EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY

19TH EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY

13-16 SEPTEMBER 2022 MAASTRICHT, THE NETHERLANDS

ABSTRACT BOOK

DEAR COLLEAGUES AND FRIENDS

Due to the continuous pressure of COVID 19 restrictions, the ESTP was forced to cancel the annual meeting in 2020 and to organize a virtual ESTP on-line meeting in 2021. Therefore, it is with great pleasure that we welcome you to our live in-person 19th EUROPEAN CONGRESS OF TOXICOLOGIC PATHOLOGY in Maastricht, The Netherlands starting 13 September - 16 September, 2022.

The congress organizing committees (Scientific and Local) have planned an inspiring 4-day scientific program on various pathology issues in the area of the female reproductive tract which justifies the challenging title of the congress "THINK FEMALE". The details of the program (including the social and side program) can be found on our brand new website, a joint website of the ESTP, ESVP and ECVP: **www.esvp-ecvp-estp-congress.eu**

Before the start of the congress on Tuesday morning, 13 September, a half-day workshop is organized by the International Academy of Toxicologic Pathology (IATP) on Alzheimer's disease. This IATP Satellite Symposium includes new insights into the pathogenesis, treatment modalities, and translational models and will be closed by a panel discussion at the end of the speaker's presentations.

Tuesday afternoon, the ESTP Congress will officially open at 13.15 and end on Friday 16 September 12.20. The scientific program is composed of seven sessions in the area of the Female Reproductive System.
Presentations given by experts in the field vary from histopathology in the FRTS of animals and women (normal, during pregnancy, drug-induced and diseased); toxicity testing for female reproductive endpoints; hormone-related pathology in human and animals (e.g., endometriosis and breast cancer); drug discovery and new therapeutic approaches; carcinogenicity testing; an update of FRTS terminology in toxicologic pathology; new techniques and models; and endocrine disruption. At the Friday morning session several Mystery Cases will be presented. The scientific program will be closed on Friday by a presentation on Fish Pathology (INHAND). During the coffee and lunch breaks there will be time to visit the vendors and the scientific posters in the Ceramique room.

We are proud to announce that the high scientific standard of the congress will be acknowledged by presenting the following awards: The Chirukandath Gopinath Lecture Award, The Maronpot Guest Lecture, The SFTP Poster Award and the ESTP Publication award.

The support of our great Sponsors and the voluntary work of ESTP members makes it possible to organize this conference providing you opportunities to improve your scientific skills and network. Therefore, we would like to encourage each member to participate in the annual general assembly and early career pathologist to participate in the younger generation program, which are held during lunch breaks. If you are not a member of the ESTP we would warmly welcome your application for a membership.

Many of us will recognize that, after two years of COVID 19 restriction, a social program which enables informal contact with colleagues will be more than welcome. Therefore, we would like to invite you to the Welcome reception on Tuesday 13 September in the historic Maastricht City Hall.
In addition, we would be very pleased to welcome you at the Congress Dinner and Dancing on Thursday 15 September at Ipanema Restaurant.
Finally, being in the beautiful city of Maastricht, we advise you to take a look to the wonderful old buildings and architecture and do not forget to visit one of the many good restaurants, bars and terraces that Maastricht offers.

Enjoy and Tot Ziens,
On behalf of the Local and Scientific Organizing Committees
Eveline de Rijk, Ankie Lambregts, Anna-Lena Frisk and Melissa Czajkowski.

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GENERAL CONGRESS INFORMATION

PROGRAM

ABSTRACTS AND CASE PRESENTATIONS

SCIENTIFIC ORGANIZING COMMITTEE, LOCAL REPRESENTATIVES AND CONGRESS ORGANIZERS

Scientific Organizing Committee

Roger Alison
Ute Bach
Melissa Czajkowski
Eric van Esch
Anna-Lena Frisk
Sibylle Gröters
Hans Harleman
Marjolein van Heerden
Paul Howroyd
Ankie Lambregts
Rosanna Manno
Alessandro Piaia
Eveline de Rijk
Matthias Rinke
Mikala Skydsgaard
Claudine Tremblay

Local Representatives

Eveline de Rijk
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Congress Organizers

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pco PAUWELS CONGRESS ORGANISERS

USEFUL INFORMATION

Venue
The ESTP Congress will be organized in: The Crown Plaza Hotel in Maastricht Ruiterij 1, 6221 EW Maastricht, T ±31(0)43–3509191

Registration Desk
The registration area in the conference centre will be open for registration and questions on:

Tuesday 13 September:	08.00-17.00
Wednesday 14 September:	07.15-17.00
Thursday 15 September:	07.15-17.00
Friday 16 September:	08.00-13.00

Please note that The official currency at the congress is the Euro. At the registration desk cash, cheques and foreign currency are not accepted.
The registration fee includes: Admission to all scientific sessions, admission to the exhibition area, lunch Wednesday and Thursday, daily coffee breaks, final programme, welcome reception and the congress Dinner.

WIFI
You will have WIFI access on-site in the congress centre. First connect with the Crowne Plaza Hotspot
WIFI in the lecture room: Username: **bourgogne**, Password: **Bourgogne**
WIFI in the exhibition room: Username: **ceramique**, Password: **Ceramique**

Congress badges
All participants, accompanying persons and exhibitors must wear the identification badges. Entrance to meeting halls and exhibition area will not be permitted to any person without badge.

Congress rooms
The plenary lecture room is in room Bourgogne. The exhibition hall, posters and catering are located in the Ceramique rooms.

Certificate of attendance
A certificate of attendance can be downloaded after submission of the online evaluation which will be requested to be filled in after the congress. You will receive an invitation via email after the congress.

Interactive Slides
For the interactive presentations we kindly ask you to download the App Slido on your smart phone.

Poster Presentations
Posters will be exhibited during the entire Congress in room Céramique. A poster session including an award ceremony is scheduled for Thursday afternoon. Authors are kindly requested to be at their posters during the coffee breaks to answer potential questions.

Anything lost?
Please go to the registration desk.

Language
The official language of the congress is English.

Mobile phones
Please silence your mobile phones during the lectures

Photography, Videotaping, Recording Policies
Photography of poster presentations is prohibited without the specific consent of the presenter(s)/author(s). Photography of exhibitor booths and/or equipment is prohibited without the specific consent of the exhibitor. Photography, videotaping, or recording of the Scientific Sessions is not permitted.

CITY MAP OF MAASTRICHT

1. **The Crown Plaza Hotel**: Congress Venue
   Ruiterij 1, 6221 EW Maastricht
2. **City Hall**: Welcome Reception
   Markt 78, 6211 CL Maastricht
3. **Ipanema**: Congress Dinner
   Avenue Ceramique 250, 6221 KX Maastricht

EXHIBITION FLOORPLAN

AWARDS

Chirukandath Gopinath Lecture Award

The award (engraved glass award), instigated by the British Society of Toxicological Pathology in 2008, was due in part to the BSTP's involvement in the organization of the scientific program of the 2008 Annual ESTP Congress held in Edinburgh. To mark the occasion, the BSTP sponsored the keynote lecture and since then this sponsorship has become a tradition at the ESTP Congresses. The sponsored lecture was called the BSTP Chirukandath Gopinath Lecture in tribute to one of the founder members of the BSTP whose name is recognized by toxicological pathologists all over the world. The lecture is to be on a topic in pathology relevant to practicing toxicological pathologists. The speaker is an internationally recognized scientist and is chosen by the scientific organizing committee and approved by the BSTP council.

IATP Maronpot Guest Lecture Award

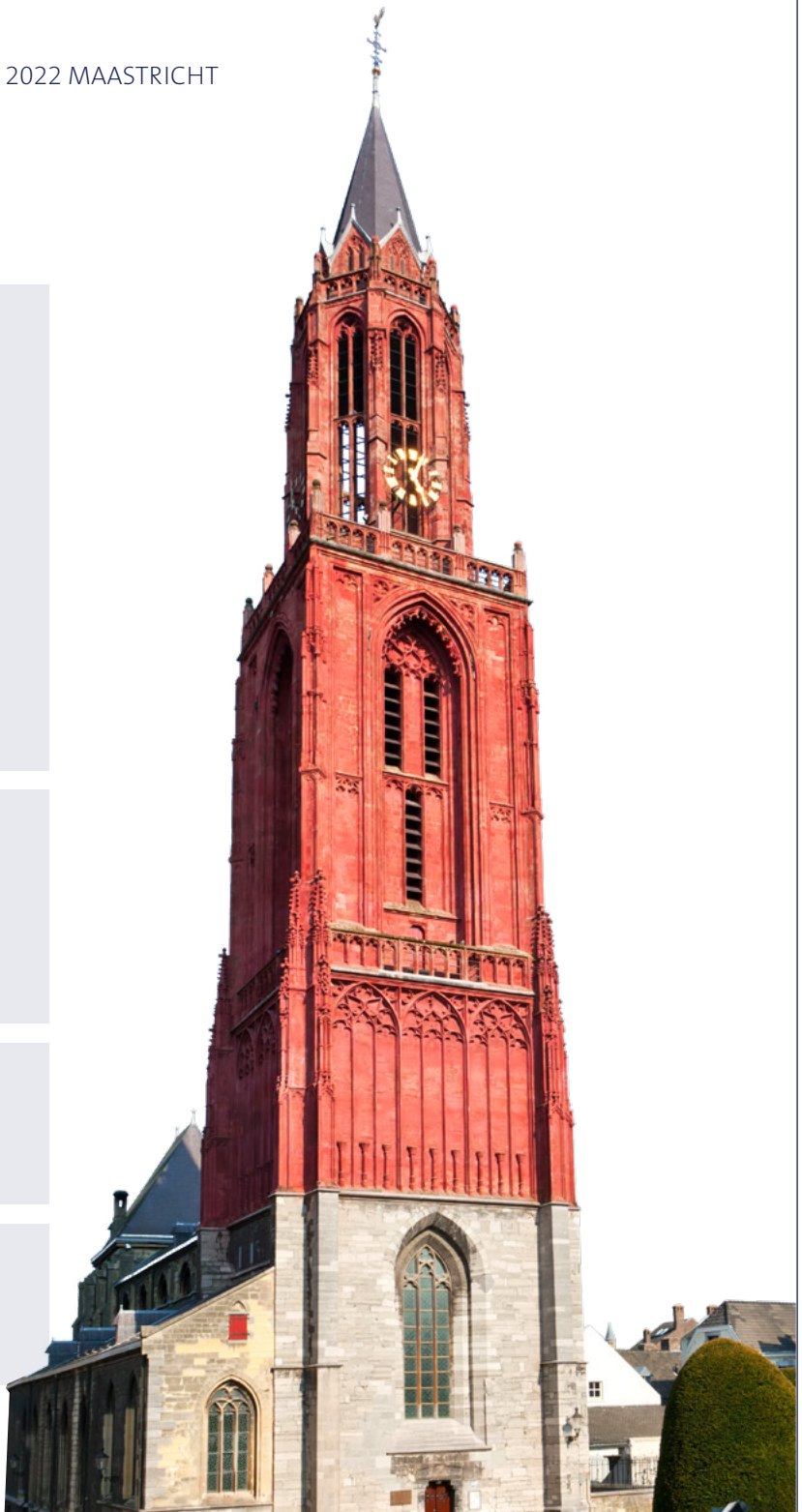
This award recognizes Dr. Robert Maronpot for his significant contributions to the field of toxicologic pathology and the advancement of the IATP. This lecture award is sponsored through an educational grant provided by The Telikicherla Higher Education Foundation.

Poster Award

Award for the Best Poster sponsored by the French Society of Toxicologic Pathology (SFPT)

Publication award

To honour advancements in the field of toxicologic pathology, impactful publications (either theses or papers) are annually awarded by the ESTP.



IATP SYMPOSIUM

Alzheimer's disease: new insights into the pathogenesis, treatment modalities, and translational models

This 4.0-hour IATP Satellite Symposium will give an overview of the many aspects of Alzheimer's Disease (AD) from understanding the manifestation of clinical disease to current therapies, translational models, and gene-environment interactions. The Symposium will give an overview of clinical disease and its pathogenesis, which will include risk factors for AD and maintenance of cognitive health. The session will further explore novel therapies and approaches, and mechanisms of action of effective treatments. Appropriate and relevant models that have comparative aspects representative and translatable to AD in human will be discussed and the pros and cons of the proposed models will be reviewed. The concept of how environmental factors interact with genetic factors, and AD and the exposome will be explored. A panel discussion will convene at the end of the speaker presentations.

Crown Plaza Hotel
Ruiterij 1, 6221 EW Maastricht,
T +31(0)43-3509191

Registration fee
IATP Symposium € 125
IATP Symposium + lunch €150
Prices are excl. 21% Dutch VAT

The registration fee includes
- Admission to the IATP scientific sessions
- Coffee break
- Lunch only upon separate registration!

TUESDAY 13 SEPTEMBER 2022

07.30-08.00 **Registration IATP Symposium**
08.00-08.05 **Opening Remarks**
08.05-08.45 **Overview of Alzheimer's Disease** *Pieter Jelle Visser, Maastricht Univerisity, Maastricht, the Netherlands*
08.45-09.30 **Novel Alzheimer's Disease therapies and approaches to cross the Blood Brain Barrier** *Kerstin Hahn & Vanessa Schumacher, Roche Innovation, Basel, Switzerland*

09.30-10.00 **Coffee break**

10.00-10.45 **Experimental Models for Alzheimer's Disease** *Donna Wilcock, University of Kentucky, USA*
10.45-11.30 **The Gero-Exposome of Alzheimer's Disease** *Caleb Finch, USC, USA*
11.30-11.55 **Panel Discussion** *All Speakers*
11.55-12.00 **Closing Remarks**

12.15-13.15 **Registration ESTP Congress**
13.15-13.25 **Welcome** *ESTP Chairman*
13.25-13.30 **Introduction Session 1: General Histopathology**
Chairs: Anna-Lena Frisk & Paul Howroyd
13.30-14.15 **Methodology in toxicologic pathology (Best Practice Histology, recognizing and recording normal immaturity, cycle stages, senescence) and spontaneous non-neoplastic pathology of the female reproductive tract in non-clinical species** *Justin Vidal*
14.15-14.45 **Comparative Aspects of Placental Histology** *Sydney Mukaratirwa*
14.45-15.30 **Patterns of toxicity in the female reproductive tract** *Justin Vidal*

15.30-16.00 **Coffee break - Posters and Exhibition**

16.00-16.05 **Introduction Session 2: Extended One Generation Studies**
Chairs: Eveline de Rijk & Maike Huizinga
16.05-16.50 **The design, conduct and rationale for Extended One-Generation Reproductive Toxicity studies** *Manon Beekhuijzen*
16.50-17.20 **Normal histology of the female reproductive tract in DART and EOGRT studies** *Paul Howroyd*
17.20-17.45 **General Discussion & Questions on the Female tract** *Justin Vidal, Manon Beekhuijzen, Paul Howroyd, Sydney Mukaratirwa*
19.00-20.00 **Welcome reception**

WEDNESDAY 14 SEPTEMBER 2022

07.30-08.35 **Registration**
07.30-08.35 **Early Bird Meeting: Mission and Vision ESTP**
07.30-08.30 **Breakfast symposium Aiforia**
08.35-08.40 **Introduction Session 3:** Human Pathology and Hormone-Related Pathology
Chairs: Ute Bach & Eveline de Rijk
08.40-09.25 **Influence of estrogens and progesterone on the endometrium: normal and pathological modifications** *Christine Bergeron*
09.25-09.55 **Is this normal? Inhabiting the limbo of the gender data gap: a patient's experience of endometriosis** *Rose George*

09.55-10.30 **Coffee break - Posters and Exhibition**

10.30-11.10 **Drug discovery & development in women's health: Don't get lost in translation** *Thomas Zollner*
11.10-11.50 **Endometriosis in macaques: Natural history and potential for new therapeutic approaches** *Mark Cline*
11.50-12.15 **Ceremony Honorary members** *ESTP - Chairman*

12.15-13.25 **Lunch - Posters and Exhibition**

12.15-13.25 **Presidents Meeting / Early Career Meeting**
13.25-13.30 **Introduction Session 4:** Mammary Gland
Chairs: Mikala Skydsgaard & Hans Harleman
13.30-14.15 **Modeling Cancer Immunotherapy in Tumor-Bearing Nonhuman Primates** *Mark Cline*
Maronpot Guest Lecture, Sponsored by IATP and the Telikicherla Higher Education Foundation (THE)
14.15-14.55 **AI and mammary gland cell proliferation** *Henning Hvid*
14.55-15.35 **Human breast cancer: a heterogeneous disease** *Trine Tramm*

15.35-16.05 **Coffee break - Posters and Exhibition**

16.00-16.05 **Introduction Session 5:** Carcinogenicity
Chairs: Matthias Rinke & Melissa Czajkowski
16.05-17.00 **INHAND female reproductive tract nomenclature (hyperplasia and/or tumors)-issues and current status of organ working group discussions**
Heike Marxfeld, BASF Frankenthal, Ute Bach, Bayer AG Wuppertal, Germany

THURSDAY 15 SEPTEMBER 2022

07.30-08.30 **Registration**

08.30-08.35 **Introduction Session 6: New Techniques and Alternative Models**
Chairs: Marjolein van Heerden & Roger Alison

08.35-09.20 **Mass Spectrometry Imaging** *Ron Heeren*

09.20-09.45 **Mass Spectrometry Imaging within the Women Health area** *Rob Vreeken*

09.45-10.15 **The zebrafish as model for osteoporosis** *Dylan Bergen*

10.15-10.45 **Coffee break - Posters and Exhibition**

10.45-11.30 **Organoids from human endometrium as cutting-edge research and screening models in reproductive biology** *Hugo Vankelecom*

11.30-11.55 **Two Validation Strategies for Deep Learning-assisted Image Analysis of Digital Study Material** *Michael Staup, Heike Antje Marxfeld*

11.55-12.15 **Pathology 2.0. - Results of Survey Artificial Intelligence** *Dirk Schaudien, Josep Monne*

12.15-14.00 **Lunch - Posters and Exhibition**

12.15-14.00 **ESTP Annual General Assembly -** pick-up your lunch before start

14.00-14.05 **Introduction Session 7: Endocrine Disruption**
Chairs: Sibylle Gröters & Ankie Lambregts

14.05-14.30 **Safety assessment of (Agro)chemicals from a pathologist's perspective** *Sibylle Gröters*

14.30-15.00 **Cracking the Egg Factor: Updates from the FREIA project** *Majorie van Duursen*

15.00-15.30 **Environmental assessment of pharmaceutical drugs with ED properties** *Daphne de Roode*

15.30-16.00 **Coffee break - Posters and Exhibition**

Chairs: Celine Thuilliez & Matthias Rinke
Chairs: Silvia Guionaud & Hans Harleman

16.00-16.30 **Poster and Publication Presentations and Award ceremony**

16.30-17.30 **Workshop Aira Matrix: Application of Deep Learning for the evaluation of reproductive toxicity end points in rodents** *Sabina Soldati, Tijo Thomas, Erio Barale*

19.30-00.30 **Congress dinner - Ipanema**

FRIDAY 16 SEPTEMBER 2022

09.10-09.15 **Introduction Session 8: Mystery cases**
Chairs: Sibylle Gröters & Ute Bach

09.15-09.55 **Mystery Cases**

09.55-10.35 **A compliant and scalable digital workflow for accelerating pre-clinical studies using Concentriq for Research** *Bettina Lawrenz, Luca Caneparo*

10.35-11.00 **Coffee break - Posters**

11.00-12.05 **Introduction Session 9: INHAND - Fish Pathology**
Chairs: Alessandro Piaia, Simone Tangermann

11.05-12.05 **Interconnection of ovary and liver in laboratory fish - INHAND terminology and morphological criteria in physiology and pathology**
Christine Ruehl-Fehlert, Heike Schmidt-Posthaus
The Chirukandath Gopinath Award (sponsored by BSTP) will be awarded to Christine Ruehl-Fehlert

12.05-12.20 **Closing Ceremony**

Room Meuse
ESTP Board Meeting
Early bird Meeting Mission and Vision ESTP
Presidents Meeting

Room Bourgogne
Lectures IATP
Lectures ESTP
Breakfast symposium Aiforia
Workshop Aira Matrix
Early Career Meeting
ESTP Annual General Assembly

Room Céramique
Registration
Exhibition
Posters
Breaks & Lunches

SOCIAL PROGRAM

Tuesday 13 September 2022

19.00-20.00 **Welcome Reception**

City Hall, Markt 78, 6211 CL Maastricht

The welcome reception will take place in the historic Maastricht City Hall (Stadhuis). The city hall, built over the years 1659-1664 under the guidance of master architect Pieter Post, has a tower dating from 1684 and a carillon with 49 bells that is still regularly played. The interior is also very attractive, with beautiful wall tapestries, stucco work, ceiling paintings, and mantelpieces.

Registration fee: Included in the registration fee for participants

On walking distance from the venue

Thursday 15 September 2022

19.30-00.30 **Congress Dinner and Dancing**

Ipanema, Avenue Ceramique 250, 6221 KX Maastricht

The congress dinner and dancing will take place in the Ipanema Tower, entrance river side.

Registration fee: Included in the registration fee for participants

On walking distance from the venue

SIDE MEETINGS

Monday 12 September

14.00–18.00 **ESTP Board Meeting - on invitation only**
Room: Meuse
Chair: Frank Chanut, ESTP Chairman

Wednesday 14 September

07.30–08.30 **Early bird Meeting Mission and Vision ESTP - open meeting**
Room: Meuse
Chair Silvia Guionaud, ESTP Designated Chairman
Since it's inception a decade ago, ESTP has made many impactful and rewarding scientific contributions to our professional lives. To keep our momentum and ensure the society remains relevant for its members, now is a good time to review the professional landscape as well as our remit and goals. Join us for a discussion on the ESTP Mission and Vison and help charting our way into the future. I am looking forward to seeing you on 14 September!

12.15–13.15 **Global Presidents Meeting - on invitation only**
Room: Meuse
Chair: Frank Chanut, ESTP Chairman

12.15–13.15 **Early Career Meeting - open meeting**
Room: Bourgogne
Chair: Simone Tangermann

The ESTP is aiming to set up a network of colleagues in an early career phase (less than 10 years in industry) which provides a platform for networking and discussion of challenges and opportunities in this career phase. The kick-off of this network will be the Early Career pathologist lunch break at the ESTP Maastricht congress in 2022. If you are an early career pathologist interested in networking with other early career colleagues, please join us on Wednesday 14 September at 12.15h. I hope to see you in Maastricht.
Simone Tangermann

TUESDAY 13 SEPTEMBER 2022

SPEAKER ABSTRACTS IATP SYMPOSIUM

- OVERVIEW OF ALZHEIMER'S DISEASE
- NOVEL ALZHEIMER'S DISEASE THERAPIES AND APPROACHES TO CROSS THE BLOOD BRAIN BARRIER
- EXPERIMENTAL MODELS FOR ALZHEIMER'S DISEASE
- THE GERO-EXPOSOME OF ALZHEIMER'S DISEASE

SPEAKER ABSTRACTS ESTP CONGRESS

- INTRODUCTION TO THE FEMALE REPRODUCTIVE SYSTEM
- COMPARATIVE ASPECTS OF PLACENTAL HISTOLOGY
- MECHANISMS AND PATTERNS OF TOXICITY IN THE FEMALE REPRODUCTIVE SYSTEM
- THE DESIGN, CONDUCT AND RATIONALE FOR EXTENDED ONE-GENERATION REPRODUCTIVE TOXICITY STUDIES (EOGRTS)
- THE REPRODUCTIVE TRACT IN BREEDING FEMALES IN RODENT DART STUDIES

TUESDAY 13 SEPTEMBER 2022 IATP SYMPOSIUM

08.05-08.45 **Overview of Alzheimer's Disease**

Pieter Jelle Visser, Maastricht University, Maastricht, the Netherlands

Alzheimer's disease is the most common cause of dementia. The key pathological features are aggregation of beta amyloid and tau proteins. The recent availability of in-vivo biomarkers for beta amyloid and tau has revolutionised our understanding of the development of the disease. It appeares that Alzheimer-type dementia is the end stage of a long disease process that has started around 15 years before with brain abnormalities. In this predementia stage of the disease, a wide range of processes beyond amyloid and tau are disregulated, such as increased neuronal plasiticity, blood brain barrier dysfunction and immune activation. Morover, there is strong evidence that individuals with predementia AD are heterogenous and differ in underlying molecular pathophysiology

The aim of this presentation is to use biomarker data from large cohorts of non-demented individuals to:
- Discuss clinical evidence for the amyloid hypothesis, which states that the disease starts with amyloid aggregation
- Discuss the role of genetic and enviromental factors on AD pathophysiology using a monozygotic twin design
- Provide evidence of molecular subtypes of Alzheimer's disease
- Discuss implications for trial design

TUESDAY 13 SEPTEMBER 2022 IATP SYMPOSIUM

08.45-09.30 **Novel Alzheimer's Disease therapies and approaches to cross the Blood Brain Barrier**

Kerstin Hahn, Vanessa Schumacher, F. Hoffmann, La Roche, Basel, Switzerland

Alzheimer's disease (AD) is a complex neurodegenerative disorder, and with an estimated 43.8 million people living with AD and other dementias, it is a growing societal burden. There is emerging clinical evidence of beneficial effects of anti-amyloid beta therapeutic antibodies, however their access to the central nervous system (CNS) is restricted by the blood-brain-barrier (BBB). The BBB is a specialized, selective feature of brain endothelial cells, pericytes, astrocytes and neuronal processes which plays a critical role in protecting the brain from pathogens. The BBB also limits the access of therapeutic antibodies, making the achievement of adequate CNS exposure challenging. Endothelial transmembrane proteins located at the BBB have been assessed as ways to facilitate therapeutic antibody transport into the brain. This talk will discuss preclinical models of AD and the BBB, and approaches for brain targeting. We will share our experiences with the Roche Brain Shuttle technology platform as an example for overcoming the challenges of delivery of large molecules to the brain.

TUESDAY 13 SEPTEMBER 2022 IATP SYMPOSIUM

10.00-10.45 **Experimental models for Alzheimer's Disease**

Donna Wilcock, University of Kentucky Lexington, United States

Alzheimer's disease (AD) is a complex neurodegenerative disorder that is progressive in nature and manifests in humans as progressive cognitive and behavioral declines. Neuropathologically, AD is characterized by the presence of amyloid plaques composed of aggregated beta-amyloid (Aβ) peptide and neurofibrillary tangles composed of aggregated, abnormally processed, hyperphosphorylated tau protein. Early-onset, familial AD results from mutations in one of three proteins involved in Aβ processing; amyloid precursor protein (APP), presenilin 1 (PS1), or presenilin 2 (PS2). Mouse models of AD were first established in the mid-1990s and incorporated mutated human genes overexpressed through transgenic expression, usually under neuronal promoters. Mouse models overexpressing mutated human APP generated elevated Aβ1-40 and Aβ1-42, and aggregated Aβ into plaques. Mice overexpressing PS1 mutations showed elevated Aβ in the brain but did not develop plaques. However, combination of APP and PS1 mutations in the same mouse showed accelerated amyloid plaque pathology and more robust cognitive deficits. There have been many variations of the APP and PS1 mutation transgenic models that will be summarized in this presentation, as some present with different patterns of pathology or different cognitive qualities. Importantly, the amyloid-depositing mice do not show significant disease progression to neurofibrillary tangles or frank neurodegeneration.

Efforts to generate tangle-forming transgenic mice turned to another related dementia, fronto-temporal dementia (FTD) for human mutations that cause pathology. Rare mutations in the microtubule-associated protein tau (MAPT) can cause familial FTD, so generation of models with tau pathology has required the transgenic over-expression of MAPT mutations such as the P301L, or P301S mutations. These mice generate neurofibrillary tangles and neurodegeneration. However, a flaw in these models is the mutated MAPT, which is not associated with AD, and many suffer from brainstem and midbrain issues that can cause motor deficits that are not AD-relevant. More recent mouse models have made efforts to evolve these transgenic overexpressing mice. There are now several "knock-in" models that essentially humanize the mouse APP sequence and introduce point-mutations to introduce those early-onset, familial AD mutations. These knock-in models have the significant benefit of endogenous gene regulation, resulting in a more physiologically relevant model. In addition, consortium efforts including MODEL-AD, are combining some of the APP, PS1, or MAPT models with AD risk-genes like the apoliporotein variant ApoE4, TREM2, and CD33, among others.

Significant challenges remain in the modeling of AD, and care should still be taken in selecting the most appropriate model for the specific pathway(s) being studied. In addition, researchers should also be cautious in over-interpreting data from mouse models, and also imparting human-like behaviors on mouse models.

TUESDAY 13 SEPTEMBER 2022 IATP SYMPOSIUM

10.45-11.30 **The Gero-Exposome of Alzheimer's Disease**

Caleb E Finch, University of Southern California, Los Angeles, CA, 91001- United States

The low heritability of later-onset AD implies a major role of environmental factors in brain aging. As a first approach to these complexities, the Alzheimer's Disease Exposome considered exogenous and endogenous domains for Gene x Environment (GxE) across the lifespan (Finch and Kulminski, Alz. Dement. 2019). The Exogenous AD exposome is illustrated by the 15 year difference in onset of AD between extremes of education and SES, and by the doubled risk of AD by elevated air pollution. Since 2019, The Endogenous AD Exposome was expanded in 2021 to include stochastic factors that interact with GxE during development and aging: the 'Tripartite Phenotype of Brain Aging (Finch and Haghani, J Gerontol, 2021). This perspective is consistent with weak correlations of postmortem AD neuropathology with cognitive status (Boyle et al. and Robinson et al. Brain 2021).

Recent papers (from 630)

- Morgan TE, Sioutas CS, Finch CE. (2011) Glutamatergic neurons in rodent models respond to nanoscale particulate urban air pollutants in vivo and in vitro. Env Health Perspect, 119: 1003-9. *Cacciottolo M, Wang X, Driscoll I, Woodward N, Saffari A, Reyes J, Serre ML, Vizuete W, Sioutas C, Morgan TE, Gatz M, Chui HC, Shumaker SA, Resnick SM, Espeland MA, Finch CE, Chen JC (2017) Particulate air pollutants, APOE alleles, and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. Transl Psych 7:e1022.
- Finch CE, Kulminski AM. 2019.The Alzheimer's Disease Exposome. Alz Dement.15:1123-1132.
- Haghani A, Thorwald M. Morgan TE, Finch CE. 2020.The APOE gene cluster responds to air pollution factors in mice with coordinated expression of genes that also differ by age in humans. Alzheimer Dement. 17(2):175-190.
- Finch CE, Haghani A. 2021 Gene-Environment Interactions and Stochastic Variations in the Gero-Exposome. J Gerontol A Biol Sci Med Sci. 13:1740-1747
- Austad SN, Finch CE. 2022. How ubiquitous is aging in vertebrates? Science. 24:1384-1385.

TUESDAY 13 SEPTEMBER 2022 ESTP CONGRESS

13.30-14.15 **Introduction to the female reproductive system: approach, recommendations and challenges for pathologists evaluating general toxicity studies**

Justin Vidal, Charles River Ashland, United States

Evaluation of the female reproductive system in a general toxicology setting can be difficult and at times frustrating for the toxicologic pathologist. The cyclic nature of the estrous and menstrual cycles drives the marked variability in the size, shape, and appearance of the reproductive organs in normal animals with the impact of puberty and reproductive senescence adding further complexity. In addition, the reproductive strategies of our commonly used laboratory animals are strikingly different. As result, the toxicologic pathologist is required to have a thorough understanding of the normal anatomy, physiology, and histology of each of these species prior to deciding what is abnormal. This session will review best practices for microscopic assessment of the female reproductive system and mammary gland in rodents and nonrodents, determination and documentation of sexual maturity and reproductive cycle stages, impact of age and reproductive senescence, and highlight common challenges, including spontaneous pathology, encountered by toxicologic pathologists tasked with evaluation of the female reproductive system.

14.15-14.45 **Comparative aspects of placental histology**

Sydney Mukaratirwa, Boehringer Ingelheim Pharma GmbH & Co. KG

The placenta act as an interface between the dam and the fetus and its primary function is to control maternal-to-fetal exchanges. The placenta also protects the embryo/fetus against xenobiotics, making it an important organ in developmental and reproductive toxicity studies (DART). The histological structure of the chorioallantoic placenta in eutherian mammals varies between different animal species. The number of cell layers in the interhemal area is considered to modify the transfer of xenobiotics between maternal and fetal blood. Three species of laboratory animals (rats, rabbit and nonhuman primates) are predominantly used in regulatory DART studies and the histology of the placenta in these animals is different from that of humans. Therefore, careful considerations should be paid to the histological structure of the interhemal area when extrapolating test animal data to the human.

TUESDAY 13 SEPTEMBER 2022 ESTP CONGRESS

14.45-15.30 **Mechanisms and patters of toxicity in the female reproductive system**

Justin Vidal, Charles River Ashland, United States

The microscopic evaluation in general toxicity studies may offer the first indication of potential effects of a test compound on the female reproductive system. While the pathology assessment plays a key role in the early hazard characterization of effects on the female reproductive system, it is often complicated by the cyclic nature of the estrous and menstrual cycles, timing of puberty and reproductive senescence, and stress-mediated effects. This session will review the basic mechanisms and patterns of toxicity that can be readily identified in the female reproductive system of rodents and non-rodents in general toxicity studies. Case examples will be provided to demonstrate the underlying pathogenesis of various effects and highlight key species differences.

16.05-16.50 **The design, conduct and rationale for extended one-generation reproductive toxicity studies (EOGRTS)**

Manon Beekhuijzen, Charles River Den Bosch, the Netherlands

The hallmark of the EOGRTS (OECD test guideline 443) is that, based on certain criteria or triggers, selected offspring are assigned at weaning to different cohorts for further investigation of sexual maturation, reproductive organ integrity and function, neuropathological and behavioral endpoints, and/or immune function. The triggers allow for a more customizable design based directly on the data, while minimizing animal usage. This design increases the number, extent and duration of F1 offspring assessments resulting in more thorough and efficient utilization of the F1 generation.
The test guideline was adopted by the OECD in 2011 and amended as test requirement under REACH in 2015. The required design of the EOGRTS is specified in the final decision letters from ECHA, and these have been published since 2017. When performing the EOGRTS, the complexity of the study should not be underestimated, and experienced and flexible testing laboratories with sufficient resources and historical control data for all parameters are essential. Since the update of this requirement, over a hundred EOGRTS have been performed worldwide, and assessment of these studies is crucial to gain insight of the pros and cons of the different study designs, and of the added value of the EOGRTS overall.
This presentation will introduce the different study designs and share experience on designing and conducting EOGRTS under REACH. Focus will be on the critical issues that ECHA published in 2021 and 2022 with emphasis on dose level selection, and several examples will be presented.

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16.50-17.20 **The reproductive tract in breeding females in rodent development and reproductive toxicity studies**

Paul Howroyd, Charles River Laboratories Edinburgh, United Kingdom
Ankie Lambregts, Charles River Laboratories Den Bosch, The Netherlands

This presentation will illustrate the histological appearance in normal pregnancy and lactation of the reproductive tract of female rats used for breeding in DART studies. This presentation should provide useful knowledge and tools for toxicologic pathologists whose main experience is with non-bred animals in general toxicology studies.

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07.30-08.30 **Breakfast symposium Aiforia**

08.40-09.25 **Influence of estrogens and progesterone on the endometrium: normal and pathological modifications**

Christine Bergeron

During reproduction time, the endometrium is subject to morphological and physiological changes characterized by a proliferation, a secretion differentiation and if there is no fertilization, a menstruation and regeneration. These endometrial morphological changes are controlled by the estrogens and progesterone secreted by the ovary, and growth factors and enzymes synthesized by these hormones. Estrogens and progesterone act through specific receptors present in the nuclei of the epithelial cells and stromal cells. Estrogens stimulate the synthesis of these receptors. Progesterone inhibits their synthesis. Estrogens allow mucosa proliferation during the proliferative period. They are also responsible for progesterone receptor synthesis and prepare the secretion differentiation. This secretion differentiation is influenced by progesterone. Such cyclical changes aim to create an appropriate environment for nidation. Understanding these morphological changes of the endometrium during the menstrual cycle is important to confirm that there is a normal action of the hypothalamus-hypophyseal-ovarian axis.

Estrogens, if given alone and for a long period of time, may induce a whole spectrum of proliferative aspects, ranging from persistent proliferative endometrium to hyperplasia and adenocarcinoma. Many epidemiological studies have clearly shown that the use of unopposed estrogens in postmenopausal women increases the risk of developing endometrial carcinoma. A simplified classification of abnormal modifications and a better expertise in « normal » modifications induced by hormonal replacement therapy should lead to a better and, especially, reproducible endometrial interpretation. From a clinical point of view, it is of no interest to differentiate simple from complex hyperplasia if there is no atypia. The term hyperplasia would lead to a much better agreement between observers and be clear for the clinician in terms of prognosis. These lesions develop under estrogens action and have a negligible or low risk of malignant transformation. On the other hand, the diagnosis of both atypical hyperplasia and well differentiated endometrioid carcinoma in biopsy or curettage specimens almost invariably leads the clinician to perform a hysterectomy except rarely for those young women who wish to preserve their potential for fertility. The examination of the hysterectomy specimen allows not only the assessment of the presence or absence of myometrial invasion but also more accurate evaluation of invasion of the stroma than in the biopsy or curettage specimens where there is considerable interobserver error in the assessment of this criterion.

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09.25-09.55 **"Is this normal?" Inhabiting the limbo of the gender data gap: a patient experience.**

Rose George

I am a 52 year-old woman with grade 4 endometriosis. I am also in the midst of my second menopause.

My experience is in many ways typical: I was diagnosed with endometriosis at age 41 only by accident during fertility investigations. Throughout my life, I had had painful periods, I had even been given prescription-strength painkillers, but not one doctor had ever thought to ask of my pain, "is this normal?" It wasn't normal, it was endometriosis. My first "treatment" was to be given a course of Prostap, which hurtled me into a menopause. The symptoms were distressing and horrible, although my consultant had dismissed them as "mild side-effects." The nurse giving me the injections once said, "he keeps prescribing this, but women hate it." This was my first taste of how women can be treated by modern medicine: with carelessness and casualness. The root of this, of course, is a massive data gap when it comes to women's sexual and gynaecological health. This was only emphasised when I began to experience perimenopausal symptoms. I was better prepared than most women, being a science journalist and having written extensively on the menopause. But even so I suffered years of debilitating depression because there is such a paucity of research on the neurological impact of oestrogen, because the menopause is usually dismissed as being just hot flushes and perhaps "low moods." So why do menopausal woman have breakdowns, divorce, leave their jobs in worrying numbers and kill themselves if it's just about hot flushes?

I am only one woman, but I have had a richness of experience as a gynaecological patient that I heartily wish I had not had. It has been fraught, fractured, difficult, expensive. And it is not over.

I will not make my talk a series of complaints, but an objective and vivid view of what it is to be a woman in the modern medical system, when most scientific and medical research excludes women because their menstrual cycles and hormones are thought to skew trials; when there is increasing research showing that medications designed using the male body work differently on the female body. It is hard, if you are a woman with a gynaecological condition, not to feel like an ongoing experiment. There must be a better way of doing things.

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10.30-11.10 **Drug discovery & development in women's health: Don't get lost in translation**
Thomas Zollner

11.10-11.50 **Endometriosis in macaques: Natural history and potential for new therapeutic approaches**
J Mark Cline, Wake Forest School of Medicine Winston Salem, United States

Endometriosis is a polygenic estrogen-dependent disorder, defined as the presence of endometrial tissue outside the uterus. It is common in humans, with an estimated prevalence of >10% in reproductive-aged women. It is associated with inflammation, pelvic and abdominal pain, dysmenorrhea, infertility, and an increased risk of ovarian cancer. Risk factors for the development of endometriosis include diethylstilbestrol exposure, low birth weight, and low body weight. Missense variants in genes encoding neuropeptide S receptor 1 have recently been described in women and macaques with endometriosis. Pathologically, endometriosis occurs most often in the lower abdomen, particularly in the caudal cul-de--sac and near the ovaries. Endometriotic lesions have also rarely been found in distant sites, including the lung. Lesions have a wide variety of gross morphologies, and the diagnostic value of a laparoscopic gross finding is low for histologic confirmation (positive predictive value <50%). The size of endometriotic lesions in women does not correlate directly with symptoms, and many affected women are asymptomatic. The pathogenesis of endometriosis remains unclear; theories include retrograde menstruation and development from embryologic remnants or metaplasia.
Histologically, endometriosis is defined by the presence of endometrial glands, endometrial-type stroma (CD10+), and hemorrhage (or hemosiderosis indicating past hemorrhage) outside the uterus. Additional clinically-relevant cellular elements include a macrophage-rich pleocellular inflammatory infiltrate, neovascularization, fibrosis, and innervation by unmyelinated sensory nerve fibers.
Treatment of endometriosis may include surgical excision of lesions, hysterectomy and/or ovariectomy, or drug treatments including GnRH agonists or antagonists; progestogens; aromatase inhibitors; selective estrogen receptor modulators; anti-inflammatory drugs; and analgesics. Old-world nonhuman primates (NHP) have a similar prevalence and biology of naturally-occurring endometriosis to women. Endometriosis has been described in macaques, baboons, great apes, and many other species. Colony prevalence rates as high as 30% have been reported for rhesus macaques. Endometriosis in NHP has also been induced by ectopic transplantation of endometrium in macaques, New-world species, and non-primate species. Naturally-occurring endometriosis in macaques most closely recapitulates the human disease, and provides an opportunity for the study and treatment of the disease, allowing longitudinal measurements, pre- and post-treatment biopsies, transcriptional and genetic profiling, assessment of the hypothalamo-pituitary-gonadal axis, and assessment of the adverse effects of treatment on other tissues. Studies to date have focused on natural biology, diagnosis and disease pathogenesis, genetic similarities to human disease, hormonal treatments, and tissue metabolism.

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13.30-14.15 **Modeling cancer immunotherapy in tumor-bearing nonhuman primates**

Maronpot Guest Lecture, sponsored by IATP and Telikicherla Higher Education Foundation

J Mark Cline , Wake Forest School of Medicine, Medical Center Boulevard, Winston Salem, NC, 27157-1040, United States

The advent of cancer immunotherapy (CI) has dramatically changed anticancer drug development, offering the promise of personalized, targeted therapy. When cures are effected, they are often durable; however, such success occurs in only ~20% of patients. Conventional preclinical drug safety and efficacy testing is poorly predictive of a favorable risk/benefit ratio for CI in humans with cancer, even if testing is done in nonhuman primates (NHP) that are genetically and physiologically similar to humans, such as Macaca fascicularis or M. mulatta.

There is potential for improving the predictive value of preclinical testing of CI in NHP, by focusing not on healthy juvenile animals, but on more mature older animals with spontaneously occurring neoplasms. Such older tumor-bearing NHP differ from typical study animals and have shared features with human patients, including (1) the presence of autologous neoplastic cells and potentially mutationally-derived neoantigens; (2) tumor-associated inflammatory changes; and (3) age-associated immune changes - including both dysfunction and antigenic priming, all of which may affect tumor and host responses to CI. Macaques share with human beings a relatively high prevalence of some neoplasms, and the biology of some tumor types is remarkably similar between human and NHP. For example, colonic/cecocolic carcinomas are the most prevalent cancer in rhesus monkeys and can be detected by fecal occult blood tests and ultrasound examination; they are usually DNA repair-deficient due to loss of MLH1, and show microsatellite instability. Breast cancer is the next-most prevalent malignancy in macaques, with a similar lifetime prevalence to humans; most cases are estrogen receptor- and progesterone receptor-positive; HER-2 and triple negative sub-types are also described. Breast cancers in NHP are particularly suited to longitudinal study because they are accessible and have a similar biology of progression from precancers to local disease to metastasis. Cervical cancer is common and is associated with oncogenic papillomavirus infection. Lymphomas are typically B-cell lymphomas, associated with lymphocryptovirus infection. Studies of tumor-bearing NHP could have high value in the development of molecularly-targeted therapies requiring high target specificity. Promising avenues of development include targeting of tumor stromal antigens such as fibroblast activation protein; activation of tumor-associated immune cells; antigens enriched in tumors, such as virally-encoded proteins or tumor neoantigens; and combined approaches such as immune priming and radioimmunotherapy. The genetic and physiologic similarities between macaques and human primates also provide an opportunity to study the off-target effects of immunotherapies, and to study CI effects on cancers in the absence of prior therapy. Challenges to the study of tumor-bearing NHPs include the low prevalence of most cancers, which limits the numbers of animals for study and necessitates a screening/recruitment approach, and confounding effects of age-associated co-morbidities or prior experimental treatments. Such animals must be sourced from existing colonies of aging animals, necessitating a collaborative relationship with investigators managing such populations. Benefits of the approach include the opportunity to treat animals that would otherwise be euthanized; to validate tumor targeting with PET imaging; and to study long-term outcomes including tumor responses and off-target effects in a large animal model with a high degree of protein-level similarity to human patients.

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14.15-14.55 **AI and mammary gland cell proliferation**

Henning Hvid, Novo Nordisk A/S, 2760 Maaloev, Denmark

Epithelial proliferation in the rat mammary gland is recommended in regulatory guidelines as an endpoint for assessment of the in vivo carcinogenic potential of insulin analogues. Epithelial proliferation is traditionally assessed by immunohistochemical staining of a proliferation marker, e.g., 5-bromo-2'-deoxyuridine (BrdU) or Ki67, followed by labour-intensive manual counting of positive and negative cells. Currently, there is a lot of interest in replacing manual evaluation of histology endpoints with image analysis tools based on artificial intelligence (AI). We explored how commercially available image analysis software based on AI (HALO AI and VIS AI) can be used to quantify epithelial proliferation in the rat mammary gland. These software packages require no programming skills from user, and are therefore attractive to use in digital pathology. The aim of our studies was therefore to develop and validate approaches for quantification of proliferation in rat mammary gland using HALO AI and VIS AI. Furthermore, the aim was to compare the markers BrdU, Ki67 and phosphorylated histone H3 (PHH3). With each software, algorithms based on AI were developed, which allowed for quantification of proliferative activity in the mammary gland epithelium. These endpoints agreed well with manually counted labelling indices, with correlation coefficients in the range ≈ 0.92-0.98. Additionally, all three proliferation markers were significantly correlated and could detect the normal variation in epithelial proliferation during the estrous cycle in female rats.
In conclusion, commercially available image analysis software based on AI can be used to quantify epithelial proliferation in the rat mammary gland and thereby replace time-consuming manual counting. Furthermore, BrdU, Ki67 and PHH3 can be used interchangeably to assess mammary gland proliferation.

14.55-15.35 **Human breast cancer: a heterogeneous disease**

Trine Tramm, Aarhus University Hospital, Department of Pathology, Palle Juul-Jensens Boulevard 99, C112, 8200 Aarhus N, Denmark

Breast cancer (BC) is heterogeneous in terms of histomorphological and molecular features as well as in metastatic spread leading to differing clinical outcome. Administration of systemic oncological treatment in BC patients is based on clinico-pathological parameters dividing the patients into categories according to expected risk of recurrence and risk of dying from BC; categories based on evidence from clinical studies and categories that match approved and available treatment schemes. The prognostic factors derived from macro- and microscopical examination of the individual tumors have not changed substantially over the last decades and are still centered around histological type, malignancy grade, tumor size and number of metastatic lymph nodes. Though known to be molecularly heterogeneous, only few targets in BC are used in daily clinical practice. One of the first and most well-described targets in BC treatment is the estrogen receptor (ER)[1]. The discovery of the ER in the 1990s led to dichotomization of BC into ER-negative (ER-) and ER-positive (ER+) cancers, which has been central for BC treatment in decades

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after. Estrogen receptor positivity predicts greater likelihood of response to antihormonal treatment and subsequently reduced breast cancer mortality[2]. ER negative cancers are in general associated with early recurrences, whereas the slower growing ER+ cancers are associated with late recurrences often up to 20 years after the initial diagnosis. ER-status is not valuable as a prognostic factor, despite a general tendency for ER-positive tumors to be associated with more favorable clinico-pathological features like e.g., lower malignancy grade. The capability of ER+ BC for late recurrences > 10 years after diagnosis is important for risk prediction in BC.

In the beginning of the 2000s, discovery of the HER2 receptor further revolutionized the diagnosis of BC leading to more refined categorization of BC patients and individualization of treatment. HER2 positivity is associated with poorer histopathological features and a poorer prognosis, but is at the same time predictive for response to anti-HER2 treatment that reverts the prognosis of HER2 positive (HER2+) cancers[3].

It has, however, become increasingly clear that BC is a highly heterogeneous disease, and that the heterogeneity extends beyond what conventional histopathology incl. ER- and HER2 status are capable of describing. It has also become evident that a fraction of patients is not benefitting from the offered adjuvant systemic treatment, but only risking side effects; a number that has been stated as high as 40%.

In an attempt to refine the categorization and individualize and hopefully deescalate treatment, several gene expression profiles (GEP) have been published over the last two decades. One of the most cited GEP studies is the study by Perou et al. from 2000[4] demonstrating that BC can be classified into molecular intrinsic subtypes based on 496 genes. The subtypes (Luminal A, Luminal B, HER2-like, Basal-like, Normal-like) has further been shown to correlate with clinical outcome and has been found to be robust over patient populations and platforms. The intrinsic subtypes as well as a limited number of other well-described GEP has been commercialized, and more GEPs are currently available for decentral testing on formalin fixed, paraffin embedded tissue.

The majority of validated GEPs in BC are prognostic and may be useful for separating patients with low or high risk of recurrence, and may as such be used to select patients in whom adjuvant systemic treatment may be spared. The available GEPs show, however, similar prognostic ability on a population-based level, but are inconsistent in risk prediction of the individual patient with 30-40% disagreement between tests[5]. This concordance may be related to the GEP being driven by differing pathways as e.g., the ER-pathway or proliferation pathway[6] or varying capability of predicting late recurrences[7]. The outcome of the individual GEPs is furthermore still categorical and not individualized, since precision needs to give way for interpretability to match the available categories of treatment.

Despite continuous search, introduction of further clinical biomarkers has not been successful in BC. Though showing prognostic/predictive value on a population-based level, several promising biomarkers have not shown sufficient reproducibility for categorizing individual patients, and thus not been optimal for clinical implementation.

Currently, attention is centered around the tumor-microenvironment, where special emphasis has been paid to tumorinfiltrating lymphocytes (TILs) used as a surrogate marker for the adaptive immune system. High levels of TILs are only found in a minority of BC (approximately 10%) but have been found to be associated with an improved response to preoperative chemotherapy in all BC types. At the same time, high level of TILs has been found to be prognostic especially in ER-/HER2- BC (so called triple negative BC) and in HER2+ BC, but with an adverse prognostic effect in ER+ tumors[8]. The reasons for this may be related to varying composition of the immune response in BC according to ER-status, or related

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to the effect of anti-hormonal treatment on the immune response[9]. The quest during the last two decades to refine categorization of the heterogeneous BC tumors in an attempt to individualize BC treatment comes as such to a large degree back to the originally discovered target, ER, and to the dichotomization into ER+ and ER- cancers.
The lecture will discuss the morphological and molecular heterogeneity of BC, but also the dilemma in striving for increasingly refinement and individualization of patient/tumor categorization, though equally individual treatment schemes are not yet available to match the patient. The importance of analytical validity of biomarkers to secure clinical utility of a biomarker driven treatment will also be discussed, and animal models will be included wherever relevant.

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16.05-17.00

INHAND female reproductive tract nomenclature (hyperplasia and/or tumors) - issues and current status of organ working group discussions

Heike Marxfeld, BASF Frankenthal, Germany, Ute Bach, Bayer AG Wuppertal, Germany

The INHAND nomenclature for female reproductive organs was published in 2014. As many questions arose in the last 10 years, the organ working group felt that some terms needed more clarification.

Therefore, a new discussion round was initiated to work on refined descriptions for hyperplastic and neoplastic lesions in the ovary as these caused the most difficulties in the daily life of a bench pathologists. The organ working group has started work end of 2021 and has met several times via video call. It quickly became apparent that major points of discussion were to be addressed.
The first new descriptions are being drafted at the moment.

This talk aims at getting input from a wider audience by showing difficult cases and inviting the audience to vote and discuss the new draft descriptions.

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SPEAKER ABSTRACTS ESTP CONGRESS

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08.35-09.20 **Mass spectrometry imaging in molecular pathology: single cells and beyond**

Ron Heeren

Modern molecular analytical technologies in the “omics” arena plays a crucial role in many scientific disciplines ranging from material sciences to clinical diagnostics. Technological advances have increased methodological sensitivity allowing researchers to acquire detailed molecular information of smaller and smaller samples. The biggest challenge is to put that concerted information in the context of the problem the samples originate from. This lecture describes how innovative mass spectrometry based molecular imaging technologies, have impacted translational clinical research. Or: how a mass spectrometer can be used as a sensitive and selective molecular microscope in modern pathology. Innovative imaging technologies now offer new insights in life’s complexity that can be employed for precision medicine, the understanding of new (bio)materials and the processes that happen on the interface of living and ‘dead’ matter. Innovations in mass spectrometry based chemical microscopes have now firmly established themselves in translational molecular research. One key aspect of translational success is the ability to obtain this molecular information on thousands of molecules on a diagnostically relevant timescale. Modern mass microscopes can now rapidly acquire images of metabolites, lipids, polymers, peptides and proteins, depending on the spatial resolution chosen. Single cells can be analyzed in great molecular detail and in the context of their native tissue. Combined this offers a truly precision multi-omics approach that reveals contextual molecular complexity in biological and material environments.

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09.20-09.45 **Mass spectometry imaging within the women health area**

Bryn Flinders[1], Benjamin Balluff[1], Farid Jahouh[2], Kathleen Allaerts[2], Loes Kooreman[3,4], Anouk AS van den Bosch[3,5], Andrea Romano[3,5], Eva Cuypers[1], Henrica MJ Werner[3,5], Marjolein van Heerden[2] and Rob J. Vreeken[1,2]

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Mass Spectrometry Imaging (MSI) is a relatively new technique, which provides a molecular view on tissue at hand. When combined with e.g. pathology and/or Immuno-histochemistry (IHC), it offers, next to the visualisation of specific markers, a detailed molecular view on tissue. The spatial resolution down to µm allow for in-depth spatial analysis of small molecules, lipids, peptides and even proteins in tissue related to specific phenotypes. Next to better understanding of these clinical phenotypes, it also serves better informed decision making in drug discovery and development.

Matrix Assisted Laser Desorption Ionisation (MALDI) is the most common used technique amongst the various techniques available. Being a technique, requiring a several ten's of hr.'s acquisition and processing of data, nowadays MALDI delivers a molecular image within hours. Being an unbiased technique, a large variety of compounds can be analysed.

In this presentation we will show some examples where MSI can be used within the context of women's health, both in a pre-clinical and a clinical setting. Examples also show the use of MALDI in view of biomarker research as well as its potential role in current drug discovery and further drug development.

Next to molecular profiling of endometrial tissue in controls opposed to endometrial carcinoma in lean and obese patients, we will look at drug distribution in view of breast cancer and cross species tissue profiling in ovaries of non-rodent and rodents.

These data clearly show the potential of this technique in i) (better) molecular (sub-)phenotyping of pre-clinical models and clinical samples, ii) distribution of drugs in tumour tissue and iii) exploration /identification of relevant biomarkers.

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09.45-10.15 **Zebrafish as a model for genetically and environmentally induced osteoporosis**

Dylan Bergen, University of Bristol Bristol, United Kingdom

The bone matrix is regulated by the metabolic balance between cells that make new bone (osteoblasts) and that degrade old bone (osteoclasts). For healthy and strong bones, these cells need to know where and when to make or degrade bone matrix. Both genetic and environmental factors can alter this balanced process. It is well described that pollutants such as iron cholates or pharmacological agents such as prednisolone can lead to early-onset osteoporosis where degradation exceeds new bone formation. Alternatively, pollutants such as sodium fluoride leads to fluorosis with increased bone mineral density. Bone is a complex tissue, coming in many forms and shapes, which makes it a relatively difficult tissue to study in the lab. Zebrafish are vertebrates that are housed in large shoals, are genetically highly amenable with the availability of many fluorescent transgenic reporter lines and generate large clutches of transparent embryos extra-maternally. As these embryos are translucent, the development of these embryos can be followed live allowing to track (fluorescently labelled) cell populations over time (Bergen et al. Front Endocrinol. (2019)). This therefore presents a relatively plenty, easy, and cheap resource of biological material for toxicology studies. Moreover, as an aquatic model, it allows easy exposure of water based (or vehicle dissolved) toxicological components that could affect juvenile and adult bone metabolism. In addition to a mineralised endoskeleton like land animals, adult zebrafish have a mineralised exoskeleton that is composed of 100s of scales that protrude from the skin. A scale is a flat mineralised collagen plate, decorated with both osteoblasts and osteoclasts. As scales sit on the flanks of an adult fish, they are easily accessible and once removed they can fully regenerate. This regeneration process involves making new mineralised bone matrix (osteo-anabolism) in a controlled manner. We showed with transcriptomic studies and gene enrichment studies that regenerating a zebrafish scale resembles an osteo-anabolic process, conserved during evolution, and that those genes expressed during regeneration are also associated with human both common and rare bone pathologies (Bergen et al. BMC Biology (2022)). This result is important, as we recently have refined the ex vivo culture of zebrafish scales. As a single transgenic adult fish can provide a couple of hundred of scales, culture in multiwell plates is possible allowing to test drugs on a larger scale. In the last part of my talk, I will show some unpublished data and the future potential of the utilities of ex vivo cultured scales for drug toxicity and environmental pollutant studies. I will also touch on gender dimensions and the effect of ageing on the zebrafish scale.

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10.45-11.30 **Organoids from human endometrium as cutting-edge research and screening models in reproductive biology**

Hugo Vankelecom, KU Leuven (University of Leuven) Leuven, Belgium

The inner lining of the uterus (endometrium) is the first contact site of the embryo and crucial for human reproduction. Knowledge on the molecular and cellular mechanisms underlying the tissue's monthly remodeling and its embryo receptivity remains poor, as well as on how these processes go awry in endometrium pathology. This limited understanding is primarily due to a lack of reliable and robust research models. Organoid technology provides an innovative tool to grow high-fidelity mini-tissues in culture. We established organoid models from both healthy and diseased human endometrium which reproduce key features of the original tissue's epithelium (Boretto et al., Development 2017; Nature Cell Biology 2019). In addition, the organoids show long-term expansion capacity while remaining genomically, transcriptomically and functionally stable. Endometrium-derived organoids phenocopy physiological responses to reproductive hormones and mimic the menstrual cycle 'in the dish'. Organoids from endometriosis and endometrial cancer recapitulate characteristics of the patients' diseased tissue and faithfully capture the clinical heterogeneity. Recently, we combined our endometrial organoid model with stem-cell derived blastocyst mimics (blastoids) to construct an in vitro human implantation model, and found that it reliably captures the first events of pregnancy (Kagawa et al., Nature 2022). Taken together, our established models provide powerful and innovative tools to study multiple aspects of reproductive biology including endometrium receptivity and (in-)fertility. Moreover, the organoids can be harnessed into (personalized) drug screening platforms, as well as into toxicity screening tools to assess the impact of chemical compounds, as present in nutrients, environment and medicines, on women's reproductive health and competence.

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11.30–11.55 **Two validation strategies for deep learning-assisted image analysis of digital study material-explainability in AI**

Heike Marxfeld, BASF Frankenthal, Germany

The manual quantification of microscopic structures is required in some toxicological studies (e.g. Differential Ovarian Follicle Count). These studies are necessary for the registration and marketing of new products . The manual evaluation of these studies is very time-consuming task requiring highly-trained personnel. Increasingly, authorities request documentation of these evaluations not only as numeric results but also as “overlays” on the evaluated pictures. Therefore, an AI solution based on YOLOv5 was established and is now being evaluated to clarify if the criteria the algorithm uses match those of human observers using state of the art explainability techniques, which will facilitate validation under GLP as a stand-alone system to evaluate this assay.

Two validation strategies for deep learning-assisted image analysis of digital study materal- Algorithm qualification

Michael Staup

Classification and quantification of ovarian follicles is a necessary analysis, requested by regulators, for reproductive toxicology studies. The traditional method of manually counting follicles is very time consuming and subject to considerable variability both within and between raters. Automated deep learning-assisted digital image analysis provides an opportunity to decrease turn around time for these studies and increase precision of the data. Validating individual algorithms for use in GLP (Good Laboratory Practice) toxicological pathology studies, however, can result in the failure to realize these labor-saving gains, as the validation process is, itself, quite time-consuming, requiring coordination of a validation committee and multiple levels of approvals that extend beyond quality of the algorithm itself, before the algorithm can be used. To avoid this obstacle, the approach we have taken at Charles River has been to validate a method of generating qualified algorithms. This method leverages the depth of expertise from our wide field of pathologists across the globe and our own team of developers. This approach is very efficient, and flexible enough to create and adapt algorithms on a study by study basis without extensive change controls, and has been applied to the generation of ovarian follicle counting algorithms.

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11.55–12.15 **Pathology 2.0 - Results of survey artificial intelligence**

Josep Monne, Roche Innovation Center Zurich Zurich Switzerland, Dirk Schaudien, Fraunhofer ITEM Hannover, Germany

The ESTP Pathology 2.0 working group has been renewed to support the scientific community by gathering and disseminating scientific and technical information on different innovative technologies. The purpose is to increase pathologists' awareness of cutting-edge technologies but also follow-up with the refinement of established techniques. The Pathology 2.0 group has identified 6 key interest areas allocated to corresponding subgroups: Mass Spectrometry Imaging, Molecular Pathology including in situ hybridization (ISH) and immunohistochemistry (IHC), Multiplexing, Artificial Intelligence (AI) and how it impacts the life of a pathologist, Spatial OMICs, and Complex In Vitro Models & Pathology. People, who are interested in joining can reach out to the Pathology 2.0 group through any of its members. Several webinars were held, and further webinars and publications are planned to disseminate information and encourage the community to active contribution. Some subgroups initiated surveys to identify information gaps and evaluate the market. In this presentation we will briefly introduce each of the subgroups, their main goals, and activities. In addition, we will expand in more details the most recent activity carried by the "AI and how it impacts the life of a pathologist".
The subgroup "AI and how it impacts the life of a pathologist" has conducted a survey within members of the ESTP, STP and BSTP societies, from June 2021 to October 2021. The main goal of the survey was to gather broad input from responders working in toxicologic pathology across different organizations to collect precompetitive baseline information showing the diversity of experience, opinion, and implementation of artificial intelligence (AI) across survey participants. Different topics such as individual experience, implementation of AI-driven approaches, AI-supported histopathological evaluations, issues, perceived business value, current developments and future applications for AI were addressed in the survey, resulting in a total of 89 questions. Valid responses were received from 107 responders representing 54 organizations.
The results of the survey will be presented, and a paper is underway.

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14.05–14.30 **Safety assessment of (Agro)chemicals from a pathologist's perspective**

Sibylle Gröters

Worldwide, regulation and registration of (agro)chemicals is a very complex process, which presumes profound knowledge of different requirements in different countries. Beside specific requirements for registration of pesticides and bulk chemicals, requested by regulatory authorities, the scope of mandatory toxicity and ecotoxicity studies will be presented from a pathologist's perspective and with special emphasis on the question of possible endocrine disruption.

14.30–15.00 **Cracking the egg factor: updates from the FREIA project**

Majorie van Duursen, Vrije Universiteit Amsterdam, the Netherlands

Currently available test methods are not fit for purpose, which is partly the reason why the effects of such endocrine disrupting chemicals (EDCs) on female reproductive health are often overlooked in regulatory chemical safety assessments. This means that women's reproductive health is at risk globally. The EU-funded project FREIA will increase our understanding of how EDCs can harm female reproductive health and use this information to provide better test methods that enable fit-for-purpose chemical regulation.
In the FREIA project, we first looked for biological characteristics (biomarkers) for female reproductive toxicity using two well understood EDCs, diethylstilbestrol (DES, a potent estrogen receptor activator) and ketoconazole (KTZ, a blocker of steroid hormone production). Next, we will assess how well our test methods and potential novel endpoints can identify EDCs that cause female reproductive toxicity.
Identification of EDCs in a regulatory context still relies heavily on rat studies. We showed that the endpoints that are currently being assessed in regulatory toxicity studies are not sufficiently sensitive to detect an endocrine disrupting effect. We found that a delay in activation of the brain to produce Gonadotropin Releasing Hormone (GnRH) is more sensitive to mark a delay in pubertal onset than the standard examination of vaginal opening (VO) in female rats that were exposed in the womb to DES and KTZ. Strikingly, the effect on GnRH was not detected when rat brain cells were exposed to DES or KTZ in a culture dish. This underlines the importance to focus on endocrine axes in the whole animal. We are investigating several additional hormone-sensitive endpoints, such as mammary gland development. A proposal to investigate this was submitted to the Organisation for Economic Co-operation and Development (OECD), a platform for international standard-setting. In addition, we found that the pups had increased blood levels of hormones like progesterone, pregnenolone, androsterone and estradiol after exposure to KTZ in the womb, and to a lesser extent DES.
Studies with cell cultures of fetal and adult human ovaries, bovine oocytes and immortalized ovarian cells all indicate an effect on steroid hormone formation and oocyte ripening by DES and KTZ, albeit in different directions. Ongoing gene expression analyses are designed to elucidate whether similar genes and pathways are affected in cell cultures and how this relates to effects in rats and

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humans. Differences in susceptibility towards effects of KTZ and DES may partly be explained by the presence of different cell types in the ovary at different ages, or the presence of different cell types in our experimental models. Nonetheless, both the rat study and the human ovary cultures show that exposure to KTZ had a stronger effect than DES, suggesting that chemicals targeting steroid hormone formation (steroidogenesis) may have worse effects on oocyte maturation and quality than those targeting the estrogen receptor (ER).
In current regulatory toxicity testing, the gold standard to study interaction with steroidogenesis is the H295R steroidogenesis assay (OECD test guideline 345). The last step in sex hormone formation, the conversion of testosterone to estradiol, is mediated by the aromatase enzyme. We have developed a computational model that can predict inhibition of aromatase with high precision and accuracy, complementary to the existing H295R assay. Nevertheless, our studies showed that effects of EDCs can also occur earlier in the steroidogenic pathway, or via alternative routes. We are now performing a study with other labs to investigate whether the H295R assay can be improved by measuring more steroid hormones. A project plan for this was submitted to the OECD. Moreover, we showed that steroid hormone profiles from human adult ovary cultures are clearly different from H295R profiles. The implication of this will be investigated further in the next phase of the FREIA project.
Considering the limited effects of known ER activator, DES, on ovarian function during early life, the ER does not seem to be a high impact target for (developmental) female reproductive effects. On the other hand, ER-beta is known to play an important role in differentiation of estradiol-producing cells surrounding the maturing oocyte. We showed that a wide variety of potential EDCs had an ER-beta activating or deactivating effect in our ER-beta assay. Notably, the chemicals that were studied for ER-beta interaction were also detected in the biological fluids surrounding oocytes, the follicular fluids, in Swedish and Estonian women undergoing fertility treatment. The total exposure to these chemicals and some chemicals specifically, decreased a woman's response to ovarian stimulation by hormones. Moreover, some chemicals in the follicular fluids of these women were associated with a reduced chance of the treatment resulting in live birth. Further studies are undertaken to investigate molecular pathways that are affected by the exposures.
Identification of molecular targets and pathways will guide the development of test methods and test strategy, provided that the steps from molecular interaction to female reproductive toxicity are defined. For example, we have developed a computational model to predict peroxisome proliferator-activated receptor (PPAR)-gamma activation, which is linked to female infertility. We have described 16 additional possible adverse outcome pathways (AOPs).
FREIA is one of the eight projects on test method development for EDC identification within the EURION cluster (www.eurion-cluster.eu). On the FREIA website (www.freiaproject.eu), general background information on EDCs and female reproductive health can be found as well as project specific information, including webinar recordings, peer-reviewed scientific publications and databases, and the FREIA factsheet (5 languages) and infographic (14 languages).
FREIA uniquely provides the opportunity to investigate hormonal processes in human ovaries from fetal to adult age in order to improve scientific knowledge on the causes of female reproductive toxicity. The FREIA approach will strongly support the work of European regulatory agencies, or even globally through the EURION cluster activities. The tools we are developing perfectly fit the needs of

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modern-day toxicity testing with a clear regulatory application in mind. Together, the FREIA outcomes will support testing, identification and assessment of EDCs that are toxic for female reproduction.

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15.00–15.30 **Environmental assessment of human drugs with endocrine disruptive properties**

Daphne De Roode, Charles River Laboratories Den Bosch BV The Netherlands

This presentation gives an overview of environmental risk assessment of human drugs, with specific attention to drugs with endocrine disrupting properties. The default environmental risk assessment that is part of a dossier for an application for marketing authorisation consists of a well-defined set of studies, some of which trigger further studies. For substances with endocrine disrupting properties, the set of data is changed to better investigate the related effects.
Attention will be given to the information that is considered to decide if a substance should be regarded as potentially displaying ED properties and to what steps need to be taken in Europe and the US. Studies focussing on effects due to ED in the environment are discussed, and their results and how these are used further in the assessment are explained.

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16.30–17.30 **Workshop Aira Matrix: application of deep learning for the evaluation of reproductive toxicity end points in rodents**

Erio Barale, Janssen R&D Beerse Belgium, Tijo Thomas, AIRA Matrix Pvt Ltd Thane India, Sabina Soldati, Pathology Experts GmbH Comano Switzerland

In this session we will present Deep Learning (DL)-based solutions that detect, quantify and potentially expedite and improve evaluation of male and female Reproductive Toxicity endpoints in rodent studies.
In male rodents, identifying and quantifying the 14 stages in the spermatogenic cycle is a difficult task but necessary to detect any toxicant effect on germ cells. We present a DL based solution that automates spermatogenic staging as well as identifies and quantifies abnormal findings in rat testes, namely, spermatic retention, giant cells, spermatogenic degeneration, and tubular vacuolation, dilation and atrophy. Our DL-based solution works on Periodic acid-Schiff (PAS) as well as hematoxylin and eosin (H&E) stained sections, potentially negating the need for special stains.
In female rodents, determination of estrus cycle provides critical information about the nature of a toxicant insult to the reproductive system, when interpreted along with other reproductive endpoints. Histopathological examination of vagina and uterus is the commonly used method for estrus staging. We present a DL-based solution based on this examination to identify the 4 phases of the estrus cycle in rodents.
Additionally, enumerating ovarian follicles is a regulatory requirement for reproductive toxicology studies in order to assess ovarian toxicity (FDA: detect qualitative depletion of the primordial follicles and quantify them; EPA: enumerate the number of primordial follicles, which can be combined with small growing follicles). Quantification of the follicles using conventional approaches is resource intensive and time consuming. We present a DL-based solution that automates enumeration and classification of follicles in H&E stained sections of rodent ovary.

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SPEAKER ABSTRACTS ESTP CONGRESS

INTERCONNECTION OF OVARY AND LIVER IN LABORATORY FISH - INHAND TERMINOLOGY AND MORPHOLOGIC CRITERIA IN PHYSIOLOGY AND PATHOLOGY

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11.05–12.05 **Interconnection of ovary and liver in laboratory fish - INHAND terminology and morphologic criteria in physiology and pathology**

The Chirukandath Gopinath Award, sponsored by the BSTP and awarded to Christine Ruehl-Fehlert.

Christine Ruehl-Fehlert, retired Wuppertal Germany, Heike Schmidt-Posthaus, University of Bern Schwitzerland.

Oocyte maturation in oviparous vertebrates such as some teleostean fish depends on the hypothalamic-pituitary-ovarian axis as in mammals and in addition on the liver as source of the yolk precursor protein vitellogenin. Vitellogenin is synthesized under hormonal control of 17ß-estradiol (E2) which is produced by the granulosa cells of ovarian follicles. Besides endogenous E2, chemicals with estrogenic properties are able to induce vitellogenin production in the liver. In testing for endocrine disruptive properties of chemicals in fish, histopathological investigation of ovary and liver are thus often combined. In addition, high-quality tissue preparation and sectioning of whole body sections of small laboratory fish species or isolated organs is an important pre-requisite for proper interpretation. To compare results of different studies and as base for regulatory decisions, standardized terminology and criteria are indispensable. In the frame of INHAND, the non-rodent fish working group guidance document on fish was developed and is now nearly complete. For proper use of pathology terminology, comprehensive understanding of normal physiology and reproduction in fish is mandatory. Fish show higher plasticity in sexual determination compared to mammals. Therefore, sex ratios may vary spontaneously or be induced. Oocyte development can be assessed by histological staging of the ovary and within a given stage by scoring proportions of follicular developmental phases. The ability of the liver to produce vitellogenin may be investigated by histological evaluation of basophilia and direct vitellogenin measurements. Distinguishing endocrine related changes in liver histology from pathology induced by other causes is therefore essential.

CASE PRESENTATIONS

CASE PRESENTATIONS

An unusual presentation of a pituitary gland tumor in a male Wistar rat

A 538 days old male Crl:WI(Han) rat died spontaneously. The rat was a control animal in an OECD 453 study (Combined Chronic Toxicity/ Carcinogenicity Study). Macroscopically a light red mass with a diameter of 11 mm was detected. The mass compressed the surrounding brain tissue. The mass was processed histotechnically and stained with HE. Additionally, the sample was stained for the following immunohistochemical markers: ACTH, CD68, GFAP, MSH, Prolactin, S100 and TSH.

Unusual lung findings in a 6 months old beagle dog

One 6 months old, male Beagle dog died spontaneously 14 days after arrival from breeder. During necropsy, lung was less collapsed and revealed a dark red discoloration. Pleural as well as pericardial surface were covered by whitish-rough material. A blood clot seemed to be attached to the left atrioventricular valve. Thymus was reduced in size. No findings were reported for any other organ. For histopathologic evaluation we received the following organs: Lung with bronchi and lung-associated lymph nodes, heart with attached pericard and aorta, liver, gall bladder, kidneys, urinary bladder, spleen and thymus. Further samples were taken from the pleural cavity for additional microbiological examination and thus, possible pathogen detection.

CASE PRESENTATIONS

A compliant and scalable digital workflow for accelerating pre-clinical studies using Concentriq for Research

Author: Dr. Bettina Lawrenz, Pathology and Clinical Pathology, Bayer AG
Co-author: Luca Caneparo, Global Application Specialist, Proscia

Introduction

Traditional approach to peer review for non-clinical studies involves a second person review of the slide, data, and interpretation requiring either the slides to be shipped to the pathologist or for the pathologist to travel for this evaluation. A digital peer review using a competent digital pathology solution enables organizations to streamline and improve efficiency significantly. However, ensuring compliance with the requirements to validate the digital pathology system for use in regulated non-clinical environment (CFR Title 21 Part 11, GLP documentation) has been a challenge for many labs.

Approach

Proscia's AI-powered digital pathology platform, Concentriq® for Research, is designed to provide support for a GLP-compliant peer review of non-clinical studies. This has been achieved by the implementation of several technical controls within Concentriq, a platform that is used by several Pharma and CROs that are intricately involved in evaluating drug candidates for safety and efficacy through their preclinical laboratory studies.

Results

The Concentriq platform was utilized to test several key aspects that are essential for compliant, digital peer-review including seamless viewing experience, live collaboration possibilities, flexibility to manage and view metadata, security, scalability, and controls (audit logs, roles & permissions) and found to fit the needs quite adequately.

Conclusion

This presentation shall highlight the capabilities of Concentriq designed to meet certain validation criteria that are essential for a GLP-compliant peer review of non-clinical safety assessment studies, as well as highlight the additional services offered by the Proscia to help ensure Concentriq is validated to meet these important compliance criteria.

POSTER PRESENTATIONS

POSTER PRESENTATIONS

P01 | Quantification of Olney Lesions using Deep Learning

Erio Barale-Thomas [1], Fauve Versaevel [1], Jogile Kuklyte [2], Fetene Tekle [1]
[1] Janssen R&D Beerse Belgium
[2] Deciphex Dublin Ireland

Introduction

Detection of Olney lesions (neuronal vacuolation and necrosis in the retrosplenial cortex (RC)) is an important part of evaluating NMDA receptor antagonists. Previously, we showed that brain levels 3 & 4 (preparing sections according to [1] and correlating them to specific planes in [2]) allowed the detection of both changes accurately, on H&E glass slides with a semiquantitative scale.

Methods

Here, we describe a deep learning (DL) method combining 4 independent approaches (brain level identification; RC selection; pixel segmentation for vacuoles at 6 hours PD and necrosis at 7 day PD; quantification) which allow a semi-automatic quantified assessment of the changes.

Results

We also show the pitfalls that affect the accuracy of the results (like slide artefacts) and provide the methods to overcome them. We explain how we compared the results of the glass slide evaluation (“slide-level ground truth”) to the results of the DL method using a statistical analysis approach.

Conclusion

We conclude by discussing the advantages of deep learning methods for toxicologic pathology (quantification and throughput); and the change of paradigm in the pathologist’s workflow (analysis of results with statistical methods; toleration of some errors by the DL method once statistical significance is attained; need to improve the slide quality). Lastly, we present the potential for the generalization of the approach to detect necrotic neurons in other brain areas; detect vacuoles in different organs; and apply the developed approach to the other species.

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POSTER PRESENTATIONS

P02 | Can Machine Learning predict the presence/absence of histological lesions in rat livers based on Clinical Chemistry? An exploratory study

Marco Tecilla [1], Benjamin Gutierrez Becker [2], Nikolaos Berntenis [2], Maria Cristina De Vera Mudry [2]
[1] *Roche Basel Switzerland*
[2] *Roche Switzerland*

Introduction
Clinical chemistry (CC) is routinely performed in toxicologic pathology assessment. Nevertheless, very little is known about the value of these data in predicting histological changes using machine learning (ML).
This study investigates the use of ML on CC data to predict the presence/absence of histopathological findings in rat livers.

Methods
CC and histopathology diagnoses from 600 rat studies over 32 years were retrieved from the archive. Reference value-based location-scale model was used to normalize numerical values; non-numerical were encoded.
Liver histopathological findings were graded according to a 1-5 severity system. Findings were considered as a score of 1 (present) or 0 (absent) if findings severity ≥ 2 or ≤ 1, respectively; if multiple liver findings, the most severe was considered.
Training and testing were split based on study ID and fed into a Random forest using histopathology score as target variable.

Results
The dataset was composed of 44087 entries, with 20 CC parameters (9 liver-specific) each, which originated from 42.51% female and 57.49% male rats. Females with histopathological findings were 82.31%, while males were 76.33%. Prediction results were, AUC of 0.80 (±0.02), Accuracy 0.76 (±0.02), F1 score 0.66 (±0.04), Precision 0.77 (±0.05), and Recall 0.58 (±0.05).

Conclusion
ML can help predict the presence/absence of liver histopathological findings in rats using CC in this dataset. Additional investigations are needed to understand the generalizability and extension to other datasets and organs (e.g., kidneys).

POSTER PRESENTATIONS

P03 | Retinal developmental neurotoxicity of trimethyltin chloride: in terms of excitotoxicity and excitatory amino acid transporters

Jae Hak PARK[1], Jin Kim[2]
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[2] *KHAV Seoul South Korea*

Introduction

Trimethyltin chloride (TMT), which is an organotin compound widely used in the agricultural and industrial fields, is a well-known neurotoxicant. In particular, excitotoxicity is suspected to be an important mechanism underlying TMT toxicity; however, the effect of TMT exposure on the retina during development and the mechanisms have not been fully elucidated to date.

Methods

Therefore, in this study, we exposed postnatal ICR mice (male and female) to TMT and performed a comprehensive analysis of the retina in terms of developmental abnormalities, histopathology, apoptosis, electrophysiological function, glutamate concentration, gene expression, and fluorescence immunostaining.

Results

Exposure to 0.75 and 1.5 mg/kg of TMT up to postnatal day 14 caused a decrease in body weight and length, delayed eye opening, and induced thinning of the inner nuclear layer of the retina. In addition, apoptosis was observed in the retinal layer along with b-wave changes and a decrease in retinal ganglion cell spiking activity in the micro-electroretinogram. This change was accompanied by an increase in the concentration of glutamate in the retina, upregulation of astrocyte-related genes, and increased expression of excitatory amino acid transporter (EAAT) 1 and 2. Conversely, EAAT 3, 4, and 5, located in the retinal neurons, were decreased, and this was consistent with the immunostaining results.

Conclusion

Our results are the first to prove that TMT induces excitotoxicity and changes in EAAT expression in the retina, and this mechanism causes functional as well as morphological retinal developmental toxicity.

POSTER PRESENTATIONS

P04 | Organ identifcation with artificial intelligence using multiple magnifications in preclinical pathology studies

Fernando Romero-Palomo[1], Citlalli Gamez Serna [2], F ilippo Arcadu [3], Jürgen Funk [1], Vanessa Schumacher[1] , Andrew Janowczyk [4]

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Introduction

Identifying organs within histology images is a fundamental and non-trivial step in toxicological digital pathology workflows as multiple organs often appear on the same whole slide image (WSI). Previous work in automated tissue classification has investigated the use of single magnifications, and demonstrated limitations when attempting to identify small and contiguous organs at low magnifications. In order to overcome these shortcomings, we present a multi-magnification convolutional neural network which employs broad context along with fine cellular detail from different magnifications to facilitate the recognition of complex organs.

Methods

For this study, we used n=320 slides generated at 3 contract research organization (CRO) laboratories, digitized to WSI using 2 scanner models. These WSI contained rat organs with and without histopathological findings. We developed artificial intelligence (AI) models that make use of multiple magnifications simultaneously to recognize 7 organs: liver, kidney, thyroid gland, parathyroid gland, urinary bladder, salivary gland, and mandibular lymph node. Our models generated visualization masks that ensured separation of organs in close proximity (e.g., thyroid vs parathyroid glands).

Results

We demonstrated state-of-the-art organ detection and segmentation performance (AUROC=0.99~1.0 for all organs, Dice≥0.9 except parathyroid (0.73)). Evaluation of our results at both inter and intra CRO levels, suggest strong generalizability performance.

Conclusion

These models offer a potential quality control tool to validate WSI organ metadata and can also serve as a preprocessing step for selective invocation of subsequent organ-specific AI use cases.

POSTER PRESENTATIONS

P05 | An E2E platform for digitale pathology: seamless journey from the lab to images and data analytics

Shanon Seger, Moritz Gilsdorf, Hoffmann-La-Roche Ltd Basel Switzerland

Introduction

At Roche Pharma Research & Early Development we created a digitally underpinned workflow from the tissue laboratory to images and analytical data. It required changing our ways of working and integration of IT solutions to gain efficiency and build the necessary capabilities to leverage our imaging and pathology data

Methods

The platform is composed of 5 components: A LIMS (Laboratory Information Management System), an image management system, a commercial universal image viewer,Toxicologic Pathology peer review software and a suite of advanced image analysis solutions (Python based script, HALO and visiopharm).

Results

Today, 2 years after the start of the project, we have a user-focused integrated landscape of Digital Pathology solutions tailored for pre-clinical studies and tissue-based laboratory workflows.

Conclusion

Among other things, the platform allows us to efficiently collaborate across sites, with external pathology consultants and with CROs (contract research organizations). With the newly implemented digital ways of working, our scientists and collaborators can work either onsite or remotely. Finally, the platform enables us to re-use our images, data and pathology findings in a F.A.I.R. (Findable, Accessible, Interoperable, Reusable) manner. This FAIR imaging database can be leveraged to initiate meaningful AI projects.

POSTER PRESENTATIONS

P06 | AI made quick and easy: How to train a deep learning algorithm for thyroid follicular cell hypertrophy in passing

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Introduction

Semiquantitative evaluation of thyroid follicular cell hypertrophy, one of the most common findings in rodent toxicity studies, requires the pathologist to consistently threshold and grade within one study - and preferably across studies – against a variable background morphology. This repetitive task may become strenuous and time consuming, often requiring "blinded" review. Automated assessment using artificial intelligence can add value to the pathologist's results by serving as a decision help and/or by adding a quantitative value to semiquantitative and subjective grading.

Methods

We describe an uncomplicated approach to train a deep learning application to detect follicular cell hypertrophy. The application was trained on 14 sections of entire, bilateral rat thyroid gland tissue, that was annotated as either normal (6 cases) or hypertrophic (6 cases) or borderline (2 cases) according to the original diagnosis.

Results

Given the simplicity of the approach, results in a test set consisting of 124 hypertrophic and normal thyroids were remarkable: whereas not all regions in all thyroids were labelled correctly from a pathologist's point of view, the percentage of "hypertrophic" versus "normal" areas within one thyroid corresponded well to the original diagnosis and grading.

Conclusion

As acknowledged by Bertani et al. (Toxicologic Pathology 2022, Vol. 50(1) 23-34), approaching thyroid hypertrophy with artificial intelligence is a useful supporting tool for the study pathologist. It may contribute to develop an objective relation between percentage of hypertrophic tissue and grading. In addition, uncomplicated approaches help to familiarize with self-training of a deep learning algorithm and to overcome reservations toward these new methods.

POSTER PRESENTATIONS

P07 | Lectin histochemistry as a tool to visualize glycosylation pattern changes in the repiratory tract of SARS-CoV-2 infected Syrian hamsters

Lea-Adriana Keller [1], Björn-Patrick Mohl [2], Sophia Harder [1], Claudia Blaurock [2], Angele Breithaupt [2], Anne Balkema-Buschmann [2], Andreas Popp [1]
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Introduction

After 2 years of fighting the SARS-CoV-2 induced COVID-19 disease, still not all parameters and pathophysiologic processes are fully elucidated. Viral entry and tropism are highly dependent on the glycan-residues on cells and the mucosal layer, not only for SARS-CoV-2, but also for other viral infections. Glycosylation is a post translational modification which is pivotal for the structure and function of glyco-conjugates. It was shown that the glycosylation pattern of glyco-conjugates such as mucins is altered under disease conditions.

Methods

Lectin histochemistry is known to be a suitable tool to visualize and examine these changes. Lectins with different glycan-specificities were used for the evaluation of the glycosylation pattern changes in the respiratory tract of SARS-CoV-2 infected Syrian hamsters.

Results

Our study revealed that the glycosylation pattern was changed during the SARS-CoV-2 infection and pathological findings were related. Lectins such as LEL and STL showed damage to pneumocytes type I while for example GSLI visualised the loss of pneumocytes type II in infected hamster lungs. Increased lectin staining of UEAI was colocalized with KI67, a proliferation marker. During SARS-CoV-2 infection an infiltration of mononuclear cells was described. Therefore, specific immune cell markers such as Iba1 and CD68 were used for double staining with selected lectins, for example GSLI, LEL and WGA.

Conclusion

The elucidation of the glycosylation pattern of glyco-conjugates and surface mucins, such as MUC1, in the respiratory tract of healthy and infected hamsters will reveal physiological and pathological aspects of the disease and will open up new possibilities for therapeutic development.

POSTER PRESENTATIONS

P08 | Spontaneous neoplasms in the uterus, vagina and cervix of rats from 104-week carcinogenicity studies

Marcia Pereira Bacares
Labcorp Drug Development Chantilly United Statess

Introduction

Carcinogenicity studies are important in identifying tumorigenic potential in animals as part of the risk assessment for new drugs. Historical control incidences are important considerations when interpreting data and useful when selecting and assessing the suitability of an animal model. This study presents and compares the incidence of neoplastic findings in three different rat models: Crl:CD(SD), Wistar Han, and Hsd:Sprague Dawley.

Methods

The incidences of tumors in the uterus, cervix and vagina were reviewed for control Crl:CD(SD) (2779-2782 females/34 studies), Wistar Han® (785-786 females/9 studies), and Hsd:Sprague Dawley®SD® rats (548-550 females/7 studies) conducted at Labcorp Drug Development during the last 10 years. Tumors were categorized as common (incidence: more than 1%) or rare (incidence: 1% or less).

Results

Endometrial stromal polyp and carcinoma were common neoplasms in Hsd:Sprague Dawley rats. Rare neoplasms included glandular polyp, hemangioma, adenoma, benign granular cell tumor, squamous cell papilloma and carcinoma, stromal sarcoma, leiomyosarcoma, malignant schwannoma, and sarcoma. Endometrial stromal polyp, malignant schwannoma, endometrial adenocarcinoma and carcinoma were common neoplasms in Harlan Han Wistar rats. Fibroma, leiomyoma and leiomyosarcoma, glandular polyp, squamous cell papilloma and carcinoma, adenoma, benign and malignant granular cell tumor, polyp, granular cell tumor, endometrial stromal sarcoma, carcinosarcoma, hemangiosarcoma, and sarcoma were considered rare. In Crl:CD(SD) rats, endometrial stromal polyp in the uterus and granular cell tumor in the vagina were common while hemangioperiocytoma, leiomyoma, glandular and vaginal polyp, hemangiosarcoma, leiomyosarcoma, benign and malignant schwannoma, stromal sarcoma, carcinoma, squamous cell carcinoma, and sarcoma were rare.

Conclusion

Benign and malignant neoplasms occurred in the uterus, cervix and vagina of all three rat strains, most neoplasms were considered rare. Endometrial stromal polyp was a common tumor among all three strains, while carcinoma was common in Han Wistar and Hsd:Sprague Dawley rats. Granular cell tumor was a common tumor in Crl:CD(SD) rats only and malignant schwannoma was a common neoplasm in Han Wistar rats only.

POSTER PRESENTATIONS

P09 | Spontaneous neoplasms in the uterus, vagina and cervix of mice from carcinogenicity studies

Marcia Pereira Bacares
Labcorp Drug Development Chantilly United Statess

Introduction

Carcinogenicity studies are important in identifying tumorigenic potential in animals as part of the risk assessment for new drugs. Historical control incidences are important considerations when interpreting data and useful when selecting and assessing the suitability of an animal model. This abstract presents and compares the incidence of neoplastic findings in two different mouse models: CD-1 and rasH2 transgenic.

Methods

The incidences of tumors in the uterus, cervix, and vagina were reviewed for 1567 to 1575 rasH2® control mice (47 studies) and for 416 to 417CD-1® IGS control mice (6 studies) conducted at Labcorp Drug Development, Madison during the last 10 years.

Results

Benign and malignant neoplasms occurred spontaneously in CD-1 and rasH2 transgenic mice. In CD-1 mice, endometrial stromal polyp was common in uterus but rare in the cervix and vagina. Hemangiosarcoma was common in uterus; and stromal sarcoma was common in the cervix and uterus. Rare benign neoplasms included hemangioma, leiomyoma, glandular polyp and keratoacanthoma in the uterus, cervix and/or vagina. No malignant neoplastic findings were noted in the vagina. In rasH2 transgenic mice, all neoplastic findings were rare. Neoplasms included glandular and endometrial polyp, adenoma, hemangioma, squamous cell papilloma, choriocarcinoma and hemangiosarcoma in the uterus and/or vagina. No neoplastic findings were noted in the cervix.

Conclusion

Historical control data help support interpretation of common and uncommon pathology findings and provides a reference for incidence of spontaneous findings. Tumor types were similar between CD-1 and rasH2 mice. However, in contrast to CD-1 mice, rasH2 transgenic mice spontaneous tumors in the uterus, cervix and vagina were considered rare.

POSTER PRESENTATIONS

P10 | Several cases of spontaneous type 2 Diabetes mellitus in stock cynomolgus monkeys

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Introduction

A number of different types of diabetes occur in nonhuman primates (NHPs), with type 2 diabetes mellitus (t2dm) being the most commonly reported. Clinical features of diabetes in NHPs are similar to those in humans (obesity, insulin resistance, dyslipidemia, pancreatic pathology and further co-morbid conditions). Type 1 diabetes mellitus has been reported in cynomolgus monkeys, but at a much lower frequency than t2dm. Experimental induction of diabetes has been achieved by partial or total pancreatectomy, alloxan, or streptozotocin administration, with the latter being the drug of choice for induction of diabetes in NHPs. Gestational diabetes mellitus has also been reported in cynomolgus and rhesus monkeys with similar complications (macrosomic infants, risk of future t2dm) as in humans.

Methods

N.A.

Results

Five spontaneous cases of type 2 diabetes mellitus in male cynomolgus monkeys ranging in age from 8 to 19 years will be presented. In addition to the classic clinical and pathological characteristics of type 2 diabetes mellitus (obesity, hyperglycemia, pancreatic islet amyloidosis, hepatic macrovesicular lipidosis, diabetic nephropathy, cataract), the four oldest animals (≥ 11 years) also display considerable myocardial changes (fragmentation/vacuolation/degeneration of cardiomyocytes, karyomegaly, fibrosis, steatosis cordis) consistent with diabetic cardiomyopathy, which's morphology is still poorly described in the literature.

Conclusion

NHPs represent a useful animal model to study the pathogenesis of diabetes, risk factors associated with t2dm, and many of the co-morbidities associated with it. The presented cases contribute to a better characterization of cardiac changes in NHPs, in order to support preclinical and translational investigations in t2dm and heart failure.

POSTER PRESENTATIONS

P11 | AI based decision support tool targeted for more efficient review of short term studies

Jogile Kuklyte [1], Eoghan Keany [1], Ross Quigley [1], Trevor D. McKee 1, Linda Mudford [1], Lise Bertrand [2], Dan Rudmann [2], Mark Gregson [1], Shane Ryan [1], Donal O'Shea [1]
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Introduction

Drug development compound screening leverages animal studies to filter out candidates demonstrating highest potential for human clinical trials. Standard study designs screen key organs for the presence of affected areas (lesions) indicating potential safety concerns. Decision support tools highlighting these lesions can improve speed and efficiency of slide review.

Methods

Pixel segmentation classifiers were developed for six organs: Liver, Kidney, Heart, Lung, Thyroid and Thymus covering 20 common lesions seen in short term studies. Lesion examples were annotated by pathologists at relevant magnification across 100 slides, selected broadly across studies to cover variations observed. The algorithm development incorporated quantitative and qualitative validation, with study level cross validation providing quantitative metrics to estimate generalisation of the classifier to different study, tissue processing or scanning protocols.

Results

Pixel level validation of the results evaluated on unseen data showed high accuracy at each level of validation. F1 scores, as well as Precision and Sensitivity for each lesion were evaluated, with the baseline goal of an F1 score at least above 0.7 at the pixel level. Sensitivity metric was used as the main criteria when evaluating lesions detection performance. Different levels of Precision were deemed acceptable depending on the lesion, with acceptance criteria determined by the pathologists participating in the annotation process.

Conclusion

Visualising highlighted regions in the study overview allows pathologists to rapidly identify organs and treatment groups with significant deviations from controls. A lesion mask overlay provides decision support information that helps to faster and more consistently estimate the severity of the lesion present.

POSTER PRESENTATIONS

P12 | Therapeutic oligonucleotides: A restrospective evaluation of non-clinical toxicology data obtained at Charles River Laboratories France

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Introduction

RNA-targeting oligonucleotides (RTO) constitute a promising class of therapeutics for the potential treatment of otherwise undruggable diseases.

Methods

A retrospective review was carried out from approximately 90 studies performed at two Charles River Laboratories sites in France during the last 15 years using different species (rat, mouse and non-human primate). The database includes therapeutic areas, chemistry platform of the tested oligonucleotides, experimental study design (study duration and route of administration), and data collected from toxicokinetic, clinical and anatomo-pathological evaluations, mainly with antisense oligonucleotides administered by the intravenous or subcutaneous route.

Results

Unscheduled termination was reported in 14% of studies, generally related to kidney or liver findings (mostly in rodents) and systemic inflammation (mostly in NHPs).
RTO-related clinical pathology changes included liver findings (increased transaminases in 40% of the studies, mostly in NHP), renal findings (increased creatinine and urea in 30% of the studies, mostly in rats), and hematology findings (thrombocytopenia in 30% of the studies, mostly in rodents). Complement activation was noted in 80% of NHP studies.
Gross findings were observed at the injection site, kidney, liver, and spleen. Microscopically, basophilic granules were observed in the kidney, liver, lymph nodes and spleen, and were considered to reflect the accumulation of the oligonucleotides. Degenerative findings were observed in the liver (80% of NHP studies, 90% in mice, 80% in rats) and kidney (85% in NHP, 75% in mice, 90% in rats).

Conclusion

The results of this retrospective review confirm that there are both similarities and species differences in the toxicity profile of RTOs in laboratory animal species.

POSTER PRESENTATIONS

P13 | A central repository of digital pathology slides to boost the development of AI

Julie Boisclair [1], Gabriele Pohlmeyer-Esch [2], Anna Lena Frisk [3], Thomas-Barale Erio [3], Fauve Versaevel [3], Moritz Radbruch [4], Brian Knight [5], Pierre Maliver [6], Xavier Palazzi [7], Caitriona Lyons [8], Holger Hoefling [9]

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Bigpicture, a pathology-led Innovative Medicines Initiative (IMI) consortium, has the vision to become the catalyst in digital transformation in toxicologic and human pathology. To achieve this vision, Europe's leaders in the field of computational pathology as well as digitalised pathology collections were brought together with 10 European Federation of Pharmaceutical Industries (EFPIA) partners. Our consortium is further strengthened by the presence of the European Society of Pathology, Digital Pathology Association, FDA and small and medium-sized enterprises (SMEs) as partners, and supported by patient advocates. Our mission is to create the first European General Data Protection Regulation (GDPR) compliant platform, in which quality-controlled Whole Slide Images (WSI) and advanced artificial intelligence (AI) algorithms will co-exist. The Bigpicture platform is built on existing assets of EU data infrastructure, including the federated technology for managing the exchange of confidential information. The IMI Bigpicture repository will host the largest and most diverse human and nonclinical WSI collection in the EU. The consortium will use Cytomine, an open-source framework to develop unique tools for access to WSI, including annotations and visualisation of algorithm results, while generic models to facilitate AI development and mining of WSI data will be developed. By engaging and building consensus with all the relevant stakeholders, this consortium will contribute to the development of a regulatory framework for digital and computational use in the field of pathology. Finally, Bigpicture envisions sustainability of its platform through a community-based model which relies on reciprocity, value creation and inclusiveness.

POSTER PRESENTATIONS

P14 | Assessment of preclinical developmental neuropathology using image analysis

Michael Staup [1], Joost Lensen [2], Odete Mendes [2], Aleksandra Zuraw [2], Virginie Piccicuto [2], Tiwanda Masinde [2], Catherine Picut [2], Kyle Takayama [2], Erica Ramaker [2]
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Introduction

The assessment of toxicological effects in the developing brain is a regulatory requirement for the pharmaceutical, industrial and agrochemical industries. These data are used, in conjunction with histopathology, to evaluate developmental neurotoxicity. Current methodology relies on a limited number of manual linear morphometric measurements that is intrinsically associated with procedural variability and bias. We present an automated solution using digitally scanned PND 21/22 and PND 71/73 rat brain samples.

Methods

A GLP (Good Laboratory Practices)-validated commercial artificial intelligence-based image analysis platform (Visiopharm®) was used to train a convolutional neural network (CNN)-based supervised deep learning model to automatically detect and measure relevant brain regions: neocortex, caudate putamen, corpus callosum, hippocampus and cerebellum. Standard morphometric measurements were automated and compared to the traditional manual measurements of these regions. Further, the area of the automatically segmented regions was used to perform a Cavalieri estimate of the total volume of these brain regions.

Results

Linear measurements automatically captured from AI generated classifications correlate well with manual measurements, and show increased precision. Area measurements do not correlate as well with the manual measurements, but generate much more precise data when paired with the more accurate Cavalieri method of volume estimation of these brain regions.

Conclusion

The automated methods presented here are highly reproducible and precise. They increase the number of quantitative endpoints collected, decrease analytical turn-around time, and improve the ability to detect morphometric changes in homologous sections, providing an invaluable regulatory compliant asset for decision-making across industries.

POSTER PRESENTATIONS

P15 | Background vacuolation of the epithelium in the Isolated chicken cornea

Peter Maslej[1], Réka Horváth[2], David John Esdaile[2], Kata Tóth-Gönczöl[2], Balázs Orovecz[2], Judit Hargitai[2]
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Introduction

The Enucleated Eye Test with isolated eyes of the chicken (harvested *post mortem*) has been recognized as a valuable alternative to the Draize eye irritation test regarding ocular corrosivity or severe eye irritancy testing. In the Isolated Chicken Eye Test (ICET), vacuolation of the corneal epithelium is the most frequently observed change, both alone and accompanying erosion/necrosis . Based on our experience (over 1000 examined eyes), vacuolation plays a key role in the final categorization of the administered compound.

Methods

Test Guideline OECD 438, 2018 is used. At Charles River Laboratories Hungary, we process the cornea in 5µm sections, and stain with Hematoxylin-Eosin/ Phloxine as standard. Vacuolation is graded as very slight/slight/moderate/severe.

Results

The negative control sections usually have 6-7 layers of corneal epithelial cells. Various sizes of small vacuoles usually distributed close to the basement membrane are recognized in the normal cornea. The poster describes the distinction between normal vacuolation and that induced by test items.

Conclusion

Vacuolation of the corneal epithelium may have an effect on the final classification of the test item. Digitalization of the cornea sections could help with routine histopathological evaluation in the near future, especially with borderline lesions.

POSTER PRESENTATIONS

P16 | How to Convey the Relevance of Clinical Pathology Changes

Results from the 9th ESTP International Expert Workshop

Pohlmeyer-Esch Gabriele, Arndt Tara, Tomlinson Lindsay, Keresztes Monika, and expert members of the 9th ESTP International Expert Workshop

In an effort to better align the positioning of clinical pathology findings in reports and regulatory documents amongst the global toxicologic (clinical) pathology community, a European Society of Toxicologic Pathology (ESTP) Workshop was formed to discuss the inconsistent utilization of terms such as “biologic relevance” and “toxicologic relevance”.

The workshop built upon previous ESTP workshops and published literature that addresses some, but not all, clinical pathology terminology. Twenty-four international experts in clinical pathology spanning the pharmaceutical and chemical industries, contract research organizations, and regulatory authorities met for 12 preparatory videoconferences and an online interactive workshop webinar to address the discrepant use of these and similar terms.

Expert discussions helped to add structure to the process of data assessment and reporting, and to the use of terms like “toxicologically relevant” or “biologically relevant”, acknowledging that there is no formula or method that can be applied to all descriptive decisions for clinical pathology. An algorithm that can be used to position effects on test systems was proposed and discussed at the workshop.

A manuscript summarizing workshop results is expected to be published in 2023

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