferative squamous cell changes accompanied by chronic inflammation associated with C. albicans infection in the forestomach in alloxan-induced diabetic rats.

Poster Abstracts

P07: Right Ventricular Cardiomyopathy in Male Rats

GA Willson, RA Miller and D Malarkey

Experimental Pathology Laboratories, Cellular and Molecular Pathology Branch, National Toxicology Program, National Institute of Environmental Health Sciences

The histopathological examination of the heart in rodent toxicity studies is an important endpoint. Spontaneous cardiomyopathy in the Sprague Dawley (SD) and F344/N rats may interfere with interpretation of treatment-related cardiotoxicity since spontaneous lesions may overlap morphologically with lesions associated with toxic effects.
There has been concern that some cardiomyopathy, observed in the right ventricular epicardium, may be uniquely positioned, dorsally, and possibly related to the gavage administration technique.
Hearts were evaluated in control male Harlan Sprague Dawley rats (gavage = 1 group, non-gavage = 4 groups; with 10 per group) and control F344/N rats (gavage = 10 groups, non-gavage = 10 groups; with mostly 10 per group). H&E sections of over 200 hearts from 90-day studies were evaluated for lesions in the right ventricle.
The percentage of gavaged SD rats with cardiomyopathy was 20% with an average severity of 1.5. The non-gavaged SD rats percentage affected was 25% with a mean severity of 1.2. The gavaged F344/N rat percentage with cardiomyopathy was 28% with a mean severity of 1.25. The non-gavaged F344/N rat percentage with cardiomyopathy was 26% with a mean severity of 1.33.
The observed cardiomyopathy was similar in HSD and F344 male rats. Morphologically it was the same regardless of the dose administration modality. This epicardial and subepicardial lesion was characterized by a minimal to mild, focal and multifocal, infiltrate of mononuclear inflammatory cells and variable degrees of fibrosis. Occasionally, there was only a thin fibrous connective tissue scaffold with few inflammatory cells along the epicardium. Occasional neutrophils and myocardial necrosis were present within the lesions. The lesions generally occurred along the dorsal one half of epicardium of the heart (towards the base) of the right ventricle.
In this sample set of control male rats, it is apparent that this epicardial lesion is a spontaneous lesion. The lesion was similar in incidence and severity in HSD and F344 male rats and not affected by the modality of exposure. In this series of rats it appears to be unrelated to the gavage technique of exposure.

Poster Abstracts

P08: Efficacy and safety of hyaluronic acid and adipose tissue derived stem cell with hyaluronic acid in treatment of steroid-induced osteonecrosis

Hye-Rim Lee, Hanjun Kim, Kyung Hyun Lee, Sun Hee Do*

College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea

Purpose of the study: Steroid-induced osteonecrosis of the femoral head is a serious complication in patients who have received steroids for the treatment of various conditions, including nephrotic syndrome, renal transplantation, and systemic lupus erythematous. As a degenerative bone disease, it leads to the collapse of the femoral head, destroying the functional hip joint and limiting patient activity. Current, treatments are limited in their ability to enhance bone repair and to prevent collapse of the articular surface and the need for hip arthroplasty. The aim of this in vivo study was to assess the potential of a novel hyaluronic acid base hydrogel with or without stem cells to stimulate bone regeneration following induction of steroid induced femoral head osteonecrosis.

Material and methods: 6 week-old age Sprague-Dawley rats were given lipopolysaccharide (20μg/kg) and methylprednisolone (MPS, 40 mg/kg) to establish the steroid induced osteonecrosis model. 4 weeks after MPS implantation, all the rats in LPS/MPS were randomized into 3 groups: 5 rats from were given hyaluronic acid (HA) hydrogel by intraarticular injection (HA group), 6 rats were given adipose tissue-derived stem cells (ADSC)/HA hydrogel (ADSC/HA group), and 3 rats received a volume of normal saline equal to the amount of HA (vehicle control group). After 8 weeks, serum biomarkers, micro-CT and histological examination were performed to compare the incidence of osteonecrosis and trabecular parameters of the femoral head.

Results: Rats in LPS/MPS showed successfully induction of osteonecrosis by steroid at 4 weeks. Following treatment for 8 weeks the HA and ADSC/HA groups had a lower incidence of osteonecrosis compared with vehicle control group.. Micro-CT and histological examination showed higher trabecular volume and trabecular thickness, and lower empty lacunae in HA and ADSC/HA group compared with vehicle control group. No statistical difference was seen between the ADSC/HA and HA groups. Serum Rankl, osteocalcin, ALP, and osteoproteogrin were not significantly different between groups

Conclusion: In this study a single intraarticular injection of HA reduced the degree of for steroid induced osteonecrosis in rats without any toxicologic and nonspecific reaction. Further studies are needed to investigate whether this is a potential therapy for osteonecrosis in humans.

Poster Abstracts

P09: Historical Background Incidence of Spontaneous Pituitary Gland Lesions of Control Rats and Charles River Mice Used in 104-Week Toxicity Studies

Claudio Petterino, Sydney Mukaratirwa, Alys Bradley

Department of Pathology, CHARLES RIVER Preclinical services

Introduction: The aim of this study was to provide the range and incidences of spontaneous lesions of the pituitary glands in control rats (Wistar Han and Sprague Dawley) and CD-1 mice from 104-week studies carried out at Charles River, Edinburgh.

Experimental Design: Data were collected retrospectively from control animals over a 12-year period giving a total of 2363 control CD-1 mice (1171 males; 1192 females), and over a period of 10 years giving a total of 1924 Wistar Han rats (933 males; 991 females) and 667 Sprague Dawley rats (333 males; 334 females).

Methods and Materials: All control animals were obtained from groups of animals that had been sham dosed with an appropriate vehicle. Tissues were examined histologically, and the findings were entered directly into a validated computerized database. Each study was subjected to an internal peer review and all data reviewed by the Quality Assurance Department. Results: In both Wistar Han and Sprague Dawley rats the incidence of proliferative lesions was higher than that of non-proliferative lesions. The incidence of non-proliferative lesions was higher in males than in female Wistar Han rats. In contrast, proliferative lesions were more common in females than in males of both rat strains. Secondary neoplastic lesions were rare in both sexes of either Wistar Han or Sprague Dawley rats. In Wistar Han rats, the incidence of pituitary cysts and cholesterol clefts was higher in males than in females. Moreover, adenoma and carcinoma of the anterior lobe had a significantly higher incidence in females than in males, and were higher in Sprague Dawley rats when compared to Wistar Han rats. In CD-1 mice non-proliferative lesions had a higher incidence in males than in females, but for proliferative lesions the incidence was higher in females than in males. Secondary neoplastic lesions were of similar incidence in both sexes, with infiltration by lymphoma the most represented secondary neoplastic lesion. Similarly to Wistar Han rats, the incidence of pituitary cysts and cholesterol clefts was higher in males than in females. In contrast, hyperplasia of the intermediate lobe and carcinoma of the anterior lobe were significantly higher in females than in males.

Conclusions: To the best of our knowledge this is the most comprehensive combined study of the incidences of background lesions in pituitary gland in control rats and CD-1 mice. Impact Statement: The results represent a useful source for the incidence of spontaneous findings in pituitary glands in control rats and CD-1 mice from 104-Week carcinogenicity studies.

Poster Abstracts

P10: Historical Background Incidence of Spontaneous Thyroid and Parathyroid Glands Lesions of Control Rats and CD-1 Mice Used in 104-Week Toxicity Studies

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Introduction: The aim of this study was to provide the range and incidences of spontaneous lesions of the thyroid and parathyroid glands in control rats (Wistar Han and Sprague Dawley rats) and CD-1 mice from 104-week studies carried out at Charles River, Edinburgh. Experimental Design: Data were collected retrospectively from control animals over a 12-year period giving a total of 2,551 CD-1 mice, 1,877 Wistar Han and 662 Sprague Dawley rats.

Methods and Materials: All control animals were obtained from groups of animals that had been sham dosed with an appropriate vehicle. Tissues were examined histologically, and the findings were entered directly into a validated computerized database. Each study was subjected to an internal peer review and all data reviewed by the Quality Assurance Department.

Results: In both strains of rats and in CD-1 mice included in the current study non-proliferative lesions of thyroid and parathyroid glands were uncommon. Similarly, secondary neoplastic lesions either in thyroid or parathyroid gland were also poorly represented. In Wistar Han rats, proliferative lesions were slightly more frequent in males than in females but in Sprague Dawley rats were of similar incidence in both sexes. Follicular cell hyperplasia, follicular cell adenoma and carcinoma, and C cell hyperplasia were significantly higher in Wistar Han than in Sprague Dawley male rats. Moreover, follicular cell hyperplasia and follicular cell adenoma occurred at significantly higher incidence in males compared to females Wistar Han rats. Interstitial fibrosis and hypertrophy of parathyroid gland were significantly more common lesions in females Sprague Dawley than in Wistar Han rats. Hypertrophy of parathyroid gland was also more common in females than males Sprague Dawley rats. Hyperplasia was the most common proliferative lesion in the parathyroid gland, and it was significantly higher in males than females Wistar Han rats. In CD-1 mice the most common non-proliferative lesions were represented by ulitmobranchial duct, follicular distension/dilation and cysts. Mononuclear cell/lymphocytic infiltration and follicular distension/dilation were significantly more common in females than in males CD-1 mice.

Conclusions: To the best of our knowledge this is the most comprehensive combined study of the incidences of background lesions in thyroid and parathyroid glands in control rats and CD-1 mice and can represent a useful source for the incidence of spontaneous findings.

Poster Abstracts

P11: Safety Assessment of Cyclodextrin Glucanotransferase: Genotoxicity Battery and 90-Day Rat Toxicity Study

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Introduction: Cyclodextrin glucanotransferase (CGTase) is used commercially in the manufacture of food, pharmaceuticals, and cosmetics. In anticipation of its use in production of alpha-glycosyl isoquercitrin, a water-soluble form of quercetin, microbiologically derived CGTase was evaluated for its toxic potential.

Methods: CGTase, produced by Bacillus pseudalcaliphilus DK-1139, was evaluated in a genotoxicity battery following OECD guidelines and included a bacterial reverse mutation assay, in vitro and in vivo micronucleus (MN) assays, and a comet assay using B6C3F1 male and female mouse tissues. These same genotoxicity assays were also conducted for sodium sulfate, a contaminant of CGTase preparation. In addition, CGTase was administered by gavage in water at daily doses of 0, 250, 500, and 1000 mg/kg/day in a 90-day toxicity study in Sprague Dawley rats.

Results: CGTase did not induce mutations with or without metabolic activation in the bacterial reverse mutation assay. Micronuclei were not induced either in in vitro with or without metabolic activation or in male or female mice. Furthermore, there was no induction of DNA damage in male or female mouse liver, stomach, or duodenum in the comet assay. Sodium sulfate also tested negative in these same genotoxicity assays. In the 90-day repeated dose rat study there were no treatment-related adverse clinical or pathological findings.

Conclusion: The genotoxicity assays and repeated dose toxicity study support the safe use of CGTase in production of alpha-glycosyl isoquercitrin.

Poster Abstracts

P12: Assessment of new preclinical liver and skeletal muscle biomarkers in Clofibrate-induced toxicity in male Wistar rats

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Background: Clofibrate, an agonist of the peroxisome proliferator activated receptor (PPAR)-α, is a known hepatoxicant in rodents. Peroxisome proliferation is mainly seen in the liver and is classically associated with hepatocellular hypertrophy, induction of the Cyp450 enzymes of the fatty acid β- oxidation system, upregulation of cellular alanine aminotransferase (ALT) activity and increased serum ALT concentration in the absence of hepatocellular damage. At toxic dose, clofibrate induces liver and skeletal muscle injury in rats (myofiber type 1).

The objective of this investigative study in Wistar rats was to assess novel biomarkers presumed to be more sensitive or specific for cellular apoptosis or necrosis (CK18 cleaved form , HMGB-1) and hepatocellular injury (GLDH, Arginase-1) in comparison to ALT/AST levels. Additional skeletal muscle biomarkers (Fatty acid binding protein 3 and myosin light chain 3) were also investigated to allow a better differentiation of liver from skeletal muscle injury.

Experimental procedure: Male Han Wistar rats received oral (gavage) doses of 0, 200, 400 or 750 mg/kg/day for up to seven consecutive days at 10 mL/kg body weight. Rats at 750 mg/kg/day dosed for 2 days and one rat at 400 mg/kg/day were sacrificed for humane reasons on days 3 or 4. Blood sampling for biomarkers and standard clinical pathology assessment was conducted on day 3/4 and 8, the day of sacrifice. Histopathological examination was performed on liver, heart, diaphragm, soleus muscle, kidney and quadriceps muscle tissues.

Results: Hepatocellular hypertrophy was noted dose dependently in all treated groups and correlated with increased ALT levels. Hepatocellular necrosis was not observed but increased CK18, Arginase-1 (and GLDH at a lesser extent) levels at 400 (transiently) and 750 mg/kg/day suggested some liver injury at ≥ 400 mg/kg/day. The absence of CK18/GLDH/Arginase-1 response at 200 mg/kg/day, despite increased AST and ALT levels, excluded liver toxicity at this dose. Skeletal muscle myofiber degeneration in the type I fiber rich Diaphragm was noted in all treated groups and was after 8 days associated with regeneration. Type I M. soleus was also affected. The degeneration correlated with a moderate increase of CK, Myl3 and AST, but a more pronounced elevation of Fabp3 on day 3/4 indicated early acute toxicity. Global decrease of skeletal and liver biomarkers levels on day 8 indicated a subsequent adaptation to the clofibrate exposure.

Conclusion: The new liver and skeletal muscle biomarkers helped in differentiating liver from skeletal muscle toxicity. Comparing classical (AST/CK) and novel skeletal muscle biomarkers, our study confirmed the higher sensitivity and dynamic range of Fabp3 to identify type I myofiber muscular injury. GLDH was less sensitive than CK18 and Arginase-1 regarding liver injury. Nevertheless, the relevance of increased CK18/Arginase-1 biomarker levels, in the absence of hepatocellular necrosis in this study, needs to be further investigated.

Poster Abstracts

P13: Evaluation of developmental neurotoxicity of cuprizone as a demyelinating agent in a framework of 28-day repeated oral toxicity study

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Introduction: Developmental neurotoxicity studies under the current testing guidelines are time consuming and require large numbers of animals to evaluate the toxicity of one chemical. Therefore, more efficient screening system of developmental neurotoxicants is necessary to be established. In the brain structure, the hippocampal dentate gyrus uniquely conducts adult neurogenesis in the subgranular zone (SGZ) during postnatal life. Of note, all of the cell populations and their inherent phenomena involved in this process may be a sensitive target of developmental neurotoxicity. Especially, self-renewal of stem cells, proliferation and migration of progenitor cells, neuritogenesis, synaptogenesis and myelinogenesis may be the vulnerable developmental processes against chemical toxicity. Thus, adult neurotoxicants targeting myelin sheath and myelination may cause developmental neurotoxicity. This study was undertaken to confirm whether cuprizone (CPZ) as a myelin toxicant could affect neurogenesis after developmental exposure and to examine whether similar effects could be obtained in a standard 28-day toxicity study using rats.

Materials and methods: In the developmental exposure study, CPZ was given to maternal SD rats at 0, 0.1 or 0.4% in diet from gestational day 6 until postnatal day (PND) 21 on weaning. In the postpubertal-stage exposure study, 5-week-old male SD rats were treated with 0, 120 or 600 mg/kg of CPZ by oral gavage for 28 days. In both studies, distribution of granule cell lineages as well as their proliferation and apoptosis in the SGZ and GABAergic interneurons in the hilus of the hippocampal dentate gyrus were analyzed at the end of exposure.

Results: Both developmental and postpubertal-stage CPZ exposure studies revealed increase in the number of TUNEL+ or cleaved caspase 3+ apoptotic cells in the SGZ, accompanied by transcript upregulation of Casp4 and Casp12 in the dentate gyrus. In addition, both studies revealed decrease in the number of TBR2+ type-2b and type-3 progenitor cells in the SGZ, and decreased density of phosphorylated TrkB+ interneurons in the dentate hilus, accompanied by transcript downregulation of Bdnf and Chrna7 in the dentate gyrus, and decreases in the number of granule cells expressing immediate-early genes (IEG), i.e., ARC and FOS, in the granule cell layer. In the postpubertal-stage CPZ exposure study, decreases in the number of DCX+ type-2b and type-3 progenitor cells and immature granule cells and NEUN+ immature and mature granule cells and PCNA+ proliferating cells were additionally detected. In the developmental exposure study, increase in the density of reelin+ interneurons in the dentate hilus was additionally detected.

Conclusion: Both developmental and postpubertal-stage CPZ exposure caused endoplasmic reticulum stress-mediated apoptosis of the granule cell lineages and impaired intermediate and late-stage neurogenesis following suppression of IEG-mediated neuronal plasticity in the rat hippocampus. These results suggest that CPZ targeting myelin sheath and myelination could affect neurogenesis as a developmental neurotoxicant. Thus, it is suggested that adult neurotoxicants could be developmental neurotoxicants and the hippocampal neurogenesis could serve as a promising evaluation endpoint for detection of developmental neurotoxicity in a scheme of 28-day toxicity study.

Poster Abstracts

P14: Rat subacute 4-week oral toxicity study in the context of translational research exemplified by four immunomodulating compounds

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In the framework of the OECD 407 subacute 4-week rodent study, two immunosuppressive drugs, cyclosporine A (CSA) and azathioprine (AZA), and two environmental pollutants, hexachlorobenzene (HCB) and benzo[a]pyrene (BAP), were investigated in rats. The compounds investigated induced different types of primary immune effects such as immunosuppression or immunostimulation. The studies were conducted to validate methods for evaluation of immune effects. Moreover, literature data were investigated regarding translation of the results to primates with specific emphasis to humans since there is a perceived gap between the knowledge on animals versus man.
Regarding the methods applied, in addition to routine immunotoxicological investigations like lymphoid organ weighing and detailed histopathological investigations of immune organs, functional testing and analysis of lymphocyte subpopulations were included.
CSA and AZA showed a high similarity of effects in animals and humans as the immune system was the most sensitive in both. The primary effect of CSA was reduced T-cell activity and the effect of AZA was suppression of bone marrow cells in rats and humans.
In contrast, for BAP and HCB the correlation between animal and human findings was less clear. Exposure rates and routes of administration in animals (subacute oral toxicity study) and humans (chronic environmental exposure via different routes of uptake) are so different, that results of the toxicity studies seem to have low relevance for humans. BAP caused bone marrow suppression and lymphoid atrophy in rats. These findings are not known to occur in humans. HCB lead to macrophage activation and inflammation mainly in skin and lungs of rats. In humans or primates, relationship to immunomodulating effects was inconclusive and toxic effects were mainly seen in the liver.
An in vitro plaque-forming cell assay, the MD (Mishell-Dutton) culture, was conducted with BAP using spleen cells from rat and human. Comparable inhibition of the in vitro immune responses was obtained with cells of both species.
In summary, the translation of effects in the immune system from animals to man is a complex terrain. A direct in vitro comparison of human and animal cells may further help to unravel the mode of action and species sensitivity regarding induced immunomodulation.

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Kemmerling J., Fehlert E, Rühl-Fehlert C., Kuper C.F., Stropp G., Jack Vogels, Krul C., Vohr H-W., (2015) The transferability from rat subacute 4-week oral toxicity study to translational research exemplified by two pharmaceutical immunosuppressants and two environmental pollutants with immunomodulating properties. Eur J Pharmacol 759, 326-342
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Poster Abstracts

P15: Resveratrol alleviates diabetes-induced testicular dysfunction by inhibiting oxidative stress and c-Jun N-terminal kinase signaling in rats

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Diabetes adversely affects reproductive functions in humans and animals. The present study investigated the effects of Resveratrol (3,5,4-trihydroxy-trans-stilbene; a natural phytoalexin) on diabetes-induced alterations in oxidative stress, c-Jun N-terminal kinase (JNK) signaling and apoptosis in the testis. Adult male Wistar rats (13-15 weeks; n=6/group) were segregated into normal control, Resveratrol-treated (5 mg/kg; ip; given during last 3 weeks), Streptozotocin-induced diabetic (this drug does not induce testicular damage) and, Resveratrol-treated diabetic groups, and euthanized on day 42 after the confirmation of diabetes. Resveratrol recovered diabetes-induced decreases in reproductive organ weights, sperm count and motility, intra-testicular levels of superoxide dismutase, catalase, and glutathione peroxidase and an increase in 4-hydroxynonenal activities ($P<0.05$). Resveratrol also recovered diabetes-induced increases in JNK signaling pathway proteins, namely, ASK1 (apoptosis signal-regulating kinase 1), JNKs (46 and 54 kDa isoforms) and p-JNK to normal control levels ($P<0.05$). Interestingly, the expression of a down-stream target of ASK1, MKK4 (mitogen-activated protein kinase kinase 4) and its phosphorylated form (p-MKK4) did not change in experimental groups. Resveratrol inhibited diabetes-induced increases in AP-1 (activator protein-1) components, c-Jun and ATF2 (activating transcription factor 2), but not their phosphorylated forms, to normal control levels ($P<0.05$). Further, Resveratrol inhibited diabetes-induced increase in cleaved caspase-3 to normal control levels. In conclusion, Resveratrol alleviates diabetes-induced apoptosis by modulating oxidative stress, JNK signaling pathway and caspase-3 in rat testis. These results suggest that Resveratrol supplementation may be a useful strategy to treat diabetes-induced testicular dysfunction.

Poster Abstracts

P16: Lead acetate exposure induces gradual hepatocyte degeneration and hepatocyte proliferation in rats

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The heavy metal- Lead adversely affects several organ systems and interferes in normal cellular functions. The present study investigated effects of 0.5% and 1% Lead exposure on ultrastructural changes and cell proliferation in the liver. Adult male and female Wistar rats were exposed to the Lead doses through drinking water for 14 and 35 days and, in each case, the rats were sacrificed the next day. Transmission electron microscopic evaluation of the livers from 0.5% group showed time-dependent hepatocyte degeneration as indicated by disappearance of glycogen granules, vacuolization, and marginalization of cellular organelles such as mitochondria, nuclear and nucleolar shrinkage and degeneration, chromatin marginalization, and degenerative changes in organelles- mitochondria, endoplasmic reticula, ribosomes, and Golgi apparatus, and degeneration of intercellular junctions. In addition, enlargement of bile canaliculi was also observed indicating possible bile stasis. Immunofluorescent microscopy for Ki-67, a cell proliferation marker, showed significant increases in hepatocyte proliferation on both sampling times, but without any dose- or time-dependent effects. In conclusion, our results indicate that Lead exposure causes severe hepatocyte degeneration in parallel with significant hepatocyte proliferation. The latter effect suggests a possibility of hepato-carcinogenic properties of Lead in rats.

Poster Abstracts

P17: Spermatogenesis in the Gottingen Minipig: A Guide for Morphological Staging

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The Gottingen minipig is a promising non-rodent species for use in preclinical safety studies and the male reproductive tract is an important potential target system to be evaluated for toxicity. Histopathology is acknowledged as a sensitive endpoint for detecting testicular toxicity and the process of spermatogenesis is now well understood and routinely assessed in rodents and, to a lesser extent, in dogs. However, detailed and up-to-date information on the organization and dynamics of spermatogenesis in minipigs is limited.
The objective of this poster is to illustrate a practical approach for morphological staging of minipig spermatogenesis and to describe common spontaneous microscopic findings routinely observed in the testes. Ten male Gottingen minipigs were supplied from Ellegaard, Denmark. They were shown to be sexually mature by sperm analysis at 5-6 months of age. At 9-10 months of age they were killed humanely and a range of tissues were taken and analysed for background pathology. The testes were fixed in Bouin's fluid and then transferred to 70% alcohol prior to processing to paraffin wax, sectioning at 4 5 µm and staining with H&E and PAS.
Tubular hypoplasia/atrophy was a common finding in this small sample. The spermatogenic cycle of the minipig has been described in 8 stages; the cellular composition of the 8 stages will be described and clear illustrative photomicrographs of each stage will form the basis of a practical guide for routine morphological staging.

Poster Abstracts

P18: The Study Pathologists' Decision-Making Process for Evaluating "Adversity" – Results from the 4th ESTP International Expert Workshop in Paris, June 8 – 9, 2015

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Toxicologic pathology plays a central role in the identification of treatment-related changes and interpretation of their adversity in the nonclinical safety assessment of chemicals and pharmaceuticals. Publications about general principles of adversity and NOAEL (No-Observed-Adverse-Effect-Level) assignments in toxicology are numerous, but do not offer sufficient practical guidance to the study pathologist. Moreover, the STP Scientific and Regulatory Policy Committee (SRPC) has drafted recommended "Best" Practices for defining and communicating adversity for nonclinical study data (2015, review by STP members), clearly requesting that adversity be discussed in pathology and toxicology reports.

Recognizing the increasing importance of this topic, the European Society of Toxicologic Pathology recently conducted a workshop comprised of 22 expert pathologists and toxicologists from the pharmaceutical and chemical industry, contract research organizations and international regulatory authorities. The aim of the workshop was to a) review currently available definitions of adversity in toxicologic pathology; b) weigh determining and qualifying factors of adversity based on practical examples; c) discuss potential differences in interpretation and consequences of adversity between the pharmaceutical and (agro)chemical industry and among world regions; d) recommend a practical approach, yet still promote the rigorous utilization of biological information, to define "adversity" in toxicology reports; and finally e) set the stage for subsequent organ- or lesion-specific "adversity" workshops.

Workshop members met during five preparatory teleconferences and a 1.5 day face-to-face workshop in Paris, 8th and 9th of June 2015. A list of subtopics was defined and presented by individual members, illustrated by anonymized case examples, and followed by group discussion. An audience of over 20 additional European toxicologic pathologists and toxicologists enriched discussions during the F2F workshop in Paris. Results of the discussions are meant to be complementary to the SRPC draft paper.

Experts found it mandatory that a holistic, weight-of-evidence, case-specific approach is followed for each adversity assessment. A working definition of adversity to be used by pathologists was developed by the group. Although adverse effects may occur at the molecular, cellular, tissue/organ or organism level, based on information/tools available to pathologists, adversity will be typically determined at a morphological level (most often the organ) in the pathology report and will refer to the test species. Assignment of the NOAEL and overall integration of target pharmacology information and consideration of human translation are typically made in the toxicology overview in the dossier.

The results of this workshop are a valuable prerequisite for future organ- or lesion-specific workshops which will be organized by the ESTP. In addition, discussions showed that there is a great demand to better elucidate to the toxicologic pathology community the different consequences of nonclinical adversity for the development process of chemical entities and new drugs.

Poster Abstracts

P19: Integrated analysis of results from studies with four immune-modulating substances to improve the understanding of effects

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Data in toxicity studies are evaluated generally per parameter. Information on the response per animal in addition to per parameter can improve the evaluation of the results. Such an integrated analysis visualizes patterns of responses and gives insight into sources of normal variability of controls. It is a kind of holistic approach, albeit that often functional and morphological data are not from the same animal.
The results from immune-toxicity studies in rats according to OECD 407 from four different immunomodulator compounds were subjected to multivariate analysis, i.c. principal component analysis (PCA) and principal component discriminant analysis (PC-DA). The selected compounds which all modulate the immune system, but with quite diverse mode of immunomodulation were two pharmaceuticals drugs: azathioprine (AZA) and cyclosporine A (CSA) and the two environmental pollutants hexachlorobenzene (HCB) and benzo(a)pyrene (BaP).
PCA illustrated the similarities between two independent studies with AZA and CSA. The PC-DA analysis did not increase substantially the information on dose levels. In general, the no-effect levels were lower upon single parameter analysis than indicated by the distances between the dose groups in the PCA. This was mostly due to the expert judgment in the single parameter evaluation, which took into account outstanding induced pathology in only one or two animals. The PCA plots did not reveal sex-related differences in sensitivity, but the key pathology for males and females differed. The observed variability in some of the control groups was largely from variation in peripheral blood parameters. The key pathology enhanced the understanding of the response of the animals to the four model compounds.
Most importantly, PCA analysis identified several animals outside the 95% confidence limit indicating high-responders; also low-to-non-responders were identified. Based on human data and/or mode of action the highest variation in response was expected especially in the AZA groups, but outstanding responders were also observed with CSA and HCB. This can be due to differences between rat and man. It may also be a reason to reconsider the expectation and re-examine the rat and human data, as multivariate analyses are tools to point to unexpected or overlooked results and correlations.

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Poster Abstracts

P20: Local infiltration pattern of pituitary carcinomas in Sprague-Dawley rats: influence of sectioning protocol

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Introduction: Pituitary neoplasms are a common finding in aged rats and the great majorities are classified histologically as adenomas. In order for a pituitary mass to be classified as a carcinoma there must be evidence of invasion of the surrounding tissues, of which generally only the brain is examined. The present study investigates the incidence and pattern of local infiltration of pituitary carcinomas in aged Sprague-Dawley (SD) rats when the sphenoid bone is examined as protocol.

Material and Methods: Data from a 104-week carcinogenicity study of SD rats in which the sphenoid bone was examined were reviewed. Incidences of pituitary tumours were compared with background data from eleven studies where a conventional sectioning protocol was followed. The invasion pattern of all pituitary carcinomas in the study was classified according to pattern (ventrolateral or dorsal) and tabulated.

Results: The control incidences of pituitary carcinomas in this study were 6/72 (8.3%) in females and 0/72 (0%) in males. When control and all treated groups were combined the total incidences were 31/288 (10.8%) in females and 2/288 (0.7%) in males. In control females 3/6 (50%) pituitary carcinomas displayed dorsal infiltration of the brain and 3/6 (50%) displayed ventrolateral infiltration of the sphenoid bone and surrounding tissues. When pituitary carcinomas from all female groups were reviewed 21/33 (63.6%) displayed a ventrolateral invasion pattern, and the remaining 12/33 (36.3%) had invaded the brain dorsally. When pituitary carcinomas from all male groups were reviewed 1/2 (50%) displayed a ventrolateral invasion pattern, and the remaining 1/2 (50%) had invaded the brain dorsally. None of the tumours displayed both invasion patterns.

Discussion: The control incidence ranges of pituitary carcinomas in carcinogenicity studies run at these laboratories during the same period were 0% in males and 0-3.1% in females, respectively. The present study reports a much higher incidence in control females as a result of examination of the sphenoid bone and surrounding tissues. When pituitary carcinomas from the entire study were reviewed, the majority infiltrated tissues ventral or lateral to the pituitary gland and did not infiltrate dorsally. None of the tumours infiltrated both dorsally and ventrolaterally. This may be due to the anatomical location of pituitary gland as it is largely covered by the sellar diaphragm of the dura mater over its dorsal aspect, which may provide a partial barrier to dorsal infiltration.

Conclusions: Pituitary carcinomas are likely to be more common in SD rats than previously recognised. Examination of the sphenoid bone and surrounding tissues can influence the diagnosis and incidence of these tumours, and can be a useful tool where incidence patterns are equivocal or pituitary tumours are induced by the test article.

Poster Abstracts

P21: INHAND and collaboration with the FDA on SEND – Background and Current Status

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The INHAND Proposal (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice) has been operational since 2005. A Global Editorial Steering Committee (GESC) manages the overall objectives of the project and the development of harmonized terminology for each rodent organ system or non-rodent species is the responsibility of the Organ Working Groups (OWG) or Non-rodent Working Groups (NRWG) respectively, drawing upon experts from North America, Europe and Japan.

Great progress has been made with 9 rodent organ systems published to date – Respiratory, Hepatobiliary, Urinary, Central/Peripheral Nervous Systems, Male Reproductive and Mammary, Zymbals, Clitoral and Preputial Glands in Toxicologic Pathology and the Integument and Soft Tissue and Female Reproductive System in the Journal of Toxicologic Pathology as supplements and on a web site – www.goReni.org. INHAND nomenclature guides offer diagnostic criteria and guidelines for recording lesions observed in rodent toxicity and carcinogenicity studies. The guides provide representative photo-micrographs of morphologic changes, information regarding pathogenesis, and key references.

During 2012, INHAND GESC representatives attended meetings with representatives of the FDA Center for Drug Evaluation and Research (CDER), Clinical Data Interchange Standards Consortium (CDISC), and the National Cancer Institute (NCI) Enterprise Vocabulary Services (EVS) to begin incorporation of INHAND terminology as preferred terminology for SEND (Standard for Exchange of Nonclinical Data) submissions to the FDA. The interest in utilizing the INHAND nomenclature, based on input from industry and government toxicologists as well as information technology specialists, suggests that there will be wide acceptance of this nomenclature.

Poster Abstracts

P22: Suitability of the minipig as non-rodent animal model to test safety of antisense oligonucleotides

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Antisense oligonucleotides (AONs) are synthetic nucleotide strands that bind to target mRNA and either catalyze RNase H mediated degradation, splice switching or block translation. Currently numerous AONs are being evaluated in clinical trials for a variety of therapeutic indications. Traditionally, the non-human primate (NHP) has been the non-rodent species used to assess toxicity of AONs and is considered predictive for toxicity in humans. As little is known about the toxicity of AONs in minipig, two short-term studies were performed in minipigs using two AONs at similar dose levels to those used in the NHP and for which clinical trials showed renal adverse effects and injection site reactions.
Two AONs were tested. In each study, the test item was administered subcutaneously on Days 1, 6, 11, and 16 to female Göttingen minipigs (n=2 for controls and n=3 for treated groups), with necropsy on Day 17. Investigations included exposure assessment, standard in-life, clinical and anatomic pathology, and also immunohistochemistry (IHC), in situ hybridization (ISH), electron microscopy of the kidney and urinary biomarkers. Data were compared with those of the reference NHP studies.
Test item-related findings were tubular dilatation and degeneration/regeneration correlating with increased creatinine and urea. One treated minipig revealed a mesangioproliferative glomerulonephritis. In addition, increased inflammation at injection sites, as well as vacuolated macrophages in lymph nodes, were observed. In contrast, in the NHP, no adverse test item-related findings had been observed in the kidney with one test item and with the second one, the findings were less severe than in the minipig. Findings observed in lymph nodes were similar in both species and injection site reactions were more pronounced in the NHP study with the second test item. IHC and ISH confirmed presence of AONs in renal tubular cells and macrophages. Ultrastructurally, an increased number of lysosomes was observed in renal tubular cells.
In summary, the findings described in the minipig are similar to those described in other animal species including NHPs. Some AONs are known to accumulate in the kidney and possibly induce proximal tubular degeneration in the monkey (Henry et al., 2012, Monteith et al., 1999). In these studies, the minipig seemed to be more sensitive to the tested AONs than the NHP in relation to AONs-induced nephrotoxicity and with this closer to the clinical findings observed with these AONs. The severity of vacuolated macrophages in the lymph nodes, a finding known to be associated with some AON treatments (Frazier, 2015), was comparable between minipigs and NHPs. Finally, under these study conditions minipigs were not better predictors than NHPs for human injection site reactions.
These studies showed the suitability of the minipig as non-rodent animal model to test AONs toxicity. Further investigations will show whether the minipig might be even a better predictor for nephrotoxicity.

Poster Abstracts

P23: Acute renal tubular degeneration in alloxan-treated rats

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Introduction: Alloxan is a well-known material to induce diabetes in experimental animals. The glycogen accumulation in distal renal tubules, so-called Ebstein-Armanni lesions, which appear in the kidney of alloxan-induced diabetic rats and mice model with prolonged hyperglycemia are well known. However, there are only a few reports about the mechanism of acute renal injury induced by alloxan. The objective of this study was to evaluate the morphological characteristics relevant to the acute renal lesions of alloxan toxicity.

Materials and methods: Seven-weeks-old male Wistar rats were intravenously treated with 50 mg/kg alloxan, and sacrificed at 8 hours, 1, 2, 4 and 7 days after treatment for histopathological examination.

Results: At 8 hours after alloxan treatment, many distal tubules showed granular degeneration at the corticomedullary junction.
These tubular lesions progressed over time. At day 4 both proximal and distal tubules showed degeneration and necrosis with dilatation of tubular lumens containing cellular debris and granular substances.
In addition, 1-2 days after treatment, mineralization was noted mainly inside the degenerated tubular epithelium and tubular lumen. At 4 days after treatment, the mineralization decreased, and remaining mineralization were sometimes enveloped by tubular epithelial cells.
At 7 days after treatment, degeneration and necrosis of renal tubules disappeared, but tubular dilatation remained in the distal tubules.

Conclusions: These findings demonstrate that alloxan initially causes degeneration to the distal tubules at the corticomedullary junction.

Poster Abstracts

P24: Maternal Exposure Effect of Ochratoxin A on Hippocampal Neurogenesis in Rat Offspring

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Introduction: Ochratoxin A (OTA) is a mycotoxin produced by Aspergillus or Penicillium species fungus in a wide variety of climates and geographical regions. The contamination with OTA was reported for various foods particularly grains, as well as dried fruits, coffee and wine. OTA is known to induce protein synthesis inhibition and oxidative stress responses to result in toxicities mainly in the kidney, as well as in other organs including central nervous system. However, there is no information with regard to the possibility of developmental neurotoxicity by OTA. Hippocampal dentate gyrus is a brain region that continues to produce new neurons throughout postnatal period. We have previously reported that maternal exposure to various neurotoxicants including a mycotoxin, T-2 toxin, affects neurogenesis in the offspring dentate gyrus using rats or mice. In the present study, we examined the developmental exposure effect of OTA on hippocampal neurogenesis using rats.

Materials and Methods: Oral doses of OTA at 0, 0.12, 0.6, 3.0 ppm in diet were given to maternal SD rats from gestational day (GD) 6 to day 21 after delivery. A part of offspring were maintained without exposure to OTA through postnatal day (PND) 77. The hippocampal dentate gyrus of offspring was immunohistochemically examined on PND 21 and PND 77. To detect the effect on hippocampal neurogenesis, granule cell populations of each differentiation stage in the subgranular zone (SGZ) and GABAergic interneuron populations in the dentate hilus, which are known to support the neurogenesis process, were analyzed.

Results: Offspring showed lower relative kidney weight at 3.0 ppm on PND 21, whereas dams did not show changes in body or organ weight. Immunohistochemical evaluation at PND 21 offspring showed decreases in the number of immunoreactive cells for PAX6, expressed in type-1 stem and type-2a progenitor cells, and TBR2, expressed mainly in type-2b cells, at 3.0 ppm, whereas the numbers of GFAP+ type-1 cells and DCX+ type-2b and type-3 cells and immature granule cells were unchanged in the SGZ. There were no differences in the number of TUNEL+ apoptotic cells and PCNA+ proliferative cells in the SGZ. In the hilus of the dentate gyrus, decrease of somatostatin+ interneurons was noted at 3.0 ppm on PND 21. These neurogenesis-related changes were disappeared on PND 77. By means of real-time RT-PCR analysis of transcript expression in the hippocampus dentate gyrus on PND 21, downregulation of Chrnb2, a gene encoding nicotinic cholinergic receptor and upregulation of Ogg1, a gene encoding oxidative stress-related DNA repair enzyme, and Bdnf, a immediate early gene in the central nervous system to support neuronal growth, were observed at 3.0 ppm.

Conclusion: Our results suggest that maternal OTA exposure reversibly affects hippocampal neurogenesis targeting type-2 intermediate-stage populations of granule cell lineages involving suppression of interneuronal support on neurogenesis in rat offspring at 3.0 ppm in diet, a level translating to 0.20 mg/kg body weight/day during gestation and 0.38 mg/kg body weight/day during lactation, respectively. Transcript expression analysis revealed induction of oxidative stress-mediated DNA damage and affection of interneuronal cholinergic signals in relation with the disruption of neurogenesis, as well as involvement of compensatory mechanism of BDNF signaling on the disruptive neurogenesis by OTA.

Poster Abstracts

P25: Neuroprotective effects of kyneurinine in in vivo and in vitro studies

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Background: It is well known that the neurotoxicity effects of Glutamate (L-Glu) involve the activation of glutamate receptors. However, during periods of cerebral ischemia there is also an activation of the Kyneurinine (KYN) pathway of tryptophan metabolism, which generates the NMDA receptor-glycine site antagonist Kyneurinic Acid (KYNA). We studied the effects of pretreatment of peripheral KYN on acute-L-Glu-induced neurotoxicity. N-methyl-D-aspartate (NMDA) receptors are supposed to play a crucial role in Glutamate (L-Glu) neurotoxicity.

Materials and Methods: To evaluate the effects of the astrocyte-derived tryptophan metabolite kynurenic acid (KYNA), on L-Glu neurotoxicity, adult male rats were pretreated with Kynurenine (KYN) which is a precursor of KYNA, at a dose of 30 mg or 300 mg/kg bw i.p., 2 h before administration of a stereotactic L-Glu bolus (1µmole/1µl) into the cerebral cortex. Key oxidant and anti- oxidant parameters were estimated in both brain homogenates and isolated cortical neurons. Cerebral cortex homogenate was prepared in phosphate buffer (0.1 M, pH 7.4, 10% w/v). To obtain individual neurons, finely chopped cerebral cortex tissue was treated with dispase (1000 protease units/ml) for 45 min at 370C. After enzymatic treatment, the dissociated neurons were passed through a filter (mesh diameter ~ 53 µm) to remove large neurons and tissue fragments. HEPES–Tyrode solution (145 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 5 mM glucose, 5 mM HEPES, pH 7.4) was used to prepare a homogenous suspension of individual neurons. Viability test was done by trypan blue and 90% viable population of neurons was obtained.

Results: In hematoxylin and eosin-stained coronal sections, the cytoarchitecture of the cerebral cortex was studied using the images in control and L-Glu administered rats. The percentage of condensed nuclei was calculated and data were represented as means ± SEM. Data show that pyramidal neurons were significantly smaller ($P<0.001$) in size in L-Glu-injected rats with respect to control neurons. Acute L-Glu administration increased the rate of lipid peroxidation, nitric oxide and decreased key antioxidant parameters such as SOD, catalase, total glutathione and glutathione reductase in brain homogenates. In isolated cortical neurons, we observed increased levels of reactive oxygen species, calcium along with decreased mitochondrial membrane potential.

Conclusion: Peripheral loading of 30 mg/kg dose of KYN had no protective effects on L-Glu induced neurotoxicity, but a dose of 300 mg/kg dose prevented the toxic effects following intracortical L-Glu.

Poster Abstracts

P26: Characterization of mouse model of human diseases: focus on X- Chronic Granulomatous Disease

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Animal models are considered an important resource for biomedical research, providing relevant insight in mechanisms of diseases and as powerful tools for the evaluation of the efficacy and safety of new therapy. In the last years the growing debate on animal usage and model predictability for pharmacological and toxicological effects of drugs in man, led regulatory authorities to develop guidelines to better validate animal models. In the development of Advanced Therapy Medicinal Products (drugs based on gene therapy, cell therapy or tissue engineering), regulators suggest to use animal models that are relevant to the human disease. To meet the regulatory expectations a proper characterization of the model is required.
X-CGD (Chronic Granulomatous Disease) is a recessive inherited disorder caused by a defective phagocyte respiratory burst oxidase (NADPH oxidase). 70% of patients with CGD present a X-linked inheritance due to mutations on the gp91phox gene (X-CGD) and they are prone to develop recurrent fungal and bacterial infections and granulomas.
The X-CGD mouse model is characterized by lack of phagocyte superoxide production, increased susceptibility to bacterial and fungal infection and altered inflammatory response in induced peritonitis. An extensive characterization of the baseline immunological, inflammatory state and histopathological features of the X-CGD mouse model will be important to study the physiopathology of the disease and adopt the model for investigating novel therapies. To this purpose, 7-9 months old X-linked CGD animals and age-matched wild type (WT) C57Bl/6 animals were evaluated.
At necropsy, terminal body weight, macroscopic findings and selected organ weights were recorded. A full tissue list was collected and evaluated microscopically. Electron microscopy was performed on kidney and bone marrow. Thymus, spleen and bone marrow were further characterized by immunohistochemistry (CD3, B220, F4/80 Caspase-3 cleaved, Ter 119, MPO, CD31) on paraffin sections. Flow cytometry was performed on bone marrow and spleen in order to evaluate the hematopoietic subpopulation. Pro-inflammatory cytokines were measured on bone marrow and lung homogenates (Bioplex assay).
The preliminary data on characterization of the X-CGD mouse model will be presented and discussed in terms of relevance with regards to the human disease and supports its use in preclinical efficacy and safety studies.
All animal studies were approved by Animal Care and Use of Committee of the San Raffaele Hospital and communicated to the Ministry of Health and local authorities according to Italian law.

Poster Abstracts

P27: Late Effect of Developmental Exposure to 3,3'-Iminodipropionitrile on Neurogenesis in the Hippocampal Dentate Gyrus in Mice

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Introduction: 3,3'-Iminodipropionitrile (IDPN) is a neurotoxic compound known to cause proximal axonopathy in the nervous system of rodents. In our previously study, we have shown that maternal IDPN exposure to rat offspring reversibly affects late-stage differentiation of granule cell lineages in the hippocampal neurogenesis involving increase of neuronal plasticity as evident by immediate-early gene responses in the granule cell layer. However, it remains unclear whether IDPN affects mouse hippocampal neurogenesis after the developmental exposure similar to rats. In the present study, we examined the effect and reversibility of developmental exposure to IDPN on neurogenesis in the hippocampal dentate gyrus in mice.

Materials and methods: Pregnant ICR mice were treated with IDPN at 0, 600 or 1200 ppm in the drinking water from gestational day (GD) 6 until weaning on postnatal day (PND) 21. Dams were subjected to measurements of body weight, food and water consumption during GD 6 and GD 21 and during the postnatal exposure period of lactation. The offspring were also weighed until PND 77. Male offspring were subjected to necropsy on PND 21 and PND 77, and brain were weighed and immunohistochemically examined for neurogenesis-related parameters in the hippocampal dentate gyrus.

Results: Dose-related decreases in the body weight, food and water consumption in dams were observed at 600 ppm during the postnatal exposure period and at 1200 ppm during the gestational and lactational exposure periods. The decreases of absolute brain weight were observed in both dams and pups at 1200 ppm on PND 21. Immunohistochemically, decreases in the number of cells immunoreactive for activity-regulated cytoskeleton-associated protein (Arc) and FBJ osteosarcoma oncogene (Fos), which are protein group of the immediate-early genes involved in neuronal plasticity in the granular cell layer (GCL) were observed at 1200 ppm as compared with the 0 ppm controls at PND 77. Decreases in glial fibrillary acidic protein (GFAP)+ type-1 stem cells (radial glial cells), T box brain protein 2 (Tbr2)+ type-2b and type-3 progenitor cells, doublecortin (Dcx)+ type-2b and type-3 progenitor cells and immature granule cells, and proliferating cell nuclear antigen (PCNA)+ cells were observed in the dentate subgranular zone (SGZ) at 1200 ppm on PND 77. The number of Arc+, Fos+, GFAP+, Tbr2+ and PCNA+ cells were unchanged on PND 21 in both IDPN-exposed groups.

Conclusion: Our results suggest that maternal IDPN exposure during gestational and lactational period induces late effect on hippocampal neurogenesis of offspring at the adult stage in mice, different from the reversible effects in rats. While involvement of immediate-early gene responses was similar to rats, response was inversely to decrease the neuronal plasticity in mice, in contrast to the transient increase in rats. It is also suggested that the impact on granule cell lineages was stronger in mice affecting whole population of granule cell lineages as compared to the affection of late-stage differentiation in rats. The late effect by developmental IDPN exposure in mice may be related to alteration in epigenetic gene control involving stem cells.

Poster Abstracts

P28: A Simple Method for Collection and Examination of the Cardiac Conduction Tissue in the Dog

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Although it is not a typical protocol tissue in the standard drug nonclinical toxicity studies, the cardiac conduction tissue (CCT) is required to be examined microscopically in the case of drugs with potential cardiac toxicities. Most methods described in the literature require performing laborious serial sections for the examination of CCT. This study aimed to develop a simple method for GLP labs to collect and examine the canine CCT microscopically. Six intact canine hearts, with the superior/inferior vena cava, the pulmonary trunk and the ascending aorta attached, were used in this study. By using the sulcus terminalis and the Koch triangle as the reference marker for locating the sinoatrial node (SAN) and atrioventricular node (AVN), respectively, the SAN, AVN and Purkinje's fibers were easily collected and sectioned for microscopic examination. For the optimal SAN section, a longitudinal cut was made along the sulcus terminalis and both cut tissue pieces were embedded together with their cut faces downwards. Similarly, an appropriate section of the AVN can be obtained by cutting along the line linking the middle of the inner edge of the orifice of the coronary sinus and the tip of the Koch triangle, with both cut faces embedded downwards. Parallel to this line, 2 caudal cuts were made at 1.5 and 2.0 cm deep, respectively, and two pieces of cut tissue across the interventricular septum were embedded with the cranial cut faces downwards, which included His bundle and Purkinje's fibers for microscopic examination. This method had been successfully used to evaluate the potential cardiotoxic effect of two artemisinins derivatives on CCT of 64 dogs.

Poster Abstracts

P29: Spontaneous background findings in albino hartley guinea pigs at mpi research

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Guinea pigs are increasingly promoted for use in ototoxicity studies in addition to models of certain human diseases. A retrospective study of histologic findings was performed to document spontaneous background findings in albino Hartley guinea pigs at MPI Research, a large preclinical CRO. Findings were tabulated for 85 animals (26 males and 59 females) less than six months of age. Findings were most prevalent in the kidneys (85.9%), liver (50.6%), heart (41.2%), testes (41.18%), and lungs (30.8%). Mineralization (74.1%) and tubular basophilia (48.2%) were the two most prevalent findings in the kidneys. Mononuclear cell infiltrate (47.0%) and myofiber vacuolation (16.5%) were the most prevalent findings in the liver and heart, respectively. Seminiferous tubule degeneration/atrophy (30.7%) was the most prevalent finding of the testes. Hemorrhage (11.7%) was the most prevalent finding of the lungs. Male guinea pigs had increased incidence of findings compared to females in the lacrimal glands (13-fold), mesenteric lymph node (3-fold), salivary mandibular glands (2-fold), lung (1.5-fold), and skeletal muscle (1.5-fold). Female guinea pigs had approximately 2-fold increases in findings of the thymus and larynx compared to males. Some previously described background findings of guinea pigs may be lacking as these studies utilized juvenile guinea pigs. Documenting frequency of spontaneously occurring findings will aid in discernment of compound-related effects in subsequent toxicity studies. The prevalence of several findings varies with gender and differences are potentially related to housing, behavior, or institutional factors.

Poster Abstracts

P30: Onset analysis of carcinogen-specific disruptive cell cycle facilitation during the early stage of repeated administration of renal carcinogens in rats

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Background: We have previously reported that carcinogens which facilitate cell proliferation of carcinogenic target cells increased populations expressing cell cycle-related molecules, especially of G2/M or M phase, and facilitated apoptosis after 28-day treatment in rats. In addition, carcinogen-treatment also induced aberrant expression of ubiquitin D (UBD), a M-phase progression-related molecule, from G2 phase, suggestive of cell cycle aberration involving spindle checkpoint.

Aim: The present study was performed to determine the time response of renal tubular epithelial cells to undergo carcinogen-specific facilitation of cell cycle aberration and proliferation after renal carcinogen-treatment for up to 28 days. For comparison, cellular responses after treatment with non-carcinogenic renal toxicants were examined.

Materials and Methods: A total of 210 male 6-week-old F344 rats were divided into 7 groups of untreated controls, renal carcinogen-treatment [nitrofurantoin (NFT), 1-amino-2,4-dibromoantraquinone (ADAQ), 1,2,3-trichloropropane (TCP)], and non-carcinogenic renal toxicant-treatment [1-chloro-2-propanol (CPN), triamterene (TAT), carboxin (CBX)] (30 rats per group). Among renal carcinogens, NFT has previously shown to induce weak renal carcinogenicity, while ADAQ and TCP have shown apparent renal carcinogenicity. At day 3, 7 and 28 of treatment, one third of the animals in each group was killed and kidneys were removed and subjected to immunohistochemistry using antibodies against the following proteins: Ki-67, a cell proliferation marker, topoisomerase II alpha (TOP2A), acting at G2/M phase, phosphorylated histone H3 (p-Histone H3), acting at M phase, and UBD. In addition, kidneys in the NFT, ADAQ, TCP and CBX groups, in which increase of cell proliferation was observed at day 28, were also subjected to double immunohistochemistry for UBD with TOP2A or p-Histone H3.

Results: At day 3, NFT, TCP and CBX increased tubular epithelial cell proliferation and cells expressing G2/M or M phase molecules. In contrast, ADAQ and TAT decreased tubular epithelial cells expressing G2/M or M phase molecules. At day 7 of treatment, NFT and TCP increased tubular epithelial cell proliferation, cells expressing G2/M or M phase molecules and apoptosis. In contrast, ADAQ, CPN and TAT decreased tubular epithelial cells expressing G2/M or M phase molecules. At day 28, NFT, ADAQ, TCP and CBX increased tubular epithelial cell proliferation and cells expressing G2/M or M phase molecules. CBX also increased apoptosis at this time point. In addition, renal carcinogenic ADAQ and TCP decreased the p-Histone H3+ cells within UBD+ cell population at day 28. On the other hand, renal carcinogenic NFT and non-carcinogenic renal toxicant CBX did not decrease the p-Histone H3+ cells within UBD+ cell population at day 28.

Discussion: In the present study, renal carcinogen-specific cell cycle responses were not observed during the treatment period up to 28 days by single immunohistochemistry. On the other hand, among chemicals facilitating cell proliferation at day 28, renal carcinogenic ADAQ and TCP specifically reduced UBD expression at M phase. However, renal carcinogenic NFT did not induce this change, probably due to its weak carcinogenic potential as compared with ADAQ and TCP. These results suggest that renal carcinogens showing apparent renal carcinogenic potential increase cell proliferation and cause aberrant UBD expression at G2 phase, leading to disruption of spindle checkpoint function at day 28. It may take 28 days to induce renal carcinogen-specific disruption of cell cycle regulation.

Poster Abstracts

P31: Immunohistochemical Detection of Aberrant Neuronal Development Induced by 6-Propyl-2-thiouracil (PTU) in the Framework of Developmental Neurotoxicity Study and 28-day Toxicity Study using Rats

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Background: Developmental neurotoxicity (DNT) testing is a field requiring a rapid screening system because testing one chemical with current guidelines is time consuming with needs of hundreds of animals to conduct one study. Utilizing hypothyroidism rat model to disrupt neuronal/glial development, we previously revealed gene expression profiles suggestive of disruption of neuronal/glial development in different brain regions including the hippocampal dentate gyrus during developmental exposure to propylthiouracil (PTU), an anti-thyroid agent, and in the hippocampal dentate gyrus during adult PTU exposure. The results suggest possibility to detect DNT in the hippocampal dentate gyrus in the framework of a 28-day toxicity study.

Aim: This study was performed to measure the sensitivity of molecular markers to detect DNT by developmental exposure, and to examine the utility of their markers in a scheme of 28-day repeated oral dose toxicity study to detect DNT by means of immunohistochemical analysis of molecules related to neuronal development.

Materials and Methods: In the developmental exposure study, pregnant SD rats were treated with 0, 1, 3, 10 ppm of PTU in the drinking water from gestational day 6 to postnatal day (PND) 21. Pups were euthanized on PND 21 and PND 77. In the adult exposure study, male SD rats were treated with 0, 0.1, 10 mg/kg of PTU by oral gavage for 28 days from postnatal week 5. Brain samples from both studies were subjected to immunohistochemical analysis.

Results: In the developmental exposure study on PND 21, decreases in glial fibrillary acidic protein+ cells, paired box 6 (PAX6)+ cells and doublecortin (DCX)+ cells, which are neuronal cell stage-defining markers of granule cell lineage in the subgranular zone (SGZ) of the hippocampal dentate gyrus, were detected at ≥ 3 or 10 ppm, compared to 0 ppm controls. Regarding molecules associated with synaptic plasticity, decreases in cyclooxygenase-2+ cells, Eph receptor A4 (EPHA4)+ cells, and activity-regulated cytoskeleton-associated protein (ARC)+ cells were observed in the dentate granule cell layer (GCL) at ≥ 3 or 10 ppm, compared to 0 ppm controls. Regarding GABAergic interneuron population, increases in reelin (RELN)+ cells, calretinin (CALB2+) cells and somatostatin (SST+) cells and decrease in parvalbumin (PVALB+) cells were observed in the dentate hilus at ≥ 3 or 10 ppm, compared to 0 ppm controls. In the cerebral cortex, increase in RELN+ cells and decreases in PVALB+ cells and neuropeptide Y+ cells were observed at ≥ 3 or 10 ppm, compared to 0 ppm controls. In the cerebellum, increases in RELN+ cells and SST+ cells, and decrease in PVALB+ cells were also observed at ≥ 3 or 10 ppm, compared to 0 ppm controls. On PND 77, decrease in PAX6+ cells in the SGZ, increases in CALB2+ cells and SST+ cells in the dentate hilus, increases in EPHA4+ cells and ARC+ cells in the GCL were observed at ≥ 3 or 10 ppm. In the adult exposure study, decrease in DCX+ cells in the SGZ and increases in RELN+ cells and SST+ cells in the hilus were observed at 10 mg/kg.

Discussion: By means of immunohistochemical analysis, developmental exposure to PTU revealed aberration in hippocampal neurogenesis accompanying changes in interneuron subpopulations and in synaptic plasticity. Changes in interneuron subpopulations in various brain regions suggest the utility of immunohistochemical approaches on these interneurons as useful markers for detection of DNT by developmental exposure under the current DNT guidelines. Adult exposure study of PTU also revealed aberration in neurogenesis accompanying changes in interneuron subpopulations, which indicates hippocampal dentate gyrus as a useful region to detect developmental neurotoxicity in the framework of a 28-day toxicity study.

Poster Abstracts

P32: Different Toxicity Targets in the Hippocampal Neurogenesis by Developmental and Postpubertal-stage Exposure to Valproic Acid in Rats

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Introduction: Valproic acid (VPA) has been extensively used as an antiepileptic drug, which can inhibit GABA transaminase and increase GABA in the brain. Maternal administration of VPA may increase the risk of autism to their offspring and maternal VPA injection in experimental animals provides an autism model. Hippocampus plays an important role in the process of learning and memory. It is supposed that autism is related to aberrations in developmental neurogenesis involving neuronal mismigration. In the present study, we examined developmental VPA exposure effect on adult neurogenesis of the hippocampal dentate gyrus in rat offspring. Because hippocampal neurogenesis continues throughout the life, we also examined the effect of adult stage exposure to VPA on hippocampal neurogenesis using rats in a scheme of 28-day toxicity study.

Materials and Methods: In the developmental exposure study, oral doses of VPA at 0, 667 or 2000 ppm in the drinking water were given to maternal SD rats from gestational day 6 to postnatal day (PND) 21 after delivery. Neurogenesis-related parameters in the dentate gyrus of male offspring were analyzed immunohistochemically at the end of exposure on PND 21 and also on PND 77 to examine reversibility. In the postpubertal-stage exposure study, 5-week-old male SD rats were treated with VPA at 0, 200 or 900 mg/kg by oral gavage for 28 days, and then, hippocampal neurogenesis-related parameters were analyzed.

Results: In the developmental exposure study, the number of reelin+ GABAergic interneurons in the dentate hilus reduced at ≥ 667 ppm compared to 0 ppm in offspring on PND 21. On PND 77, the number of PCNA+ proliferative cells in the subgranular zone (SGZ) and NeuN+ postmitotic granule cells in the granule cell layer (GCL) of the dentate gyrus increased at ≥ 667 ppm compared to 0 ppm. Granule cells expressing ARC and COX2, which are protein group of the immediate-early genes involved in neuronal plasticity, increased in the GCL at ≥ 667 ppm compared to 0 ppm. In the postpubertal-stage exposure study, the number of GFAP+ type-1 stem cells increased and the number of DCX+ type-2b and type-3 progenitor cells and immature granule cells decreased in the SGZ at 900 mg/kg compared to 0 mg/kg. The number of TBR2+ type-2b and type-3 progenitor cells did not change in the SGZ. There were no changes in the neurogenesis-related parameters as observed in the developmental exposure study.

Conclusion: We revealed that developmental VPA exposure reversibly affects neuronal migration in the hippocampal dentate gyrus of offspring. Interestingly, late effect was evident at the adult stage after cessation of developmental VPA exposure, causing facilitation of SGZ cell proliferation to cause increase of granule cells accompanying increased neuronal plasticity. On the other hand, postpubertal-stage VPA exposure caused an increase in type-1 stem cells and a decrease in immature granule cells in the SGZ. However, there were no changes in interneuron subpopulations and neuronal plasticity-related parameters. These results suggest that developmental and postpubertal-stage VPA exposure revealed different toxicity targets in the hippocampal neurogenesis. Of note, the late effect by developmental VPA exposure may be related to alteration in epigenetic gene control involving stem cells, because VPA is known as a histone deacetylase 1 inhibitor.

Poster Abstracts

P33: Anti-inflammatory activity of lectin purified from Morus nigra against lipopolysaccharide (LPS) induced renal stress in rats.

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The study was designed to investigate the possible protective role of lectin of Morus nigra in lipopolysaccharide renal inflammatory, by using biochemical approaches. The effects of lectin of Morus nigra on LPS induced oxidative and renal stress were evaluated by serum creatinine, urea and uric acid levels, lipid peroxidation, GSH levels, SOD, GSH-Px and GST activities in kidney tissue. Administration of LPS induced significant increase in serum: creatinine, urea and uric acid concentration showing renal inflammatory. LPS also induced oxidative stress, as indicate by decreased of GSH level, SOD, GSH-Px and GST activities in kidney tissue along with increased lipid peroxidation. Furthermore, treatment with LPS caused a marked increased kidney weight and decreased body weight. In Histological studies, LPS induced various pathological alterations in kidney of rats; these alterations were characterized by renal tubular damage, indicating by tubular necrosis. Treatment with lectin of Morus nigra markedly reduced elevated: creatinine, urea and uric acid levels and counteracted the deleterious effects of LPS on oxidative stress markers and attenuated histological changes caused by LPS in kidney.

Our results indicate that lectin of Morus nigra could have a beneficial role against LPS induced nephrotoxicity and oxidative stress in rat.

Poster Abstracts

P34: Trimethyltin chloride inhibits neuronal cell differentiation in zebrafish embryo neurodevelopment

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Trimethyltin chloride (TMT) is a neurotoxicant widely present in the aquatic environment, primarily from effluents of the plastic industry. It is known to cause acute neuronal death in the limbic-cerebellar system, particularly in the hippocampus. However, relatively few studies have estimated the effects of TMT toxicity on neurodevelopmental stages. In this study, we confirmed the dose-dependent effects of TMT on neurodevelopment through morphological changes and a fluorescent analysis using HuC-GFP transgenic zebrafish embryos. In addition, we analyzed the expression of genes related to neurodevelopment. Exposure of embryos to TMT for 4 days post fertilization (dpf) elicited a concentration-related decrease in body length and increase in axial malformation. TMT affected the fluorescent CNS structure of HuC-GFP transgenic zebrafish. In addition, it significantly modulated the expression patterns of Sonic hedgehog a (Shha), Neurogenin1 (Ngn1), Embryonic lethal abnormal vision like protein 3 (Elavl3), and Glial fibrillary acidic protein (Gfap). The overexpression of Shha and Ngn1, and downregulation of Elavl3 and Gfap, indicate repression of proneural cell differentiation. Our study demonstrates that TMT inhibits specific neurodevelopmental stages in zebrafish embryos and suggests a possible toxic mechanism of TMT in vertebrate neurodevelopment.

Poster Abstracts

P35: Quantifying PD-L1 spatial distribution signatures for patient selection approaches

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Inhibitors of inflammatory checkpoints (e.g. PD-L1 inhibitors) have demonstrated great promise in preclinical and clinical studies. They focus on controlling natural inflammatory checkpoints to stimulate an elevated inflammatory response against tumors through increased anti-tumor inflammatory cell infiltrates in the tumor microenvironment (TME; stroma) and tumor epithelium. Cells positive for biomarkers of inflammatory cells or mechanisms (e.g. PD-L1, CD8, etc.) are often assessed qualitatively or semi-quantitatively using immunohistochemistry in a subset of representative microscopy fields. However, the locale of inflammatory biomarkers, such as PD-L1, may be more revealing than estimating tumor-wide dispersion of biomarker-positive cells. Unfortunately, the spatial relationships and complex distribution of inflammatory cells in tissue context pose significant challenges for a meaningful manual evaluation by a pathologist.
We have developed an approach which quantified spatial relationships in tissue context. PD-L1-positive cells were quantified with CellMap™ software relative to: 1) the total number of cells in the tumor and TME tissue compartments, and 2) the number of cells within distance ranges from the tumor/TME interface. No clear sample stratification was identified with total cell analysis. However, samples could be preliminarily stratified by PD-L1+ cell density profile relative to the tumor/TME interface. This proof-of-concept study demonstrated a unique quantitative contextual assessment of inflammatory cell infiltrates in tumors that could be used to gain new insights into 1) inflammatory cell type distributions and interactions in tumors, 2) inflammatory cell spatial responses to oncology therapies, and 3) novel patient selection criteria for traditional and immuno-oncology therapeutics.

Poster Abstracts

P36: Current status of Chinese toxicology pathology community

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Following the fast development of Chinese pharmaceutical R&D, the toxicological facilities have been growing rapidly in China during recent years. The expansion of global pharmaceutics and bioethics in this country furthermore presses on the need of qualified local CROs. To help the development of toxicological pathologists we recently organized the 4th toxicology pathology training workshop in Chengdu, China. There were 8 experienced pathologist speakers coming from Europe, US, and China, and 110 participates from China and Korea. During the 3 lecture days we collected a questionnaire survey from 85 participates including 69 pathologists, 6 study directors/toxicologists and 10 non-classified participants. The survey results helped us to understand the current status of Chinese toxicology pathology community as well as the need of Chinese toxicological pathologists.

Organisations: These 85 participants came from 55 facilities including Chinese pharmaceuticals, CROs, research institutes and universities. Among these facilities, 42 can provide domestic CRO service while 14 of them can provide international service. These larger CROs are facilitated for different types of toxicological studies from single dose study to 2 year carcinogenesis studies. A few of these CROs in big cities (Beijing, Shanghai, Chengdu, etc) are SFDA/FDA/OECD compliant.

Pathologists: These were 145 pathologists in total working in these 55 participants' facilities currently. They had education as Veterinarian (about 50%) or Medical Doctor (about 44%). Most of them received their education and working experience in China. More than half of them are young pathologists with less than 5 years of experience. About 8.2% of Chinese toxicological pathologists had overseas working experience in pharmaceuticals/universities/research institutes.

Slide reading: According to the survey, the annual a slides reading was variable, about 1/3 pathologists read less than 3000 slides, 1/3 read 3000-8000 slides, 1/3 read 8000-15000 slides. Less than 10% pathologists read more than 15000 slides.

Pathology peer review: Pathology peer review is applied by 84% participants' facilities. About 33% of them follow STP recommendations, for both internal and external peer review. Other participants follow their local SOPs. The larger CROs with international studies often invite independent pathologists or sponsor's pathologists to perform the pathology peer review.

The survey results were used to understand the current status of Chinese toxicology pathology community. The data indicate that there is a great shortage of experienced toxicological pathologists within China. More advanced training in toxicological pathology and more interaction with international toxicological pathology societies will help to speed up the development of Chinese toxicological pathologists.

Poster Abstracts

P37: Ex-vivo Compact Magnetic Resonance Imaging (MRI) of the Brain with Histopathology Validation in a Rat Model of Huntington Disease

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Introduction: Ex-vivo Magnetic Resonance Imaging (MRI) of fixed biological samples allows the thorough examination of the entire specimen using multiple digital slices, leaving the specimen intact for subsequent investigations, like histopathology. We tested MRI in a rat model of Huntington's disease and validated the results by histopathology.

Materials and Methods: The classical neurotoxin 3-nitropropionic acid (3NP) was administered at 55 mg/kg/day for 8 days to 3 Lewis rats via osmotic mini-pump (i.p.) in order to induce lesions in the nucleus caudatus and putamen of the brain corresponding to the striatum in humans in which Huntington's disease associated lesions are most prominent. Three rats served as untreated controls. Formalin perfusion was applied at necropsy. Sixty-four transverse digital sections of each brain were acquired by a compact MRI system (Aspect Imaging). In addition, transverse sections stained with Hematoxylin-Eosin and Cresyl violet-Luxol Fast Blue were prepared for histopathologic examination.

Results: Ex-vivo MRI targeted the location and the size of neuropathological lesions with good precision. Histopathologically, the lesions were described as neuro¬degeneration, characterized by hemorrhage and spongiosis in the nucleus caudatus and putamen. Some unclear MRI signals were identified as sampling artifacts.

Conclusion: Ex-vivo MRI was successful in localizing the 3NP-induced neurotoxic lesions, allowing the quantification of the damage and the correlation to histopathology. The MRI technique is able to cover the whole rat brain and, being non-invasive, can be used for time course observations of the neurotoxic changes.

Poster Abstracts

P38: GSK's Nonclinical Experience with ADA SCID Gene Therapy; The Story So Far

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In October 2010 GSK entered into a Research, Development alliance with Fondazione Telethon (TIGET) and San Rafaele Hospital in Milan "to promote the development and commercialization of gene therapy (GT) for the treatment of genetic inherited diseases". TIGET's GT platform consists of a number of programs targeting primary immune deficiencies and other serious monogenic disorders amenable to ex vivo transduction of haematopoietic stem cells using retroviral (gamma- and lenti-) vectors.
A gamma-retroviral based approach for the treatment of Severe Combined Immune Deficiency caused by Adenosine Deaminase Deficiency (ADA-SCID), then in development at TIGET, was in-licensed into GSK at the same time (now known as GSK2696273). A nonclinical package had been constructed by TIGET prior to initiating a clinical trial on this program in 2001. Since then clinical evidence of efficacy and safety has been built up in a population of 18 patients with this rare disease. In order to submit an MAA (and subsequently a BLA) TIGET and then GSK have discussed regulatory expectations for the nonclinical sections with EMA and FDA, respectively, on a number of occasions. Topics to be addressed centered around insertional mutagenesis and biodistribution of transduced stem cells and their progeny. This poster will discuss these regulatory interactions, the efforts made to develop in vivo models for addressing the above topics and in vitro alternatives considered.

Poster Abstracts

P39: Busulfan-induced lymphoproliferative lesions: a study to evaluate incidence and localization.

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Busulfan, an alkylating agent, has myeloablative properties and activity against non-dividing marrow cells. Its clinical use has been well established in the treatment of haematological malignancies (chronic myeloid leukaemia and other myeloproliferative syndromes). It is also commonly used (with or without cyclophosphamide) as a conditioning regimen for haematopoietic stem cell transplantation (HSCT) preclinically and clinically.
Lymphoproliferative lesions have been reported to be induced by the administration of busulfan in mice. These lesions can interfere with the interpretation of preclinical toxicity and tumorigenicity studies performed to support the human clinical studies using allogeneic HSCT where such proliferations could potentially originate from the transplanted cells. As such, it is important to understand the background incidence of busulfan-induced lymphoid cell proliferations and their preferred tissues of origin.
We present preliminary results from a study where B6C3F1 mice were given 4 intraperitoneal injections of Busulfan (40 mg/kg, on days 0, 14, 28 and 42). Animals were sacrificed on days 126, 168, and 252. The incidence of lymphoproliferative disorders will be discussed.
All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

Poster Abstracts

P40: Epididymal Histiocytic Sarcomas Identified in B6C3F1 Mouse Carcinogenicity Studies Conducted by the National Toxicology Program

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Primary or metastatic neoplasms of the mouse epididymis are uncommonly reported from National Toxicology Program (NTP) carcinogenicity studies. However, low numbers of epididymal histiocytic sarcomas (HS) have been diagnosed as either a primary neoplasm or part of a multi-site neoplastic disease. Histiocytic sarcomas in mice are usually recognized as focal tumors primarily in organs such as the liver, lung, mesenteric lymph node, skin, and the uterus. In some cases, HS becomes widespread involving many sites. This study was undertaken to investigate the presence and incidence of epididymal HS in NTP carcinogenicity studies using the B6C3F1 mouse. The histologic and immunohistochemical staining pattern of epididymal HS from NTP cases was consistent with previous reports of the appearance and immunohistochemical patterns of HS. In general, epididymal HS, in the B6C3F1 mouse, appears to have a strong predilection for the epididymal cauda as observed in other mouse strains.

Poster Abstracts

P41: A case of hepatic malignant mesenchymoma consisting of leiomyosarcomatous and osteosarcomatous differentiations in a beagle dog

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Hepatic malignant mesenchymoma are extremely rare in dogs. We encountered a spontaneous hepatic malignant mesenchymoma in a 6-year-old female beagle dog and examined its morphological and immunohistochemical characteristics. A large endoceliac mass (approximately 20 x 10 x 10 cm) originating from the left lateral hepatic lobe was detected. On cut section, the mass was composed of hematoid fluid-filled cysts and white to grayish solid tissue with sporadic gritty areas. Histopathologically, the mass consisted of two different mesenchymal components. One form was predominantly spindle cells arranged in interlacing fascicles which were immunohistochemically positive for smooth muscle actin (SMA) and negative for S-100, indicating leiomyosarcomatous differentiation. The other form was composed of short spindle cells which were positive for S-100 and negative for SMA, and was producing various amounts of eosinophilic osteoid-like and trabecula-like matrices, indicating osteosarcomatous differentiation. In addition, invasive proliferation at the edge of the lesion into adjacent hepatic parenchyma and mild cell atypia of tumor cells were observed. Based on these findings, the mass was diagnosed as hepatic malignant mesenchymoma.
There is only one case of hepatic malignant mesenchymoma in dogs reported in the literature, and this tumor showed rhabdomyosarcomatous and hemangiosarcomatous differentiation. This is the first case of hepatic malignant mesenchymoma with leiomyosarcomatous and osteosarcomatous differentiations in dogs.

Poster Abstracts

P42: In vitro multi-parameter hepatotoxicity assay of phytochemicals using HepG2 cells with phase I and phase II metabolic systems.

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Background: Phytochemicals including polyphenols shows effectiveness for human by strong antioxidant ability. Some of phytochemicals are known to have hazardousness for human from the result of an in vitro assay and the intraperitoneal administration to animals. However, both in vitro assay and i.p. administration are not reflecting the metabolic reactions (cytochrome P450-mediated metabolism and conjugation) in the intestine. Thus, these methods could not properly assess the cell toxicity of phytochemicals. We examined the in vitro multi-parameter assay system using human hepatocellular carcinoma cell line (HepG2) with phase I and phase II metabolic activation condition to evaluate phytochemical-induced hepatotoxicity.

Methods: Nine phytochemicals including flavonoids with rat S9 and/or UDPGA and PAPS (co-factor of glucuronidation and sulfate conjugation, respectively) was added in the plate having the HepG2 cells seeded on its surface. Within 24 hours treatment with test compounds, the media was exchanged with fresh media. Intracellular staining with ROS, GSH, marker of mitochondrial membrane potential induction, and Hoescht or nuclear count, an index of cell number, were measured after 24 hours of post culture in HepG2 cells and analyzed using high content imaging system.

Results: Among nine evaluated phytochemicals, some compounds of phenylpropanoids and flavonoids showed toxicity to HepG2 cell in a non-metabolic activation condition. On the other hand, they did not display toxicity under the phase I and II metabolic activation condition. Usnic acid and aloe emodin showed cytotoxicity with or without metabolic activation. Coumarin showed hepatotoxicity only under metabolic activation condition. Furthermore, the mitochondrial dysfunction was consistent with in vivo gavage study.

Conclusion: These results correlated with the report of adverse effects in human, and the in vivo toxicity with the oral administration of these nine phytochemicals. It is suggest that the in vitro cytotoxic assay with phase I and II metabolic system was suitable to predict the toxicity of phytochemicals, particularly polyphenols.

Poster Abstracts

P43: Automated Assessment of Dystrophin Expression in Muscular Dystrophy

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Quantification of skeletal muscle fiber parameters is challenging, but important for the evaluation of a variety of neuromuscular diseases. Various endpoints from individual fiber characteristics to whole tissue determinations may be required to better understand disease biology and/or treatment efficacy. In the context of muscular dystrophy studies, assessment of dystrophin expression in muscle fibers has largely been qualitative or semi-quantitative assessments of representative areas of an entire tissue section. To provide more robust quantitation of muscle fiber endpoints, an automated method to quantify a number of group and individual muscle fiber parameters was created and applied to a test cohort of dystrophic muscle biopsies. Duchenne and Becker muscular dystrophy (DMD and BMD, respectively) are rare genetic diseases that result from mutations in the DMD gene, which encodes the dystrophin protein, leading to progressive muscle degeneration, muscle weakness and fatigue, and premature death. Muscle biopsy cryosections derived from DMD and BMD patients and from healthy control individuals were assessed using the image analysis algorithm. Numerous parameters relating to staining intensity, membrane staining completeness, and morphometric presentation of dystrophin in individual muscle fibers were quantified in dual label, immunofluorescence-stained sections. A number of parameters, including mean dystrophin staining intensity and dystrophin membrane staining completeness, were significantly different in DMD and BMD tissue when compared to normal controls, and are promising biomarkers for understanding biology of the disease. From early drug development to clinical trials, automated quantification of dystrophin expression in muscle fibers is an attractive endpoint given the mechanism of action for current promising therapies.

Poster Abstracts

P44: Tissue-based biomarkers for porcine Davidson's fixed paraffin embedded eyes

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In toxicology studies, the minipig is used increasingly as non-rodent species. Routinely, the eye is included into the histopathological evaluation, and is usually fixed in (modified) Davidson to ensure good ocular morphology. So far, most of the tissue markers to characterize structures and lesions in the porcine eye have only been established for paraformaldehyde-fixed cryosectioned eyes. Here we describe a list of tissue based markers of neuronal, glial and inflammatory cells, retinal pigment epithelium and vasculature that can easily be applied for better characterization of ocular findings in porcine Davidson's fixed paraffin embedded eyes. Both immunohistochemistry and in situ hybridization techniques were used.

Poster Abstracts

P45: Genotoxic Escherichia coli as a threat to intestinal stem cells?

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It is now recognized that the intestinal microbiota is a major environmental risk factor for colorectal cancer (CRC) and that certain commensal bacterial strains can be genotoxic and pro-oncogenic. This is the case with Escherichia coli (E. coli), one of the first commensal bacterium that colonizes the intestinal tract of newborns and persists in adults as a long-term colonizer. Indeed, some commensal strains of E. coli produce a genotoxin named colibactin. This bacterial toxin induces DNA double-stranded breaks, chromosomal instability and genetic mutations in mammalian cells. In the AOM/IL10–/– mouse model, monocolonization with a colibactin-producing E. coli strain promotes CRC. It is worth noting that all colibactin-producing E. coli strains belong to the phylogenetic group B2 that appears to be predominant in industrialized countries, with more than 50% B2 strains isolated from infant stools. Therefore, we have examined the consequences of the neonatal colonization by these genotoxic E. coli. A colibactin-producing E. coli strain, or its non-toxic isogenic mutant, was administrated by oral route to 8-day-old or 21-day-old newborn mice. Gut tissues were collected 6 hours after exposure to localize bacteria by FISH and DNA-damaged γH2AX+ intestinal cells by IHC. We observed E. coli bacteria mainly in their niche, the colic mucus gel layer, and some bacteria interacting with upper epithelial cells. Although direct interaction is required for genotoxic effect in vitro, we found γH2AX+ cells in the basal crypt region, especially at day 8. Although DNA damage cannot be seen any more at adulthood, abnormal mitosis figures persisted and the renewal of the epithelium was enhanced. Thus, the perinatal period is a critical period when the intestinal epithelium is directly exposed to genotoxic primo-colonizer bacteria that can leave a persistent footprint in intestinal stem cells.

Poster Abstracts

P46: GLP Principles in Gene Therapy: Experience and Challenges

Nicola Carriglio[1], Ilaria Visigalli[1], Michela Vezzoli[1], Francesca Sanvito[2], Samanta Scaramuzza[1], Aisha Sauer[1], Francesca Ferro[1], Stefania Delai[1], Raisa Jofra Hernandez[1], Giacomo Mandelli[1], Francesca Tiboni[1], Claudia Rossi[1], Chiara Villa[2], Paola Albertini[1], Edoardo Stradella[2], Vanda Parezanovic[2], Alessandro Aiuti[1,4], Alessandra Biffi[1], Giuliana Ferrari[1,4], Luigi Naldin[1,4] and Patrizia Cristofori[1,3].

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Gene therapy is now emerging as a medical reality with clinical efficacy demonstrated in a number of gene therapy trials and an increasing number of products entering phase ll and lll trials each year.
For the promise and potential of a gene therapy medicinal product (GTMP) to be fully realized it is important to address regulatory expectations. Guidelines for GTMP progression in clinical trials and marketing authorizations are available to facilitate a harmonized approach in the EU and US. Non-clinical studies have the primary objective of providing sufficient information for a proper risk assessment for the product's use in human subjects. The paradigm described in ICH M3 for safety evaluation of conventional pharmaceuticals is recognized as not always appropriate or relevant to GTMPs. Non-clinical studies should be designed on a case by case basis, understanding the relevant aspects of the science underpinning that product and need for specific expertise beyond the traditional pharmaceutical field. The combination of expertise of personnel trained in research, experimental pathology, safety assessment and quality assurance has enabled to set up GLP Test Facilities in an academic environment. The objective is to maximize the early collection of proof-of-concept data that address regulatory expectations, with assurance of scientific integrity, validity and reliability. Challenges in designing non-standard study protocols to investigate in vivo fate of genetically modified cells (biodistribution) and toxicological and tumorigenicity potential are discussed focusing on the definition of test item, characterization, test system (animal model), sample traceability, duration of treatment and endpoints.
Accordingly, fit for purpose study designs for GTMP safety assessment were developed and adopted to GLP conditions. HSR-TIGET has successfully completed several safety studies for gene therapy of immunodeficiency, metabolic and blood disorders. The obtained information support discussion with regulatory boarding allows early collection of proof of concept data and minimizes the use of animals (3Rs). Evaluating the biosafety of GTMPs and in GLP provides results meeting the highest regulatory standards and outstanding scientific significance.

Poster Abstracts

P47: Recombinant human thioredoxin attenuates the gastric mucosal injury induced by aspirin and ethanol through different action mechanisms in mice.

Soo-Min Lee[1], Jin-Woo Oh[1], Guen-Suek Lee[1], Sae-Byel Hong[1], Byoung O O[2], Sang-Uk Nham[2], Jeong-Hee Han[1], Byung-IL Yoon[1], Byung-Woo Lee[1]

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Thioredoxin (Trx) is a potent redox regulating protein which has antioxidant properties and other functions for cell survival by inhibiting apoptosis and inflammatory cytokine production. Aspirin and ethanol can cause gastrointestinal toxicities, particularly in the stomach, which are associated with triggering apoptotic signaling and generation of reactive oxygen species (ROS).
Therefore, we tested the hypothesis that exogenous recombinant human Trx (rhTrx) would attenuate aspirin and ethanol-induced gastric injuries, and investigated its action mechanisms. Male C57BL/6 mice (8 week old) were orally administered with single dose of aspirin (300 mg/kg/b.w.) or 70 % ethanol to induce gastric injury, respectively. rhTrx was intraperitoneally injected at 30 min before and after the treatments at the concentrations of 0, 0.5, 1.5 and 10 mg/kg/b.w. . Five or four hours after the last treatment of either aspirin or ethanol, all mice were sacrificed. The stomachs were grossly and histopathologically examined, and TUNEL assay and immunohistochemistry for 4-hydroxynoneal (4-HNE, an indicator of ROS) and cyclooxygenase-2 (COX-2) were also performed.
As results, aspirin and ethanol-induced gastric injuries, characterized by mucosal disruption with cell apoptosis and necrosis and inflammation, were significantly attenuated in the rhTRX-treated mice at the doses of 1.5 and 10 mg/kg/b.w. for the aspirin group and at the dose of 10 mg/kg/b.w. for the ethanol group ($p<0.05$). Gastric mucosal cell apoptosis was notably reduced by rhTrx- treatment in both gastric injury models. In terms of the underlying mechanisms, immunohistochemistry for 4-HNE showed that ROS was deeply associated with the gastric injury by ethanol but not by aspirin. Pre-treatment with rhTrx decreased ROS in the stomach of the ethanol-treated mice. Meanwhile, COX-2 immunoreactivity was diminished in the areas injured by aspirin, which suggested that COX-2 dysregulation was likely related to the aspirin-induced gastric injury. Taken together, our results indicate that rhTrx protects against the gastric injury induced by aspirin or ethanol through different action mechanisms, maintaining COX-2 expression in cells and antioxidant effects, respectively.

Poster Abstracts

P48: Exogenous recombinant human thioredoxin-1 prevents the hepatotoxicity of acetaminophen and ethanol via regulation of free radicals and JNK cell death signaling

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Thioredoxin-1 (Trx-1) is a potential therapeutic material against various diseases related to oxidative stress and apoptosis. Acetaminophen (APAP) and ethanol are hepatotoxicants inducing liver injury through oxidative stress-related cell death signaling. In the present study, we investigated the preventive effects of exogenous recombinant human Trx (rhTrx-1) against liver injury induced by APAP and ethanol in mice.
For the APAP study, C3H/he mice (8 weeks old, male) were given rhTrx-1 intraperitoneally (0, 10, 50 and 100 mg/kg/b.w.) 1 h after single oral administration of APAP (300 mg/kg), then necropsied at 6 h after the APAP treatment. To see the rhTrx-1 effect against the APAP lethality, 10 mg/kg/b.w. rhTrx-1 was administered i.p. before the administration of a lethal dose (400 mg/kg/b.w.) of APAP. For the ethanol study, C57BL/6 mice were injected 3 times i.p. with rhTrx (0, 0.1, 1 and 10 mg/kg/b.w., respectively) 1h before 3 oral administrations of 25% ethanol (4.5g/kg/b.w.). Immunohistochemistry for 4-hydroxynonenal and nitrotyrosine (3-NT), TUNNEL assay and Oil red O stain were performed as well as gross and histological examinations. Western blots were also carried out for 3-NT, Trx-1 and p-JNK/JNK.
As results, rhTrx-1 treatment at 10 mg/kg/b.w. significantly reduced the APAP-induced liver injury, characterized by centrilobular necrosis and apoptosis,. Correspondingly, increases of 3-NT and p-JNK/JNK and down-regulation of Trx-1, which are the critical liver toxicity-related molecular changes occurring post-APAP treatment, were significantly inhibited by rhTrx-1 treatment at 10 mg/kg/b.w. Our results indicated that exogenous rhTrx-1 at 10mg/kg/b.w.is also preventive against the ethanol-induced periportal microvesicular liver steatosis. Those inhibitory effects of rhTrx-1 against APAP and ethanol toxicity were most likely associated with regulation of free radicals and cell death signaling, JNK activity.

Poster Abstracts

P49: Exogenous treatment of recombinant human thioredoxin-1 inhibits atrophy of glomeruli and apoptotic cell death induced by chronic exposure to cyclosporine A in the mouse kidney via reducing production of reactive oxygen species

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[*] Hong and Oh contributed equally for this study.

Thioredoxin-1 (Trx-1), a small molecule, has various cellular functions including anti-oxidant, anti-inflammation and anti-apoptosis. Thus it has been expected as a potential therapeutic agent against various inflammatory diseases. Cyclosporine A (CsA), calcineurin inhibitor, which has been widely used for immune suppression after organ transplantation and in autoimmune disorders, can cause nephrotoxicity.

In the present study, we investigated the preventive effect of exogenous recombinant human Trx-1 (rhTrx-1) against CsA-induced nephrotoxicity and explored its protective action mechanisms. Male C57BL/6 mice (8 weeks old) were injected intraperitoneally daily with rhTrx-1 (0, 1, 5 and 10 mg/kg/b.w.) for 4 weeks before daily subcutaneous treatment of CsC (30 mg/kg/b.w.) for 4 weeks. Throughout the experimental period, the mice were subjected to a low salt diet to promote CsA-inducing nephrotoxicity. The mice were sacrificed at 4 hours after the last CsA treatment for gross and histopathological examination of the kidneys. In the histopathological examination, total number of atrophic glomeruli was counted in the full area of each tissue section and the results from each group were compared. In addition, TUNEL assay and immunostaining for 4-Hydroxynonenal (4-HNE, a maker of reactive oxygen species (ROS)) were performed on the kidneys.

The CsA-induced nephrotoxicity included significantly increased numbers of atrophic glomeruli, tubular degeneration, and often cortical haemorrhage. The increase in atrophic glomeruli was significantly blocked by 10 mg/kg/b.w. treatment of rhTrx; the number of the atrophic glomeruli was similar to that in the vehicle control group. The protective effect of rhTrx against CsA-induced nephrotoxicity was further supported by significant reduction of apoptotic cells in the mouse kidney by pre-treatment with 10 mg/kg/b.w. rhTrx-1. Pre-treatment with rhTrx-1 reduced ROS level in a dose-dependent manner. Taken together, our results demonstrated that exogenous rhTrx-1 is preventive against CsA-induced chronic nephrotoxicity, and we propose that rhTrx-1 could be a therapeutic agent against CsA-induced kidney toxicity.

Poster Abstracts

P50: Induced Interstitial Pulmonary Fibrosis (IPF) Model: Unlabeled Bleomycin Distribution and Early IPF Markers Identification by MALDI Imaging.

David Bonnel[1], Mary Mcelroy[2], Emeline Falaux[1], Gaël Picard De Muller[1], Fabien Pamelard[1], Alain Heron[1], Stephen Madden[2] and Jonathan Stauber[1]

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Interstitial Pulmonary Fibrosis (IPF) is a chronic and progressive lung disease. It is currently believed that fibrosis is caused by aberrant alveolar epithelial cell activation and repair leading to fibroblastic/myofibroblastic foci, accumulation of extracellular matrix and irreversible destruction of lung tissue. Combined with classical histological staining, Mass Spectrometry Imaging (MSI) was used to improve the understanding of the Bleomycin IPF rat model and to identify several potential early biomarkers of this pathology. Rats were administered seven doses of Bleomycin delivered to the lungs at 1 mg/kg. Several fresh lung sections were prepared and analyzed by MSI. We describe a process which combines MSI and classical staining approach directly on tissue to follow the distribution of molecules implicated in fibrosis, and allow a better understanding of lung damage and repair. Indeed most of the identified biomarkers were located in extracellular medium and in the plasma membrane, as some upregulated lipids and novel lipids not previously associated with fibrosis. These lipids are known to be involved in cell signaling, chemotaxis or membrane stability, which might be associated with dysregulated alveolar epithelial repair and/or fibrosis. Combination of MSI and histological staining provides information regarding molecules distribution and identification in the tissue by studying their co-distribution and by comparing their relative abundance at active sites of fibrosis. Here we identified a number of biomarkers which may be useful diagnostic and/or therapeutic targets and therefore useful tools to help understand the efficacy and safety of novel treatments in IPF.

Poster Abstracts

P51: Evaluation of Drug Phototoxicity in Skin Layers by Multimodal Molecular Imaging Techniques

Gregory Hamm[1]; Pierre-Maxence Vaysse[1]; Aaron M. Sargeant[2]; Fabien Pamelard[1], Alain Heron[1], David Bonnel[1] and Jonathan Stauber[1]

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Phototoxicity is an inflammatory skin reaction caused by exposure to a chemical and subsequent exposure to sunlight or ultraviolet radiation. This phenomenon is mainly a concern for drugs and pharmaceuticals that are either ingested or applied directly to the skin, for example, as a cream. Today, the FDA requires Drug induced phototoxicity studies for each new compound on the market. In this context, Mass Spectrometry Imaging (MSI) enables spatially resolved and unlabeled imaging of the drug and its photo-metabolites directly in their skin micro-environment. MSI offers also the possibility of discovery of new molecular biomarkers that are regulated by a photo-irradiation and that are specific to skin substructures. In a proof of concept experiment, multimodal molecular Imaging technique was used to study the absorption, distribution and metabolism of a drug, Ketoprofen, its photo-metabolites as well as some potential biomarkers of toxicity or inflammatory process within dermal layers. A minipig (WT) was treated with different sites of topical administration of Ketoprofen solution. For comparison, a minipig was treated with vehicle alone. After 5 days of administration and photosensitization, skin biopsies were removed for each condition and snap-frozen before being cryo-sectioned and mounted on ITO glass slides (4 per group). MS images were obtained in both positive and negative detection mode using a SolariX MALDI-FTICR 7.0T Mass Spectrometer (Bruker Daltonics) at high spatial resolution (20-40 µm). Data were generated and analyzed using MultimagingTM and QuantinetixTM softwares (ImaBiotech, France). A Quantitative measurement of Ketoprofen and its photo-metabolites was performed using a specific MSI protocol [(including the use of an internal standard and the spotting of a dilution range near targeted tissue, to locally evaluate the amount of each targeted molecule within skin substructures (epidermis, dermis and deep dermis)]. Multimaging can also provide a penetration profile of the drug through these layers. Moreover, the distribution of some inflammatory biomarkers such as lysoPC or LysoPA can be assessed and compared with drug localization and quantification in the same histological structures of the skin. LC-MS was used on the same samples to validate this approach and compare quantitative measurement of both MS based techniques. Immunohistochemical (IHC) staining was used on adjacent skin sections to label several cells involved in skin defense mechanism. Notably, we are able to follow Langerhans cells within the epidermis using CD1a labeling. These cells have specific behaviors in regards to danger signal (in our case the phototoxicity) based on molecular changes. MS images were then correlated with IHC results to establish a relationship between cell types and molecular species modulation. We present here a new approach to evaluate the phototoxicity of a drug, to discover toxicity biomarkers and to interpret biological or pharmacological mechanisms within tissues based on MS image analysis that link quantitative assessment of ions in the target material to the tissue microscopic anatomy.

Poster Abstracts

P52: NASH mechanism understanding using MS Imaging: Discover New Disease State Biomarkers

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The liver is the main organ of concern in drug discovery to assess efficacy or safety of a treatment. NASH (Non Alcoholic Steato Hepatitis) is emerging as a real health corner stone in the progression from simple steatosis to more severe liver pathologies like cirrhosis. The characterization of this pivotal step using predictive biomarkers can improve the knowledge on the pathogenesis of the disease in various aspects such as inflammation, fibrosis, oxidative stress or modifications of lipid metabolism. As the hallmarks required for the diagnosis of NASH (steatosis, lobular inflammation, hepatocellular ballooning or pericellular fibrosis) are still established on liver biopsy, the identification of biomarkers using MSI approach associated with histological staining has a very interesting role to play in this field.
E3L.CETP and LDLR-/- mice fed with different diets were sacrificed and livers (12 tissues in each group) were removed. Snap frozen liver collected in N2 or Formaldehyde-Paraffin-embedded were sectioned and thaw mounted on ITO glass slide. DHB (40 mg/mL in MeOH/W+0.1% TFA) and 9AA (10 mg/mL in MeOH) were respectively used as MALDI matrices for positive and negative detection mode. Two matrix applications were used, the classical way with SunCollect sprayer device (SunChrom) and the sublimation process using home-made apparatus. Mass spectrometric images were performed in positive or negative detection mode using a SolariX MALDI-FTICR 7.0T Mass Spectrometer (Bruker Daltonics). Histological experiment involved H&E, Oil Red O and Masson's Trichrome staining.
In this study, we investigated the targeted metabolite profiling in two animal models of metabolism disorders exposed to various diets to assess liver histological specificities. A multimodal approach to focus on various molecular classes (small metabolites & lipids) and using different polarities was used to describe liver modifications in NAFLD and NASH models. Potential disease or histological related biomarkers were observed especially at the level of lipids, phospholipids (PC) or lysophospholipids classes (LPA or LPC). Smaller molecules have been detected such as biliary acids near portal vein area or the GSH/GSSG couple. All these biomarkers were characterized on-tissue section using MS/MS experiments (SORI-CID mode) and high mass accuracy measurement (below ppm level). Potential correlation with fibrosis and inflammation process has been advanced at a biological point of view. Molecular distribution was correlated with H&E (for classical histology), Oil Red O (for lipids droplets detection) and Masson's Trichome (for fibrosis area observation) staining on adjacent tissue section to highlight histological specificities related to metabolites levels. In conclusion, MSI was used to achieve a better understanding of underlying process occurring in liver disease development linking to metabolites changes.

Poster Abstracts

P53: New Biomarkers Discovery Approach Based on Morphometric Evaluation of Mass Spectrometry Imaging (MSI) Dataset: A Case Study on Bile Acids Transporter Inhibition

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The discovery and qualification of new biomarkers to evaluate the efficacy or the toxicity of a treatment within a tissue is of great interest in pharmaceutical research. In this context, MSI enables spatially resolved imaging of different metabolites directly in their micro-environment and the discovery of new molecular biomarkers that are regulated by a treatment. In a proof of concept experiment, comparison of ions in treated and control liver tissues shows that the distribution of certain bile acids is a biomarker of transporter inhibition with better significance than that obtained from the global ion intensity in the images. MS image analysis shows a differential localization of bile acids, especially TCA, under the different treatment conditions. Interestingly, morphometry reveals a biomarker that would not be found on the basis of global intensity or concentration in the tissue. For example, 26 TCA objects were detected in the image from livers with high level inhibition compared with only 1 object in control tissue, but the images have similar global ion intensities. TCA is mainly concentrated in the portal area in control liver but is more widely distributed throughout the parenchyma in livers from both low and high level inhibition conditions – a differential localization which may be attributed to biliary transporter inhibition. In conclusion, this morphometric analysis of ions can be applied to a variety of OMICS studies for pharmaceutical or diagnostic purposes, the assessment of biological barrier crossing by a drug, or the discovery of disease state biomarkers.

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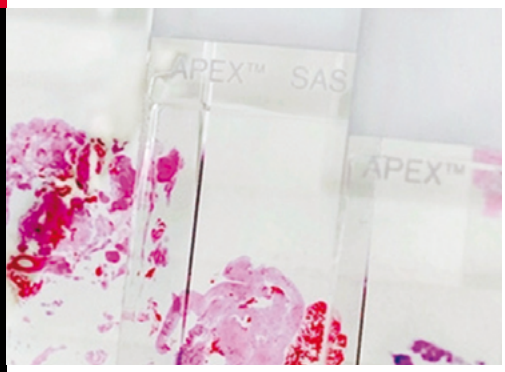

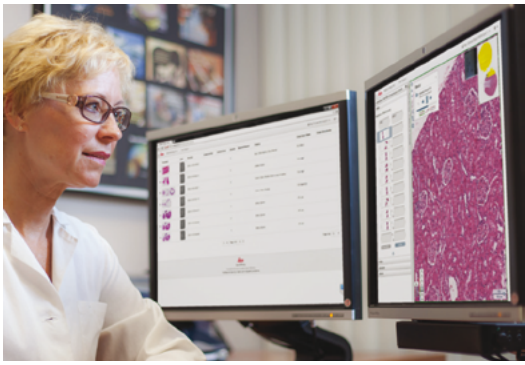

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